

# Antibiotic resistance genes detected in lichens: insights from *Cladonia stellaris*

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## Running title

Lichens as reservoirs of antibiotic resistance

## 21 **ABSTRACT**

### 22 **Background and Aims**

23 Antibiotics are natural compounds produced by microorganisms that have long existed in  
24 ecosystems. However, the widespread clinical and agricultural use of antibiotics has intensified  
25 selective pressures on bacteria, leading to the proliferation of antibiotic resistance genes (ARGs).  
26 The increasing prevalence of these genetic elements in clinical and environmental settings now  
27 poses a major global health threat. While ARGs are well documented in anthropogenically  
28 influenced environments, their distribution and origins in remote ecosystems, such as the boreal  
29 forests, remain poorly understood. Here, we investigate the occurrence, diversity, and potential  
30 origins of ARGs in the boreal lichen *Cladonia stellaris*.

### 31 **Methods**

32 We conducted the first targeted assessment of ARGs in lichens by analyzing 42 *C. stellaris* samples  
33 from northern and southern lichen woodlands (LWs) in eastern Canada. Using high-throughput  
34 quantitative PCR, we screened for 33 ARGs and three mobile genetic elements (MGEs), quantifying  
35 their relative abundance. Bacterial community composition was characterized via 16S rRNA gene  
36 sequencing. Statistical analyses evaluated geographical patterns, co-occurrence between ARGs and  
37 bacterial taxa, and the influence of latitude on ARG distribution.

### 38 **Key Results**

39 Ten ARGs conferring resistance to four antibiotic classes (aminoglycosides, beta-lactams,  
40 quinolones and sulfonamides), along with one MGE, were detected. The ARGs *blaCTX-M-1*,  
41 *qnrB*, and *qepA* were highly prevalent, with *qepA* often surpassing 16S rRNA gene abundance.  
42 Only *qnrB* showed significantly higher abundance in southern samples. Latitude significantly  
43 influenced ARG profiles, whereas bacterial community composition did not.

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## 44 **Conclusions**

45 Our findings demonstrate that *C. stellaris* harbors diverse ARGs in remote boreal ecosystems  
46 with limited anthropogenic influence. Proposed explanations for ARG presence include long-  
47 distance dispersal via bioaerosols and endogenous development within lichen microbiomes, yet  
48 these remain speculative. Future work incorporating bacterial isolation, whole-genome  
49 sequencing, metatranscriptomics, air sampling, and metabolomic profiling is necessary to  
50 unravel the ecology and evolution of ARGs in natural habitats.

51

## 52 **Key words**

53 Antibiotic resistance genes, beta-lactamase, bioaerosols, boreal ecosystems, *Cladonia stellaris*,  
54 coevolution, lichen microbiome, lichen woodlands, mobile genetic elements, quinolone resistance.

55

## 56 **Introduction**

57 Antibiotics are a class of secondary metabolites naturally produced by microorganisms, such as  
58 bacteria or fungi, or chemically synthesized analogous compounds (Demain and Sanchez 2009).  
59 In nature, they serve various ecological roles, acting as signaling molecules and defense  
60 mechanisms. They provide producing organisms with a competitive advantage by inhibiting the  
61 growth of rival microbes, thus securing resources and space (Waksman and Woodruff 1940).  
62 According to the arms-shield hypothesis, the production of antibiotics and the development of  
63 resistance to them is an ongoing evolutionary battle (Han *et al.* 2022). Some microorganisms  
64 have evolved the ability to produce antibiotics as a means of defense, while their competitors  
65 have developed resistance mechanisms through natural selection, enabling them to survive

66 antibiotic exposure. This resistance capability is conferred by antibiotic resistance genes (ARGs),  
67 which are segments of DNA encoding proteins that enable bacteria to survive exposure to  
68 antibiotics. ARGs can develop intrinsically within the bacterial genome or acquired through  
69 horizontal gene transfer (HGT) (Thomas and Nielsen 2005; Barlow 2009) from other bacteria via  
70 mobile genetic elements (MGEs) often associated with plasmids (Partridge *et al.* 2018; Razavi *et*  
71 *al.* 2020; Ni *et al.* 2023), or via transduction from bacteriophages (Balcazar 2014; Penadés *et al.*  
72 2015). Their existence in the environments predates the anthropogenic use of antibiotics (Wright  
73 2007; Martínez 2008) with evolutionary evidence tracing their development from thousands  
74 (D'costa *et al.* 2011) to billions of years (Hall and Barlow 2004).

75 Since the 1940s, when antibiotics began to be used in medicine, their application has  
76 expanded to sectors such as agriculture (e.g. Anderson and Gottlieb 1952; McManus *et al.* 2002),  
77 aquaculture (e.g. Cabello 2006; Chen *et al.* 2020), and animal husbandry (e.g. Hong *et al.* 2013;  
78 Busch *et al.* 2020; Karwowska 2024). Such extensive, and at times inappropriate, use of  
79 antibiotics has led to the proliferation of ARGs and ARG-carrying bacteria in both natural and  
80 anthropogenic ecosystems. The World Health Organization has identified antibiotic resistance as  
81 one of the top public health threats of the 21<sup>st</sup> century. In 2019 alone, over 4.95 million deaths  
82 were linked to antibiotic resistance, with approximately 1.27 million directly attributed to  
83 infections caused by antibiotic-resistant bacteria (Murray *et al.* 2022). At the national level,  
84 antimicrobial resistance was responsible for the deaths of approximately 5,400 Canadians in 2018.  
85 If current trends continue, resistance rates are projected to increase from 26% in 2018 to 40% and  
86 potentially up to 396,000 cumulative deaths by 2050, with economic losses ranging from 13 to 21  
87 billion CAD (Council of Canadian Academies 2019).

88 The spread of ARGs in the environment occurs not only through local contamination near  
89 anthropogenic sources, such as the direct release of ARG-carrying bacteria via wastewater  
90 discharge or agricultural runoff (Almakki *et al.* 2019; Junaid *et al.* 2022), but also by atmospheric  
91 processes that enable their dispersal over much greater distances. Bioaerosols, which are airborne  
92 particles of biological origin encompassing bacteria, viruses, and fungi, can carry ARGs over  
93 distances ranging from meters to hundreds of kilometers (Brunet *et al.* 2017; Griffin *et al.* 2017).  
94 Bioaerosols are transported by atmospheric currents (Jin *et al.* 2022; Wang *et al.* 2022), before  
95 settling out of the air through dry deposition (gravity-driven settling onto surfaces) or precipitation  
96 into terrestrial or aquatic environments (Di Cesare *et al.* 2017; O'Malley *et al.* 2023). Such  
97 deposition can reach even remote ecosystems, which thus become valuable settings for  
98 investigating the potential long-distance dispersal of ARGs. In this context, LWs offer a suitable  
99 system, as their open canopies provide little physical obstruction to airborne particles, allowing  
100 microorganisms suspended in the atmosphere to settle directly onto the ground layer. LWs cover  
101 approximately 2 million km<sup>2</sup> of Canada, including nearly 300,000 km<sup>2</sup> in the province of Quebec  
102 (Payette and Delwaide 2018). They are situated north of the closed-crown forest zone and south  
103 of the forest-tundra zone, between 52.00°N and 55.00°N, in remote regions characterized by low  
104 human population density and limited anthropogenic activities (Bhiry *et al.* 2011). An exception  
105 to the typical distribution of LWs occurs in Parc National des Grands-Jardins, Quebec (PNGJ;  
106 47.68°N, 70.85°W), where a LW is found 500 km south of its usual range (Jasinski and Payette  
107 2005).

108 Within LWs, the ground layer is dominated by a continuous and exposed cover of lichens  
109 (Payette *et al.* 2001; Girard *et al.* 2017), with *Cladonia stellaris* (Opiz) Pouzar & Vězda being  
110 the dominant and most representative species. Lichens are well known for their ability to absorb

111 and accumulate substances from their surrounding environment, including airborne particles.  
112 Beyond their role as passive accumulators, lichens also produce a variety of antimicrobial  
113 compounds (Burkholder *et al.* 1944; Yilmaz *et al.* 2004; Rankovič *et al.* 2010; Shrestha and St.  
114 Clair 2013), which exert selective pressure on their associated bacterial communities (Grube *et al.*  
115 2009; Grimm *et al.* 2021), and may promote the development of resistance, as proposed for the  
116 lichen species *Rhizocarpon geographicum* (Miral *et al.* 2022). In this context, LWs provide a  
117 unique system to investigate ARG dynamics, encompassing both the deposition of airborne ARGs  
118 and the potential development of endogenous resistance mechanisms within lichen-associated  
119 bacterial communities.

