New entities in *Physcia aipolia*–*P. caesia* group (Physciaceae, Ascomycetes): an analysis based on mtSSU, ITS, group I intron and betatubulin sequences

Katileena Lohtander¹,* Leena Myllys¹, Mari Källersjö², Roland Moberg³, Soili Stenroos¹ & Anders Tehler⁴

¹) Botanical Museum, Finnish Museum of Natural History, P.O. Box 7, FI-00014 University of Helsinki, Finland
²) Swedish Museum of Natural History, P.O. Box 50007, SE-104 05 Stockholm, Sweden
³) The Botany Section, Museum of Evolution, Norbyvägen 16, SE-752 36 Uppsala, Sweden
⁴) Department of Cryptogamic Botany, Swedish Museum of Natural History, P.O. Box 50007, SE-104 05 Stockholm, Sweden

* current address: Finnish Environment Institute, Chemicals Division P.O. Box 140, FI-00251 Helsinki, Finland (e-mail: katileena.lohtander-buckbee@ymparisto.fi)

Received 13 Mar. 2008, revised version received 2 May 2008, accepted 6 May 2008


We have revisited the phylogenetic evaluation of the *Physcia aipolia*–*P. caesia* lichen group (sect. *Caesiae*; Physciaceae, Lecanorales) in order to investigate whether new sequence data and extensive sampling can help us to understand the phylogenetic relationships in that group. We combined partial mtSSU DNA data with two previously used nuclear gene regions (betatubulin, ITS) and a group I intron. We also compared the resulting phylogenies with chemical and morphological characters. Altogether 52 specimens of the *P. aipolia*–*P. caesia* group were analysed. Direct optimization of the molecular data revealed several well-supported groups. Our results essentially agreed with those of the earlier studies, and we were able to confirm the independent taxonomic status of some controversial morphotaxa. We also discovered at least two distinct clades that potentially represent species new to science. A new nomenclatural combination, *Physcia alnophila* (Vain.) Loht., Moberg, Myllys & Tehler, is proposed.

**Key words:** betatubulin, intron, ITS, lichen, mtSSU rDNA, phylogeny, *Physcia*

**Introduction**

The lichen family Physciaceae (Ascomycetes, Lecanorales) has a worldwide distribution. The genus *Physcia*, one of the approximately 30 genera in Physciaceae, contains about 50 species worldwide (Moberg 1994). *Physcia* is separated from the other genera in the family by the *Physcia*-type ascospores, subcylindrical pycnoconidia and the presence of atranorin in the upper cortex (Moberg 1977).

The genus *Physcia* has been divided into sections (e.g., sect. *Physcia, Caesiae, Fusicporae*, and *Stellares*) by Moberg (1977), based on
morphological and chemical characters, such as the presence or absence of marginal cilia and K+ yellow colour reaction in the medulla. Our goal in the present study was to investigate the section Caesiae, which is characterized by maculate surface of the thallus, lobes without cilia, K+ yellow reaction in medulla, and the presence of zeorin in the thallus (Moberg 1977). The section Caesiae contains the widespread species P. aipolia, P. caesia and P. phaea (Moberg 1977). Also several other Physcia species, such as P. alba, P. albata, P. alboblumbea, P. cinerea, P. convexa, P. coronifera, P. decorcicata, P. dilatata, P. lanciatula, P. lopezii, P. nubila, P. pachyphylla, P. poncinsii, P. rolfi, P. sinuosa, P. tribacoides, P. undulata, and P. verrucosa have a K+ yellow medulla reaction and they contain zeorin. Hence they have been included in the section Caesiae as well (Moberg 1986, 1989).

The ITS phylogeny by Helms et al. (2003) indicated that at least P. alba, P. phaea and P. undulata do not belong in the P. aipolia–P. caesia group. Furthermore, our ongoing study of the phylogeny of the genus Physcia indicates that at least P. convexa, P. lancinatula, P. nubila, P. poncinsii, P. rolfi, P. sinuosa, and P. tribacoides do not form a monophyletic group with P. aipolia and P. caesia (authors’ unpubl. data).

