DEPARTAMENTO DE BIOLOGÍA VEGETAL II
FACULTAD DE FARMACIA
UNIVERSIDAD COMPLUTENSE DE MADRID

PhD dissertation

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

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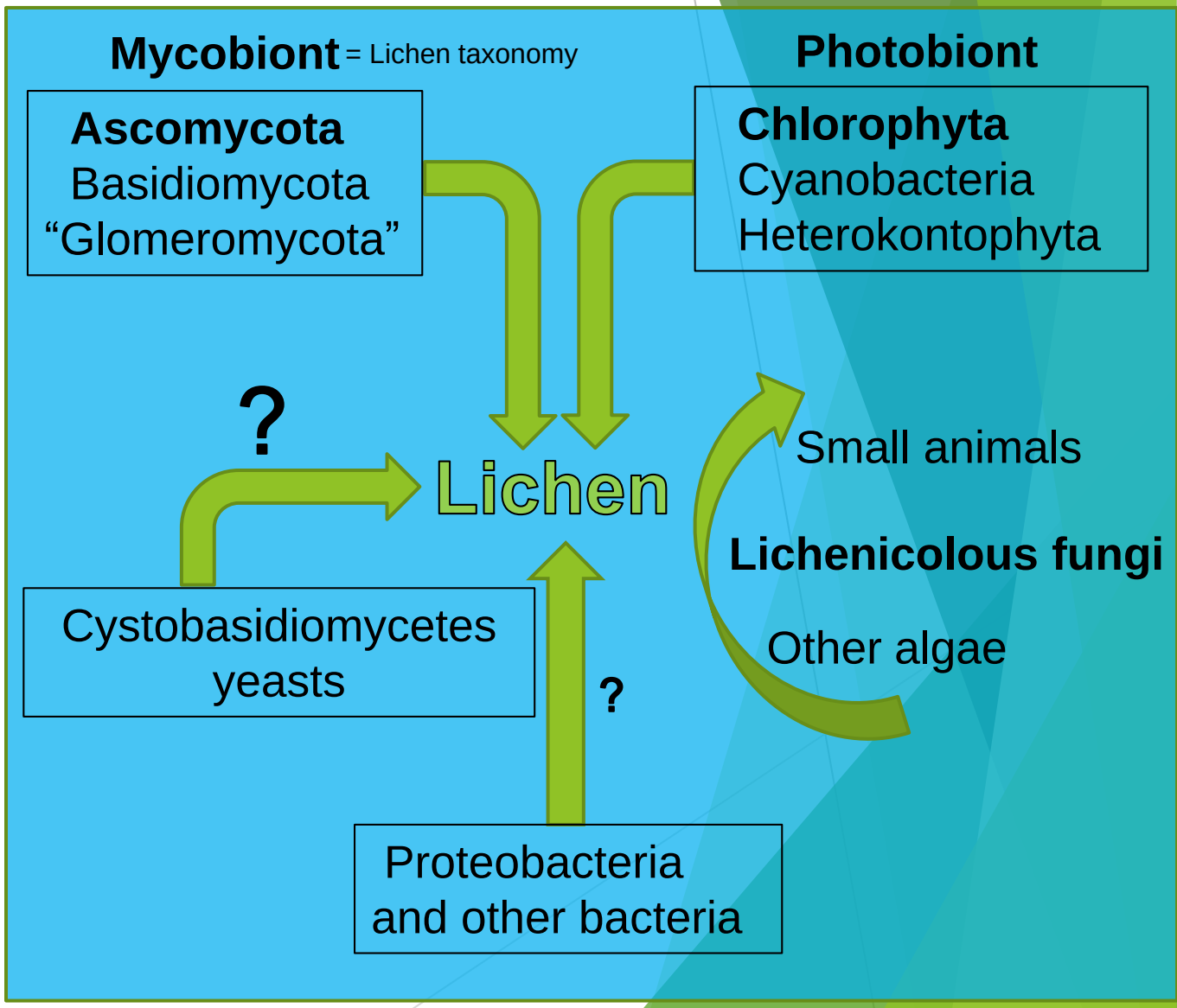
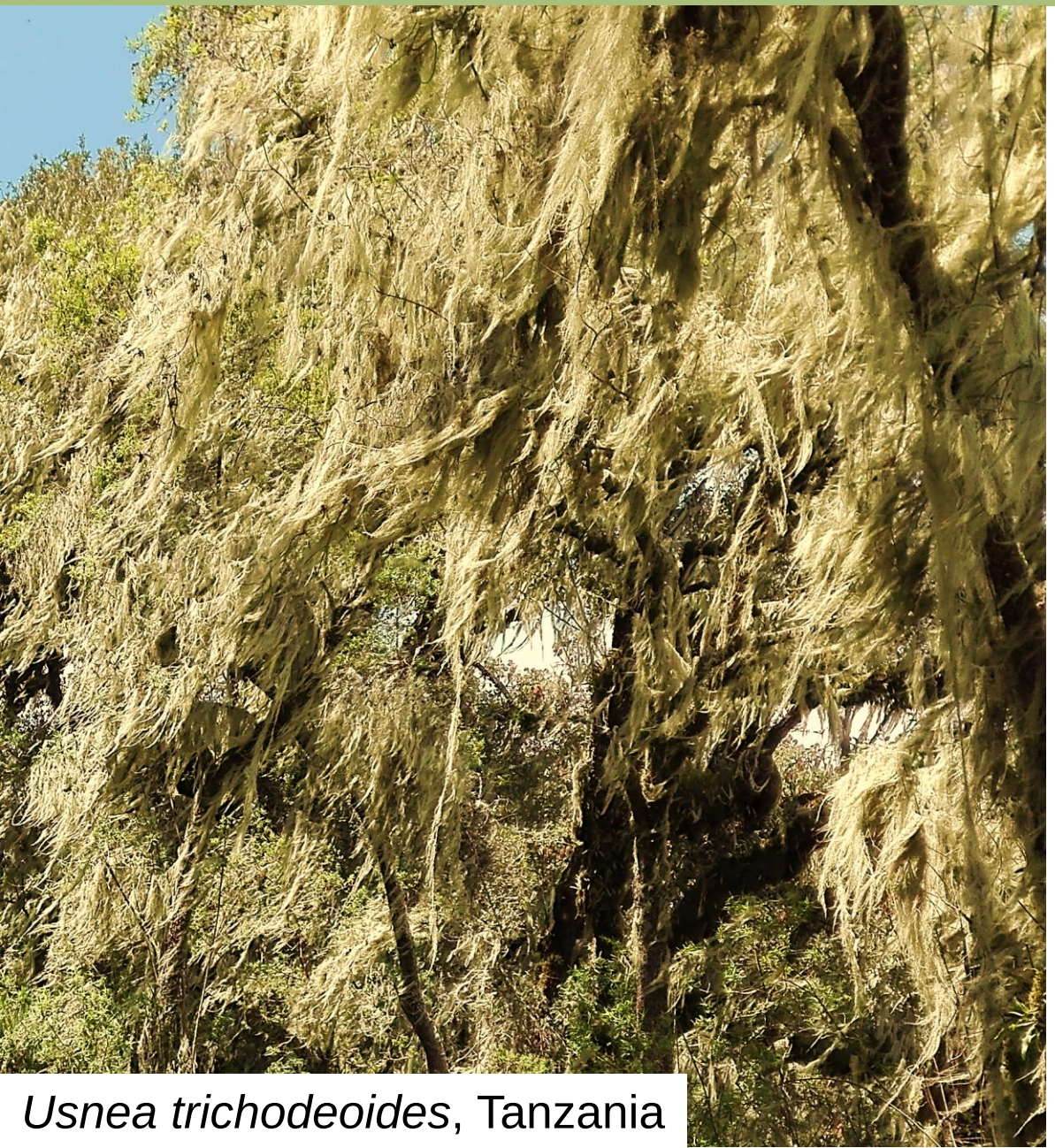
Introduction

