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# Phylogenetic analysis of ITS and group I intron sequences from European and North American samples of cetrarioid lichens

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Phylogenetic analyses were carried out on 23 samples representing 10 cetrarioid taxa from Europe and North America, representing 10 cetrarioid taxa; they were compared in parsimony analyses. The study is focused on ITS sequences of the ribosomal DNA. Intervening sequences, most likely representing group I introns, were found in four taxa. The results from the phylogenetic analysis are congruent with species delimitations derived from morphological characters, regardless of the geographic origin of the material. ITS and group I intron sequences from populations within the same species usually differ at very few base positions. The inclusion of *Cetraria aculeata* (Schreb.) Fr. in *Cetraria* Ach. and the exclusion of *Melanelia hepatizon* (Ach.) A. Thell, *Tuckermannopsis chlorophylla* (Willd.) Hale, and the genera *Cetrelia* W. L. Culb. & C. F. Culb., *Flavocetraria* Kärnefelt & A. Thell, and *Platismatia* W. L. Culb. & C. F. Culb. from *Cetraria* is supported by the analyses, if the genus *Vulpicida* J.-E. Mattsson and M. J. Lai is maintained in this grouping.

Key words: Ascomycotina, *Cetraria*, group I intron, ITS, phylogeny, rDNA

## INTRODUCTION

Recently 135 species were included in a second updated world list of cetrarioid lichens, a polyphyletic group of macrolichens in the Parmeliaceae (Randlane *et al*. 1997). This assembly is morphologically highly heterogeneous, and cannot be delimited without difficulty. An attempt was made in the latest world list: all species were included that belong to *Cetraria* Ach. at present or have been placed there earlier. Some of the listed species have been transferred to, or described in, any of the 21 allied segregates (Randlane *et al*. 1997). A large majority of the cetrarioid species occur on the ground or on barks in the arctic, alpine and boreal zones of the Northern Hemisphere.

The genus *Cetraria* in the strict sense, composed of 15 species, was delimited by Kärnefelt *et al*. (1993). Two further species were added later (Kärnefelt & Thell 1993, van den Boom & Sipman 1994).

The utility of the internal transcribed spacer

(ITS) of the ribosomal RNA genes for phylogenetic analyses within cetrarioid lichens was recently demonstrated in the genera *Platismatia* W. L. Culb. & C. F. Culb. and *Tuckermannopsis* Gyelnik (Thell 1998, Thell *et al*. 1998). The small subunit, SSU, has been the more commonly used tool for recent phylogenetic studies in lichens (Gargas *et al*. 1995a), whereas the sequences from the ITS region are suitable for resolution of closely related taxa (Mattsson & Wedin 1998, Lutzoni 1997), or for studies at a population level (Crespo *et al*. 1997). Thus, the present study is to further extend our knowledge of the ITS region within cetrarioid lichens for systematic investigations, and to compare European and North American representatives of species occurring in both continents.

The ITS of the rRNA is highly variable but can be amplified from genomic DNA using primers specific for conserved sequences flanking the ITS itself (White *et al*. 1990, DePriest 1993, Palumbi 1996). Primers ITS4 and ITS5 are typically used to amplify a region 550 to 600 bp in length, including the non-coding sections ITS 1 and ITS 2, the 3´ end of the SSU, the 5´ end of the large subunit and the entire 5.8 S rRNA gene.

## MATERIAL AND METHODS

### The material

Fresh material was collected to represent the taxa selected for this study, now archived in the herbaria BG, H, LD, TDI (TerraGen Diversity Inc.) and UBC. The extracted DNA belongs to two different series. DNA#AT is kept at LD, while DNA#VM is kept at TDI (Table 1).

### Extraction methods

Lichen genomic DNA from the #AT series was extracted using a CTAB (hexadecyl trimethylammonium bromide) detergent buffer, followed by chloroform separation and isopropanol precipitation (Thell *et al*. 1998). DNA of the #VM series was obtained from ground lichen samples following a standard method used for filamentous fungi: this method includes lysis in a sodium lauroyl sarcosine solution, isopropanol precipitation in ammonium acetate, and phenol/chloroform extraction followed by ethanol precipitation (Miao *et al*. 1991).

### Amplification

The ITS region was amplified from genomic DNA by the polymerase chain reaction (PCR) using the primers ITS5 and ITS4 (White *et al*. 1990). A 30 cycle PCR was performed with a denaturation temperature of 94°C for 1 min., an annealing temperature of 48°C for 1 min., and an extension temperature of 72°C for 45 seconds. PCR products were electrophoretically purified through agarose twice (Thell *et al*. 1998), and sequenced with four primers, ITS2, ITS3, ITS4 and ITS5 (White *et al*. 1990) using an automatic sequencer, ABI Prism 377, and an ABI Prism Dye Terminator Cycle Sequencing Ready Reaction Kit from Perkin Elmer was used for the sequencing PCR (ABI Perkin Elmer).

### Sequencing and phylogeny

Sequence data were aligned with SeqApp/CAP 2 (Huang 1992, Gilbert 1993), slightly adjusted by hand, and transferred to PAUP 3.1.1. (Swofford 1993). Bootstrap analyses with 500 standard parsimony bootstrap replicates, using the heuristic searches with the tree bisection-reconnection method of swapping branches, were performed. A preliminary parsimony analysis without branch-swapping was processed for the ITS matrix to find and exclude identical sequences or samples that had no unique characters of their own, thus identical to reconstructed ancestors. Ten sequences were excluded (Fig. 1, Tables 2 and 3). Removing these sequences does not affect the bootstrap results but considerably speeds up the computation process for the bootstrap replicates. *Cetrelia cetrarioides* (Duby) W. L. Culb. & C. F. Culb. and *Melanelia hepatizon* (Ach.) A. Thell were selected as outgroups in the two parsimony analyses.