Physcia is commonly cited to contain so-called species pairs. The concept of species pairs (Poelt 1970, 1972) is based on the suggestion that lichen taxa that differ from each other only or primarily by their dispersal methods may be grouped as pairs. The so-called “primary species” produces fruiting bodies and sexual spores, while its counterpart, the “secondary species”, is vegetatively dispersed, usually by means of soredia (Poelt 1972). The different counterparts of species pairs have traditionally been treated and named as separate species. The study by Lohtander et al. (2000) showed that based on the ITS and group I intron sequences P. aipolia and P. caesia did not form a separate group in the phylogenetic tree. Moreover, Myllys et al. (2001) included an additional gene region (betatubulin gene) to the ITS and intron data set. The phylogenetic tree in that study had a well-resolved and supported internal structure, and the specimens of P. aipolia and P. caesia formed several, intermixed but well-supported groups.

Physcia aipolia and P. caesia have different morphotypes that are sometimes treated as species or varieties. For example in P. aipolia Moberg (1977) recognised two varieties: var. aipolia and var. alnophila. The varieties are morphologically rather distinctive. Physcia aipolia var. alnophila forms smaller, narrow-lobed thalli and smaller spores than var. aipolia, as well as smaller, crowded apothecia. Physcia aipolia var. alnophila also usually grows on trees such as Alnus incana, Salix spp. and Sorbus aucuparia, while var. aipolia seems to prefer Populus tremula (Moberg 2002). Furthermore, P. aipolia var. alnophila has a more northern distribution than var. aipolia. The studies by Lohtander et al. (2000) and Myllys et al. (2001) showed that var. alnophila and var. aipolia form separate groups in phylogenetic trees and are thus obviously distinct species, but the nomenclatural combination “P. alnophila” was not validly published.

Similarly, Physcia caesia has a morphotype, P. subalbinea (P. wainioi, P. caesioalba) that many authors have recognised, but that Moberg (1977) treated as a synonym of P. caesia. Typical P. caesia has capitate laminal soralia while P. subalbinea has soralia located primarily at the lobe-tips (Moberg 1977).

We wanted to find out whether the phylogeny of the Physcia aipolia–P. caesia group in the studies by Lohtander et al. (2000) and Myllys et al. (2001) could be further clarified using an additional gene region (mtSSU), the secondary chemistry of the specimens and more comprehensive sampling.

We used DNA data and phylogenetic methods to (1) study the status of certain species assigned to the section Caesiae, (2) study the species pairs and try to clarify the species boundaries in the section, and (3) find out whether chemical characters and additional molecular data can discriminate between the morphotypes in P. aipolia and P. caesia.

While studying the morphology of the specimens, we found that certain P. aipolia thalli had slight morphological differences as compared with the rest of the material representing the same species. For example, most of the North American specimens have apothecia that are located only in the centre of the thallus, while the rest had at least some apothecia close to the
thallus margins. Furthermore, some Russian *P. aipolia* var. *alnophila* specimens had conspicuously minute thalli compared to the rest of the var. *alnophila* material. We especially wanted to find out whether the deviating specimens form distinct phylogenetic groups and thus perhaps represent unrecognised species.

In order to scrutinize these issues, we added several new specimens to the material of our previous studies and sequenced a partial mitochondrial small subunit (mtSSU) for most of the specimens. Partial betatubulin sequences of many of the specimens were already available in the GenBank (see Myllys et al. 2001). Similarly, internal transcribed spacers (ITS) of the nuclear ribosomal DNA, as well as a nucleotide insertion identified as a group I intron at position 1516 in SSU rDNA for many of the specimens had already been sequenced (Lohtander et al. 2000, Myllys et al. 2001).

**Methods**

Several new ITS, intron and betatubulin sequences were prepared for the present study and the secondary chemistry of all available specimens was studied using thin-layer chromatography.

**TLC**

The secondary chemistry of the *Physcia* specimens was determined by thin-layer chromatography (TLC) using standard methods (solvents A and B; see Culberson 1972).