120 To investigate these patterns, we conducted the first targeted assessment of ARGs in  
121 lichen samples using high-throughput quantitative PCR (HT-qPCR). The objectives of this study  
122 are to i) test the presence of ARGs in lichens from both northern and southern LWs, ii) quantify  
123 their abundance, iii) compare the diversity and abundance of ARGs between northern and  
124 southern LWs, and iv) explore the potential relationship (correlations) between their ARG  
125 profiles and constituent bacterial communities. To frame our investigation, we consider two  
126 potential explanations for the presence of bacteria-carrying ARGs in *C. stellaris*: first, that long-  
127 distance dispersal by bioaerosols introduces ARGs to LWs, by transporting bacteria-carrying  
128 ARGs from urban and agricultural environments where antibiotics are frequently used (Kormos  
129 *et al.* 2022; George *et al.* 2022); and second, that ARGs in lichen-associated communities may  
130 be indigenous, developing as a response to selective pressures imposed by the antimicrobial  
131 compounds produced by lichens (Francolini *et al.* 2004; Goga *et al.* 2021; Zorrilla *et al.* 2022).  
132 As this is the first exploratory assessment of ARGs in lichens, these explanations remain  
133 hypotheses that will need to be tested in future targeted studies.

# 134 Material and methods

## 135 Study areas and sampling

136 This study builds upon data previously collected by Alonso-García et al. (2021); Alonso-García  
137 and Villarreal A. (2022), which investigated the factors shaping the bacterial community  
138 composition of lichens across Quebec, Canada. *Cladonia stellaris* samples were collected from  
139 two locations: the first near Kuujjuarapik-Whapmagoostui (55.28, -77.75) in the north and from  
140 PNGJ (47.68, -70.85) in the south. Figure 1 shows the LW ecosystem and a close-up of the  
141 lichen species. Kuujjuarapik-Whapmagoostui has a mean annual temperature of -3.6°C and a  
142 mean annual precipitation of 640 mm (Prairie Climate Centre University of Winnipeg 2022). In  
143 this region, LWs develop on sandy terraces and wind-affected areas, where *Picea glauca*  
144 (Moench) Voss and *Picea mariana* (Mill.) Britton, Sterns & Poggenb. coexist with a ground  
145 cover dominated by *C. stellaris* and ericaceous shrubs such *Betula glandulosa* Michx.,  
146 *Rhododendron groenlandicum* Retzius, and *Salix glauca* L. (Bhiry et al. 2011). These open  
147 forests grow on acidic, nutrient-poor, well-drained soils (Payette 1993; Payette et al. 2001).

148 The PNGJ has a mean annual temperature of 2°C, and an annual precipitation of  
149 approximately 1200 mm (Payette et al. 2000; Prairie Climate Centre University of Winnipeg  
150 2022). The park features a mosaic of closed-crown spruce-moss forests and open-crown lichen  
151 woodlands dominated by *P. mariana*, Ericaceae species and *C. stellaris* (Jasinski and Payette  
152 2005), thriving on acidic, nutrient-depleted moraine-derived soils and granitic outcrops (Payette  
153 1992; Payette and Morneau 1993). Although both regions are relatively remote compared to  
154 urban centers, potential sources of anthropogenic contamination exist near both study sites. In  
155 Kuujjuarapik-Whapmagoostui, there is a possibility that domestic waste, healthcare services, and  
156 potential wastewater seepage may introduce antibiotics or resistant organisms into nearby

157 ecosystems. The PNGJ has protected status, restricting human activities. However, it is located  
158 within the Charlevoix region, where agriculture and livestock farming are widespread (MRC de  
159 Charlevoix 2022), and its proximity to the town of Baie-Saint-Paul (less than 30 km away),  
160 could expose the park to anthropogenic contaminants.

161 Sampling was performed in 2018 using sterilized steel forceps, targeting thalli of *C.*  
162 *stellaris*, which were immediately stored at -20°C. Further methodological details are available  
163 in Alonso-García et al. (2021). In the present study, 42 samples were selected for analyses: 18  
164 from seven sites around Kuujjuarapik-Whapmagoostui (northern LW), and 24 from PNGJ  
165 (southern LW) (Table S1). Each sample corresponded to one individual thallus, with no technical  
166 replicates performed.

167

## 168 Data processing and analysis of 16S rRNA genes

169 To characterize bacterial community composition, we used amplicons of V3-V4 region of the 16S  
170 rRNA gene generated by Alonso-García and Villarreal A., (2022). These data were passed through  
171 an established DADA2 pipeline (Callahan *et al.* 2016) in the R version 4.4.2 (R Core Team 2021)  
172 for processing amplicon sequence variants (ASVs, Callahan et al. 2017). We transformed ASV  
173 counts to relative abundances using the phyloseq package version 1.38 (McMurdie and Holmes  
174 2013) for comparisons of biodiversity between northern and southern LWs. To calculate the  
175 Shannon diversity index, we first normalized the ASV table using the DESeq2 package version 1.38  
176 (Love *et al.* 2014), applying the poscounts method. This approach estimates size factors using the  
177 median-of-ratios method while excluding zero counts, making it particularly suitable for sparse  
178 amplicon data typical of microbial community profiles. We tested for differences in bacterial alpha  
179 diversity between LWs using unpaired t-tests, after confirming normality and homogeneity of

180 variances using the Shapiro-Wilk and Levene's tests, respectively. To analyze beta diversity, we  
181 built a Bray-Curtis distance matrix based on relative abundances and performed a PCoA (Principal  
182 Coordinates Analyses). We assessed differences in bacterial community composition between LWs  
183 with PERMANOVA (Permutational Multivariate Analysis of Variance) test, preceded by a check of  
184 the homogeneity of dispersions using the betadisper function from the vegan package version 2.6  
185 (Oksanen *et al.* 2024). We used the Bray-Curtis distance matrix from the relative abundance data to  
186 perform db-RDA to evaluate the influence of the latitude of LW on the bacterial community  
187 composition, fitting the model with the capscale function in vegan package (Oksanen *et al.* 2024),  
188 with LW as explanatory variable. The significance of the db-RDA model was assessed using  
189 permutation tests.

190

### 191 SmartChip high-throughput quantitative PCR

192 Bacterial biomass was assessed via qPCR using the 16S rRNA marker gene. Primers and probe  
193 as described in Bach *et al.* (2002). We conducted qPCR analyses using a Bio-Rad CFX-384  
194 Touch™ Real-Time PCR Detection System (Bio-Rad, Montreal, CA) under the following  
195 thermoprotocol: 95 °C for 3 min; followed by 40 cycles of 95 °C for 20 s and 62 °C for 1 min.  
196 Results were validated only if the accompanying standard curves showed efficiency values  
197 between 90% and 110%.

198 We employed the SmartChip Real-Time PCR System (Takara Bio USA, Inc.) to screen  
199 for the presence of ARGs and MRGs. The HT-qPCR was performed following the  
200 manufacturer's protocol. Each 250 µl reaction contained the appropriate primers for the targeted  
201 ARGs, along with the lichen DNA extracted previously in Alonso-García and Villarreal A.  
202 (2022).The selection of ARGs was guided by the framework established George *et al.* (2022),

203 ensuring comparability with previous global studies. We targeted 33 ARGs and three MGEs  
204 (Table 1). We included positive controls consisting of synthetic target gene sequences at  
205 concentrations of  $10^6$ ,  $10^3$ , and  $10^1$  copies per  $\mu\text{l}$ , along with negative (no-template) controls, to  
206 verify specificity and the absence of contamination. Each sample represented a single thallus  
207 (biological replicate), and ARG detection was performed in technical triplicates. A gene was  
208 considered present if detected in at least two out of the three technical replicates. The Ct values  
209 from these replicates were averaged for further analysis.

210 The relative abundance of each ARG/MGE compared with 16S rRNA was calculated  
211 using the  $2^{-\Delta Ct}$  method, where  $\Delta Ct = Ct(ARG \text{ or } MGE) - Ct(16S \text{ rRNA})$ ; hereafter referred  
212 to as ARG relative abundance. To visualize the variation of ARGs across the lichen samples, we  
213 created a heat map using the relative abundance values. A logarithmic transformation ( $\log_{10}$ ) was  
214 applied to the abundance values to better represent the wide range of abundances in the heat  
215 map. We compared ARG relative abundance between northern and southern LWs using  
216 exclusively ARGs with a prevalence greater than 50% across all samples. To test for statistical  
217 differences in ARG abundance between northern and southern LWs, we performed Wilcoxon  
218 rank-sum tests with Benjamini-Hochberg (BH) correction due to non-normal distribution of the  
219 data revealed by Shapiro-Wilk test. Prior to testing, abundance values of zero were excluded  
220 from the dataset to avoid skewing the results. Additionally, we assessed the overall ARG  
221 abundance in each lichen sample, by summing the relative abundances of all ARGs for each  
222 sample, and we applied a Wilcoxon rank-sum test to compare it between the two LWs. We  
223 assessed the influence of the latitude of LW on the relative abundance of the eleven ARGs  
224 detected in *C. stellaris* performing a db-RDA on the Bray-Curtis distance matrix of ARG relative  
225 abundance as described above.

