Species, Phylogeography and Extrolite Production in Bryoria and Pseudephebe (Parmeliaceae)

Carlos Galán Boluda

Madrid, July 6th 2017

Study conducted under the supervision of:

Dr. Víctor Jiménez Rico
Dr. David L. Hawksworth
• Introduction
• Objectives
• Materials and Methods
• Chapters:
  • 1. *Pseudephebe* species are cryptic with an environmentally modified morphology.
  • 2. Molecular studies reveal a new species of *Bryoria* in Chile.
  • 3. *Bryoria fuscescens* s. l. show a mismatch between haplotypes and chemotypes.
  • 4. Fluorescence microscopy as a tool for the visualization of lichen substances within *Bryoria* thalli.
  • 5. Characterization of microsatellite loci in lichen-forming fungi of *Bryoria* section *Implexae*.
  • 6. Towards an integrative taxonomy of *Bryoria* sect. *Implexae*.
  • 7. Phylogeography and evolution of *Bryoria fuscescens* s. str.
• Conclusions
Introduction

Tundra community, Norway

Mycobiont = Lichen taxonomy

Ascomycota
Basidiomycota
“Glomeromycota”

? Lichen

Photobiont

Chlorophyta
Cyanobacteria
Heterokontophyta

Small animals

Lichenicolous fungi

Cystobasidiomycetes yeasts

Proteobacteria and other bacteria

Other algae
Introduction

Usnea trichodeoides, Tanzania

At least 15 independent origins of lichenized fungi
Introduction

Divakar et al. 2015

Parmeliaceae

Bryoria

Pseudephebe

Nodobryoria

Bryocaulon

Alectoroid

Alectoriorid

Psiloparmelioid

Hypogymnioid

Cetrarioid

Anzioiid

Usneoid

Aelectoria

Parmelioid
Introduction

Intermediate specimens between species in:

Pseudephebe

Pseudephebe minuscula → Pseudephebe pubescens

Bryoria section Implexae

Velmala et al. 2014

Brodo & Hawksworth 1977
Main Objectives

- Perform an integrative taxonomical study of \textit{Bryoria} section \textit{Implexae} and \textit{Pseudephebe}.

- Study the extrolite composition, location and taxonomical utility in \textit{Bryoria} and \textit{Pseudephebe}.

- Study the interaction between \textit{Bryoria fuscescens} and the environment.

- Understand the evolutive processes that are producing the phenotypical variability observed in \textit{Bryoria fuscescens} s. str.
Materials and Methods

Sampled areas

*Pseudephebe*: aprox. 120 specimens

*Bryoria*: aprox. 2,100 specimens
Materials and Methods

Example of three sampled regions

Norway

Norway

Portugal
# Materials and Methods

## Main data

<table>
<thead>
<tr>
<th>Distribution</th>
<th>Phenotypical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphological characters</td>
<td>Chemical characters</td>
</tr>
</tbody>
</table>

**Molecular**
- 7 standard DNA markers (6 +1)
- 5 new DNA markers
- 18 microsatellite markers

## Main lab. techniques

- Fluorescence microscopy
- Thin layer chromatography
- PCR
- Sequencing
- 545 pyrosequencing

---

## Main analyses

- **Phenograms**: “R”.
- **Recombination detection**: RDP, GENECONV, Chimaera, Maxchi, Bootscan, SiScan, PhylPro, 3Seq.
- **Phylogenetic reconstruction**: RAxML, MrBayes, MAFFT, Partitionfinder, Mega, jModeltest, Beast, Figtree, CADM test.
- **Divergence time estimation**: Beast.
- **Species delimitation programs**: ABGD, GMYC, PTP, BP&P, DISSECT.
- **Genepool detection**: PCoA, DAPC, haplotype network, STRUCTURE.
- **Genetic diversity**: “R”, ADZE, GenAlEx, KGTESTS, Arlequin.
- **Population dynamics**: Beast.
- **Spatial analyses**: Migrate-n, ibd.
- **Potential distribution**: Maxent.

---

*Bryoria: Pseudephebe:*
Chapter 1

*Pseudephebe* species are cryptic with an environmentally modified morphology
Chapter 1 *Pseudephebe* species concept

---

**Species**

- *Pseudephebe pubescens*
- *P. minuscula*
- *Bryoria mariensis*

---

**Phylogenetic tree using**

- *nuITS*, *RPB1 & MCM7*

---

**120 specimens**

- **37 specimens + 25 outgroup**

---

**Characters not reported in bibliography**

- Not three phenotypic groups can be obtained

---

**Table**

<table>
<thead>
<tr>
<th>Species</th>
<th>DNA code</th>
<th>Extrolites</th>
<th>SSU intron</th>
<th>Internodes length (mm)</th>
<th>Branches width (mm)</th>
<th>Pseudephelae</th>
<th>Profusely branched tips</th>
<th>Old branches appressed</th>
<th>Flattened branches</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bryoria menensis</em></td>
<td>5077</td>
<td>Norstotic</td>
<td>+</td>
<td>&lt; 1 - 7</td>
<td>0.26 (0.1 - 1)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>B. minuscula</em></td>
<td>5075</td>
<td>Norstotic</td>
<td>+</td>
<td>&lt; 1 - 7</td>
<td>0.20 (0.1 - 0.4)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Pseudephebe minuscula</em></td>
<td>4339</td>
<td>Norstotic</td>
<td>+</td>
<td>&lt; 1</td>
<td>0.18 (0.1 - 0.3)</td>
<td>-</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td><em>P. pubescens</em></td>
<td>4338</td>
<td>Norstotic</td>
<td>+</td>
<td>&lt; 1</td>
<td>0.18 (0.1 - 0.2)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td><em>P. minuscula</em></td>
<td>4764</td>
<td>Absent</td>
<td>+</td>
<td>&lt; 1</td>
<td>0.32 (0.2 - 0.5; crusty)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>P. pubescens</em></td>
<td>4781</td>
<td>Norstotic</td>
<td>+</td>
<td>&gt; 1</td>
<td>0.10 (0.1 - 0.2)</td>
<td>perforated</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>P. pubescens</em></td>
<td>4363</td>
<td>Norstotic</td>
<td>+</td>
<td>&lt; 1 - 2</td>
<td>0.17 (0.1 - 0.3)</td>
<td>±</td>
<td>+</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td><em>P. pubescens</em></td>
<td>4362</td>
<td>Greyish pale spot</td>
<td>+</td>
<td>&lt; 1</td>
<td>0.17 (0.1 - 0.2)</td>
<td>-</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
</tbody>
</table>

---

**Image**

- A: Pseudocyphellae
- B: Pseudocyphellae
- C: Pseudocyphellae

---
Chapter 1 *Pseudephebe* species concept

26.5 Mya

9.5 Mya

4.3 Mya

1.25 Mya

ML and bayesian phylogenetic tree from the concatenated matrix of ITS, RPB1 and MCM7.
Chapter 1 *Pseudephebe* species concept

**New species concept:**

**P. minuscula**

*Syn:* *Bryoria mariensis*

**P. pubescens**

ML and Bayesian phylogenetic tree from the concatenated matrix of ITS, RPB1 and MCM7.