**Molecular techniques**

The total DNA of lichen samples was extracted using the QIAamp Tissue Kit (Qiagen) according to the manufacturer’s instructions with slight modifications described in Lohtander et al. (2000).

**mtSSU**

The small subunit of the mitochondrial ribosomal repeat (mtSSU) was amplified using the primers mtSSU1-KL and mtSSU2-KL (Lohtander et al. 2002). The products were sequenced using the same primers and two additional primers (mtSSU2 and mtSSU2r; see Zoller et al. 1999).

**Betatubulin**

Betatubulin primers for PCR and sequencing were Bt3-LM and Bt10-LM (for further information, see Myllys et al. 2001).

**ITS and intron**

The internal transcribed spacers ITS1 and ITS2 and the 5.8S rDNA of the nuclear ribosomal DNA, as well as insertions at position 1516 in the SSU rDNA region were sequenced using the primers ITS1-F (Gardes & Bruns 1993) and ITS4 (White et al. 1990). In cases where products of different lengths appeared after PCR, a rerun of samples was performed using primers ITS1-LM (Myllys et al. 1999) and ITS4. By using the primer ITS1-LM we were able to omit introns at the end of the SSU rDNA region and thus get a single product. All the resulting PCR products (consisting of ITS and intron) were sequenced using primers ITS5 (White et al. 1990) and ITS2-KL (Lohtander et al. 1998). However, most of the ITS sequences used in this study were obtained from our previous studies of the family Physciaceae (Lohtander et al. 2000, Myllys et al. 2001) (see Table 1 for the GenBank numbers).