226

## 227 Comparison of ARGs and bacterial communities in lichens

228 We assessed how much of the variance in the relative abundance of ARGs could be explained by the  
229 relative abundance of bacterial families. The relative abundances of ARGs served as the response  
230 variables, while the relative abundances of bacterial families were the explanatory variables. To check  
231 for multicollinearity among the explanatory variables, we calculated Spearman's correlation  
232 coefficients. We used a threshold of 0.4 for correlations to identify and remove highly co-correlated  
233 bacterial families from the analysis. The removed families were: Bdellovibrionaceae,  
234 Sphingomonadaceae, Isosphaeraceae, SM2D12, Caulobacteraceae, and Microbacteriaceae. Given that  
235 the linearity assumption between ARGs and bacterial families was not confirmed, we opted to use  
236 Canonical Correspondence Analysis (CCA), which can handle non-linear relationships. The  
237 significance of the CCA model was tested using ANOVA with permutation.

238 To explore potential relationships between ARGs and bacterial taxa associated, we  
239 conducted network analyses. First, we prepared a dataset combined ARG relative abundances with  
240 bacterial genera abundances, transformed using centered log-ratio to standardize the data. We  
241 calculated Spearman correlation coefficients ( $|\rho|$ ) and corresponding p-values using the `rcorr`  
242 function from the `Hmisc` package in R (Harrell J. 2024). We set significance thresholds at a  
243 correlation coefficient greater than 0.2 and an adjusted p-value below 0.05. To control for multiple  
244 testing, we applied BH correction to the p-values. Significant correlations were retained, and their  
245 values were used to construct the network, where nodes represent ARGs or bacterial taxa, and  
246 edges represent significant correlations between them. It is important to note that these correlations  
247 indicate co-variation patterns and do not confirm direct genetic linkage between ARGs and  
248 bacterial taxa. The network was generated using the `igraph` package in R (Csardi and Nepusz

249 2006), with edge weights corresponding to the absolute values of the Spearman correlation  
250 coefficients. The direction of the correlation (positive or negative) was annotated, and correlation  
251 strength was categorized as weak, moderate, strong or very strong based on the absolute value of  
252 the correlation coefficient (ranges: 0.2-0.4, 0.4-0.6, 0.6-0.8, 0.8-1.0, respectively). For further  
253 visualization and editing, we exported the network data, including node attributes and edges with  
254 adjusted p-values (BH correction), to a CSV file compatible with Cytoscape software version 3.10  
255 (Shannon *et al.* 2003).

256

## 257 Results

### 258 Bacterial community differs between LW

259 A total of 481 unique ASVs were retained after quality and prevalence filters from 48 lichen samples,  
260 a number consistent with previous reports for lichen- and moss-associated bacterial communities  
261 using similar prevalence thresholds (Alonso-García and Villarreal A. 2022; Escolástico-Ortiz *et al.*  
262 2023). The ASVs were assembled into ten phyla. Proteobacteria was the most abundant, accounting  
263 for 242 ASVs. The next most abundant phyla were Acidobacteriota (86 ASVs), Planctomycetota (78  
264 ASVs), followed by Verrucomicrobiota (42 ASVs) (Figure S1A). Among the ASVs assigned to  
265 genera, *Tundrisphaera* (65 ASVs), *Granulicella* (41 ASVs), and LD29 (33 ASVs) were the most  
266 abundant, while genera such as *Conexibacter*, *Terriglobus*, and *Novosphingobium* were represented  
267 by only one or two ASVs each (Figure S1B, Table S2). Unpaired t-test revealed that alpha diversity  
268 was higher in northern than in southern LWs ( $p = 0.0001$ ) (Figure 2A). The Bray-Curtis distance  
269 based PCoA showed that bacterial communities of *C. stellaris* differ between LWs, with no  
270 overlap, with axes 1 and 2 explaining 22% and 10.5% of the total variance, respectively (Figure

271 2B). PERMANOVA results confirmed that the separation between LWs was statistically  
272 significant ( $R = 0.143$ ,  $p = 0.001$ ). The db-RDA model revealed that the latitude of LW  
273 explained 14.23% of the variance in the bacterial community composition. The global  
274 permutation test confirmed that the model was statistically significant ( $F = 6.634$ ,  $p = 0.001$ ).

## 276 Characterisation of ARGs present in lichens

277 Among the 33 ARGs and three MGEs tested, ten ARGs and one MGE were detected in *C.*  
278 *stellaris* samples, corresponding to resistance to aminoglycosides (*aac(6')-Ib* and *aac(3')*), beta-  
279 lactams (*blaCTX-M-1*, *blaMOX/CMY*, *blaTEM* and *blaVIM*), quinolones (*qepA*, and *qnrB*), and  
280 sulfonamides (*sul1* and *sul2*), as well as one MGE (*int1-a-marko*). The relative abundances of  
281 the detected ARGs and MGE are shown in Figure 3 and Table S3. While most ARGs and the  
282 MGEs exhibited lower abundance compared to the 16S rRNA gene, *qepA* was an exception,  
283 displaying higher relative abundance in most samples (Figure 3 **Error! Reference source not**  
284 **found.**).

285 The three most prevalent ARGs, *blaCTX-M-1*, *qnrB* and *qepA*, were detected in more  
286 than 50% of the samples. Specifically, *blaCTX-M-1* was found in 35 samples (16 from the  
287 northern LW and 19 from the southern LW), *qnrB* in 34 samples (14 northern LW, 20 southern  
288 LW), and *qepA* in 23 samples (14 northern LW, 9 southern LW) (Figure 3, Table S3) **Figure 3.**  
289 Heat Map of ten antibiotic resistance genes (ARGs) and one mobile genetic element (MGE)  
290 found in *Cladonia stellaris* samples. Each tile represents the logarithmic ( $\log_{10}$ ) abundance of a  
291 specific ARG/MGE in each lichen sample, with darker colors indicating higher abundance  
292 and white tiles indicating an absence of the gene. Samples are grouped by lichen woodland (LW)  
293 latitude, on the y-axis, while the ARGs/MGE are displayed on the x-axis.. Comparisons of the

294 relative abundance of these three ARGs between northern and southern LWs revealed a significant  
295 difference only for *qnrB* (adjusted  $p = 0.027$ ), which was higher in southern LW (Figure 4).  
296 However, when considering the total relative abundance of all detected ARGs combined, no  
297 significant differences were observed between the two regions (adjusted  $p = 0.37$ ) (Figure S2).

298 Finally, the influence of LW latitude on ARG composition was assessed using db-RDA.  
299 Latitude explained 12.61% of the variance in the relative abundance of ARGs associated with *C.*  
300 *stellaris*, and the global permutation test confirmed that the model was statistically significant ( $F =$   
301  $5.696$ ,  $p$ -value = 0.005).

302

### 303 Relationship between ARGs and bacterial communities in lichens

304 The CCA did not yield statistically significant results ( $F = 1.7$ ,  $p = 0.139$ ), indicating that the  
305 relative abundances of bacterial families did not account for a significant proportion of the  
306 variance observed in ARG relative abundances. Network analysis displayed the relationships  
307 between bacterial genera and the two quinolone ARGs (*qnrB* and *qepA*), with no connexions  
308 observed for *blaCTX-M-1*. The overall network comprised 32 nodes and 147 edges, with an  
309 average of 9.19 neighbors per node, a clustering coefficient of 0.527, and a network density of  
310 0.296 (Table 2). These metrics indicate a moderately interconnected network with a cohesive  
311 structure, where no bacterial genera or ARGs were isolated (Figure 5).