Species delimitation programs
Chapter 1 *Pseudepehebe* species concept

Pseudocyphellae

P. pubescens

No discriminatory characters between species

<table>
<thead>
<tr>
<th>Character</th>
<th><em>Pseudepehebe minuscula</em></th>
<th><em>Pseudepehebe pubescens</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Habit</td>
<td>Rarely subcrustose, small to large fruticose</td>
<td>Never subcrustose, small to medium fruticose</td>
</tr>
<tr>
<td>Thallus size (diameter)</td>
<td>Usually less than 3 cm, but reaching more than 8 cm</td>
<td>Less than 5 cm</td>
</tr>
<tr>
<td>Internode length</td>
<td>Usually less than 1 mm, but reaching 7 mm</td>
<td>Usually 1–3 mm, but sometimes less than 1 mm</td>
</tr>
<tr>
<td>Compressed old branches</td>
<td>Frequent</td>
<td>Rare</td>
</tr>
<tr>
<td>Flattened branching</td>
<td>Frequent</td>
<td>Rare, but never very flattened</td>
</tr>
<tr>
<td>Richly branched tips</td>
<td>Frequent</td>
<td>From absent to present in the same specimen</td>
</tr>
<tr>
<td>SSU-3’ 1516 intron</td>
<td>Frequent</td>
<td>Absent</td>
</tr>
<tr>
<td>Spore size</td>
<td>8.1-8.9 x 5.9-6.8 μm</td>
<td>9.0-9.5 x 5.4-6.0 μm</td>
</tr>
</tbody>
</table>

*P. minuscula* and *P. pubescens* are cryptic species
Chapter 2

Molecular studies reveal a new species of *Bryoria* in Chile
Chapter 2 *Bryoria araucana* sp. nov.

- Morphological study
- Thin layer chromatography
- nuITS, mtSSU, MCM7

Maximum likelihood tree

Bayesian tree

*B. glabra / fuscescens*  
*Bryoria aff. trichodes*  
or *B. sect. Implexae*
Chapter 2 **Bryoria araucana** sp. nov.

*B. araucana* sp. nov. is polyphyletic.
## Table 2. Main diagnostic features for *Bryoria araucana* and phylogenetically related species, based on literature (Brodo & Hawksworth 1977; Bystrek 1969; Mylly et al. 2011a; Wang & Chen 1994) and our observations.

<table>
<thead>
<tr>
<th>Character/Species</th>
<th><em>Bryoria araucana</em></th>
<th><em>B. nadovornikana</em></th>
<th><em>B. poetii</em></th>
<th><em>B. trichodes</em></th>
<th><em>B. furcellata</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Main chemistry</strong></td>
<td>Fum</td>
<td>Fum</td>
<td>Fum</td>
<td>Fum</td>
<td>Fum</td>
</tr>
<tr>
<td>Thallus</td>
<td>Pendent</td>
<td>Caespitose (base) to pendent</td>
<td>Pendent</td>
<td>Caespitose</td>
<td>Caespitose</td>
</tr>
<tr>
<td>Pseudocellae</td>
<td>Inconspicuous, dark grey-brown</td>
<td>Inconspicuous, white</td>
<td>Conspicuous, dark brown-black</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td>Soralia</td>
<td>Absent</td>
<td>Tuberculate to fissural, white</td>
<td>Tuberculate to fissural, white</td>
<td>Rare, fissural, white</td>
<td>Fissural, white, spinulose (tufts)</td>
</tr>
<tr>
<td>Sporocarps or spinoles branches</td>
<td>On terminal portions, sparse</td>
<td>Lateral, sparse to frequent</td>
<td>Spore, also on soralia</td>
<td>Lateral, sparse</td>
<td>Spore to frequent</td>
</tr>
<tr>
<td>Colour</td>
<td>Dark grey-brown, base usually darker</td>
<td>Pale to dark brown-violet, base generally black</td>
<td>Dark brown to black</td>
<td>Pale to dark brown</td>
<td>Pale to dark brown, base often darker</td>
</tr>
<tr>
<td>Distribution</td>
<td>Chile, South America</td>
<td>Europe, Africa, Asia, Hawaii, North America</td>
<td>Himalayas</td>
<td>Asia, North America</td>
<td>Europe, Macaronesia, Asia, Oceania, North and Central America</td>
</tr>
</tbody>
</table>

---

Bryoria fuscescens s. l. show a mismatch between haplotypes and chemotypes
Chapter 3 *Bryoria fuscescens* mismatch

45 morphologically invariable *B. fuscescens* specimens from three close localities.

Each specimen was divided into four regions:
- Base
- Branches (without soralia)
- Soralia
- Tips

TLC & nuITS
Chapter 3 *Bryoria fuscescens* mismatch

nuITS phylogenetic tree

nuITS Haplotype network showing the chemotypes

<table>
<thead>
<tr>
<th>Specimen 1</th>
<th>Specimen 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base</td>
<td>Fum</td>
</tr>
<tr>
<td>Branch</td>
<td>Fum</td>
</tr>
<tr>
<td>Tips</td>
<td>Fum</td>
</tr>
<tr>
<td>Soralia</td>
<td>Fum-Pso</td>
</tr>
</tbody>
</table>
Chapter 4

Fluorescence microscopy as a tool for the visualization of lichen substances within *Bryoria* thalli

Autofluorescence of psoromic acid crystals
Chapter 4 Fluorescence microscopy

Fumarprotocetraric acid

Pseudocyphellae Pd+ red

Chlorophyll

Fumarprotocetraric acid

Norstictic acid

Pseudocyphellae K+ red

UV 450-490 nm
Chapter 4 Fluorescence microscopy

White light

UV 450-490 nm

Atranorine

Usnic acid

Pseudocyphellae

Chlorophyll

Atranorine

Salazinic acid

*Parmelia sulcata* UV 450-490 nm
Fluorescence microscopy is a good tool to locate and sometimes identify extrolites inside the lichen thallus.
Chapter 5

Characterization of microsatellite loci in lichen-forming fungi of *Bryoria* section *Implexae*
Chapter 5 Mirosatellites development

**Bryoria sect. *Implexae***
- Spanish specimens
- Swiss specimens
- Finnish specimens
  - 30 morphologically variable specimens

**18 microsatellites**

**454 Pyrosequencing**
- 533,962 reads
  - (average length 812 bp)

**MSATCOMMANDER**
- (Scanning for microsatellites)
  - 6,329 putative microsatellites
    - Unfavorable primers
    - Duplicates

**44 putative microsatellites**

**58 putative microsatellites**

**397 putative microsatellites**

**NCBI**

**Treouxia cultures**

**36 flanking regions**

**5 flanking regions**

**Unfavorable primers**
- Invariable regions
Chapter 6
Towards an integrative taxonomy of *Bryoria* sect. *Implexae*
Tabla 2. Sustancias químicas diagnóstico que junto con caracteres adicionales permiten distinguir las especies de *Bryoria* sect. *Implexae*. Los caracteres adicionales incluyen la coloración del talo, los ángulos de ramificación, las características de los soralios y pseudocifelas y la distribución.