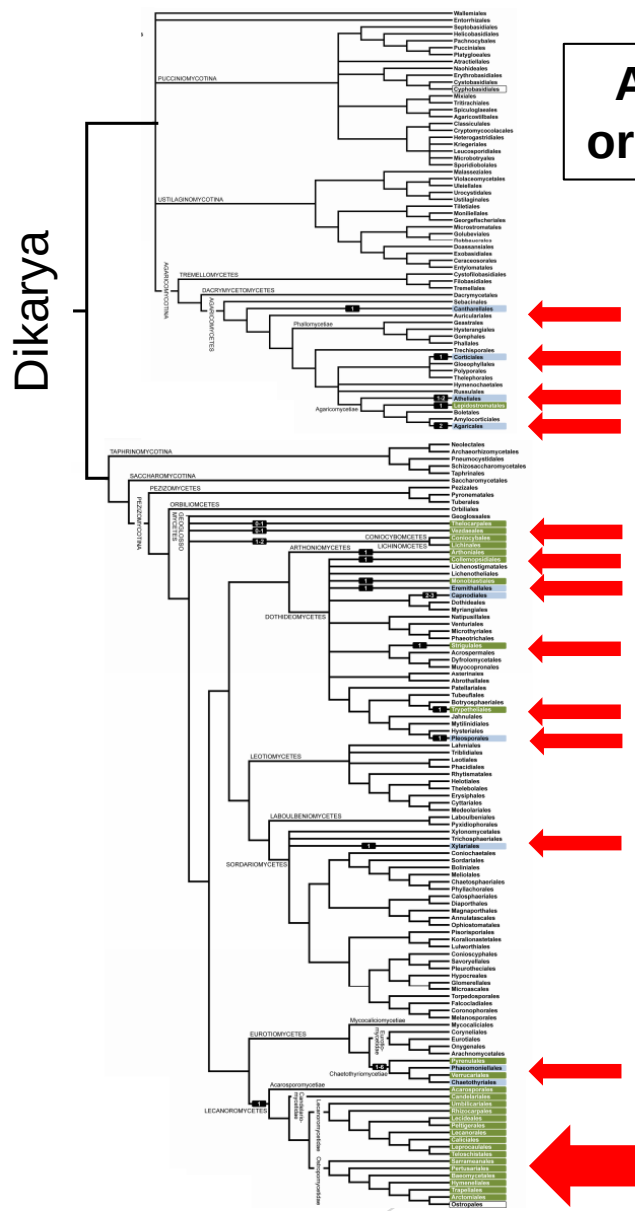
Tundra community, Norway



Introduction



Usnea trichodeoides, Tanzania