312 Among the genera identified, *Conexibacter* (represented by 2 ASVs) emerged as a key  
313 node within the bacterial community of *C. stellaris*. It exhibited the highest number of  
314 connections (20) and high centrality indices (closeness centrality = 0.67, betweenness centrality  
315 = 0.219) (Figure 5, Table S4). This genus was positively correlated with *qnrB* ( $|r| = 0.54$ ),  
316 indicating co-variation in relative abundance across samples. *Granulicella* (41 ASVs) and

317 *Novosphingobium* (1 ASV), though differing in abundance, showed similar patterns in the  
318 network. Both genera showed moderate positive correlations with *qnrB* ( $|\rho| = 0.54$  for  
319 *Granulicella* and  $|\rho| = 0.43$  for *Novosphingobium*) and had relatively low betweenness centrality  
320 (0.010 for *Granulicella* and 0.027 for *Novosphingobium*). Additionally, *Granulicella* contributed  
321 eight connections to the network, indicating a moderate role in the network structure, while  
322 *Novosphingobium* had 16 connections, reflecting a stronger presence in the network (Figure 5,  
323 Table S4). Two different genera were positively correlated with *qepA*: *Tundrisphaera* ( $|\rho| = 0.43$ )  
324 and *Terriglobus* ( $|\rho| = 0.45$ ), indicating co-occurrence patterns. While *Tundrisphaera* (65 ASVs)  
325 had 11 connections, *Terriglobus* (1 ASV) had only three. Both genera displayed relatively low  
326 betweenness centrality (0.089 for *Tundrisphaera* and 0.007 for *Terriglobus*) (Figure 5, Table S4),  
327 suggesting a more localized role in linking different parts of the network. A weak but significant  
328 negative correlation was observed between *qnrB* and *qepA* ( $|\rho| = -0.40$ ) (Figure 5, Table S4),  
329 which may indicate that these two resistance genes do not co-occur frequently in the same bacterial  
330 community. However, given that *qepA* exhibits much higher relative abundance across samples  
331 compared to *qnrB*, this negative correlation may also reflect the influence of their relative  
332 abundance differences, which could affect their co-occurrence patterns.

333

## 334 Discussion

335 Bacteria associated with *C. stellaris* from southern and northern LWs in Quebec harbored ten  
336 genes conferring resistance to four classes of antibiotics: aminoglycosides (*aac(6')-Ib* and  
337 *aac(3')*), beta-lactams (*blaCTX-M-1*, *blaMOX/CMY*, *blaTEM* and *blaVIM*), quinolones (*qepA*,  
338 and *qnrB*), and sulfonamides (*sul1* and *sul2*), as well as one MGE (*int1-a-marko*). One beta-  
339 lactam resistance gene (*blaCTX-M-1*) and two quinolones (*qepA*, and *qnrB*) were detected in

340 over 50% of the samples, with *qepA* showing a particularly high relative abundance, surpassing  
341 the abundance of 16S rRNA gene in most samples. Comparisons between northern and southern  
342 LWs indicated a trend toward higher ARG relative abundance in southern samples; however,  
343 only the *qnrB* gene showed a statistically significant difference. Redundancy analyses revealed  
344 that LW latitude explained almost 13% of the variance in ARG profiles, suggesting a spatial  
345 effect. In contrast, no significant influence of bacterial family composition on ARG abundance  
346 was detected. Additionally, we identified positive correlations between *qnrB* and the bacterial  
347 genera *Connexibacter*, *Granulicella*, and *Novosphingobium*, and between *qepA* and  
348 *Tundrisphaera* and *Terriglobus*. These correlations reflect co-occurrence patterns across samples,  
349 but do not provide direct evidence that these bacteria carry the ARGs. Our data confirm the  
350 presence of ARGs in *C. stellaris* across both northern and southern LWs nevertheless, the  
351 mechanisms underlying the arrival and persistence of ARGs in these ecosystems remain unclear.  
352 In the following sections, we explore two main hypotheses: i) long-distance dispersal via  
353 bioaerosols, and ii) endogenous development driven by coevolutionary dynamics between the  
354 lichen host and its associated bacteria (arms-shields race hypothesis). These interpretations are  
355 discussed here only as possible scenarios to contextualize our findings, but the current dataset  
356 does not allow us to evaluate their likelihood. Further research will be essential to address the  
357 unresolved questions raised by our findings.

358

### 359 Influence of LW latitude on bacterial community and ARGs distribution

360 Consistent with the findings of Alonso-García and Villarreal A., (2022), our results revealed  
361 significant differences in the diversity and composition of bacterial communities associated with  
362 *C. stellaris* between northern and southern LWs, with northern LWs exhibiting higher bacterial

363 diversity. Latitude accounted for around 14% of the variation in the bacterial community of  
364 lichen samples and about 13% of the variation in ARG relative abundance, suggesting that local  
365 factors specific to each LW influence both bacterial community and ARG abundance. While the  
366 impact of the local abiotic factors on bacterial community composition is well established  
367 (Cardinale *et al.* 2012; Klarenberg *et al.* 2020; Paulsen *et al.* 2024), recent studies have also  
368 demonstrated its influence shaping ARGs abundance and distribution. Soil physicochemical  
369 properties, for example, have been shown to structure soil ARG profiles (Song *et al.* 2021; Wang  
370 *et al.* 2024). In addition to abiotic factors, biotic drivers such as microbial community  
371 composition can also influence ARGs abundance (Bahram *et al.* 2018; Yan *et al.* 2021), although  
372 the strength and direction of this effect vary by habitat type. For instance, bacterial community  
373 structure influences ARG abundance in soils, whereas no such effect was observed in the  
374 phyllosphere of plants (Xiang *et al.* 2020). Our results are consistent with this complexity: while  
375 LW latitude explained a small but significant portion of the variation in ARG abundance,  
376 bacterial community composition itself had no detectable influence on abundance of those genes,  
377 suggesting that additional, yet unidentified, factors are also driving ARG abundance in LWs.  
378 Notably, no previous studies have directly applied high-throughput qPCR to assess ARGs in  
379 lichens or other cryptogams, precluding direct comparison of our results with related systems.  
380 Future studies integrating environmental, climatic, and anthropogenic data with microbial and  
381 ARG profiling, including comparative analyses across lichens, and adjacent soils, will be  
382 essential to identify the factors and mechanisms driving ARG distribution in boreal lichen  
383 woodlands.

384

## 385 Frequent and abundant ARGs: beta-lactam and quinolone resistance

### 386 Beta-lactam resistance in lichen-associated bacteria

387 Beta-lactams antibiotics are among the most widely used antimicrobial agents and are naturally  
388 produced by certain microorganisms, such as the fungi *Penicillium chrysogenum* or the bacteria  
389 *Agrobacterium radiobacter* (Sykes *et al.* 1981). In this study, we detected four beta-lactamase  
390 resistance genes (*blaMOX/CMY*, *blaTEM*, *blaVIM* and *blaCTX-M-1*) in *C. stellaris* samples.  
391 Three of these genes were sporadically detected, exclusively in the southern LW (PNGJ). This  
392 sporadic detection may be related to nearby anthropogenic activities, such as agriculture and  
393 livestock farming, as beta-lactam ARGs are frequently enriched in agricultural and urban  
394 environments (Chen *et al.* 2018; Yan *et al.* 2019; Xiang *et al.* 2020). Bioaerosol transport from  
395 these areas could contribute to their subsequent deposition in PNGJ (Bai *et al.* 2022; George *et*  
396 *al.* 2022; Yang *et al.* 2023).

397 Conversely, *blaCTX-M-1* was by far the most prevalent ARG, found in 83% of the *C.*  
398 *stellaris* samples. We hypothesize that *blaCTX-M-1* may be stably integrated within the lichen-  
399 associated microbiome. Although lichens are not known to produce beta-lactams, they synthesize  
400 a wide array of antimicrobial compounds (Burkholder *et al.* 1944; Aslan *et al.* 2006; Ranković *et*  
401 *al.* 2010; Mitrović *et al.* 2011; Shrestha and St. Clair 2013; Kosanić *et al.* 2014; Kosanić and  
402 Ranković 2015), which could exert selective pressures favoring *blaCTX-M-1* retention over  
403 evolutionary timescales. Noël *et al.* (2021) demonstrated that lichen-associated bacteria  
404 developed mechanisms to survive in the presence of antibacterial compounds produced by  
405 lichens, and Agersø *et al.* (2019) suggested that some ARGs may be intrinsic to bacteria and  
406 originated from ancient resistomes rather than recent contamination.

407 In our dataset, *blaCTX-M-1* did not show significant correlations with specific bacterial  
408 genera, a pattern also reported for *blaTEM* (Jang *et al.* 2022) and *aac-(6)-Ib* (Yan *et al.* 2017). A

409 plausible explanation for this lack of association is the frequent linkage of these genes to MGEs  
410 (Cantón *et al.* 2012; Partridge *et al.* 2018; Cormier *et al.* 2022), which are known to facilitate  
411 HGT among diverse bacterial taxa. Such mechanisms may enable *bla*CTX-M-1 to disseminate  
412 widely across microbial communities without producing detectable genus-level co-variation  
413 (Zhu *et al.* 2017; Li *et al.* 2023), potentially contributing to its broad occurrence across both  
414 northern and southern LWs.