<table>
<thead>
<tr>
<th>Especie</th>
<th>Sustancia diagnóstico</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. austromontana</em></td>
<td>Ácido fumarprotocetríaco</td>
</tr>
<tr>
<td><em>B. capillaris</em></td>
<td>Ácido barbatólico</td>
</tr>
<tr>
<td><em>B. friabilis</em></td>
<td>Ácido girofórico</td>
</tr>
<tr>
<td><em>B. fuscescens</em></td>
<td>Ácido fumarprotocetríaco</td>
</tr>
<tr>
<td><em>B. glabra</em></td>
<td>Ácido fumarprotocetríaco</td>
</tr>
<tr>
<td><em>B. implexa</em></td>
<td>Ácido psórómico</td>
</tr>
<tr>
<td><em>B. inactiva</em></td>
<td>Sin sustancias</td>
</tr>
<tr>
<td><em>B. kockiana</em></td>
<td>Ácido psórómico</td>
</tr>
<tr>
<td><em>B. pikei</em></td>
<td>Ácido barbatólico</td>
</tr>
<tr>
<td><em>B. pseudofuscescens</em></td>
<td>Ácido norestíctico</td>
</tr>
<tr>
<td><em>B. salazinica</em></td>
<td>Ácido salazínico</td>
</tr>
<tr>
<td><em>B. vrangiana</em></td>
<td>Ácido fumarprotocetríaco</td>
</tr>
</tbody>
</table>

Main key characters:
- Extrolites
- Soralia
- Pseudocyphellae
- Thallus colour
- Branching angles
Tabla 2. Sustancias químicas diagnóstico que junto con caracteres adicionales permiten distinguir las especies de *Bryoria* sect. *Implexae*. Los caracteres adicionales incluyen la coloración del talo, los ángulos de ramificación, las características de los soralios y pseudocifelas y la distribución.

<table>
<thead>
<tr>
<th>Especie</th>
<th>Sustancia diagnóstico</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. austromontana</em></td>
<td>Ácido fumarprotocetrárico</td>
</tr>
<tr>
<td><em>B. capillaris</em></td>
<td>Ácido barbatólico</td>
</tr>
<tr>
<td><em>B. friabilis</em></td>
<td>Ácido girofórico</td>
</tr>
<tr>
<td><em>B. fuscescens</em></td>
<td>Ácido fumarprotocetrárico</td>
</tr>
<tr>
<td><em>B. glabra</em></td>
<td>Ácido fumarprotocetrárico</td>
</tr>
<tr>
<td><em>B. implexa</em></td>
<td>Ácido psórómico</td>
</tr>
<tr>
<td><em>B. inactiva</em></td>
<td>Sin sustancias</td>
</tr>
<tr>
<td><em>B. kockiana</em></td>
<td>Ácido psórómico</td>
</tr>
<tr>
<td><em>B. pikeli</em></td>
<td>Ácido barbatólico</td>
</tr>
<tr>
<td><em>B. pseudofuscescens</em></td>
<td>Ácido norestítico</td>
</tr>
<tr>
<td><em>B. salzinica</em></td>
<td>Ácido salazínico</td>
</tr>
<tr>
<td><em>B. vrangiana</em></td>
<td>Ácido fumarprotocetrárico</td>
</tr>
</tbody>
</table>

- **Morphology** (incl. lichenicolous)
- **Chemistry**
- **DNA sequences**
  - nuITS, IGS, GAPDH
  - FRBi15, FRBi16 (not showed)
- **Microsatellites**

Chapter 6 *Bryoria* sect. *Implexae* taxonomy

142 specimens from:
The species concept in *Bryoria* sect. *Implexae*, established mainly using septentrional specimens, break down when meridional specimens are studied.
Chapter 6 *Bryoria* sect. *Implexae* taxonomy

ML and Bayesian tree form the concatenated matrix (ITS, IGS & GAPDH)

**Bryoria fuscescens agg.**

**Bryoria sect. Implexae**

Tree backbone well supported
Chapter 6

ML and Bayesian tree form the concatenated matrix (ITS, IGS & GAPDH)

Tree backbone well supported
Chapter 6

Extrolite composition
Chapter 6

DISSECT

<table>
<thead>
<tr>
<th>Method</th>
<th>ITS</th>
<th>IGS</th>
<th>GAPDH</th>
<th>Concatenated</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABGD</td>
<td>2 = G + (Ko, NA, WD)</td>
<td>2 = G + (Ko, NA, WD)</td>
<td>4 = G + Ko + NA + WD</td>
<td>4 = G + Ko + NA + WD</td>
</tr>
<tr>
<td>PTP</td>
<td>2 = G + (Ko, NA, WD)</td>
<td>2 = G + (Ko, NA, WD)</td>
<td>4 = G + Ko + NA + WD</td>
<td>5 = G + Ko + NA + WD</td>
</tr>
<tr>
<td>GMYC6s</td>
<td>4 = G + (Ko, NA, WDg) + WDr + WDr</td>
<td>3 = G + (Ko, WDr) + NA</td>
<td>4 = G + Ko + NA + WD</td>
<td>6 = G + Ko + NA + pik5 + WDr + WDg</td>
</tr>
<tr>
<td>GMYCm</td>
<td>4 = G + (Ko, NA, WDg) + WDr + WDr</td>
<td>4 = G + (Ko, WDr) + NA1 + NA2</td>
<td>4 = G + Ko + NA + WD</td>
<td>5 = G + Ko + NA + WDr + WDg</td>
</tr>
</tbody>
</table>

DISSECT

WD
NAE
pik5
G
Chapter 6

Haplotype network

gaps missing
95% connection limit
Chapter 6

Principal coordinate analyses using microsatellites
Chapter 6

STRUCTURE using microsatellites

- B. glabra
- B. kockiana (Ko Clade)
- B. capillaris
- B. inermis
- B. kummerowiae
- B. vranjacana

K2
K3
K4
K5
K6

G
NA
Ko
WD
Chapter 6

Divergence time estimation

- WD Clade
- NA Clade
- Ko Clade
- G clade
- B. furcellata

<table>
<thead>
<tr>
<th>Node</th>
<th>Million years ago</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6.9 (95% HPD = 3.5-10.8)</td>
</tr>
<tr>
<td>B</td>
<td>1.0 (95% HPD = 0.3-2.2)</td>
</tr>
<tr>
<td>A</td>
<td>6.5 (95% HPD = 2.2-11.4)</td>
</tr>
<tr>
<td>B</td>
<td>0.6 (95% HPD = 0.2-1.5)</td>
</tr>
</tbody>
</table>