## RESULTS

### The ITS region and group I introns of cetrarioid lichens

The majority of the PCR products recovered after amplification of the ITS were in the expected range, between 550–570 base pairs. In four of the nine species studied, sequences of almost 800 base pairs occurred. Alignment of the sequences to each other and with reference to the *Escherichia coli* rRNA (Gutell *et al*. 1994) showed that the extra length in each case was caused by the insertion of single tracts about 220 base pairs, between positions 1516 and 1517 of the SSU (Gargas *et al*. 1995b). Using the insertion sequence of *Cetraria ericetorum* Opiz ssp. *ericetorum* (DNA AT#212, Table 4)

Table 1. Locality data for the specimens.

| Species | European samples | North American samples |
|---|---|---|
| *Cetraria aculeata* (Schreb.) Fr. | Sweden, Scania, Olseröd, Nyagrop, 9.VI.1996, *A. Thell SK-9606*, DNA#AT11 (LD). | Canada, British Columbia, Mayne Island, Mt. Parke, 7.IV.1996, *T. Taylor*, DNA#AT156 (TDI#217). |
| *Cetraria ericetorum* Opiz ssp. *ericetorum* | Finland, Nyland, Helsingfors, Viksbacka, 11.III.1997, *A. Thell FIN-9706*, DNA#AT212 (LD). | |
| *Cetraria ericetorum* ssp. *reticulata* (Räs.) Kärnefelt | | Canada, British Columbia, Pemberton, DNA#AT187 (TDI#225). British Columbia, Kamloops, Jacko Lake, 16.VIII.1996, *A. Thell & C. Veer BC-9644*, DNA#AT44 (LD). |
| *Cetraria islandica* ssp. *islandica* (L.) Ach. | Iceland, Nordu-Thingeyar sysla, Asheidi, 3.IX.1995, *O. Andrésson*, DNA#AT#94 (TDI#151). Sweden. Scania, Vitaby, Kivik, 10.VI.1996, *A. Thell SK-9607*, DNA#AT41 (LD). | Canada, British Columbia, Pemberton, 7.VIII.1995, S. Delcardayre, DNA#AT93 (TDI#125). |
| *Cetraria sepincola* (Ehrh.) Ach. | Austria, Styria, Seetaler Alpen, 29.VIII.1996, *I. Kärnefelt* ÖS960411, DNA#AT139 (LD). Sweden, Scania, Örkened, Esseboda, 2.VI.1996, *A. Thell SK-9608*, DNA#AT03 (LD). | Canada, British Columbia, Clearwater, 1996-10, *T. Goward TG-961347*, DNA#AT151 (UBC). |
| *Cetrelia cetrarioides* (Duby) W. L. Culb. & C. F. Culb. | Austria, Styria, Schladminger Tauern, Kleinsölktal, Breitlahnalm, 30.VIII.1996, *I. Kärnefelt ÖS960606*, DNA#AT140 (LD). | Canada, British Columbia, Harrison Mills, Tappadera Farm, 21.II.1997, *C. Olson*, DNA#AT214 (TDI#230). USA, Washington, S. of Tacoma, Pack Forest, 20.III.1996, *V. Miao*, DNA#AT154 (TDI#206). |
| *Flavocetraria cucullata* (Bellardi) Kärnefelt & A. Thell | Austria, Styria, Seetaler Alpen, 29.VIII.1996, *I. Kärnefelt ÖS960410*, DNA#AT138 (LD). | Canada, Alberta, Cardinal Divide, V. Miao, DNA#VM149 (TDI#122). |
| *Flavocetraria nivalis* (L.) Kärnefelt & A. Thell | Sweden, Scania, Rinkaby rifle range, 1995-05, *J.-T. Johansson*, DNA#AT#01 (LD). | Canada, Alberta, Berland River (N of Jasper), *V. Miao*, DNA#VM153 (TDI#126). |
| *Melanelia hepatizon* (Ach.) Thell | Finland, Nyland, Sjundeå, Västerby, 15.IV.1997, *M. Kuusinen FIN-9713*, DNA#AT224 (LD). | Canada, British Columbia, Brew Lake, 1.IX.1996, *S. Delcardayre*, DNA#AT183 (TDI#223). |
| *Platismatia glauca* (L.) W. L. Culb. & C. F. Culb. | Sweden, Scania, Örkened, S. Esseboda, 2.VII.1996, *A. Thell SK-9613*, DNA#AT42 (LD). | Canada, British Columbia, Chilliwack, Vedder Peak, 1.IX.1996, *S. Hayden*, DNA#AT159 (TDI#218). British Columbia, Vancouver, U. B. C., Student Union Bldg., 27.XII.1996, *A. Thell BC-96191*, DNA#AT191 (LD). |
| *Platismatia norvegica* (Lynge) W. L. Culb. & C. F. Culb. | Norway, Hordaland, Sund, Sotra Tælavåg, 14.III.1997, *S. Ekman 3007*, DNA#AT217 (BG, LD). | Canada, British Columbia, Garibaldi Lake, 7.VII.1995, *V. Miao*, DNA#AT172 (TDI#204). USA, Washington, Mt. Baker, 8.V.1995, *S. Delcardayre*, DNA#VM57 (TDI#40). |
| *Tuckermannopsis chlorophylla* (Willd.) Hale in Egan | Sweden, Scania, Eslöv, Trollsjön, 30.VI.1996, *A. Thell SK-9602*, DNA#AT39 (LD). Scania, Örkened, Esseboda, 2.VI.1996, *A. Thell SK-9603*, DNA#AT04 (LD). | Canada, British Columbia, Garibaldi Prov. Park, Rubble Creek parking area, 26.IX.1996, *V. Miao & T. Taylor*, DNA#AT78 (TDI#207). British Columbia, Vancouver, U. B. C., Vancouver School of Theology, 12.XII.1996, *A. Thell BC-96185*, DNA#AT185 (LD). |
| *Vulpicida pinastri* (Scop.) J.-E. Mattsson & M. J. Lai | Sweden, Scania, Örkened, Ulfshult, 23.VI.1996, *A. Thell SK-9604*, DNA#AT02 (LD). | Canada, Alberta, Rock Lake, 30.VII.1995, *V. Miao*, DNA#VM391 (TDI#180). |