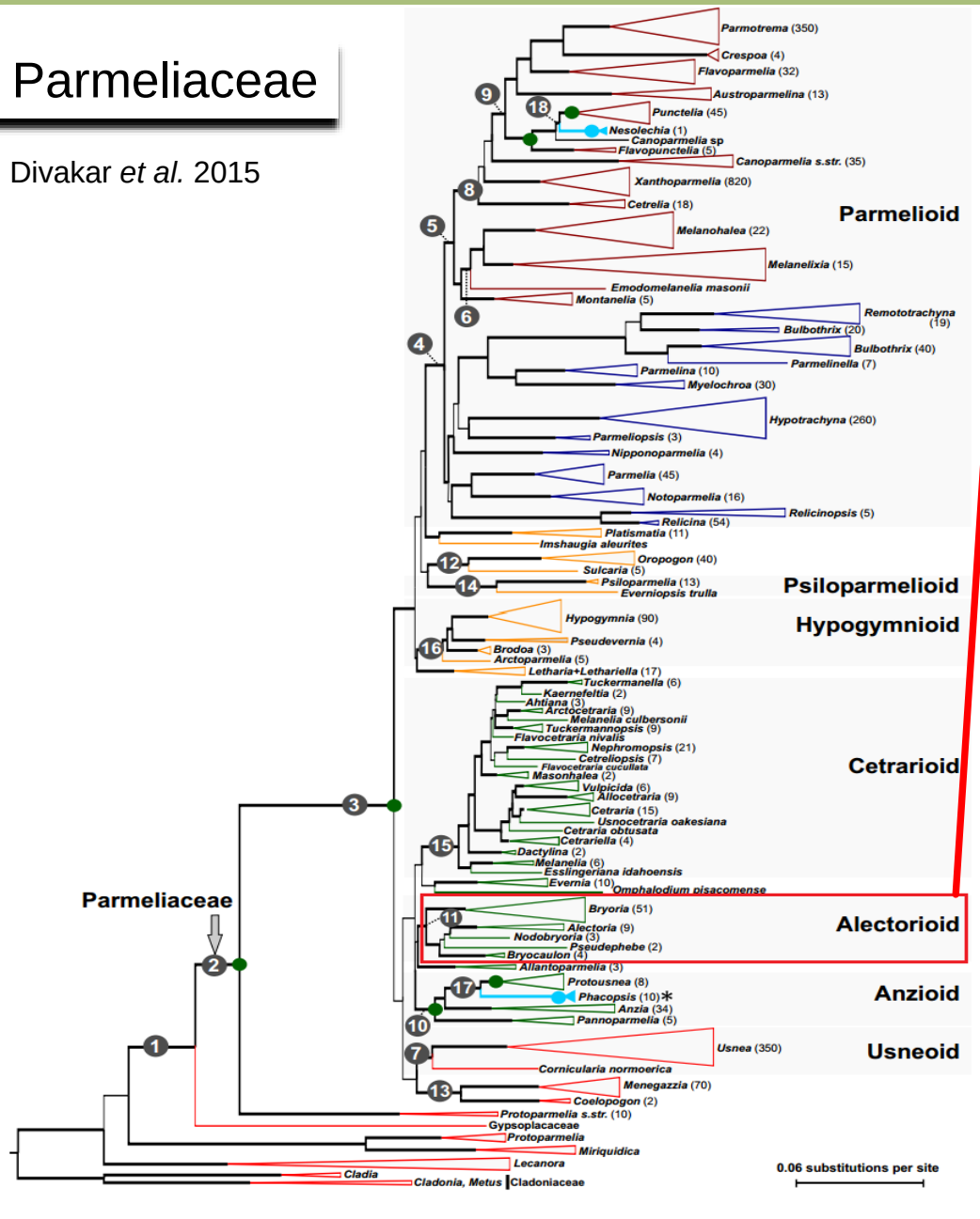
At least 15 independent origins of lichenized fungi