All PCR reactions were performed using Amersham Pharmacia Biotech Inc. Ready To Go PCR beads following a procedure described in Lohtander et al. (2000). The PCR profile for each reaction was 60 sec at 95° (denaturation), 60 sec at 58° (annealing), and 60 sec at 72° (extension), 30 cycles, followed by 7 min at 72°. PCR products were purified with the PCR Puri fication Kit (Qiagen). The amplified DNA products were sequenced using the ABI Prism Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer). Sequence reactions were then purified using the AutoSeq™ G-50 columns of Amersham Pharmacia Biotech Inc. The purified samples were then run on an ABI Prism 377 automated sequencer (PE Biosystems).
<table>
<thead>
<tr>
<th>No.</th>
<th>species</th>
<th>Collector</th>
<th>Collection no.</th>
<th>Herb.</th>
<th>mtSSU</th>
<th>Bt</th>
<th>ITS</th>
<th>Intron</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Physaeophyscia ciliata</em></td>
<td>Himelbrant</td>
<td>K-04-23</td>
<td>LECB</td>
<td>EU682114</td>
<td>EU682154</td>
<td>EU682182</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><em>Heterodermia erinacea</em></td>
<td>Moberg</td>
<td>12212</td>
<td>UPS</td>
<td>EF582789</td>
<td>EU682155</td>
<td>EU582746</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><em>Anaptychia isidiza</em></td>
<td>Himelbrant</td>
<td>K-04-10</td>
<td>LECB</td>
<td>EF582628</td>
<td>EU682156</td>
<td>EU582780</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td><em>Physica stellaris</em></td>
<td>Moberg</td>
<td>12012</td>
<td>UPS</td>
<td>EU682115</td>
<td>EU682157</td>
<td>EU682183</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td><em>Physica adscendens</em></td>
<td>Moberg</td>
<td>12260</td>
<td>UPS</td>
<td>EU682116</td>
<td>EU682158</td>
<td>EU682184</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td><em>Physica aipolia</em></td>
<td>Hernandez &amp; Sicilia</td>
<td>XII.2002</td>
<td>UPS</td>
<td>EU682117</td>
<td>EU682159</td>
<td>EU682185</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td><em>Physcia subalbinea</em></td>
<td>Pykälä</td>
<td>9712</td>
<td>H</td>
<td>EU682118</td>
<td>EU682160</td>
<td>EU682186</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td><em>Physcia subalbinea</em></td>
<td>Moberg</td>
<td>12019</td>
<td>UPS</td>
<td>–</td>
<td>AF264090</td>
<td>AF224384</td>
<td>AF224385</td>
</tr>
<tr>
<td>9</td>
<td><em>Physcia subalbinea</em></td>
<td>Lohtander</td>
<td>316</td>
<td>S</td>
<td>EU682119</td>
<td>AF264089</td>
<td>AF224385</td>
<td>AF224385</td>
</tr>
<tr>
<td>10</td>
<td><em>Physcia subalbinea</em></td>
<td>Haikonen</td>
<td>21103</td>
<td>H</td>
<td>EU682120</td>
<td>EU682161</td>
<td>EU682187</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td><em>Physcia subalbinea</em></td>
<td>Vitikainen</td>
<td>14417</td>
<td>H</td>
<td>EU682121</td>
<td>EU682162</td>
<td>EU682188</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td><em>Physcia subalbinea</em></td>
<td>Haikonen</td>
<td>12522</td>
<td>H</td>
<td>EU682122</td>
<td>EU682163</td>
<td>EU682189</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td><em>Physcia aipolia</em></td>
<td>Tehler</td>
<td>7894b</td>
<td>S</td>
<td>EU682123</td>
<td>AF264099</td>
<td>AF224395</td>
<td>AF224395</td>
</tr>
<tr>
<td>14</td>
<td><em>Physcia aipolia</em></td>
<td>Tehler</td>
<td>7906</td>
<td>S</td>
<td>–</td>
<td>AF264097</td>
<td>AF224397</td>
<td>AF224397</td>
</tr>
<tr>
<td>15</td>
<td><em>Physcia aipolia</em></td>
<td>Tehler</td>
<td>7891</td>
<td>S</td>
<td>EU682124</td>
<td>AF264096</td>
<td>AF224396</td>
<td>AF224396</td>
</tr>
<tr>
<td>16</td>
<td><em>Physcia aipolia</em></td>
<td>Lohtander</td>
<td>404</td>
<td>S</td>
<td>–</td>
<td>AF264103</td>