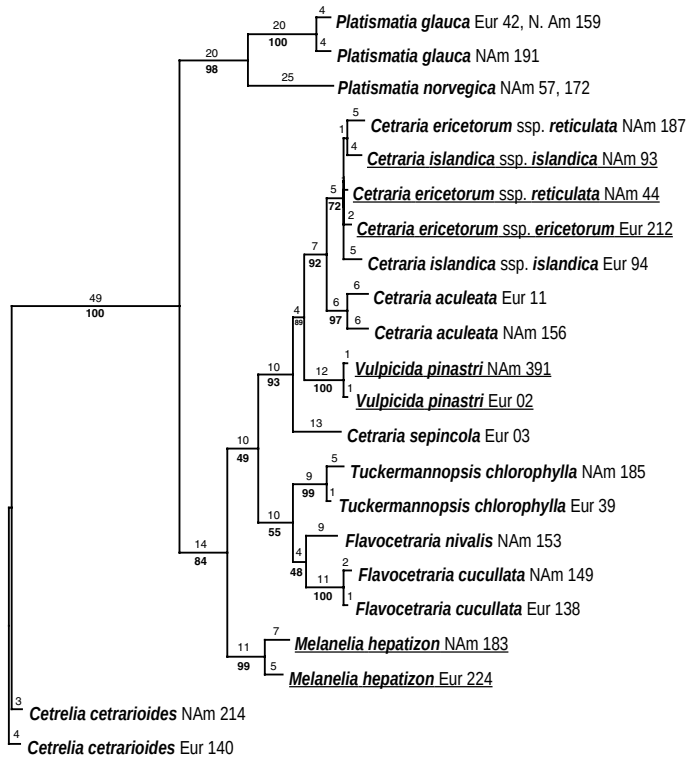


Fig. 1. Consensus tree derived from 66 most parsimonious trees based on 577 base positions from the ITS region (Tables 2–3), length = 317, CI = 0.669, generated using heuristic searches in PAUP 3.1.1. The numbers above or to the right of the branches are substitution numbers per branch. Branch lengths are proportional to substitution numbers. Underlined samples indicate presence of group I introns (Fig. 2, Table 4). Bootstrap percentages are in bold face (from 500 replicates).

for comparison, a search of a public database at National Center for Biotechnology Information, NCBI, homepage: http://www.ncbi.nlm.nih.gov, using the BLASTN algorithm identified a segment of 59 bp with greater than 90% identity to sequences recognized as group I introns (Altschul *et al*. 1990). The sequence from *C. ericetorum* could be aligned with and folded to closely resemble a group I intron, CcgSSUI-5, from *Cladonia grayi* (DePriest & Been 1992). This sequence, as well as the other very similar insertion sequences in this study (Table 4) were therefore interpreted as presumptive group I introns.

After removing intron sequences, all remaining data were easily aligned (Tables 2 and 3). All sequences are identical until position 33, the 3´ end of the SSU:

5´ GTTTCCGTAG GTGAACCTGC GGAAGGATCA TTA 3´

The ITS 1 section extends from position 34 to 224 and is highly variable. The conserved 5.8S rRNA gene starts at position 225, and the sequences are, except for the *Melanelia* and *Platismatia* samples at position 288 and for *Platismatia glauca* (L.) W. L. Culb. & C. F. Culb. at position 371, identical up to position 382 (Thell *et al*. 1998: Fig. 2):

5´AAAACT TTCAACAACG GATCTCTTGG TTC CAGCATC ATGAAGAACG CAGCGAAATG CG ATAACTAA TGTGAATTGC AGAATTCATG AA TCATCGAG TCTTTGAACG CACATTGCGC CC CTCGGTAT TCCGGGGGGC ATGCCTGTTC GA GCGTCATT 3´

The ITS 2 section lies between positions 383 and 537. The last part is the 5´ end of the LSU, having identical sequences for all samples:

5´TTG ACCTCGGATC AGGTAGGGAT ACCCGCT GAA CTTAAGC 3´

ITS and group I intron sequences from populations within the same species are either identical or usually differ at very few base positions, regardless of the geographic origin of the material (Tables 2–4).

### The species included in the study

Thirty-two samples divided between twelve species, occurring in both Europe and North America, were included in the study. Sequences from the ITS regions of *Cetrelia cetrarioides*, *Platismatia glauca* and *P. norvegica* (Lynge) W. L. Culb. & C. F. Culb., presented earlier by Thell *et al.* (1998) were used here as an outgroup (*Cetrelia cetrarioides*) or included for comparison with other species in the phylogenetic analysis (Fig. 1). Sequences of *Cetraria aculeata* (Schreb.) Fr., *C. ericetorum* Opiz, *C. islandica* (L.) Ach., *C. sepincola* (Ehrh.) Ach., *Flavocetraria cucullata* (Bellardi) Kärnefelt & A. Thell, *F. nivalis* (L.) Kärnefelt & A. Thell, *Melanelia hepatizon* (Ach.) A. Thell, (Willd.) Hale and *Vulpicida pinastri* (Scop.) J.-E. Mattsson & M. J. Lai are presented here for the first time.

Group I introns occurred in eight samples representing the species *Cetraria ericetorum, C. islandica, Melanelia hepatizon* and *Vulpicida pinastri*. In *Cetraria* it was, however, absent in one of the *C. ericetorum* ssp. *reticulata* samples and in the material of *C. islandica* collected in Iceland (Table 4).

## DISCUSSION

### Selection of outgroups

*Cetrelia cetrarioides* was thought to have an isolated position among cetrarioid lichens, having a *Parmelia*-form of ascus not shared by other species included here (Kärnefelt & Thell 1992, Thell *et al*. 1995). Thus, we selected *C. cetrarioides* as the outgroup in the ITS analysis. ITS sequences from one specimen collected in Austria and two from western North America were previously reported by Thell *et al*. (1998).

*Melanelia hepatizon* was selected as the outgroup in the phylogenetic analysis of the group I introns (Fig. 2). Compared with *Cetraria s. str.* it is the most distantly related taxon among those containing introns — according to the phylogenetic tree based on ITS sequences (Fig. 1).