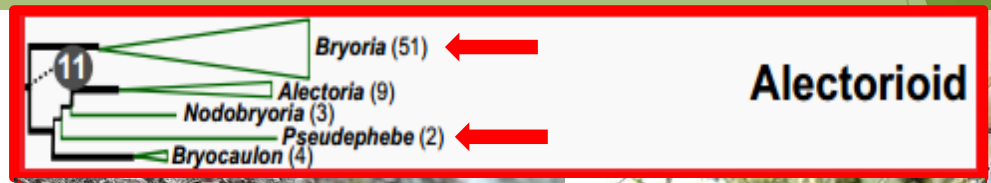
Lecanoromycetes

Introduction

Parmeliaceae

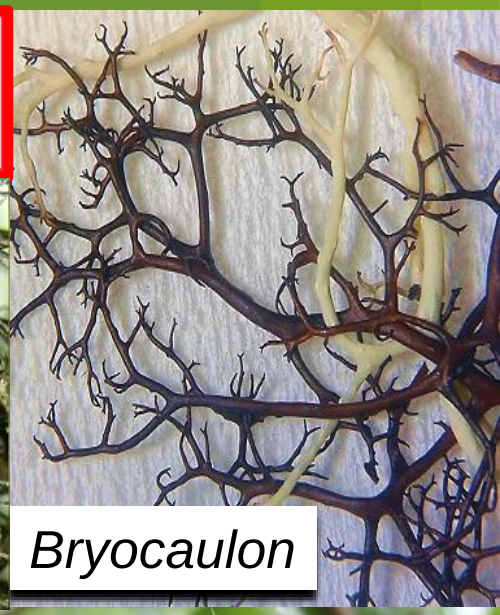
Divakar *et al.* 2015





Pseudephebe

Nodobryoria



Bryocaulon

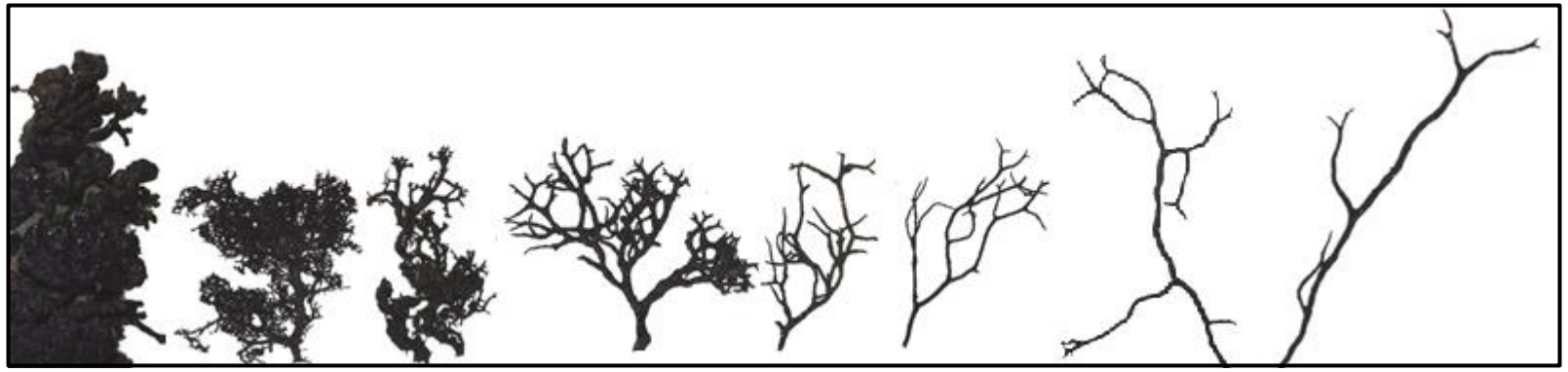
Alectoria

Bryoria

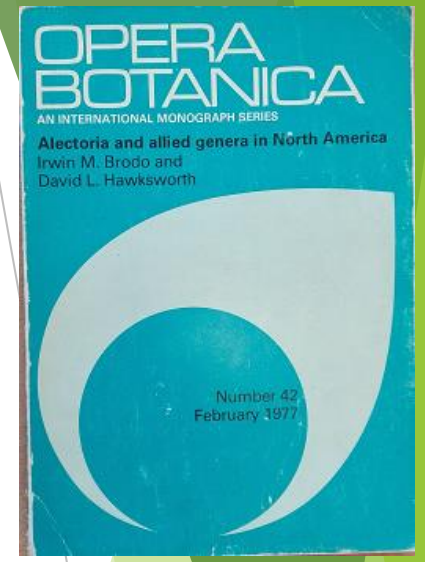
Introduction

Intermediate specimens between species in:

Pseudephebe

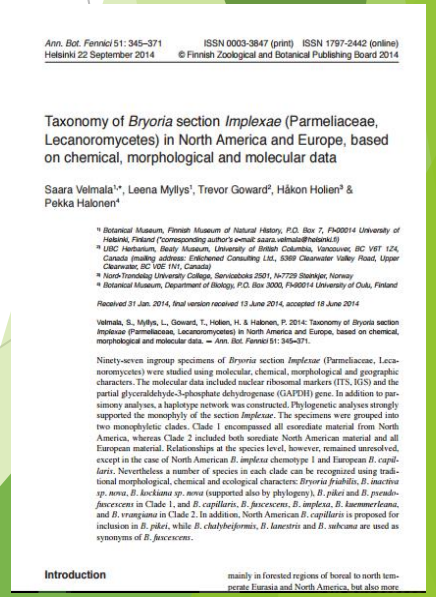


Pseudephebe minuscula → ***Pseudephebe pubescens***



Brodo & Hawksworth 1977

Bryoria section *Implexae*



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Taxonomy of *Bryoria* section *Implexae* (Parmeliaceae, Lecanoromycetes) in North America and Europe, based on chemical, morphological and molecular data

