Short Communication: Co-occurring *Lobaria pulmonaria* and *Ricasolia quercizans* share green algal photobionts: Consequences for conservation

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Photobiont sharing among lichens within communities is a well-documented phenomenon, especially among closely related fungal species that form photobiont-mediated guilds (Dal Grande et al. 2014; Rikkinen et al. 2002). Species of *Lobaria* and other closely related genera that associate with *Symbiochloris reticulata* are one example of a photobiont-mediated guild that have been the subject of extensive research (Dal Grande et al. 2014). In their study, Dal Grande et al. (2014) compared algal multi-locus genotypes (MLGs) at one site in Taiwan and one site in Madeira among four species of *Dendriscosticta*, 16 species of *Lobaria*, and one species of *Ricasolia*, and, using pairwise FST values, found the species did not associate with significantly different green algal partners when they co-occurred. Green algal symbiont sharing has also been documented between the predominantly sexually reproducing *Umbilicaria spodochroa* and predominantly vegetatively reproducing *Lasallia pustulata* (Hestmark et al 2016). Werth (2012) recovered a more complex pattern of compartmentalization in *Ramalina menziesii* and adjacent lichen species. Photobiont sharing may also occur with co-occurring non-lichen organisms, such as liverworts (Cornejo & Scheidegger 2016).

Here we aimed to test the photobiont sharing capacity of one iconic eastern North American species, *Ricasolia quercizans*, with *Lobaria pulmonaria* (Fig. 1). We sampled 41 individuals of *R. quercizans* and 66 individuals of co-occurring *L. pulmonaria* at five sites in the southeastern United States (Table 1). We amplified and sequenced nine microsatellite markers (LPh1, LPh2, LPh3, LPh4, LPh5, LPh6, LPh7, LPh8 and LPh9; Supplementary Table S1) for *S. reticulata* from both species using established protocols (Dal Grande et al. 2010; Widmer et al. 2010). The R packaged poppr was used to analyze multi-locus genotypes (MLGs) among the samples (Kamvar et al. 2014). There were no shared identical MLGs among any of the samples within four of the five populations sampled. In one population (CRG) four individuals, two *L. pulmonaria* and two *R. quercizans*, shared photobionts with identical MLGs. To test if genetic distances among photobionts associated with co-occurring individuals of *L. pulmonaria* and *R. quercizans* were significantly different within or between each site, a PERMANOVA was performed using the adonis function in the vegan package with 999 permutations (Oksanen et al. 2019). The results suggest that genetic distances of photobionts associated with co-occurring *L. pulmonaria* and *R. quercizans* were not significantly different at the site level (R² = 0.0131, p = 0.184). Our finding that *S. reticulata* associated with co-occurring *L. pulmonaria* and *R. quercizans* are not significantly genetically distinct in southeastern North America concurs with results from Dal Grande et al. (2014) on other *Ricasolia* species.

Our study provides an additional example of a photobiont mediated guild of *Symbiochloris reticulata* where *Lobaria pulmonaria* is considered the core species of this guild, actively spreading the photobiont by means of vegetative propagules. In *Ricasolia quercizans* the fungal partner reproduces sexually with ascospores and relichenization depends on available *S. reticulata* which is dispersed only by

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means of vegetative propagules of *L. pulmonaria*. Most of the remaining populations of *L. pulmonaria* in the southeastern United States are very small and genetically depauperate (Allen et al. 2021). Selective logging practices have led to significant declines in both abundance and fertility of both *L. pulmonaria* and *R. quercizans* in eastern North America (Edman et al. 2007). A continued decline of *L. pulmonaria* is expected to result in an even more dramatic decline of *R. quercizans* in this region because of the declining availability of the shared photobiont, leading to an extinction vortex of lichen species and its shared photobiont. In Switzerland the continuous decline of *L. pulmonaria* (Vulnerable) over at least two centuries (Scheidegger et al. 2002) resulted in a reported dramatic decline of the two fringe species *R. amplissima* (Endangered) and *R. virens* (Regionally Extinct) (Scheidegger et al. 2015).

Table 1. Sampling locations and sizes of *Symbiochloris reticulata* from co-occurring *Lobaria pulmonaria* (*Sr – Lp*) and *Ricasolia quercizans* (*Sr – Rq*).

<table>
<thead>
<tr>
<th>Identifier</th>
<th>Locality</th>
<th>Lat.</th>
<th>Long.</th>
<th><em>Sr – Lp</em></th>
<th><em>Sr – Rq</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>CRG</td>
<td>U.S.A., West Virginia, Pocahontas County, Monongahela National Forest</td>
<td>38.2025</td>
<td>-80.2768</td>
<td>9</td>
<td>5</td>
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<tr>
<td>POT</td>
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<td>38.1707</td>
<td>-80.2747</td>
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<td>3</td>
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<td>RBB</td>
<td>U.S.A., North Carolina, Jackson County, Pisgah National Forest</td>
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<td>-82.9697</td>
<td>31</td>
<td>19</td>
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<tr>
<td>RMT</td>
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<td>-82.1105</td>
<td>14</td>
<td>10</td>
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<tr>
<td>WIR</td>
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<td>38.3279</td>
<td>-80.2132</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>
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LITERATURE CITED


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Supplementary documents online:
Supplementary Table S1. All data underlying the analyses conducted in this research. Data includes both microsatellite marker data for all photobionts and locality data.