### Monophyletic groups

When using *Cetrelia cetrarioides* as the outgroup taxon, *Platismatia* appears as a sister group to other cetrarioid species (Fig. 1). The phylogeny of *Platismatia* was discussed earlier while the ITS sequences of the remaining 10 taxa are presented here for the first time. This group was supported by a bootstrap value of 84%. The two populations of *Melanelia hepatizon* constitute a sister group to the remaining nine taxa, which were loosely kept together by a bootstrap value of 49% (Fig. 1). *Melanelia hepatizon* belonged to the *Cetraria commixta* group, now accomodated in *Melanelia* (Esslinger 1977, Thell 1995).

Two monophyletic lines are observed in the sister group. The first line, supported by a bootstrap value of 55%, contains *Tuckermannopsis chlorophylla, Flavocetraria cucullata*, and *F. nivalis*. We do not believe in a very close relationship between these two genera, and this group is an item for an extended analysis of species assigned to *Tuckermannopsis* (Thell 1998). *Tuckermannopsis chlorophylla* was together with several other species transferred from *Cetraria* by Hale (in Egan 1987). The genus *Tuckermannopsis* was briefly described by Gyelnik (1933) and not commonly used until it was reinstated by Lai (1980). Several taxa that received a new position in *Tuckermannopsis* by Lai and Hale are now removed but the genus is still not monophyletic.

The second monophyletic group has a much stronger support, bootstrap value 93%. *Cetraria s. str*., *Vulpicida pinastri* and *Cetraria sepincola* belong here (Fig. 1). Both the *Cetraria* and *Lecanora* ascus types — the two latter taxa — are represented in this group as well.

### Delimitation of *Cetraria* in the strict sense

*Cetraria s. str*. is represented by four taxa in the study, *C. aculeata, C. ericetorum* ssp. *ericetorum*, *C. ericetorum* ssp. *reticulata* and *C. islandica* ssp. *islandica*. *Cetraria aculeata* was transferred back to *Cetraria* together with three other species from *Coelocaulon* Link (Kärnefelt *et al*. 1993). The two genera differed in the appearance of the lobes only,

Table 2. ITS 1 sequences from European (Eur.) and North American (N. Am.) populations of 12 cetrarioid species. The numbers refer to DNA numbers in Table 1.

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                                                    40         50         60         70         80         90
Cetraria aculeata Eur.11                        5´  CTGAGAG AGGGGCTTCG CGCTCCCGGG GGCCTCGGCC CCCTACTCTT CCCCC-TTTG TGTACC-
Cetraria aculeata N. Am. 156                        ....... .......... .......... ..T...C... ..TA...... .A........ .......
Cetraria ericetorum ssp. ericetorum Eur. 212        ....... .......... .......... ..T...C... ..TA...... .A........ .......
Cetraria ericetorum ssp. reticulata N. Am. 44       ....... .......... .......... ..T...C... ..TA...... .A........ .......
Cetraria ericetorum ssp. reticulata N. Am. 187      ....... .......... .......... ..T...C... ..TA...... .A........ .......
Cetraria islandica Eur. 41                          ....... .......... .......... ..T........ ..TA...... .A........ .......
Cetraria islandica Eur. 94                          ....... .......... .......... ..T........ ..TA...... .A........ .......
Cetraria islandica N. Am. 93                        ....... -......... .......... .......... ..TA...... .A........ .......
Cetraria sepincola Eur. 03                          ....... -......... ......-... ..T........ ...A...... .A....A... AC.....
Cetraria sepincola Eur. 139, N. Am. 151             ....... -......... .......... ..T........ ...A...... .A....A... AC.....
Cetrelia cetrarioides Eur. 140                      .C..... C........A T..C.....A ...T...... T.TA...... .A...CA... CC..T.T
Cetrelia cetrarioides N. Am. 154                    .C..... C........A T..C.....A ...T...... T.TA...... .A...CA... CC..T.T
Cetrelia cetrarioides N. Am. 214                    .C..... C........A T........A ...T...... T.TA...... .A...CA... CC..T.T
Flavocetraria cucullata Eur. 138                    ....... .......... .......... ..TT...... T.TAG..... .G....A... .C...A.
Flavocetraria cucullata N. Am. 149                  ....... .......... .......... ..TT...... T.TAG..... .A....A... .C...A.
Flavocetraria nivalis Eur. 01                       ....... .......... .......... ...T...... T..A...... .A....A... .C.....
Flavocetraria nivalis N. Am. 153                    ....... .......... .......... ...T...... T..A...... .A....A... .C.....
Melanelia hepatizon Eur. 224                        ....... .......... .......... ..T...A-T. ...A...... .A....A... .C..T..
Melanelia hepatizon N. Am. 183                      .C..... .......... .......... ......A-.. ...A...... .A....A... .C..T..
Platismatia glauca Eur. 42, N. Am. 159              .C..... -......-.. ...C...... ...TC..... ...G.....C .A....A... C......
Platismatia glauca N. Am. 191                       ....... -.....-... ...C...... ...TC..... ..GG.....C .A....A... C......
Platismatia norvegica Eur. 217                      .C..... -......... ...C...... ....C..... ...G.....C .A....G... CAC....
Platismatia norvegica N. Am. 57, 172                .C..... -......... ...C...... ....C..... ...G.....C .A....G... CAC....
Tuckermannopsis chlorophylla Eur. 02, N. Am. 78     ....... .........- ---------- ..T.------ ...A...... .A....A... .C.....
Tuckermannopsis chlorophylla Eur. 39                ....... .........- ---------- ..TT------ ...A...... .A....A... .C.....
Tuckermannopsis chlorophylla N. Am. 185             ....... .........- ---------- ..T.------ ...A...... .A....A... .CC....
Vulpicida pinastri Eur. 02                          ....... .......... ..T....... ..T....... ...A...... .A....A... .G.....
Vulpicida pinastri N. Am. 391                       ....... .......... ...C...... ..T....... ...A...... .A....A... .G.....