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Velmala, S., Myllys, L., Goward, T., Holien, H. & Halonen, P. 2014: Taxonomy of *Bryoria* section *Implexae* (Parmeliaceae, Lecanoromycetes) in North America and Europe, based on chemical, morphological and molecular data. — *Ann. Bot. Fennici* 51: 345–371.

Ninety-seven ingroup specimens of *Bryoria* section *Implexae* (Parmeliaceae, Lecanoromycetes) were studied using molecular, chemical, morphological and geographic characters. The molecular data included nuclear ribosomal markers (ITS, IGS) and the partial glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene. In addition to parsimony analyses, a haplotype network was constructed. Phylogenetic analyses strongly supported the monophyly of the section *Implexae*. The specimens were grouped into two monophyletic clades. Clade 1 encompassed all esorediate material from North America, whereas Clade 2 included both sorediate North American material and all European material. Relationships at the species level, however, remained unresolved, except in the case of North American *B. implexa* chemotype 1 and European *B. capillaris*. Nevertheless a number of species in each clade can be recognized using traditional morphological, chemical and ecological characters: *Bryoria fuhrilis*, *B. inactiva sp. nova*, *B. kockiana sp. nova* (supported also by phylogeny), *B. pikei* and *B. pseudofuscescens* in Clade 1, and *B. capillaris*, *B. fuscescens*, *B. implexa*, *B. luemmerleana*, and *B. vrangiana* in Clade 2. In addition, North American *B. capillaris* is proposed for inclusion in *B. pikei*, while *B. chalybeiformis*, *B. lanestris* and *B. subcana* are used as synonyms of *B. fuscescens*.

Introduction

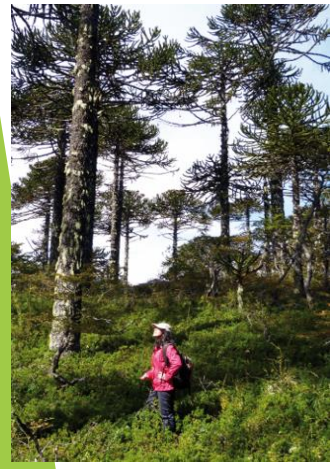
mainly in forested regions of boreal to north temperate Eurasia and North America, but also more