<td>AF224392</td>
<td>AF224392</td>
</tr>
<tr>
<td>17</td>
<td><em>Physcia aipolia</em></td>
<td>Lohtander</td>
<td>314</td>
<td>S</td>
<td>–</td>
<td>AF264102</td>
<td>AF224390</td>
<td>AF224390</td>
</tr>
<tr>
<td>18</td>
<td><em>Physcia aipolia</em></td>
<td>Lohtander</td>
<td>400</td>
<td>S</td>
<td>–</td>
<td>AF264100</td>
<td>AF224394</td>
<td>AF224394</td>
</tr>
<tr>
<td>19</td>
<td><em>Physcia aipolia</em></td>
<td>Hermansson</td>
<td>3021a</td>
<td>UPS</td>
<td>EU682125</td>
<td>AF264101</td>
<td>AF224443</td>
<td>AF224443</td>
</tr>
<tr>
<td>20</td>
<td><em>Physcia aipolia</em></td>
<td>Urbanavichine</td>
<td>C-01333</td>
<td>LECB</td>
<td>EU682126</td>
<td>EU682164</td>
<td>EU682190</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td><em>Physcia aipolia</em></td>
<td>Moberg</td>
<td>12008</td>
<td>UPS</td>
<td>EU682127</td>
<td>–</td>
<td>AF224391</td>
<td>AF224391</td>
</tr>
<tr>
<td>22</td>
<td><em>Physcia caesia</em></td>
<td>Nordin</td>
<td>3847</td>
<td>UPS</td>
<td>EU682128</td>
<td>AF264092</td>
<td>AF224438</td>
<td>AF224438</td>
</tr>
<tr>
<td>23</td>
<td><em>Physcia sp. 1</em></td>
<td>Moberg</td>
<td>10137</td>
<td>UPS</td>
<td>EU682129</td>
<td>AF264095</td>
<td>AF224431</td>
<td>AF224431</td>
</tr>
<tr>
<td>24</td>
<td><em>Physcia sp. 1</em></td>
<td>Tehler</td>
<td>7915</td>
<td>S</td>
<td>–</td>
<td>AF264094</td>
<td>AF224393</td>
<td>AF224393</td>
</tr>
<tr>
<td>25</td>
<td><em>Physcia sp. 1</em></td>
<td>Nordin</td>
<td>5160</td>
<td>UPS</td>
<td>EU682130</td>
<td>AF264093</td>
<td>AF224432</td>
<td>AF224432</td>
</tr>
<tr>
<td>26</td>
<td><em>Physcia sp. 1</em></td>
<td>Nordin</td>
<td>5251</td>
<td>UPS</td>
<td>EU682131</td>
<td>EU682165</td>
<td>EU682191</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td><em>Physcia sp. 1</em></td>
<td>Nordin</td>
<td>5183</td>
<td>UPS</td>
<td>EU682132</td>
<td>EU682166</td>
<td>EU682192</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td><em>Physcia caesia</em></td>
<td>Mattson</td>
<td>3341b</td>
<td>UPS</td>
<td>EU682133</td>
<td>AF264104</td>
<td>AF224436</td>
<td>AF224436</td>
</tr>
<tr>
<td>29</td>
<td><em>Physcia caesia</em></td>
<td>Hansen</td>
<td>ex.782</td>
<td>UPS</td>
<td>EU682134</td>
<td>EU682167</td>
<td>EU682193</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td><em>Physcia caesia</em> (non-sorediate)</td>
<td>Otnyukova</td>
<td>21</td>
<td>UPS</td>
<td>–</td>
<td>EU682168</td>
<td>EU682194</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td><em>Physcia caesia</em> (non-sorediate)</td>
<td>Otnyukova</td>
<td>7/24/97</td>
<td>UPS</td>
<td>–</td>
<td>EU682169</td>
<td>EU682195</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td><em>Physcia caesia</em></td>
<td>Moberg</td>
<td>10810</td>
<td>UPS</td>
<td>–</td>
<td>AF264109</td>
<td>AF224436</td>
<td>AF224436</td>
</tr>
<tr>
<td>33</td>
<td><em>Physcia caesia</em></td>
<td>Tehler</td>
<td>7887</td>
<td>S</td>
<td>–</td>
<td>–</td>
<td>AF224387</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td><em>Physcia caesia</em></td>
<td>Hermansson</td>
<td>3472a</td>
<td>UPS</td>
<td>EU682135</td>
<td>AF264107</td>
<td>AF224347</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td><em>Physcia caesia</em></td>
<td>Lohtander</td>
<td>346</td>
<td>S</td>
<td>EU682136</td>
<td>–</td>
<td>AF224389</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td><em>Physcia caesia</em></td>
<td>Myllys</td>
<td>190</td>
<td>S</td>
<td>EU682137</td>
<td>AF264105</td>
<td>AF224379</td>
<td>AF224379</td>
</tr>
<tr>
<td>37</td>
<td><em>Physcia caesia</em></td>
<td>Zhurbenko</td>
<td>96305</td>
<td>LECB</td>
<td>EU682138</td>
<td>EU682170</td>
<td>EU682196</td>
<td></td>
</tr>
</tbody>
</table>
Alignment and phylogenetic analysis