WD

NA

Ko

G
Chapter 6

Past population dynamics

X = Million years ago
Y = Population size
Chapter 6

Proposed species concept

**Bryoria fuscescens**

Syn: *B. capillaris, B. implexa, B. kuemmerleana, B. vrangiana.*

**Bryoria pseudofuscescens**

Syn: *B. friabilis, B. inactiva, B. pikei.*

**Bryoria kockiana**

Syn: *B. sp.*

**Bryoria glabra**

Cryptic

1 Mya of divergence
Chapter 7

Phylogeography and evolution of *Bryoria fuscescens*
Chapter 7 *Bryoria* phylogeography

*Bryoria* morphospecies growing together
What is producing that phenotypes?
Chapter 7 *Bryoria* phylogeography

**Phenotype-*capillaris***
- Usually pale
- With barbatolic acid
- Soralia rare
- Angles usually acute

**Bryoria fuscescens s. str.**

**Phenotype-*fuscescens***
- Usually dark
- Without barbatolic acid
- Soralia frequent
- Angles variable
Chapter 7 *Bryoria* phylogeography

1.400 specimens, 64 populations, 18 microsatellites

35 specimens used for a phylogenetical reconstruction (3 standard loci, and 5 new loci)

Table 1. Amplified and analysed SSRs. Left: Number of specimens with successful amplification for each locus, and its respective number of alleles. Right: Selected loci and specimens for the analyses after remove unexpected alleles and specimens with missing data.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Specimens</th>
<th>Alleles</th>
<th>Specimens</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bi01</td>
<td>1384</td>
<td>22</td>
<td>Not used</td>
<td>Not used</td>
</tr>
<tr>
<td>Bi02</td>
<td>1123</td>
<td>6</td>
<td>Not used</td>
<td>Not used</td>
</tr>
<tr>
<td>Bi03</td>
<td>1391</td>
<td>5</td>
<td>1359</td>
<td>5</td>
</tr>
<tr>
<td>Bi04</td>
<td>1388</td>
<td>8</td>
<td>1359</td>
<td>7</td>
</tr>
<tr>
<td>Bi05</td>
<td>1359</td>
<td>14</td>
<td>1359</td>
<td>10</td>
</tr>
<tr>
<td>Bi06</td>
<td>1366</td>
<td>22</td>
<td>1359</td>
<td>21</td>
</tr>
<tr>
<td>Bi07</td>
<td>1368</td>
<td>6</td>
<td>1359</td>
<td>6</td>
</tr>
<tr>
<td>Bi08</td>
<td>1385</td>
<td>5</td>
<td>1359</td>
<td>5</td>
</tr>
<tr>
<td>Bi09</td>
<td>597</td>
<td>3</td>
<td>Not used</td>
<td>Not used</td>
</tr>
<tr>
<td>Bi10</td>
<td>1393</td>
<td>5</td>
<td>1359</td>
<td>3</td>
</tr>
<tr>
<td>Bi11</td>
<td>1391</td>
<td>12</td>
<td>1359</td>
<td>10</td>
</tr>
<tr>
<td>Bi12</td>
<td>1399</td>
<td>22</td>
<td>1359</td>
<td>21</td>
</tr>
<tr>
<td>Bi13</td>
<td>1359</td>
<td>18</td>
<td>1359</td>
<td>18</td>
</tr>
<tr>
<td>Bi14</td>
<td>1391</td>
<td>4</td>
<td>1359</td>
<td>3</td>
</tr>
<tr>
<td>Bi15</td>
<td>1071</td>
<td>3</td>
<td>Not used</td>
<td>Not used</td>
</tr>
<tr>
<td>Bi16</td>
<td>1360</td>
<td>6</td>
<td>1359</td>
<td>6</td>
</tr>
<tr>
<td>Bi18</td>
<td>1359</td>
<td>9</td>
<td>1359</td>
<td>9</td>
</tr>
<tr>
<td>Bi19</td>
<td>1388</td>
<td>8</td>
<td>1359</td>
<td>6</td>
</tr>
</tbody>
</table>

1.359 specimens
14 microsatellites
No missing data
Chapter 7 *Bryoria* phylogeography

Higher diversity within populations than among. Similar diversity in trunks and twigs.

Table S6. Global Analysis of Molecular Variance (AMOVA) using 14 loci, 1359 individuals, 64 populations and the 7 geographical regions from Fig. 1. $F_{SC} = 0.21381$, $F_{ST} = 0.23187$ and $F_{CT} = 0.02297$, statistically significant with $P \leq 0.035$.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Sum of squares</th>
<th>Variance components</th>
<th>% of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among regions</td>
<td>6</td>
<td>211.80</td>
<td>0.889</td>
<td>2.30</td>
</tr>
<tr>
<td>Among populations within regions</td>
<td>57</td>
<td>1146.23</td>
<td>0.808</td>
<td>20.89</td>
</tr>
<tr>
<td>Within populations</td>
<td>1295</td>
<td>3846.72</td>
<td>2.970</td>
<td>76.81</td>
</tr>
<tr>
<td>Total</td>
<td>1358</td>
<td>5204.75</td>
<td>3.867</td>
<td></td>
</tr>
</tbody>
</table>

**AMOVA**

Table S5. Allelic richness (AR) and private allelic richness (PAR) detected in each type of substrate. Standard deviations are showed in brackets.

<table>
<thead>
<tr>
<th></th>
<th>Twigs</th>
<th>Branches</th>
<th>Trunks</th>
<th>Rock</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AR</td>
<td>PAR</td>
<td>AR</td>
<td>PAR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AR</td>
<td>PAR</td>
</tr>
<tr>
<td></td>
<td>AR/n</td>
<td>-</td>
<td>AR/n</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AR/n</td>
<td>-</td>
</tr>
<tr>
<td>n:203</td>
<td>5.53</td>
<td>6.57</td>
<td>3.57</td>
<td>6.07</td>
</tr>
<tr>
<td></td>
<td>(1.15)</td>
<td>(0.21)</td>
<td>(0.53)</td>
<td>(0.07)</td>
</tr>
<tr>
<td>n:30</td>
<td>3.57</td>
<td>6.07</td>
<td>0.07</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>(0.07)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n:256</td>
<td>6.07</td>
<td>0.68</td>
<td>0.37</td>
<td>2.79</td>
</tr>
<tr>
<td></td>
<td>(0.07)</td>
<td></td>
<td>(0.39)</td>
<td></td>
</tr>
<tr>
<td>AR/n</td>
<td>0.029</td>
<td>-</td>
<td>AR/n</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AR/n</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.119</td>
<td>-</td>
<td>0.024</td>
<td>-</td>
</tr>
<tr>
<td>n:37</td>
<td>0.39</td>
<td></td>
<td>AR/n</td>
<td>0.075</td>
</tr>
<tr>
<td></td>
<td>(0.39)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Allelic richness
Chapter 7 *Bryoria* phylogeography