Velmala et al. 2014

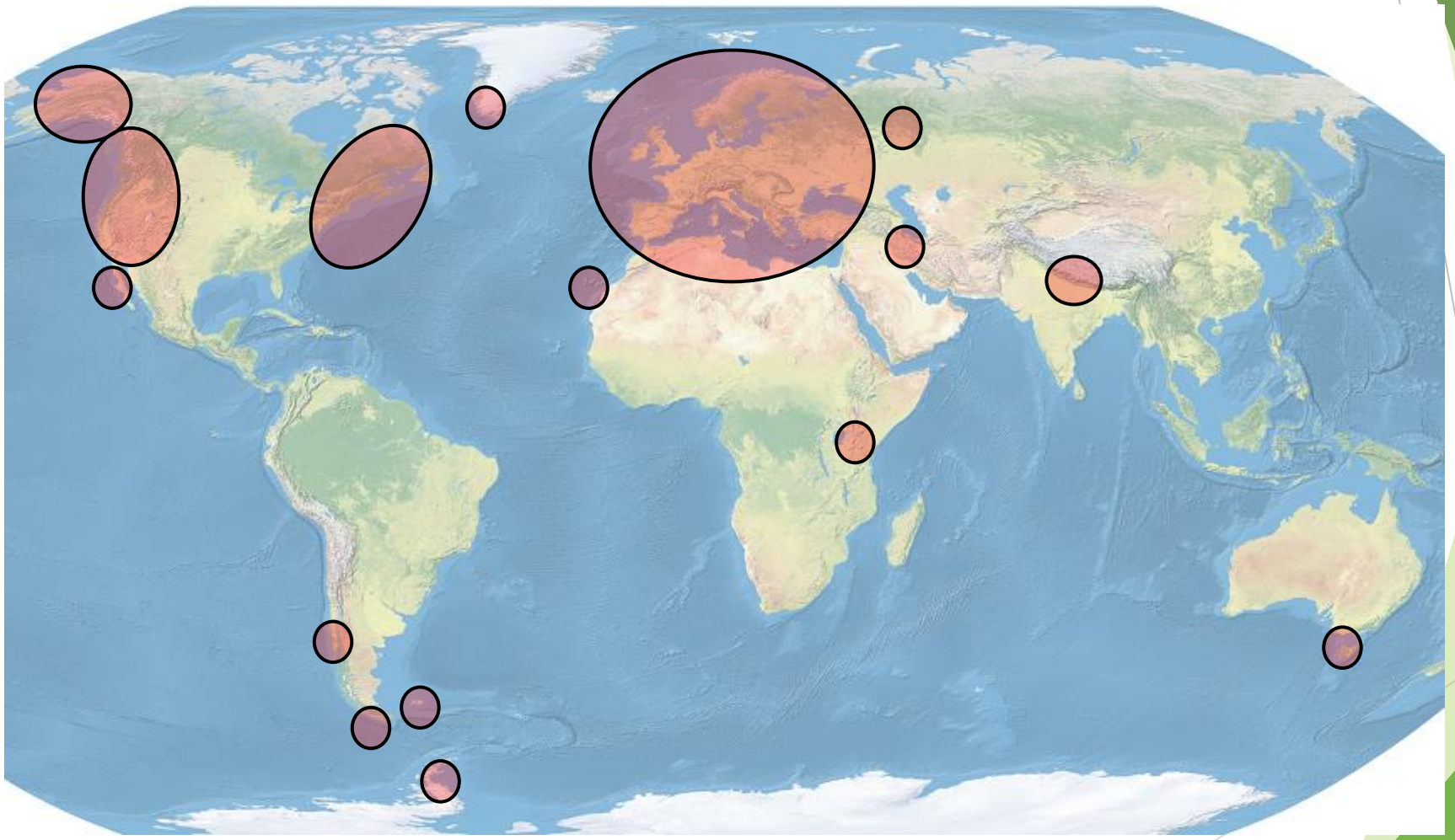
Main Objectives

- **Perform an integrative taxonomical study of *Bryoria* section *Implexae* and *Pseudephebe.***
- **Study the extrolite composition, location and taxonomical utility in *Bryoria* and *Pseudephebe*.**
- **Study the interaction between *Bryoria fuscescens* and the environment.**
- **Understand the evolutive processes that are producing the phenotypical variability observed in *Bryoria fuscescens* s. str.**