#### 415 416 Quinolone resistance in lichen-associated bacteria

417 Quinolone are synthetic antibiotics introduced in the 1960s, with widespread use established by  
418 the 1980s (Hooper and Jacoby 2015). They are extensively employed in human and veterinary  
419 medicine, as well as in food production (World Health Organization 2018), contributing to  
420 growing contamination of natural environments (Cattoir *et al.* 2008; Ben *et al.* 2019; Zhai *et al.*  
421 2024). In this study, we detected two quinolone resistance genes in *C. stellaris* samples: *qnrB*  
422 and *qepA*.

423 The *qnrB* gene exhibited a particularly high prevalence, being present in 81% of the  
424 lichen samples. It showed significantly higher relative abundance in southern lichens, which  
425 could be consistent with introductions via bioaerosol dispersal (Pilote *et al.* 2019; George *et al.*  
426 2022) from anthropogenically influenced areas such as nearby agricultural and urban centers.  
427 However, this remains speculative and cannot be directly tested with our current dataset.  
428 Network analysis revealed positive correlations between *qnrB* and several bacterial genera,  
429 including *Connexibacter*, *Granulicella*, and *Novosphingobium*. Interestingly, *Connexibacter*,  
430 which is known for its resistance to quinolones (Monciardini *et al.* 2003), showed the strongest  
431 correlation with *qnrB*. While this does not provide evidence that *Connexibacter* harbors the gene,  
432 it reflects a pattern of co-variation within the lichen-associated microbial community, possibly

433 driven by shared ecological or selective pressures. *Novosphingobium* also showed a weaker but  
434 positive correlation with *qnrB*, consistent with previous reports linking *Novosphingobium* to  
435 other *qnr* genes (Yan *et al.* 2017).

436 Both *qnrB* and *blaCTX-M-1* were detected together in 77% of the *C. stellaris* samples,  
437 however our network analysis did not reveal significant co-occurrence between the two genes.  
438 Previous studies have reported their joint presence in mobile genetic elements, such as *IncN*  
439 plasmids flanked by the MGE *IS26* (Juraschek *et al.* 2022), suggesting that these genes can be  
440 mobilized together. A similar mechanism could potentially contribute to their frequent co-  
441 detection in *C. stellaris*, but our data do not provide direct evidence to support this hypothesis.

442 The *qepA* gene, encoding a quinolone efflux pump, was detected in 57% of the *C.*  
443 *stellaris* samples, with no significant differences in relative abundance between northern and  
444 southern LWs. Notably, *qepA* exhibited high relative abundance, often exceeding that of the 16S  
445 rRNA gene. This pattern is striking, since 16S rRNA genes typically occur in multiple copies per  
446 bacterial genome (ranging from 1 to >10 (Louca *et al.* 2018; Pan *et al.* 2023)), whereas *qepA* is  
447 usually found as a single copy on plasmids or other MGE. The unusually high abundance of  
448 *qepA* could reflect i) inflation due to plasmid multicopy replication, ii) the persistence of  
449 extracellular DNA containing *qepA* in the lichen thalli, or iii) a genuine ecological enrichment,  
450 where bacteria carrying *qepA* are selectively favored within the lichen microbiome. Our data do  
451 not allow discrimination among these alternatives. The third explanation, ecological enrichment,  
452 is consistent with the known roles of efflux pumps, which serve diverse physiological roles for  
453 bacteria, particularly detoxification of potentially harmful compounds (Piddock 2006; Martínez  
454 *et al.* 2009; García-León *et al.* 2014). For instance, Alonso *et al.* (1999) demonstrated that  
455 environmental isolates of *Pseudomonas aeruginosa* collected prior to the widespread use of

456 synthetic antibiotics could already expel quinolones, suggesting that these mechanisms originally  
457 evolved as defenses against naturally occurring compounds. In the context of lichen symbioses,  
458 efflux pumps may therefore provide a key advantage by protecting bacteria against the diverse  
459 array of antimicrobial metabolites occurring in the environment, including those produced by  
460 lichens themselves (Ranković *et al.* 2007; Studzińska-Sroka *et al.* 2015; Taylor *et al.* 2023). In  
461 this framework, *C. stellaris* provides a relevant case study. While there is currently no evidence  
462 that this species synthesizes quinolone-type compounds, its diverse secondary metabolite profile  
463 could nonetheless favor the retention of versatile detoxification mechanisms like *qepA*,  
464 potentially explaining both its prevalence and elevated abundance in *C. stellaris* associated  
465 bacteria. This interpretation remains plausible given the ecological functions of efflux pumps,  
466 but it remains speculative in the absence of transcriptional, functional, and genomic evidence  
467 linking *qepA* activity to lichen-associated selective pressures.

468 Network analysis revealed positive correlations between *qepA* and the genera  
469 *Tundrisphaera* and *Terriglobus*. These correlations do not provide direct evidence that these  
470 genera carry *qepA*, but they indicate that the relative abundances of these taxa co-vary with the  
471 abundance of this efflux pump gene across *C. stellaris* samples. This pattern may reflect  
472 ecological co-variation, where both the gene and these genera thrive under similar environmental  
473 conditions within the lichen microbiome.

#### 475 Occasional ARGs: aminoglycosides, sulfonamides, and *int1-a-marko*

476 In addition to the dominant ARGs discussed above, we detected the occasional presence of other  
477 resistance genes and the integron marker gene *int1-a-marko* in *C. stellaris*. Specifically, the  
478 aminoglycoside resistance genes *aac(6')-Ib* and *aac(3')*, the sulfonamide resistance gene *sull*,

479 and the integron marker gene *int1-a-marko* were identified at low frequencies, predominantly in  
480 samples from the southern LW. Aminoglycosides are natural compounds produced by soil  
481 bacteria (Schatz *et al.* 1944), and resistance to these compounds is widespread across diverse  
482 natural environments (Li *et al.* 2015; Yan *et al.* 2017; Zhuang *et al.* 2021; Urban-Chmiel *et al.*  
483 2022). In contrast, sulfonamides are synthetic antibiotics first introduced in the 1930s, widely  
484 used in both human and veterinary medicine (Landers *et al.* 2012), with resistance genes now  
485 also broadly disseminated in the environment (Nunes *et al.* 2020). The geographic clustering of  
486 these ARGs in southern LW samples is consistent with a possible influence from nearby urban  
487 areas, potentially mediated by bioaerosol dispersal. However, the very limited number of  
488 detections make it difficult to support this hypothesis, and alternative explanations, such as co-  
489 evolution, cannot be excluded. Additionally, we detected *sul2* in more than 40% of samples from  
490 both northern and southern regions. The frequent detection of *sul2* may reflect widespread  
491 resistance. Sulfonamide resistance gene have been documented in bioaerosols (Zhang *et al.*  
492 2018; Han and Yoo 2020; Qu *et al.* 2024). However, it has also been detected in forest soils with  
493 no history of exposure to these synthetic drugs (Willms *et al.* 2019). These observations suggest  
494 that, although sulfonamides may not naturally occur in these environments, the genetic  
495 mechanisms for resistance can exist within microbial communities. Such persistence may  
496 indicate an adaptive capacity explaining *sul2* persistence within the *C. stellaris*, despite limited  
497 exposure to antibiotics from anthropogenic uses. Nevertheless, our observations do not provide  
498 direct evidence on the factors driving *sul2* maintenance in *C. stellaris*, and targeted studies will  
499 be required to elucidate its origin and ecological role.

500

## 501 Conclusion

502 To our knowledge, this is the first study to directly quantify and confirm the presence of ARGs  
503 in lichens using high-throughput quantitative PCR. We detected ten ARGs and one MGE in the  
504 lichen *C. stellaris* from northern and southern LWs in eastern Canada, with predominance of  
505 beta-lactam and quinolone resistance genes. Our results revealed differences in ARG relative  
506 abundances between northern and southern sites, although only *qnrB* showed significant  
507 variation. Furthermore, the bacterial community composition, while distinct between LWs, did  
508 not appear to drive ARG distribution.

509 Interpretations of ARG–taxa relationships should be viewed with caution, as they are  
510 based solely on co-occurrence patterns from 16S rRNA data and cannot establish direct host  
511 carriage. Future studies combining bacterial isolation with whole-genome sequencing or  
512 applying genome-resolved metagenomics (including MAG reconstruction and plasmid-focused  
513 assemblies), will be essential to determine the genomic context of the detected ARGs and to  
514 validate potential host–gene associations.