<table>
<thead>
<tr>
<th>Region</th>
<th>specimens</th>
<th>AR</th>
<th>PAR</th>
<th>specimens</th>
<th>AR</th>
<th>PAR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>North</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Great Britain</td>
<td>32</td>
<td>2.642</td>
<td>0.000</td>
<td>588</td>
<td>7.500</td>
<td>1.357</td>
</tr>
<tr>
<td>Scandinavia</td>
<td>556</td>
<td>7.428</td>
<td>1.357</td>
<td>(1.207)</td>
<td></td>
<td>(0.452)</td>
</tr>
<tr>
<td><strong>Central</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alps</td>
<td>189</td>
<td>5.357</td>
<td>0.357</td>
<td>(1.014)</td>
<td></td>
<td>(0.199)</td>
</tr>
<tr>
<td>Carpathians</td>
<td>179</td>
<td>5.428</td>
<td>0.000</td>
<td>(0.976)</td>
<td></td>
<td>(0.000)</td>
</tr>
<tr>
<td>Iberia</td>
<td>273</td>
<td>5.571</td>
<td>0.285</td>
<td>(0.976)</td>
<td></td>
<td>(0.125)</td>
</tr>
<tr>
<td><strong>South</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Africa</td>
<td>65</td>
<td>4.571</td>
<td>0.428</td>
<td>(0.947)</td>
<td></td>
<td>(0.227)</td>
</tr>
<tr>
<td>Mediterranean</td>
<td>65</td>
<td>4.214</td>
<td>0.071</td>
<td>(0.575)</td>
<td></td>
<td>(0.071)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>130</td>
<td>5.571</td>
<td>0.500</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(1.087)</td>
<td></td>
<td>(0.251)</td>
</tr>
</tbody>
</table>

**Scandinavia:** High diversity

**Alps:** High diversity

**Iberia:** High diversity

**Carpathians:** Low diversity

**Great Britain:** Low diversity
Chapter 7 *Bryoria* phylogeography

- Less clonality than expected
- Saxicolous populations are genetically poor.
- Apotheciated populations are not significantly more diverse.
- Human activities can increase genetic diversity.
- Recent colonization signals
- Putative sexual reproduction in non-apotheciated populations.

<table>
<thead>
<tr>
<th>Population number</th>
<th>n</th>
<th>Non-clonal specimens</th>
<th>Polymorphic loci (%)</th>
<th>u/h mean (stand. error)</th>
<th>rBarD</th>
<th>AR</th>
<th>PAR</th>
<th>K-test</th>
<th>Putative population disturbances</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pops. 1-13 not showed</td>
<td>14</td>
<td>19</td>
<td>16</td>
<td>100%</td>
<td>0.525 (0.033)</td>
<td>0.224 (0.350)</td>
<td>3.214 (0.350)</td>
<td>0.003 (0.003)</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>22</td>
<td>16</td>
<td>100%</td>
<td>0.550 (0.053)</td>
<td>0.128 (0.571)</td>
<td>3.428 (0.621)</td>
<td>0.129 (0.088)</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>20</td>
<td>20</td>
<td>100%</td>
<td>0.573 (0.033)</td>
<td>0.126 (0.350)</td>
<td>3.285 (0.350)</td>
<td>0.071 (0.071)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>14</td>
<td>6</td>
<td>71%</td>
<td>0.255 (0.056)</td>
<td>0.221 (0.289)</td>
<td>2.071 (0.289)</td>
<td>0.071 (0.071)</td>
<td>14 expansion</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>23</td>
<td>16</td>
<td>100%</td>
<td>0.510 (0.080)</td>
<td>0.175 (0.571)</td>
<td>3.285 (0.571)</td>
<td>0.000 (0.000)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>23</td>
<td>13</td>
<td>100%</td>
<td>0.356 (0.053)</td>
<td>0.031 (0.289)</td>
<td>2.642 (0.289)</td>
<td>0.000 (0.000)</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>9</td>
<td>1</td>
<td>0%</td>
<td>0.000 (0.000)</td>
<td>NA</td>
<td>1.000 (0.000)</td>
<td>0.000 (0.000)</td>
<td>14 expansion Saxicolous</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>10</td>
<td>1</td>
<td>0%</td>
<td>0.000 (0.000)</td>
<td>NA</td>
<td>1.000 (0.000)</td>
<td>0.000 (0.000)</td>
<td>14 expansion Saxicolous</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>23</td>
<td>4</td>
<td>71%</td>
<td>0.275 (0.050)</td>
<td>0.380 (0.289)</td>
<td>1.926 (0.289)</td>
<td>0.000 (0.000)</td>
<td>11 expansion Village close, apothecia present Near pastures</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>23</td>
<td>5</td>
<td>86%</td>
<td>0.356 (0.057)</td>
<td>0.183 (0.148)</td>
<td>2.000 (0.148)</td>
<td>0.000 (0.000)</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>9</td>
<td>5</td>
<td>64%</td>
<td>0.317 (0.079)</td>
<td>0.258 (0.286)</td>
<td>2.071 (0.286)</td>
<td>0.000 (0.000)</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>23</td>
<td>14</td>
<td>71%</td>
<td>0.375 (0.072)</td>
<td>0.069 (0.289)</td>
<td>2.214 (0.289)</td>
<td>0.002 (0.002)</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>23</td>
<td>21</td>
<td>100%</td>
<td>0.505 (0.045)</td>
<td>0.268 (0.357)</td>
<td>3.357 (0.357)</td>
<td>0.000 (0.000)</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>23</td>
<td>21</td>
<td>93%</td>
<td>0.451 (0.023)</td>
<td>0.023 (0.412)</td>
<td>3.071 (0.412)</td>
<td>0.000 (0.000)</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>23</td>
<td>18</td>
<td>100%</td>
<td>0.486 (0.041)</td>
<td>0.336 (0.350)</td>
<td>3.214 (0.350)</td>
<td>0.002 (0.002)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>23</td>
<td>23</td>
<td>100%</td>
<td>0.494 (0.057)</td>
<td>0.144 (0.357)</td>
<td>3.571 (0.357)</td>
<td>0.008 (0.008)</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>22</td>
<td>20</td>
<td>86%</td>
<td>0.337 (0.072)</td>
<td>0.034 (0.289)</td>
<td>2.857 (0.289)</td>
<td>0.008 (0.008)</td>
<td>12 expansion Apothecia present</td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>23</td>
<td>20</td>
<td>100%</td>
<td>0.566 (0.048)</td>
<td>0.184 (0.612)</td>
<td>3.765 (0.612)</td>
<td>0.000 (0.000)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>21</td>
<td>19</td>
<td>93%</td>
<td>0.350 (0.058)</td>
<td>0.044 (0.289)</td>
<td>2.642 (0.289)</td>
<td>0.000 (0.000)</td>
<td>7</td>
</tr>
</tbody>
</table>

Pops. 33-64 not showed
Chapter 7 *Bryoria* phylogeography

- Less clonallity than expected
- Saxicolous populations are genetically poor.
- Apotheciated populations are not significantly more diverse.
- Human activities can increase genetic diversity.
- Recent colonization signals
- Putative sexual reproduction in non apotheciated populations.