                                                    100        110        120        130        140        150        160
Cetraria aculeata Eur. 11                           AAC CTTTGTTGCT TT-GGCGGGC C-CGAGGACC -CCTCGCGCC GCGT-AC-AA ACCGGCGAGC
Cetraria aculeata N. Am. 156                        ... .......... .......... .......... .T........ .T........ ..........
Cetraria ericetorum ssp. ericetorum Eur. 212        ... .......... .........T ..T....... .T........ ..CC.....G G.........
Cetraria ericetorum ssp. reticulata N. Am. 44       ... .......... .........T .......... .T........ ..CCC....G G.........
Cetraria ericetorum ssp. reticulata N. Am. 187      ... .......... .........T .......... .T........ ..CC.....G G.........
Cetraria islandica Eur. 41                          ... .......... .........T .......... .T........ ..CC.....G G.........
Cetraria islandica Eur. 94                          ... .......... .........T .......... .T........ ..CC.....G G.........
Cetraria islandica N. Am. 93                        ... ..C....... .........T .......... .T........ ..CC.....G G.........
Cetraria sepincola Eur. 03                          T.. .......... .......... .C........ .T........ .........G G.........
Cetraria sepincola Eur. 139, N. Am. 151             T.. .......... .......... .C........ .T........ .........G G.........
Cetrelia cetrarioides Eur. 140                      T.. .......... .......... .T.....T.. C-........ .ACCCT..GG GT........
Cetrelia cetrarioides N. Am. 154                    T.. .......... .......... .T.....T.. C-........ .ACCCT..-G GT........
Cetrelia cetrarioides N. Am. 214                    T.. .......... .......... .T.....TT. C-........ .ACCCT..GG GT........
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Flavocetraria cucullata Eur. 138                  T.. ......... ......... .T..G.C... GT.C..T.T. .AC.G...TG GT........
Flavocetraria cucullata N. Am. 149                T.. ......... ......... .T..G.C... GT.C..T.T. .AC.G...TG GT........
Flavocetraria nivalis Eur. 01                     T.. ..A...... ......... .T..G.T... AT.C..T.T. .GCCT...CG GT........
Flavocetraria nivalis N. Am. 153                  T.. ..A...... ..C...... .T..G.T... AT.C..T.T. .GCCT...CG GT........
Melanelia hepatizon Eur. 224                      T.. ......... ......... .CT.G..T.T ........T. .GC.TTG..G G.........
Melanelia hepatizon N. Am. 183                    T.. ......... ......... .C..G..TTT ........T. .GC.TT...G G.........
Platismatia glauca Eur. 42, N. Am. 159            G.. .......A.. ........A. .C..G..T.T C..C.....T .GC.CC..GG G.........
Platismatia glauca N. Am. 191                     G.. ......... ........A. .C..G..T.T C..C.....T .GC.TC.TGG G.........
Platismatia norvegica Eur. 217                    T.. ......... ........A. .C..G..TT. T..C.....T .GCC.C.TGG G.........
Platismatia norvegica N. Am. 57, 172              T.. ......... ........A. .C..G..TT. T..C.....T .GCC.C.TGG G.........
Tuckermannopsis chlorophylla Eur. 02, N. Am. 78   T.. ..A...... ......... .T..G.T.T. AT.C..T.T. .GC.T...CG GT........
Tuckermannopsis chlorophylla Eur. 39              T.. ..A...... ......... .T..G.T.TT AT.C..T.T. .GC.T...CG GT........
Tuckermannopsis chlorophylla N. Am. 185           T.. ..A...... ......... .T..G.T.T. AT.C..T.T. .GC.T...CG GT........
Vulpicida pinastri Eur. 02                        T.. ......... ......... .C.......T -T.......T ..C......G GG........
Vulpicida pinastri N. Am. 391                     T.. ......... ......... .C.......T -T.......T ..C......G GG........