515 Similarly, our DNA-based approach establishes the presence of ARGs but does not reveal  
516 whether these genes are functionally active or represent extracellular or relic DNA persisting in  
517 the lichen thalli. DNA-based detection alone cannot distinguish between relic DNA and active  
518 transcription, which can only be demonstrated using RNA-based approaches such as Reverse  
519 Transcription quantitative PCR (RT-qPCR) or metatranscriptomics. Establishing whether the  
520 detected ARGs are expressed in situ, or instead represent non-functional remnants of past genetic  
521 events, will be essential to clarify their ecological relevance within the lichen microbiome.

522 Among the ARGs identified, we hypothesize that *blaCTX-M-1* and *qepA* may reflect  
523 ancient co-evolutionary processes within lichen-associated microbiomes, whereas the higher

524 relative abundance of *qnrB* in southern samples may suggest more recent introductions  
525 influenced by anthropogenic activity. These scenarios remain speculative and required targeted  
526 validation. Future studies applying air sampling and atmospheric monitoring, chemical profiling  
527 of *C. stellaris* metabolites combined with antimicrobial susceptibility assays, and comparative  
528 analyses of co-occurring across lichens and soils will be critical to disentangle long-distance  
529 dispersal from endogenous selective processes.

530 Taken together, our results demonstrate that ARGs occur in *C. stellaris* across both  
531 northern and southern boreal lichen woodlands, including remote sites with limited direct human  
532 influence. This exploratory study establishes a framework for characterizing the lichen resistome  
533 and highlights key avenues for future research. As antibiotic resistance continues to pose a major  
534 global health challenge, understanding how resistance genes persist and spread even in isolated  
535 environments such as LWs represents an open and relevant scientific question.

536

## 537 Author contributions

538 **Marta Alonso-García:** Conceptualization; Funding acquisition; Investigation; Methodology;  
539 Data analysis; Writing – original draft; Writing – review & editing. **Paul B.L. George:** Funding  
540 acquisition (supporting); Data analysis (supporting); Writing – review & editing. **Samantha**  
541 **Leclerc:** Investigation (supporting); Methodology (supporting). **Marc Veillette:** Supervision  
542 (laboratory coordination); Methodology (advice on experimental procedures). **Caroline**  
543 **Duchaine:** Funding acquisition (supporting), Supervision; Writing – review & editing. **Juan**  
544 **Carlos Villarreal A.:** Conceptualization; Funding acquisition, Supervision; Writing – review &  
545 editing.

546

## 547 Conflict of interest

548 The authors declare that the research was conducted in the absence of any commercial or  
549 financial relationships that could be construed as a potential conflict of interest.

550

## 551 Data availability

552 The 16S rRNA sequencing data analyzed in this study were generated in a previous project and  
553 are publicly available in the NCBI Sequence Read Archive under BioProject accession numbers  
554 PRJNA593044. Detailed sample metadata, including BioSample accession numbers, geographic  
555 coordinates, and collection details, are provided in Table S1. The R script used for the analyses is  
556 publicly available at Zenodo: <https://doi.org/10.5281/zenodo.16967960>

557

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## 862 Tables

863 **Table 1.** List of gene targets used for HT-qPCR analyses.

Gene name	Gene type
<i>aac(6')-II, aac(6')-Ib, aac(3')</i>	Aminoglycoside resistance
<i>blaCTX-M-1, blaGES, blaIMP, blaMOX/blaCMY, blaOXA, blaTEM, blaVEB, blaVIM</i>	Beta-lactam resistance
<i>mcr1</i>	Colistin resistance
<i>ermB, ermF, ermT, ermX, erm(35)</i>	Macrolide resistance
<i>qnrB, qepA</i>	Quinolone resistance
<i>sul1, sul2</i>	Sulfonamides resistance
<i>tet32, tetA, tetC, tetL, tetM, tetO, tetQ, tetS, tetW, tetX</i>	Tetracyclines resistance
<i>vanA, vanB</i>	Vancomycin resistance
<i>IS26</i>	Mobile genetic element (transposase gene)
<i>tnpA</i>	Mobile genetic element (transposase gene)
<i>int1-a-marko</i>	Mobile genetic element (integrase gene)

864

865 **Table 2.** Summary of key network metrics of bacterial communities and antibiotic resistance  
866 genes (ARGs) associated with *Cladonia stellaris*.

Summary Statistics	All samples
Number of nodes	32
Number of edges	147

Avg. number of neighbors	9.188
Network diameter	5
Network radius	3
Characteristic path length	2.109
Clustering coefficient	0.527
Network density	0.296
Network heterogeneity	0.66
Network centralization	0.372
Connected components	1

867

## 868 Figure captions

869 **Figure 1. A.** Representative lichen woodland dominated by *Cladonia stellaris*. **B.** Close-up of  
 870 the lichen *Cladonia stellaris*, showing the thallus morphology characteristic of this species  
 871 (photo credits: **A.** Claude Morneau, **B.** Philip Bell-Doyon).

872

873 **Figure 2. A.** Alpha diversity Shannon index of bacteria associated with *Cladonia stellaris* from  
 874 northern and southern lichen woodlands (LW). The median (horizontal line), quartiles (edges of  
 875 the box) and 1.5X the interquartile range of (whiskers) are displayed. Points represent individual  
 876 observations. Student's t-test results indicated a significant difference in alpha diversity between  
 877 the two LWs (p-value < 0.05). **B.** Principal coordinates analysis (PCoA) of bacteria associated  
 878 with *Cladonia stellaris* from northern and southern LW. Samples are colored according to LW  
 879 latitude. PERMANOVA results indicated a significant difference in the bacterial community  
 880 composition between the two LWs (R = 0.14303, p-value < 0.01).

881

882 **Figure 3.** Heat Map of ten antibiotic resistance genes (ARGs) and one mobile genetic element  
 883 (MGE) found in *Cladonia stellaris* samples. Each tile represents the logarithmic ( $\log_{10}$ )  
 884 abundance of a specific ARG/MGE in each lichen sample, with darker colors indicating  
 885 higher abundance and white tiles indicating an absence of the gene. Samples are grouped by  
 886 lichen woodland (LW) latitude, on the y-axis, while the ARGs/MGE are displayed on the x-axis.