### Table 2. Results of the analyses for each population, indicating the number of specimens (n), number of non-clonal specimens, percentage of polymorphism, and number of loci with negative values in the K-test (in bold = significant values with a p-value < 0.05). Some characters are also indicated in the 4th column (in bold).

<table>
<thead>
<tr>
<th>Population number</th>
<th>n</th>
<th>% Polymorphism</th>
<th>K-test</th>
<th>Putative population disturbances</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8 (0.003)</td>
<td>7</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>8 (0.088)</td>
<td>8</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>11 (0.071)</td>
<td>4</td>
<td>14 expansion</td>
<td>Some tourism, apothecia present</td>
<td></td>
</tr>
<tr>
<td>11 (0.071)</td>
<td>14 expansion</td>
<td>Apothecia present</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (0.000)</td>
<td>5</td>
<td>Apothecia present</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (0.000)</td>
<td>10</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (0.000)</td>
<td>14 expansion</td>
<td>Saxicolous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (0.000)</td>
<td>14 expansion</td>
<td>Saxicolous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (0.000)</td>
<td>14 expansion</td>
<td>Village close, apothecia present</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (0.000)</td>
<td>Near pastures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (0.000)</td>
<td>10</td>
<td>Artificial forest</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (0.002)</td>
<td>7</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (0.002)</td>
<td>7</td>
<td>Some tourism, apothecia present</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 (0.008)</td>
<td>10</td>
<td>Human activities and constuctions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (0.002)</td>
<td>Tourism</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 (0.008)</td>
<td>10</td>
<td>Apothecia present</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (0.000)</td>
<td>5</td>
<td>Apothecia present</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (0.000)</td>
<td>7</td>
<td>Apothecia present</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Pops. 33-64 not showed
- Less clonality than expected
- Saxicolous populations are genetically poor.
- Apotheciated populations are not significantly more diverse.
- Human activities can increase genetic diversity.
- Recent colonization signals
- Putative sexual reproduction in non-apotheciated populations.

<table>
<thead>
<tr>
<th>Population number</th>
<th>n</th>
<th>Non-clonal specimens</th>
<th>Polymorphic loci (%)</th>
<th>uh mean (stand. error)</th>
<th>rBarD</th>
<th>AR</th>
<th>PAR</th>
<th>K-test</th>
<th>Putative population disturbances</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pops. 1-13 not shown</td>
<td></td>
<td></td>
<td>100%</td>
<td>0.525 (0.033)</td>
<td>0.224 (0.303)</td>
<td>3.214 (0.350)</td>
<td>0.003 (0.003)</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>19</td>
<td>16</td>
<td>100%</td>
<td>0.559 (0.053)</td>
<td>0.126 (0.571)</td>
<td>3.286 (0.350)</td>
<td>0.129 (0.088)</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>22</td>
<td>16</td>
<td>100%</td>
<td>0.573 (0.033)</td>
<td>0.221 (0.303)</td>
<td>3.286 (0.350)</td>
<td>0.071 (0.071)</td>
<td>4</td>
<td>Some tourism, apothecia present</td>
</tr>
<tr>
<td>16</td>
<td>20</td>
<td>20</td>
<td>71%</td>
<td>0.519 (0.053)</td>
<td>0.175 (0.571)</td>
<td>3.286 (0.350)</td>
<td>0.129 (0.088)</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>14</td>
<td>6</td>
<td>100%</td>
<td>0.517 (0.053)</td>
<td>0.173 (0.571)</td>
<td>3.286 (0.350)</td>
<td>0.071 (0.071)</td>
<td>4</td>
<td>Some tourism, apothecia present</td>
</tr>
<tr>
<td>18</td>
<td>23</td>
<td>16</td>
<td>100%</td>
<td>0.510 (0.053)</td>
<td>0.358 (0.289)</td>
<td>2.642 (0.289)</td>
<td>0.000 (0.000)</td>
<td>5</td>
<td>Apothecia present</td>
</tr>
<tr>
<td>19</td>
<td>23</td>
<td>13</td>
<td>100%</td>
<td>0.510 (0.053)</td>
<td>0.358 (0.289)</td>
<td>2.642 (0.289)</td>
<td>0.000 (0.000)</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>9</td>
<td>1</td>
<td>0%</td>
<td>0.000 (0.000)</td>
<td>NA</td>
<td>1.000</td>
<td>0.000 (0.000)</td>
<td>14 expansion</td>
<td>Saxicolous</td>
</tr>
<tr>
<td>21</td>
<td>10</td>
<td>1</td>
<td>0%</td>
<td>0.000 (0.000)</td>
<td>NA</td>
<td>1.000</td>
<td>0.000 (0.000)</td>
<td>14 expansion</td>
<td>Saxicolous</td>
</tr>
<tr>
<td>22</td>
<td>23</td>
<td>4</td>
<td>71%</td>
<td>0.276 (0.039)</td>
<td>0.380 (0.148)</td>
<td>1.926 (0.148)</td>
<td>0.000 (0.000)</td>
<td>7</td>
<td>Village close, apothecia present</td>
</tr>
<tr>
<td>23</td>
<td>23</td>
<td>5</td>
<td>86%</td>
<td>0.356 (0.057)</td>
<td>0.183 (0.148)</td>
<td>2.000 (0.148)</td>
<td>0.000 (0.000)</td>
<td>7</td>
<td>Artificial forest</td>
</tr>
<tr>
<td>24</td>
<td>9</td>
<td>5</td>
<td>64%</td>
<td>0.317 (0.079)</td>
<td>0.256 (0.286)</td>
<td>2.071 (0.286)</td>
<td>0.000 (0.000)</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>23</td>
<td>14</td>
<td>71%</td>
<td>0.375 (0.072)</td>
<td>0.090 (0.317)</td>
<td>2.214 (0.317)</td>
<td>0.002 (0.002)</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>23</td>
<td>21</td>
<td>100%</td>
<td>0.505 (0.045)</td>
<td>0.268 (0.357)</td>
<td>3.357 (0.357)</td>
<td>0.000 (0.000)</td>
<td>7</td>
<td>Some tourism, apothecia present</td>
</tr>
<tr>
<td>27</td>
<td>23</td>
<td>21</td>
<td>93%</td>
<td>0.451 (0.062)</td>
<td>0.023 (0.412)</td>
<td>3.071 (0.412)</td>
<td>0.000 (0.000)</td>
<td>8</td>
<td>Human activities and constructions</td>
</tr>
<tr>
<td>28</td>
<td>23</td>
<td>18</td>
<td>100%</td>
<td>0.486 (0.041)</td>
<td>0.336 (0.350)</td>
<td>3.214 (0.350)</td>
<td>0.002 (0.002)</td>
<td>6</td>
<td>Tourism</td>
</tr>
<tr>
<td>29</td>
<td>23</td>
<td>23</td>
<td>100%</td>
<td>0.494 (0.057)</td>
<td>0.144 (0.571)</td>
<td>3.571 (0.571)</td>
<td>0.008 (0.008)</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>22</td>
<td>20</td>
<td>86%</td>
<td>0.337 (0.072)</td>
<td>0.034 (0.350)</td>
<td>2.857 (0.350)</td>
<td>0.008 (0.008)</td>
<td>12 expansion</td>
<td>Human constructions, apothecia present</td>
</tr>
<tr>
<td>31</td>
<td>23</td>
<td>20</td>
<td>100%</td>
<td>0.506 (0.045)</td>
<td>0.184 (0.612)</td>
<td>3.756 (0.612)</td>
<td>0.000 (0.000)</td>
<td>5</td>
<td>Apothecia present</td>
</tr>
<tr>
<td>32</td>
<td>21</td>
<td>19</td>
<td>93%</td>
<td>0.330 (0.030)</td>
<td>0.044 (0.247)</td>
<td>2.462 (0.247)</td>
<td>0.000 (0.000)</td>
<td>7</td>
<td>Apothecia present</td>
</tr>
<tr>
<td>Pops. 33-64 not shown</td>
<td></td>
<td></td>
<td>100%</td>
<td>0.525 (0.033)</td>
<td>0.224 (0.303)</td>
<td>3.214 (0.350)</td>
<td>0.003 (0.003)</td>
<td>7</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2. Results of the analyses for each population, indicating the number of specimens (n), number of non-clonal specimens, percentage of polymorphic loci, unbiased haploid genetic diversity (uh), unbiased measure of linkage disequilibrium (rBarD), in bold = significant values with a p-value of 0.001, rarefied allelic richness (AR), rarefied private allelic richness (PAR), number of loci with negative values in the K-test (in bold = significant values with a p-value of 0.05), and putative population disturbances (→ well-preserved more or less uniform forest).
Chapter 7 *Bryoria* phylogeography