Initially, several phylogenetic analyses of aligned data sets of the separate gene regions were performed in order to study tree topologies based on different data sets and to study the effect of indels (insertions and deletions). The sequences were aligned using the program ClustalW (Thompson et al. 1994). Phylogenetic trees were obtained using the heuristic search option in PAUP 4.0 (Swofford 2000), with random addition sequence and TBR. Support for each node was estimated using bootstrapping (5000 reps.), as implemented in PAUP (results not shown).

For analysing the combined DNA data set we used optimization alignment (Wheeler 1996) as implemented in POY (Gladstein & Wheeler 1997). The basic principle of optimization alignment is that alignment and search for the optimal topology are made simultaneously. This is to find topology(-ies) and alignment(s) that minimize transformations of the terminals. POY analyses were performed using the IBMSC parallel supercomputer, located at CSC — Scientific Computing Ltd., Espoo, Finland. Insertions and deletions were counted equally as transformations between sequences. Phaeophyscia ciliata, Heterodermia erinacea, Anaptychia isidiosa, as well as Physcia adscendens and P. stellaris were chosen as outgroups based on earlier studies of the family (e.g., Lohtander et al. 2000, Grube et al. 2001, Helms et al. 2003).

Results

Secondary chemistry

Physcia stellaris and P. adscendens contained atranorin only, while all the ingroup specimens contained atranorin and zeorin. There were also several unknown substances present in some of the specimens (see below).

mtSSU

The sequenced mtSSU rDNA region was 1095–1112 bp long. Most of the P. aipolia var. aipolia and P. subalbinea specimens had a 14–15 bp
deletion in the mtSSU rDNA region as compared with the other sequences (including the outgroup).

**Betatubulin**

The Bt sequences were 711–718 bp long, and they contained two introns (see also the study of Myllys et al. 2001) that were included in the analyses. Some Physcia aipolia var. alnophila specimens (11–16) contained a deletion of 7 bp in the second intron region compared to the other sequences.

**ITS**

The ITS region of the specimens was 478–483 bp long, but the length differences were due to few short insertions/deletions. Therefore the ClustalW alignment was rather unambiguous.

**Intron**

Most of the Physcia specimens possessed insertions in their nuclear SSU rDNA at position 1516. The length of the insertions varied from 223 to 237 nucleotides. However, the intron sequence is not available for all specimens, since some of the specimens appeared to have two types of the SSU rDNA region, which resulted in two DNA-bands of different length in PCR amplification. In such cases we used PCR primers that omit the intron region altogether, and only the ITS region was amplified and sequenced.

When visually inspected, there was a significant incongruence between the PAUP trees based on nuclear and mitochondrial data. However, the separate analyses each produced a tree that had the same terminal groups than those in the combined analysis (Fig. 1). The main discrepancy between the analyses was the order of the deep nodes. In all trees based on nuclear data sets (betatubulin, ITS, intron) P. aipolia var. alnophila formed a sister group to the rest of the specimens in the section Caesiae, while in the mitochondrial tree P. subalbinea and P. aipolia formed a sister group to the rest of the specimens.

**Separate and combined phylogenies**

We performed two kinds of POY analyses. The first data set (Fig. 1) contained all the sequenced gene regions. POY analysis with 58 taxa resulted in 12 equally parsimonious optimizations (trees) of 2019 steps (CI 48; RI 96).

Since the use of introns may give misleading information (Simon et al. 2005) we also performed a second analysis, in which the intron data set was excluded. That tree had a slightly poorer resolution and support.

The ingroup (sect. Caesiae) of the phylogenetic tree (Fig. 1) consists of six main groups, which are discussed below.

**Group I: Physcia subalbinea**

The group had a Bremer support value of 6, and the sorediate specimens form a subgroup that has a support value of 21. All specimens in this group have soralia that are located at lobe tips (hence typical P. subalbinea), except for specimen 6, which contains apothecia. The specimens were collected in Finland, Macaronesia (apothecial specimen 6), Russia and Sweden. The specimens were growing on Chamaecytisus (specimen 6) or rocks (mostly granite). This group is especially rich in secondary substances. All sorediate specimens in the group contain atranorin and zeorin (the apothecial specimen 6 was not available for TLC). There are also additional substances. Specimens 7, 9, 10 and 11 contain the substance “X-unknown” that is slightly fluorescent (blue) and with Rf class values of 2 (solvent A) and 1.5 (solvent B). Many of the specimens also contain an unknown substance that is only observable in the solvent B (Rf 5) or two unknown UV+ substances that have Rf values of 4 (solvent A) and 5.5 (solvent B); 6.5 (solvent A) and 6.5 (solvent B).