                                                         170         180         190         200         210         220
Cetraria aculeata Eur. 11                         GCCCGCCAGA GGCCCATTAA AATCTGCTTA TTAGTGATGT CCGAGCGAAA AACACAATCA AT— 3´
Cetraria aculeata N. Am. 156                      .......... .......... .......... .......... .......... ..-.....G ....
Cetraria ericetorum ssp. ericetorum Eur. 212      .......... .......... .-........ .......... .......... ........A. ....
Cetraria ericetorum ssp. reticulata N. Am. 44     .......... .......... .......... .......... .......... ........A. ....
Cetraria ericetorum ssp. reticulata N. Am. 187    .......... .......... .......... .......C.. .......... ........A. ....
Cetraria islandica Eur. 41                        .......... .......... .......... .......... .......... ........A. ....
Cetraria islandica Eur. 94                        .T........ .......... ......T... .C........ .......... ........A. ....
Cetraria islandica N. Am. 93                      .......... .......... .......... .......... .......... ........A. ....
Cetraria sepincola Eur. 03                        .......... ........C. .T....T... .C........ .....T.-.. .......... ...C
Cetraria sepincola Eur. 139, N. Am. 151           .......... ........C. .T....T... .C........ .....T.-.. .......... ...C
Cetrelia cetrarioides Eur. 140                    .T..G..... ..T....... .T...AT..- .-....G... .....TC... .C......AG -..C
Cetrelia cetrarioides N. Am. 154                  .T..G..... ..T....... .T...AT..- .-....G... .....TC... .C......AG -..A
Cetrelia cetrarioides N. Am. 214                  ....G..... ..T....... .T...AT... .C....G... .....TC... .C......AG -..A
Flavocetraria cucullata Eur. 138                  .....T.... ....A..C.. .TT..AT... .C........ ......-.....A. -..C
Flavocetraria cucullata N. Am. 149                .....T.... ....A..C.. .TT..AT... .C........ ......-... ...G....A. -..C
Flavocetraria nivalis Eur. 01                     .....T.G.. ....A..C.. .TC..AT... .......... .....T-... ........AG -..C
Flavocetraria nivalis N. Am. 153                  .....T.G.. ....A..C.. .TC..AT... .......... .....T-... ........AG -..C
Melanelia hepatizon Eur. 224                      .....T.... ...T...... .T....T... .C........ ......G... -CT.....-. ...A
Melanelia hepatizon N. Am. 183                    .....T.... ...T..A... .T....T... .C........ ......G... -C......-. ...C
Platismatia glauca Eur. 42, N. Am. 159            .T...T.... .......C.. .T..CAT..T CC.-C.-C.. .....T.... .CAC-....A T...
Platismatia glauca N. Am. 191                     .T...T.... .......C.. .T..CAT..T CC.-C.CC.. .....T.... .CAC-....G T...
Platismatia norvegica Eur. 217                    .T...T.... .......C-. .....CG..T CC....CC.. .....TA... .C..A....G T.AA
Platismatia norvegica N. Am. 57, 172              .T...T.... .......C-. .....CG..T CC....CC.. .....TA... .C..A....G T..A
Tuckermannopsis chlorophylla Eur. 02, N. Am. 78   .....T.... .......... .T....T... .C.A...C.. .T....-... ........-. ...C
Tuckermannopsis chlorophylla Eur. 39              .....T.... .......... .T....T... .C.A...C.. .T....-... ........-. ...C
Tuckermannopsis chlorophylla N. Am. 185           .....T.... .......... .T....T... .C.A...C.. .T....-... ........-. ...C
Vulpicida pinastri Eur. 02                        .......... .......C.. ......T... .C.A..G... .......... .-......-. ...C
Vulpicida pinastri N. Am. 391                     .......... .......C.. ......T... .C.A..G... .......... .-......-. ...C
```

being dorsiventral in *Cetraria* and isodiametric in *Coelocaulon*. The inclusion of *Coelocaulon* in *Cetraria s. str*. is supported by the ITS sequences (Fig. 1).

*Cetraria ericetorum* and *C. islandica* are weakly separated taxa, and molecular data suggest they constitute a species complex. *Cetraria ericetorum* is distinguished from *C. islandica* by the marginal position of the pseudocyphellae. Two distinct subspecies are recognized in the Northern Hemisphere, *C. ericetorum* ssp. *ericetorum* and *C. ericetorum* ssp. *reticulata*. The former differs from the latter in having more smooth, narrow and canaliculate lobes (Kärnefelt 1979). *Cetraria islandica* is one of the most well-known and widely distributed of all lichens. A large number of infraspecific taxa have been described; most of them, however, turned out to be environmental modifications and were treated as synonyms by Kärnefelt (1979), who recognized four subspecies.

*Cetraria sepincola* and *Vulpicida juniperina* are more distant to *Cetraria s. str*. than *C. aculeata* (Fig. 1). This was expected because of the *Cetraria*-type of ascus for *C. aculeata* and *Lecanora*-type ascus for *Cetraria sepincola* and *Vulpicida pinastri* (Mattsson 1993, Thell *et al*. 1995). To maintain *Vulpicida* as a separate segregate, which seems reasonable to us, *C. sepincola* has to be excluded from *Cetraria*, if following data obtained from the ITS rRNA region.

## ITS data vs. morphological characters

The results from the phylogenetic analysis of the ITS data are, with exception of the *Cetraria islandica-ericetorum* complex, congruent with species delimitations derived from morphological characters, regardless of the geographic origin of the material (Figs. 1 and 2). Thus, although there are some genetic differences among samples, the extent of variation observed in representatives even from widely separated localities did not adversely affect or confound the phylogenetic analysis of the cetrarioid lichens. Indeed, use of such sampling strengthens the conclusions that one may draw from the analyses.

The material of *Cetraria sepincola* differed considerably in morphology. The North American specimen was a typical representative from its region, having tiny, narrow lobes, forming

Table. 3. ITS 2 sequences from European (Eur.) and North American (N. Am.) populations of 12 cetrarioid species. The numbers refer to DNA numbers w Table 1.

```
                                  390       400       410       420       430       440       450       460
Cetraria aculeata Eur. 11      5´ ATA-CCCC TCAAGCGTAG CTTGGTATTG GGTCTCGCCC CCGTGGCGTG CCCGAAAAGC AGTGGCGGTC CGGGGCGACT
Cetraria aculeata N. Am. 156      ...A.... .......... .......... .........T .......... .......... .....A.... .......... ..........
Cetraria ericetorum ssp. ericetorum Eur. 212 .......... .......... .......... .C........ .......... .......... .......... ..........
Cetraria ericetorum ssp. reticulata N. Am. 44 .......... .......... .......... .C........ .......... .......... .......... ..........
Cetraria ericetorum ssp. reticulata N. Am. 187 .......T .......... .......... .C........ .......... .......... .......... ..........
Cetraria islandica Eur. 41 .......... .......... .......... .C........ .......... .......... .......... .....T....
Cetraria islandica Eur. 94 .......... .......... .......... .C........ .......... .......... .......... .....T....
Cetraria islandica N. Am. 93 .......... .......... .......... .C........ .......... .......... .......... .....T....
Cetraria sepincola Eur. 03 .C........ .......... .......... .CC....... .......... .......... .......... ...T..T.G..
Cetraria sepincola Eur. 139, N. Am. 151 .C........ .......... .......... .CC....... .......... .......... .......... ...T..T.G..
Cetrelia cetrarioides Eur. 140 .C........ .......... .......... ....-.T.T.. .T.A...... .......GTT .......... ....C.T....
Cetrelia cetrarioides N. Am. 154 .C........ .......... .......... ..-..T.T.. ...A...... .......GTT .......... ....C.T....
Cetrelia cetrarioides N. Am. 214 .C........ .........C. .......... ...T...T.. .T.A...... .......GTT .......... ....C.T....
Flavocetraria cucullata Eur. 138 .C........ .......... .......... ...T...... ...C...... .......... .......... ...T.....
Flavocetraria cucullata N. Am. 149 .C........ .......... .......... ...T...... ...C...... .......... .......... ...T.....
Flavocetraria nivalis Eur. 01 .C........ .......... .....C.... ..C....... ...C...... .......... .......... ...T.....
Flavocetraria nivalis N. Am. 153 .C........ .......... .....C.... ..C....... ...C...... .......... .......... ...T.....
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Melanelia hepatizon Eur. 224                .C..... ......... ......... ..C..... ...C..... ........T. ......... ..CT...G..
Melanelia hepatizon N. Am. 183              .C..... ......A... ......... ..C..... ...C..... ........T. ......... ..CT...G..
Platismatia glauca Eur. 42, N. Am. 159      .C..... ........C. ......C... .ACG..... ...A....C. ........T. ......... ...T......
Platismatia glauca N. Am. 191               .C..... ........C. ......C... .ACG..... ...C....C. ........T. ......... ...T......
Platismatia norvegica Eur. 217              .C..... .....TAG.A ......CC.. ..CG.....T TTCC..... ........A. ......... ...T.T....
Platismatia norvegica N. Am. 57, 172        .C..... .....TAG.A ......CC.. ..CG.....T TTCC..... ........A. ......... ...T.T....
Tuckermannopsis chlorophylla Eur. 04, N. Am. 78 GC....T. ......... ......... ..C..... ...C..... ......... ......... ...A......
Tuckermannopsis chlorophylla Eur. 39        GC..... ......... ......... ..C..... ...C..... ......... ......... ...A......
Tuckermannopsis chlorophylla N. Am. 185     GC....T. ......... ......... ..C..... ...C..... ......... ......... ...A......
Vulpicida pinastri Eur. 02                  .T..... ......... ......... ..C..... ......... ......... ......... .........
Vulpicida pinastri N. Am. 391               .T..... ......... ......... ..C..... ......... ......... ......... .........