Best genepools:

**DAPC K3**

**Genepool 1**
Soralia: Frequent
Fum.: Variable
Ph. *capillaris*: 13%
Ph. *fuscescens*: 87%

**Genepool 2**
Soralia: Absent
Fum.: No
Ph. *capillaris*: 87%
Ph. *fuscescens*: 13%

**Genepool 3**
Soralia: Absent
Fum.: No
Ph. *capillaris*: 100%
Ph. *fuscescens*: 0%
Chapter 7 *Bryoria* phylogeography

nuITS, IGS & GAPDH
Phylogenetic tree

**Best genepools:**
- **DAPC K3**
  - **Genepool 1**
    - Soralia: Frequent
    - Fum.: Variable
    - Ph. *capillaris*: 13%
    - Ph. *fuscescens*: 87%
  - **Genepool 2**
    - Soralia: Absent
    - Fum.: No
    - Ph. *capillaris*: 87%
    - Ph. *fuscescens*: 13%
  - **Genepool 3**
    - Soralia: Absent
    - Fum.: No
    - Ph. *capillaris*: 100%
    - Ph. *fuscescens*: 0%

**Phenotype**
- Ph. *capillaris*
- Ph. *fuscescens*
Chapter 7 *Bryoria* phylogeography

**Best genepools:**
- **DAPC K3**
- **Genepool 1**
  - Soralia: Frequent
  - Fum.: Variable
  - Ph. *capillaris*: 13%
  - Ph. *fuscescens*: 87%
- **Genepool 2**
  - Soralia: Absent
  - Fum.: No
  - Ph. *capillaris*: 87%
  - Ph. *fuscescens*: 13%
- **Genepool 3**
  - Soralia: Absent
  - Fum.: No
  - Ph. *capillaris*: 100%
  - Ph. *fuscescens*: 0%
Chapter 7 *Bryoria* phylogeography

Analysis to detect genetic isolation by geographic distance between pairs of populations

**Best genepools:**

**DAPC K3**

**Genepool 1**
- Soralia: Frequent
- Fum.: Variable
- Ph. *capillaris*: 13%
- Ph. *fuscescens*: 87%

**Genepool 2**
- Soralia: Absent
- Fum.: No
- Ph. *capillaris*: 87%
- Ph. *fuscescens*: 13%

**Genepool 3**
- Soralia: Absent
- Fum.: No
- Ph. *capillaris*: 100%
- Ph. *fuscescens*: 0%

**Soralia are not favouring dispersion**
Chapter 7 *Bryoria* phylogeography

Potential distribution prediction for each Genepool using Maxent and 11 bioclimatic layers

<table>
<thead>
<tr>
<th>Genepool 1</th>
<th>Genepool 2</th>
<th>Genepool 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phenotype</strong>-fuscescens</td>
<td><strong>Phenotype</strong>-capillaris</td>
<td><strong>B. fuscescens-pseudofuscescens</strong></td>
</tr>
</tbody>
</table>

**Best genepools:**

**DAPC K3**

- **Genepool 1**
  - Soralia: Frequent
  - Fum.: Variable
  - Ph. *capillaris*: 13%
  - Ph. *fuscescens*: 87%

- **Genepool 2**
  - Soralia: Absent
  - Fum.: No
  - Ph. *capillaris*: 87%
  - Ph. *fuscescens*: 13%

- **Genepool 3**
  - Soralia: Absent
  - Fum.: No
  - Ph. *capillaris*: 100%
  - Ph. *fuscescens*: 0%
Chapter 7 *Bryoria* phylogeography

Potential distribution prediction for the main chemotypes using Maxent and 11 bioclimatic layers.

### Genetically fixed
- Barbatolic acid
- Fumarprotocetraric acid

### Environmentally influenced
- Gyrophoric acid
- Norstictic acid
- Psoromic acid

### Best genepools: DAPC K3

<table>
<thead>
<tr>
<th>Genepool 1</th>
<th>Soralia: Frequent</th>
<th>Fum.: Variable</th>
<th>Ph. <em>capillaris</em>: 13%</th>
<th>Ph. <em>fuscescens</em>: 87%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genepool 2</td>
<td>Soralia: Absent</td>
<td>Fum.: No</td>
<td>Ph. <em>capillaris</em>: 87%</td>
<td>Ph. <em>fuscescens</em>: 13%</td>
</tr>
<tr>
<td>Genepool 3</td>
<td>Soralia: Absent</td>
<td>Fum.: No</td>
<td>Ph. <em>capillaris</em>: 100%</td>
<td>Ph. <em>fuscescens</em>: 0%</td>
</tr>
</tbody>
</table>
Chapter 7 *Bryoria* phylogeography

Potential distribution prediction for *Bryoria fuscescens s. str.* using Maxent and 11 bioclimatic layers

---

**Glacial refugia candidates:**

- East of British Isles
- Northwest of Iberian Peninsula
- Alps lowlands
- Black sea
Chapter 7 *Bryoria* phylogeography