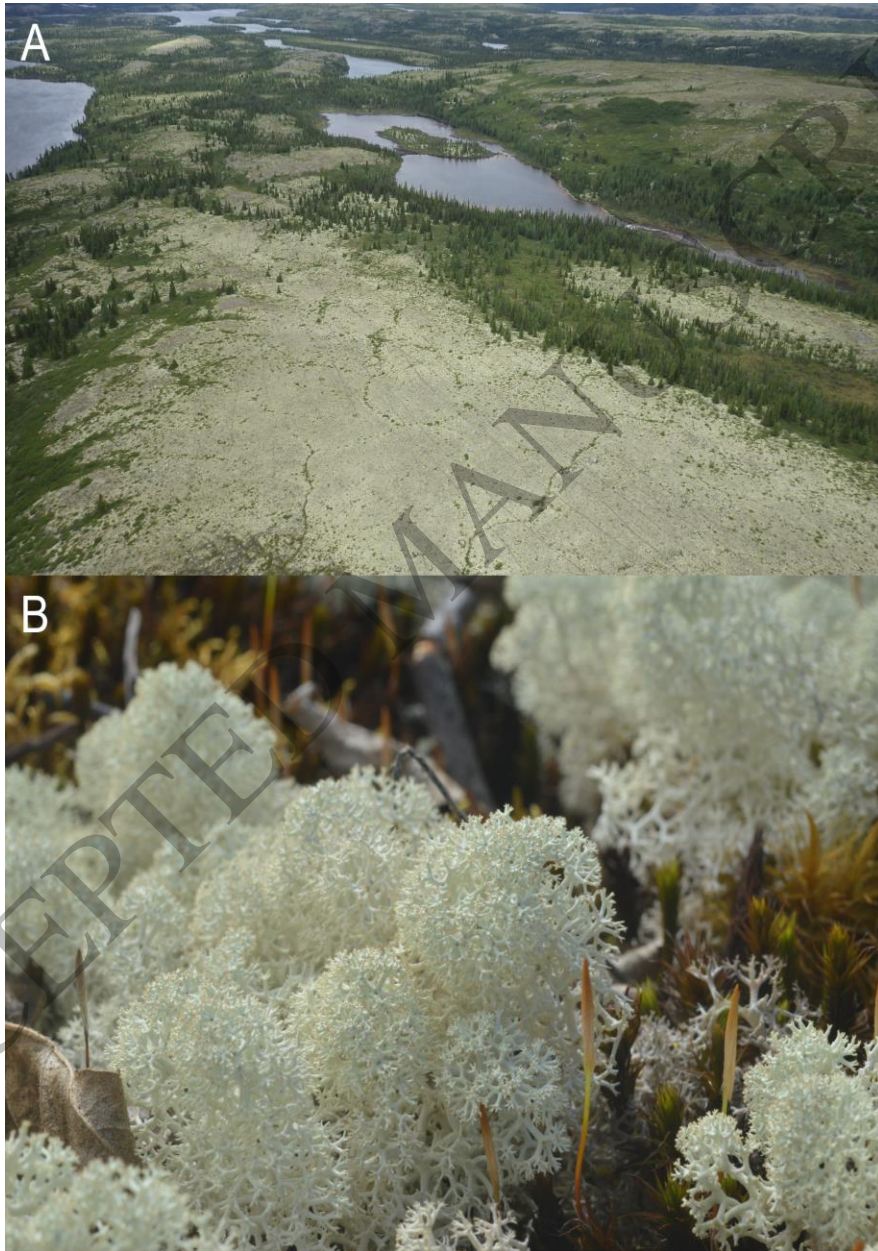
887

888 **Figure 4.** Relative abundance of three prevalent antibiotic resistance genes (ARGs) in *Cladonia*  
 889 *stellaris* from northern and southern lichen woodlands (LWs). The median (horizontal line),  
 890 quartiles (edges of the box) and 1.5X the interquartile range of (whiskers) are displayed. Points  
 891 represent individual observations. A dashed red horizontal line at  $y = 1$  indicates the reference  
 892 value, where values equal to 1 represent abundance equivalent to the 16S rRNA gene. Values  
 893 greater than 1 indicate higher abundance compared to the 16S rRNA gene, while values less than  
 894 1 indicate lower abundance. Wilcoxon rank-sum results indicated significant differences in the  
 895 relative abundance of *qnrB* between the northern and southern LWs (adjusted p-value = 0.0274).

896

897 **Figure 5.** Network analysis of correlations between antibiotic resistance genes (ARGs) and  
 898 bacterial genera associated with *Cladonia stellaris*. The network was constructed using

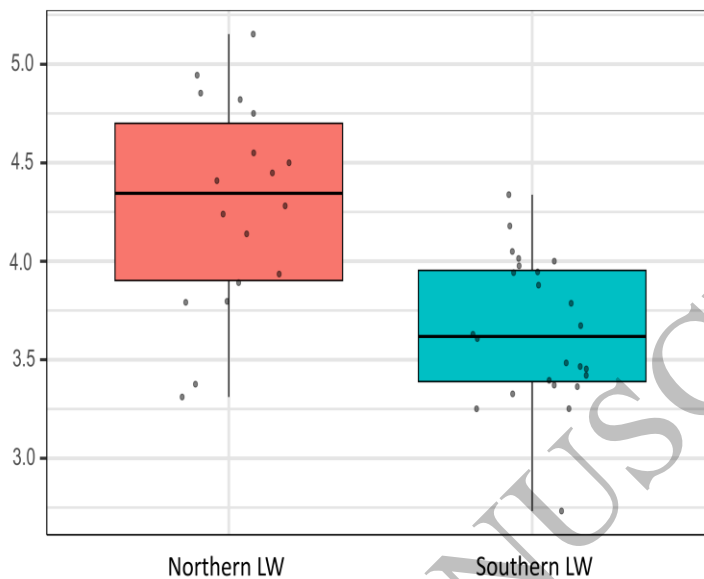
899 Spearman correlation coefficients, displaying significant correlations ( $|\rho| > 0.2$  and adjusted p-  
900 value  $< 0.05$ ) between ARGs and bacterial genera. Nodes represent ARGs (diamonds) or  
901 bacterial genera (rectangles), with edges depict significant correlations. Edge color indicates  
902 correlation strength, categorized as weak ( $0.2 < |\rho| \leq 0.4$ ), moderate ( $0.4 < |\rho| \leq 0.6$ ), or strong ( $|\rho|$   
903  $> 0.6$ ). Solid edges indicate positive correlation, and dotted edges represent negative correlations.  
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905



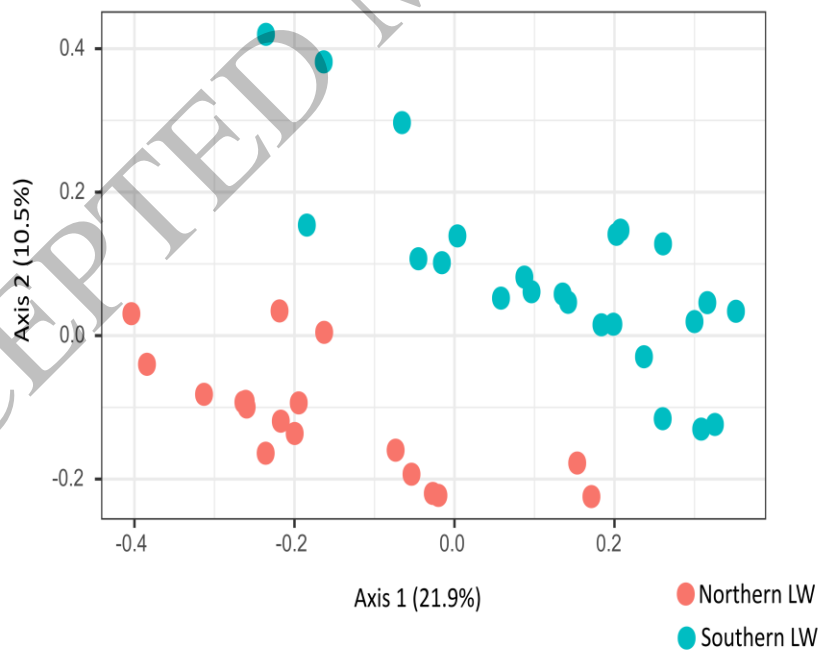
906  
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Figure 1  
170x209 mm ( x DPI)

A. Alpha diversity of bacterial communities in northern vs southern lichen woodlands (LW)



B. PCoA of bacterial community composition across lichen woodlands (LW)



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912

Figure 2  
240x298 mm ( x DPI)

Antibiotic resistance genes (ARGs) and mobile genetic element (MGE) detected in *Cladonia stellaris*