                                                  470        480        490        500        510        520        530
Cetraria aculeata Eur. 11                   TTAAGCGTAG TAAAAACCAA TCCCGCTTTG AAAGTTCGCG TCGTGGCCGG CCAGATAACC CCGTACATTT CAAATCA 3´
Cetraria aculeata N. Am. 156                .......... .....-.... .......... .......... .......... .....C.... T......... ....C..
Cetraria ericetorum ssp. ericetorum Eur. 212 .......... .....-.-T. .......... .....C...C ........T. .....C.... ......... .......
Cetraria ericetorum ssp. reticulata N. Am. 44 .......... .....T--T. .......... .........C ........T. .....C.... .......C.. .......
Cetraria ericetorum ssp. reticulata N. Am. 187 .......C.. .....T--T. .......... .........T ........T. T........ .......... .......
Cetraria islandica Eur. 41                  .......... .....T--T. .......... .........C ........T. .....C.... .......... .......
Cetraria islandica Eur. 94                  .......... .....T--T. .......... .........C ........T. .....C.... .......... .......
Cetraria islandica N. Am. 93                .......... .....T--T. .......... .........C ........T. .....T.... .......A.. .......
Cetraria sepincola Eur. 03                  .C........ .....T--C. .......... ....C...TC .......... .....C.... ..A..TC..C ..T....
Cetraria sepincola Eur. 139, N. Am. 151     .C........ .....T--C. .......... ....C...TC .......... .....C.... ..A..TC..C ..T....
Cetrelia cetrarioides Eur. 140              .......... .....TTT.T C..GC....- ..-....... C.......C. .....C..TA ATT.TT.... TCC..A.
Cetrelia cetrarioides N. Am. 154            .......... .....TTT.T C..GC....- ..-....... C.......C. .....C..TA ATT.TT.... TCC..A.
Cetrelia cetrarioides N. Am. 214            .......... .....TTT.T C..GC....- ..-....... C.......C. .....C..TA ATT.TT.... TCC..A.
Flavocetraria cucullata Eur. 138            .......... .....-TTTC. .......... .........C C......T.. .....C.... ..A...-..- ...-.A.
Flavocetraria cucullata N. Am. 149          .......... .....-TTTT. .......... .........C C......T.. .....C.... ..A...-..- ...-.A.
Flavocetraria nivalis Eur. 01               .......... .....-TTTC. .......... .........C C......... .....C.... ..A.-..... ...-.A.
Flavocetraria nivalis N. Am. 153            .......... .....-TTTC. .......... .........C C......... .....C.... ..A.-..... ...-.A.
Melanelia hepatizon Eur. 224                .......... .....CTTTC. .......... .....C.... C....A...G .....C.... ..AATT.... ...-...
Melanelia hepatizon N. Am. 183              .......... .....CTTTC. .......... .....C.... C....A.... .....C.... .TAATT.... ...-...
Platismatia glauca Eur. 42, N. Am. 159      ..GC...... .....-TTTCT C..GCG..-. .....C...C C..C...... .....C.... ..CAG-...- ...-C..
Platismatia glauca N. Am. 191               ..GC.T.... .....-TTTCT C..GCG..-. .....C...C C..C...... .....C.... ..CAG-...- ....-..
Platismatia norvegica Eur. 217              .......... .....-T--CG .......... .....-C.C.C C..C...... ....TC.... .GTAG..... ...-.A.
Platismatia norvegica N. Am. 57, 172        .......... .....-T--CG .......... .....-C.C.C C..C...... ....TC.... .GTAG..... ...-.A.
Tuckermannopsis chlorophylla Eur. 04, N. Am. 78 .......... .....-TTTC. .......... .........C C..C...... .....C.... ....TAC..C -...-.A.
Tuckermannopsis chlorophylla Eur. 39        .......... .....-TTTC. .......... .........C C.......... .....C.... ..A.TAC..C -...-.A.
Tuckermannopsis chlorophylla N. Am. 185     .......... .....-TTTC. .......... .........C C..C...... .....C.... ....TAC..C -...-.A.
Vulpicida pinastri Eur. 02                  .......... .....T--C. .......... .....C...C ...C...... .....C.... ..A....... T...-..
Vulpicida pinastri N. Am. 391               .......... .....T--C. .......... .....C...C ...C.A.... .....C.... ..A....... T...-..
```

Table 4. Sequences from group I introns of seven cetrarioid samples representing four species. The numbers refer to DNA numbers in Table 1.

```
                                  10        20        30        40        50        60        70
Cetraria ericetorum ssp. ericetorum Eur. 212  5´ GAATGGGTTT GCCTCCGGAA CGCCCCAGCA GCGACTCTAA ACAACTGCAT TAGTCGCCCT GAACGGGCTG GCAAC
Cetraria ericetorum ssp. reticulata N. Am. 44 .......... .......... .......... .......... .......... .......... .......C. .....
Cetraria islandica Eur. 41                    .......... .......... .......... .......... .......... .......... ......... .....
Cetraria islandica N. Am. 93                  .......... .......... .......... .......... .......... .......... ......... .....
Melanelia hepatizon Eur. 183, N. Am. 224      ...C-..... ....A.TAG. A..T.TC... .......... .....G..A. ..C....... AGCT..... .....
Vulpicida pinastri Eur. 02                    ...C-..... ....G.T.G. T...TTG... .......A.. -...A..... .......... .....C T.TG....A. .....
Vulpicida pinastri N. Am. 391                 ...C-..... ....G.T.G. T.T.TTG... .......A.. -...A..... .......... .....C T.TG....A. .....