Results from Migrate analysis
Chapter 7 *Bryoria* phylogeography

Results from Migrate analysis

2Nm
- < 25
- 25-50
- 50-75
- 75-100
- 100-250
- > 250

Glacial refugia candidates according to Maxent
Chapter 7 *Bryoria* phylogeography

Results from Migrate analysis

<table>
<thead>
<tr>
<th>2N_m</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 25</td>
<td></td>
</tr>
<tr>
<td>25-50</td>
<td></td>
</tr>
<tr>
<td>50-75</td>
<td></td>
</tr>
<tr>
<td>75-100</td>
<td></td>
</tr>
<tr>
<td>100-250</td>
<td></td>
</tr>
<tr>
<td>&gt; 250</td>
<td></td>
</tr>
</tbody>
</table>

Glacial refugia candidates according to Maxent

Hypothetical migrations

Scandinavia

Carpathians

Mediterranean

Africa
Chapter 7 *Bryoria* phylogeography

Results from Migrate analysis

<table>
<thead>
<tr>
<th>2Nm</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 25</td>
<td></td>
</tr>
<tr>
<td>25-50</td>
<td></td>
</tr>
<tr>
<td>50-75</td>
<td></td>
</tr>
<tr>
<td>75-100</td>
<td></td>
</tr>
<tr>
<td>100-250</td>
<td></td>
</tr>
<tr>
<td>&gt; 250</td>
<td></td>
</tr>
</tbody>
</table>

Nunataks across Europe

Glacial refugia candidates according to Maxent

Cryptic glacial refugia

Hypothetical migrations
Chapter 7 *Bryoria* phylogeography

Results from Migrate analysis

<table>
<thead>
<tr>
<th>2Nm</th>
<th>&lt; 25</th>
<th>25-50</th>
<th>50-75</th>
<th>75-100</th>
<th>100-250</th>
<th>&gt; 250</th>
</tr>
</thead>
</table>
| Isolation by distance analysis
| Sorediate genepool |

**Nunataks across Europe**

**Glacial refugia candidates according to Maxent**

**Cryptic glacial refugia**

**Hypothetical migrations**

**Migration:**
Far pair populations much more differentiated than closer ones.

**Ancestral shared alleles:**
Far pair populations not much more differentiated than closer ones.
(usually linked to incomplete lineage sorting)
Chapter 7 *Bryoria* phylogeography

Evidence of incomplete lineage sorting
Chapter 7 *Bryoria* phylogeography

ML & Bayesian phylogenetic reconstruction of intergenic loci FRBi15 & FRBi16

Evidence of recombination
Chapter 7 *Bryoria* phylogeography

Evolutive conclusions

**Bryoria fuscescens**
- Speciating to: **Fenotype-fuscescens**
- **Fenotype-capillaris**
- GAPDH sequence
- Microsatellites

**Bryoria pseudofuscescens**
- Microsatellites

**Bryoria kockiana**
- GAPDH sequence
- Microsatellites

**Bryoria glabra**
- Morphology
- Standard DNA sequences

Around 1 million years ago

Around 7 million years ago

B. fuscescens agg.
Main Conclusions

• 1. Fluorescence microscopy is confirmed as a useful tool to locate and sometimes identify the secondary metabolites stored in the lichen thalli.

• 2. In *Bryoria fuscescens* agg. the presence-absence and composition in extrolites are variable in different thallus parts and sometimes associated with pseudocyphellae or soralia.

• 3. In *Bryoria fuscescens* s. l. specimens, there is no correlation between extrolites composition, the genetic affinity and the morphospecies.

• 4. The populations of *Bryoria fuscescens* s. l. in the Mediterranean Region show a combination of characters that does not fit with the established morphospecies concept based on boreal specimens.

• 5. New microsatellite markers specific for *Bryoria* sect. *Implexae* has been obtained to perform phylogeographical studies at population level.
Main Conclusions

• 6. Integrative taxonomy allows to develop a species concept in *Bryoria* sect. *Implexae* that do not reveal taxonomies with single approaches. Of the 14 morphospecies analyzed, only four accomplish with the phylogenetic species concept, being *Bryoria fuscescens*, *B. kockiana* and *B. pseudofuscescens* cryptic and *B. glabra* distinguishable.

• 7. The species of *Bryoria fuscescens* agg. represent the most recent speciation event known in lichens.

• 8. *Bryoria fuscescens* s. str. includes three main genepools in Europe and North Africa, two of them widely distributed, whereas one is restricted to North Scandinavia. The genetic traits of the latter are intermediate between *Bryoria fuscescens* and *B. pseudofuscescens*.

• 9. The high dispersal capacities of *Bryoria fuscescens* s. str. detected here seems influenced by an artefact of shared ancestral polymorphisms.
Main Conclusions

- **10.** The Scandinavian Peninsula, followed by the Alps and the Iberian Peninsula, have the richest genetic diversity of *Bryoria fuscescens* s. str. in Europe. The genetic diversity of the populations do not correlate with the presence or absence of apothecia.

- **11.** *Bryoria fuscescens* s. str. seems involved in an evolutionary process influenced by genetic drift towards two phenotypic groups with high levels of incomplete lineage sorting.

- **12.** The lichenicolous fungus *Raesaenenia huuskonenii* grows on *Bryoria fuscescens* agg. independently of the morphospecies, chemotype or genepool.

- **13.** The *Bryoria* specimens collected in Chile belongs to an undescribed species here proposed as *Bryoria araucana*.

- **14.** *Bryoria mariensis* must be considered a synonym with *Pseudephebe minuscula*.

- **15.** *Pseudephebe minuscula* is a very variable species whose morphology overlaps with that of *P. pubescens*, so both species must be considered cryptic.
!Muchas gracias!

Collection pictures:
¡Muchas gracias!
Introduction
Introduction

**Genetic & phenotypic diversity**

- Sexual reproduction
- Population sizes
- Predators
- Human interactions
- Photobiont
- Parasites
- Dispersal capacities
- Environment
- Environmental changes
- **Species conservation**
  - Basic knowledge
Chapter 1 *Pseudephebe* species concept

P. minuscula

P. pubescens

9.5 Mya

26.5 Mya

Fig. S1. Dated BEAST maximum clade credibility tree estimated from three-loci concatenated data. Grey bars indicating the 95% highest posterior density interval for the estimated divergence times. Posterior probabilities of interesting nodes and its divergence time as the mean posterior estimate of their age in Mya. Clades A, A’ and B indicated as in Fig. 1.
Chapter 1 *Pseudephebe* species concept

Fig. 1. Phylogenetic consensus tree based on ITS, RPB1 and MCM7 markers analyzed as a concatenated data matrix (Table 1). Whole tree in the upper left corner using *Allantoparmelia* as outgroup, with the *Pseudephebe* clade shown in detail. Tree topology depicts the results of Bayesian inference, showing significant posterior probabilities ≥0.95 and bootstrap values ≥70% obtained in the maximum likelihood analysis. Intron presence and extrolite composition are indicated on the lower left corner. RefSpec = sequenced reference specimen.