**Group II: Physcia aipolia**

Group II contains specimens that are morphologically closest to P. aipolia s. stricto, having numerous apothecia of variable size. The specimens are from Finland, Sweden and Russia, and
all but one specimen (specimen 20 from walnut) in this group have been collected on Populus. One of the specimens (19) was not available for TLC, but all the other specimens contain atranorin and zeorin. Specimen 17 also contains the substance “X-unknown” (Rf A 2, UV+ blue) that is otherwise present in the P. subalbinea group (see above).
Group III: Physcia sp. 1

Group III consists of a basal *P. caesia* from Mexico and a well-supported group (Bremer support 15) of *P. aipolia* specimens mainly from the U.S.A., but also from Romania (specimen 25) and Sweden (specimen 26). The European and American specimens group separately. The specimens in this group differ from the rest of the *P. aipolia* and *P. caesia* specimens by having crowded apothecia/soralia at the middle of the thallus and a very wide peripheral thallus margin without any dispersal organs. The specimens in this group only contain atranorin and zeorin. Specimen 24 was unfortunately not available for TLC at the moment. Group III may represent a distinct species.

Group IV: Physcia caesia

Group IV (Fig. 1) contains *P. caesia* specimens that have capitate soralia (hence typical *P. caesia*). The group has a rather poor Bremer support (only 4) and contains specimens from Greenland, Finland, Romania, Russia and Sweden. Most of the specimens were collected on bird-perching stones, limestone or stone wall (see Fig. 1). All specimens in this group contain atranorin and zeorin.

Group V: Physcia cf. phaea

Group V (Fig. 1) consists of specimens collected in Finland and the U.S.A. Saxicolous specimens 40 and 41 were determined as *P. phaea*. The specimens contain atranorin and zeorin and specimen 41 also contains an unknown substance that was only visible in the solvent B (Rf value 4.5). Specimens 46 contains an unknown substance that was only visible in the solvent B (Rf value 4.5). Specimens 48 and 49 contain two unknown substances with Rf values of 4.5 (A) and 6 (solvent B).

Since morphological characters, such as small thalli, as well as numerous, crowded, small apothecia that occur almost at the tips of the lobes (see Moberg 1977), together with the DNA sequence data and distinct distribution, uniformly support a recognition of an exclusive group with *Physcia aipolia var. alnophila* specimens, we here raise this taxon to species level.

**Physcia alnophila** (Vain.) Loht., Moberg, Myllys & Tehler, **comb. nova**


Thallus orbicular, up to 4 cm in diameter, with narrow lobes. Apothecia very common, crowded and small (up to 1.5 mm) usually with convex disc provided with white pruina. Spores 16–22(–24) × 7–10 μm.

In Scandinavia and Finland *Physcia alnophila* has a more northern distribution than *P. aipolia*, and it is often growing on *Alnus* and *Salix*, while *P. aipolia* prefers *Populus* (Moberg 1977). When collecting specimens for our study, *P. aipolia* and atranorin and zeorin. This group may represent an undescribed species.

Group VII: Physcia aipolia var. alnophila

Group VII consisted of typical *P. aipolia var. alnophila* specimens, having crowded small apothecia that occur at the tips of the thallus lobes. However, one of the specimens (51: Fig. 1) was determined as *P. phaea*, since it was collected on rock. The geographical origins of the specimens were Canada, Finland, Norway, Russia, and the U.S.A. Most of the specimens were from *Populus*, but nearly as many from *Salix*. This clade was further divided into several well-supported subgroups (see Fig. 1). All specimens contain atranorin and zeorin, but there are also some additional substances present. Specimen 46 contains an unknown substance that was only visible in the solvent B (Rf value 4.5). Specimens 48 and 49 contain two unknown substances with Rf values of 4.5 (A) and 6 (solvent B).
**Discussion**

There was a significant incongruence between the gene regions when the phylogenetic trees based on the different data sets were visually compared. Especially the nuclear and mitochondrial genes resulted in different tree topologies. The terminal groups were more or less stable in each gene tree, but the deep nodes were different between the nuclear and the mitochondrial gene trees. The addition of the mtSSU data to the rest of the data sets resulted in a tree that was different as compared with that in the study of Myllys et al. (2001).