                                   80        90       100       110       120       130       140       150
Cetraria ericetorum ssp. ericetorum Eur. 212  GCCGT CACTGTGCGC CGG-AAGTCC CCTGAACTCG GGGTCAACCA GCAGCTCCA- GCTTT-TTTG GAGTTCACAG
Cetraria ericetorum ssp. reticulata N. Am. 44 ..... .......... .......... .......... .....G.... .......... .......... ..........
Cetraria islandica Eur. 41                    ..... .......... ...A..... ..C..... .......... .....G.... .......... .......... ..........
Cetraria islandica N. Am. 93                  ..... .......... .......... ...-..... .......... .......... .....T.. .......... ..........
Melanelia hepatizon Eur. 183, N. Am. 224      ...A. ..A.C..... T..G...A.. .--...T.A. .TC....TG .......... AAG.CG.... .......... ..........
Vulpicida pinastri Eur. 02                    ..TA. T.G.C..... T..G..-.. ..C....AA. ...C.....G .......... .....GC AG.AC.CC. ..........
Vulpicida pinastri N. Am. 391                 ..TA. T.G.C..... T..G..-.. ..C....AA. ...C.....G .......... .....GC .AG.AC.CC. ..........

                                  160       170       180       190       200       210       220
Cetraria ericetorum ssp. ericetorum Eur. 212  ATCAAACGAT AGCGGCCACT TCGGTGGTT- GAGATATGAA CGGCCCCTGC TGTTACCAGC TGGAGACTTT GCG 3´
Cetraria ericetorum ssp. reticulata N. Am. 44 .......... .......... ...C..... .......... .......... .......... .......... ...
Cetraria islandica Eur. 41                    .......... .......... .......... .......... .......... .......... .......... ...
Cetraria islandica N. Am. 93                  .......... .......... .......... .......... .......... .......... .......... ...
Melanelia hepatizon Eur. 183, N. Am. 224      .......... .........A .GC..... ....C....T .....T.G.. ..CC.TT... C-...CA... ...
Vulpicida pinastri Eur. 02                    .......... ..T....G.G .T.AC..... .......... .....TG.. .....T.... .A.G.G-... ...
Vulpicida pinastri N. Am. 391                 .......... ..T....G.G .T.AC..... .......... .....TG.. .....T.... .A.G.G-... ...
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much smaller and less distinct rosettes than most European specimens. *Cetraria sepincola* is usually frequently furnished with apothecia (Esslinger 1973, Goward *et al*. 1994, Thell & Goward 1996). However, a missing C at position 57 was the only difference between the south Swedish collection and the two other samples (Table 2).

Fertile material of *Flavocetraria cucullata* from Austria, belonging to the broad-lobed var. *vainioi* (Gyelnik 1935, Räsänen 1939: 29), was compared with an "ordinary" sample from Alberta, Canada. They differed at three sites (Tables 2 and 3).

## Sexual vs. apomictic species

*Cetraria s. str.* and *Flavocetraria* both have asci of *Cetraria*-type, characterized by a narrow shape, an amyloid ring structure in the tholus and a very small axial body (Kärnefelt *et al*. 1994). The other taxa included in the analysis, among them *Tuckermannopsis chlorophylla*, have asci with large axial bodies categorized as *Lecanora*-type. This ascus type is present in a majority of the species included in the Parmeliaceae (Thell *et al*. 1995). If the *Lecanora*-type is the primitive state for the family, then the *Cetraria*-type of ascus, with its typical features, has evolved twice, convergently. Thus, the phylogeny presented here indicates different evolutionary origins for the *Cetraria* ascus type.

Poelt (1994) grouped the lichenized Ascomycotina into three main categories according to the genetic background: 1. stable sexual species, 2. genetically variable sexual species and 3. apomictic species. Most cetrarioid species are more or less apomictic, and 11 of the 12 species included here have to be classified as such. *Melanelia hepatizon*, on the other hand, is frequently fertile and shows a modest morphological variation. Although *Cetraria sepincola* is furnished

with a large number of apothecia, a study of a large number of specimens from different areas shows that its ascospore production is infrequent. The high percentage of apomictic species among cetrarioid lichens could be an explanation for the minute infraspecific variation of the ITS sequences detected in most studied taxa. The number of dissimilarities within a species is generally not larger between populations from different continents than between those collected within the province (Figs. 1 and 2, Tables 2 and 3). The difference between the two ITS sequences of *M. hepatizon*, the only typically sexual species in this study, however, is not more comprehensive than what was found in *Cetraria aculeata*, *Cetrelia cetrarioides* and *Platismatia glauca*. An ancient origin of these three taxa could explain the large genetic diversity.

## The taxonomic value of group I introns

Group I intron sequences seem to be an interesting complement to ITS data. They might be present or absent not only in the same genus but also within the same species, individual or even DNA-repeat (De Priest & Been 1992, Grube *et al*. 1995). When present, group I intron sequences could be quite informative and perhaps a useful complement to ITS based studies. An extended study of group I introns of the Parmeliaceae is in progress.

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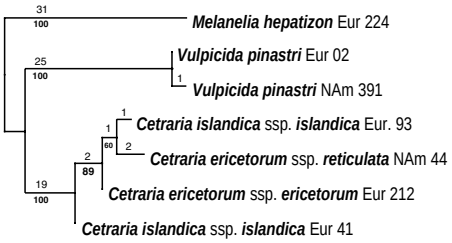


Fig. 2. The single most parsimonious tree based on group I intron sequences (Table 4), tree length 82, CI = 0.988, generated using heuristic searches in PAUP 3.1.1. The numbers above or to the right of the branches are substitution numbers per branch. Branch lengths are proportional to substitution numbers. Bootstrap percentages are in bold face (from 500 replicates). Bootstrap percentages are in bold face (from 500 replicates).

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