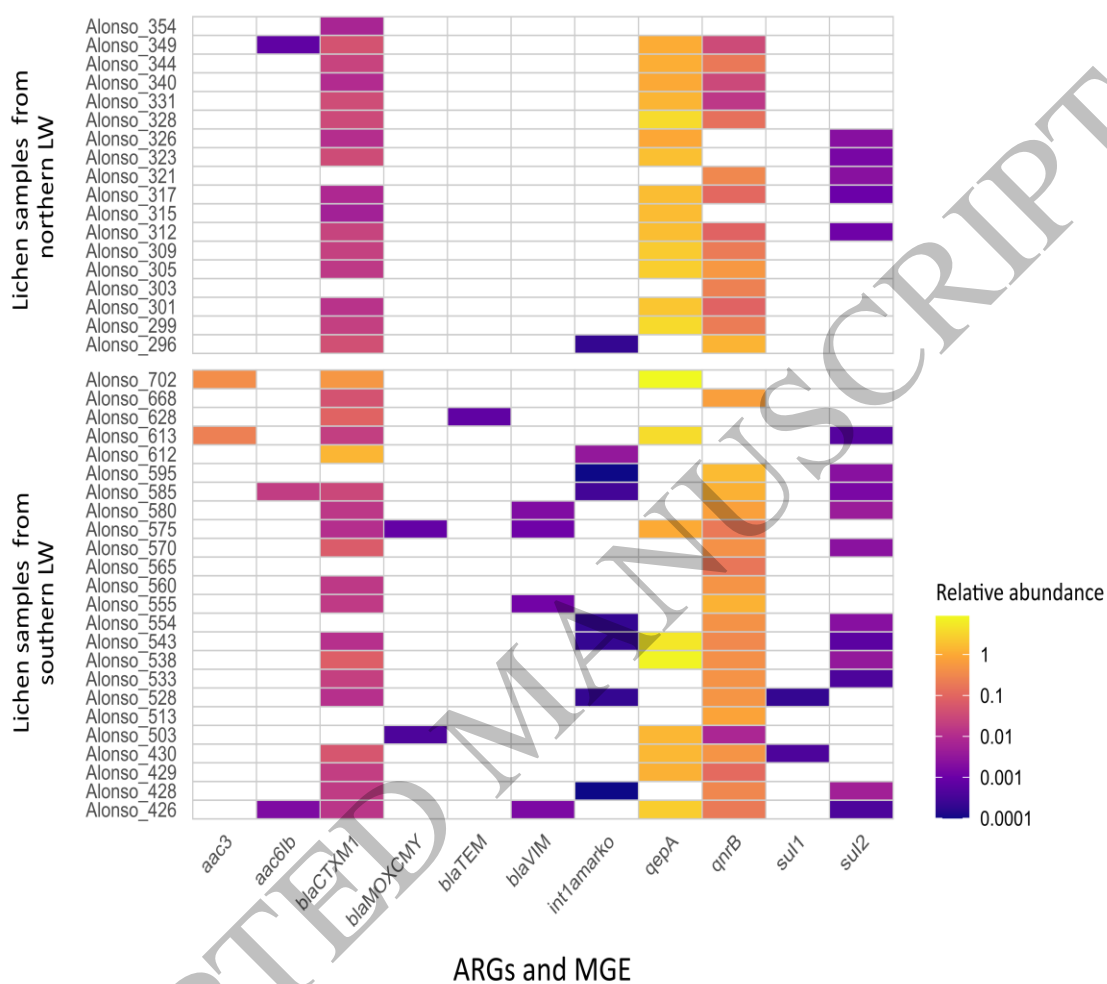


Figure 3  
225x168 mm ( x DPI)

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Comparison of relative abundance of prevalent antibiotic resistance genes (ARGs) from northern and southern lichen woodlands (LW)

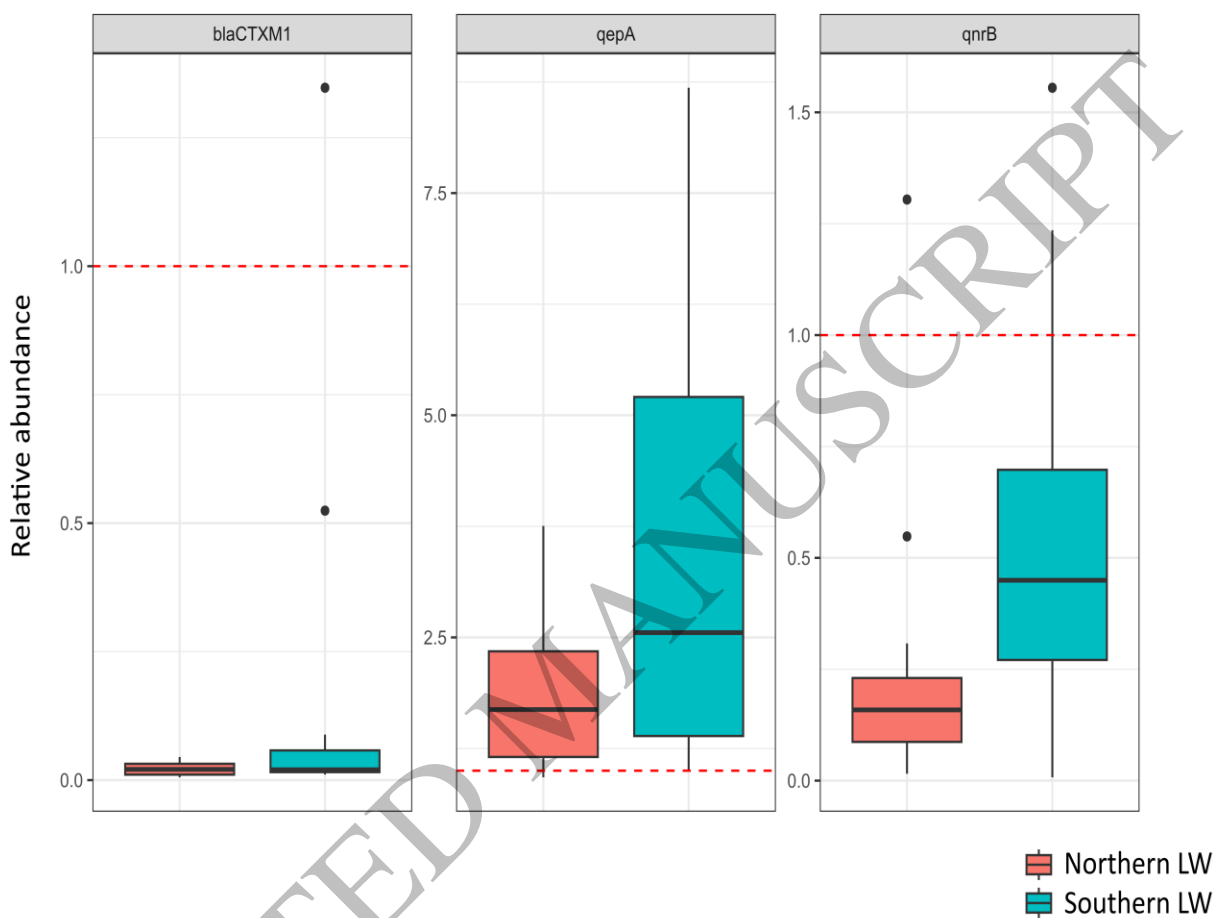
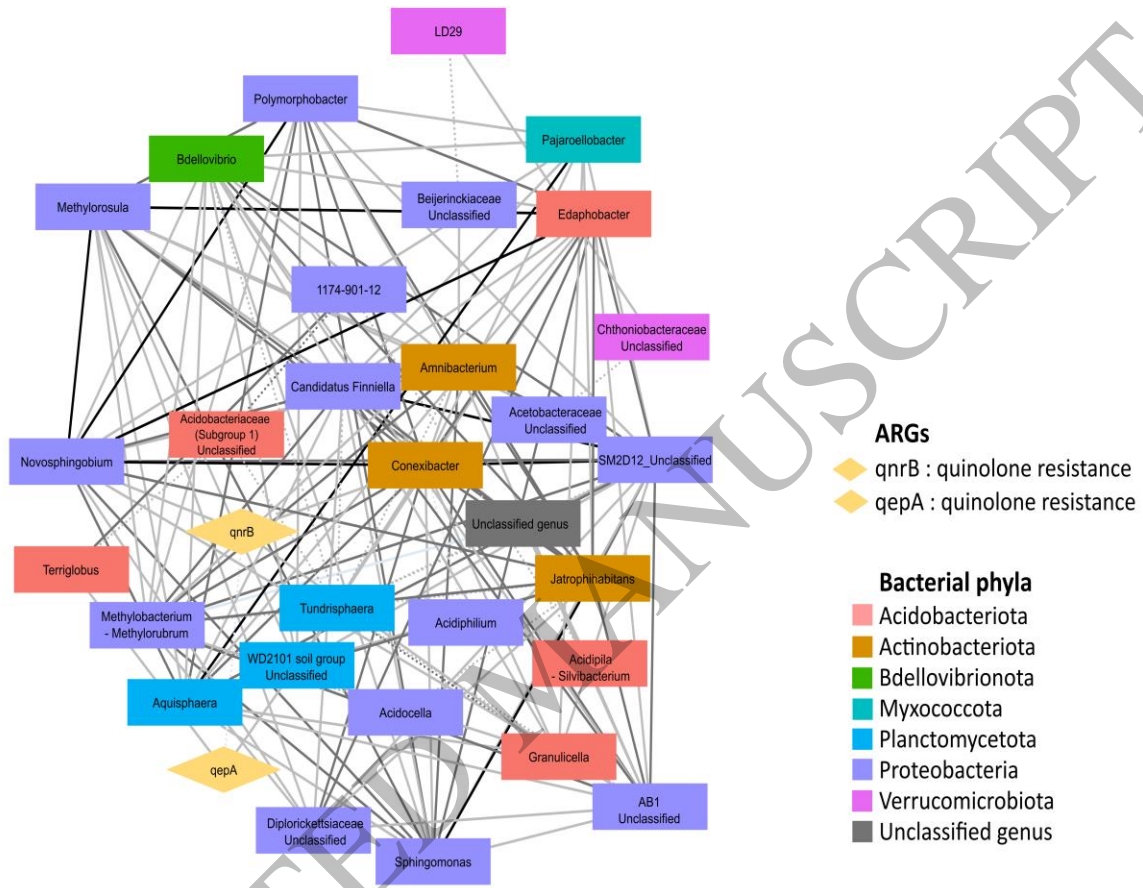


Figure 4  
247x174 mm ( x DPI)

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Correlation network between antibiotic resistance genes (ARGs) and bacterial genera in *Cladonia stellaris*



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Figure 5  
237x190 mm ( x DPI)