We already knew from the study of Myllys et al. (2001) that the betatubulin, ITS and intron data sets were significantly incongruent, although there were only few discrepancies between trees based on the different data sets, incongruence in gene trees may be due to occasional gene flow and recombination of different taxa. However, Myllys et al. (2001) combined their data sets, since the combination of seemingly deviating data sets may reveal groupings that cannot be seen in any of the trees based on separate data sets. Also in the present case (Fig. 1) the analysis of the combined data set resulted in groups that had better support than in any of the trees based on individual data sets. Furthermore, new groups that were not present in trees based on individual data sets appeared in a tree based on the combined data set. However, more information of the specimens is needed to clarify whether hybridization, introgression or lineage sorting resulting from recent diversification is responsible for the deviating results.

**Group I: Physcia subalbinea**

*Physcia subalbinea* has a secondary chemistry different from most of the other specimens in this study. Some substances present in *P. subalbinea* were also found from specimens that group outside the *P. subalbinea* clade. Several TLC-tested *P. subalbinea* specimens not included into the molecular study had the substance “X-unknown”, while the substance was absent from the tested *P. caesia* specimens. According to the molecular data (see Fig. 1) *P. subalbinea* is more closely related to *P. aipolia* than to another sorediate species *P. caesia* and thus represents a taxon distinct from *P. caesia*.

**Group II: Physcia aipolia**

Group II (Fig. 1) corresponds to typical *P. aipolia* based on morphological characters.
Group III: Physcia sp. 1

This clade contains apothecial and sorediate specimens. They all have a common feature with crowded apothecia/soredia only at the middle of the thallus and hence very wide thallus margins. The group containing the apotheciate specimens is very well supported. This clade (at least the well-supported group with the apotheciate specimens) most probably represents a species of its own.

Group IV: Physcia caesia

Group IV (Fig. 1) corresponds to P. caesia. Two of the specimens (30 and 31) had apothecia together with soralia and they were previously determined as P. phaea. This group is, however, poorly supported.

Group V: Physcia cf. phaea

All the apothecial specimens growing on rocks that were included in this study had been previously determined as P. phaea. However, only two of all these specimens (group V; Fig. 1) studied may actually correspond to P. phaea. The other “P. phaea” specimens appeared to be either P. alnophila growing on rocks or apotheciate P. caesia specimens. Specimens incorrectly determined as P. phaea have also been found from clades outside the P. aipolia–P. caesia group (authors’ unpubl. data). Species delimitation and identification of this taxon is still somewhat unclear.

Group VI: Physcia sp. 2

Group VI consists of two specimens that have conspicuously minute thalli compared to the rest of the material (see Introduction). Since the specimens in this group (Fig. 1) appeared much smaller than those of P. alnophila, and they formed a separate well supported group (Bremer support 12), they may prove to represent a species new to science. However, more specimens are needed before the species status can be confirmed.

Group VII: Physcia alnophila

Physcia aipolia var. alnophila constituted a well-defined group that was clearly separate from P. aipolia var. aipolia, which instead grouped together with P. caesia (Fig. 1). Since the molecular data was also supported by morphological and ecological characters, we find that the new combination P. alnophila is well justified.

Species pairs

Based on this study it seems apparent that P. aipolia and P. caesia do not form a species pair sensu Poelt (1972), since the two taxa are more closely related to other species than to each other (Fig. 1). However, they seem not to form a single species either, as was suggested in Lohtander et al. (2000) and in Myllys et al. (2001), neither do they represent typical cryptic species (see e.g. Kroken & Taylor 2001), since the groupings are also supported by morphological and/or chemical characters. Instead, according to the molecular data, the P. aipolia–P. caesia group contains several groups that also have morphological and/or chemical characters and that thus can be treated as species.

Acknowledgements

We thank Jaana Kekkonen who performed most of the laboratory tasks and Dr. Gennadii Urbanavichus and Dr. Dmitry Himelbrandt, who provided fresh specimens. This study was financially supported by NFR grant B-BU9702-302 (Swedish Natural Science Research Council) and the Academy of Finland (grant 106118), as well as by The Finnish Cultural Foundation.

References


Gardes, M. & Bruns, T. D. 1993: ITS primers with enhanced specificity for basidiomycetes — application to the iden-
tification of mycorrhizae and rusts. — *Molecular Ecology* 2: 113–118.


This article is also available in pdf format at http://www.annbot.net