Materials and Methods



Sampled areas

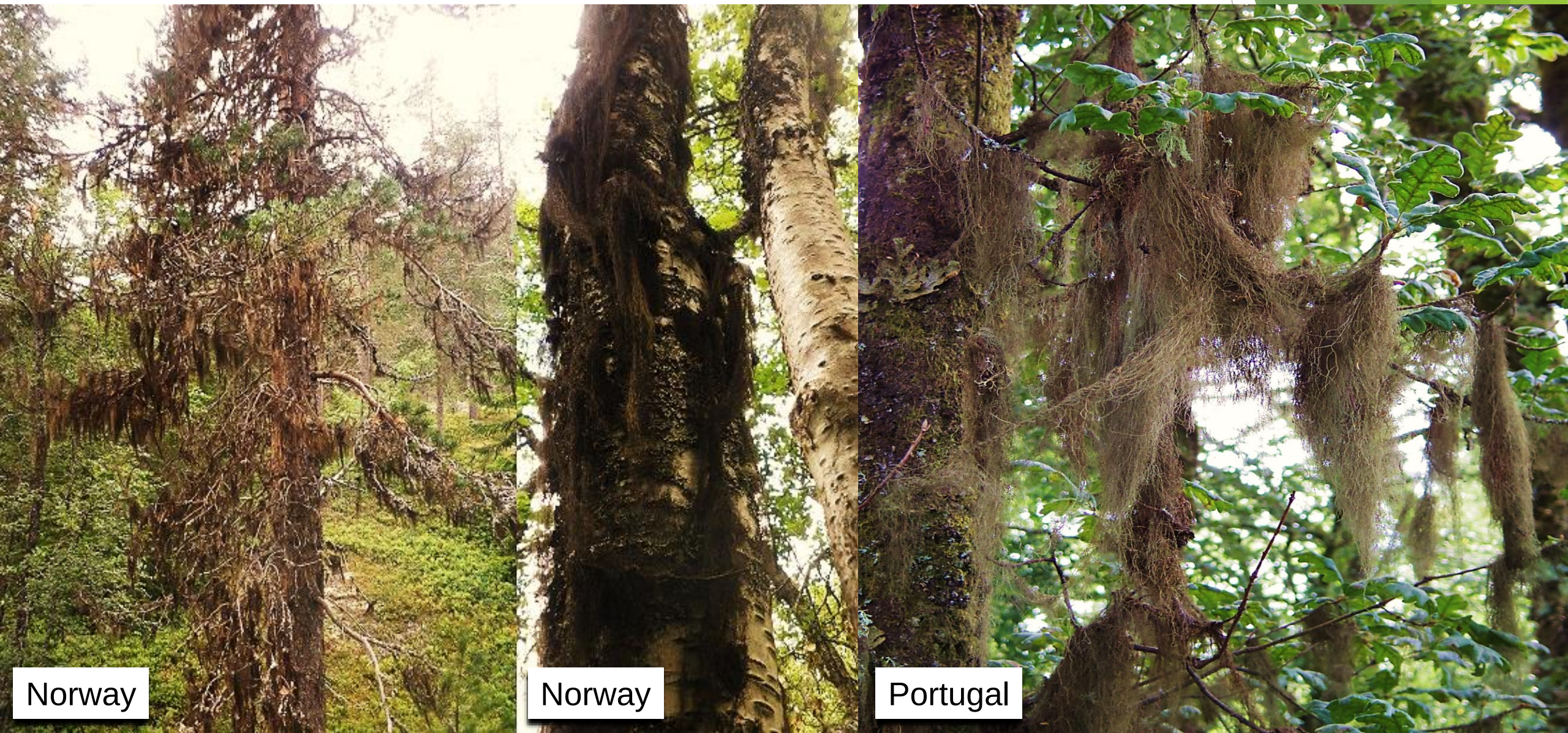


Pseudephebe: aprox. 120 specimens

Bryoria: aprox. 2.100 specimens

Materials and Methods

Example of three sampled regions



Materials and Methods

Main data

- **Distribution**
- **Phenotypical**
  - Morphological characters
  - Chemical characters
- **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

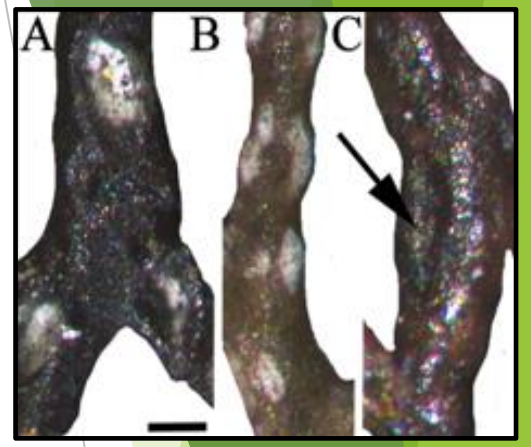
Chapter 1 *Pseudephebe* species concept

Pseudephebe pubescens

P. minuscula

Bryoria mariensis

120 specimens



Pseudocyphellae

37 specimens + 25 outgroup

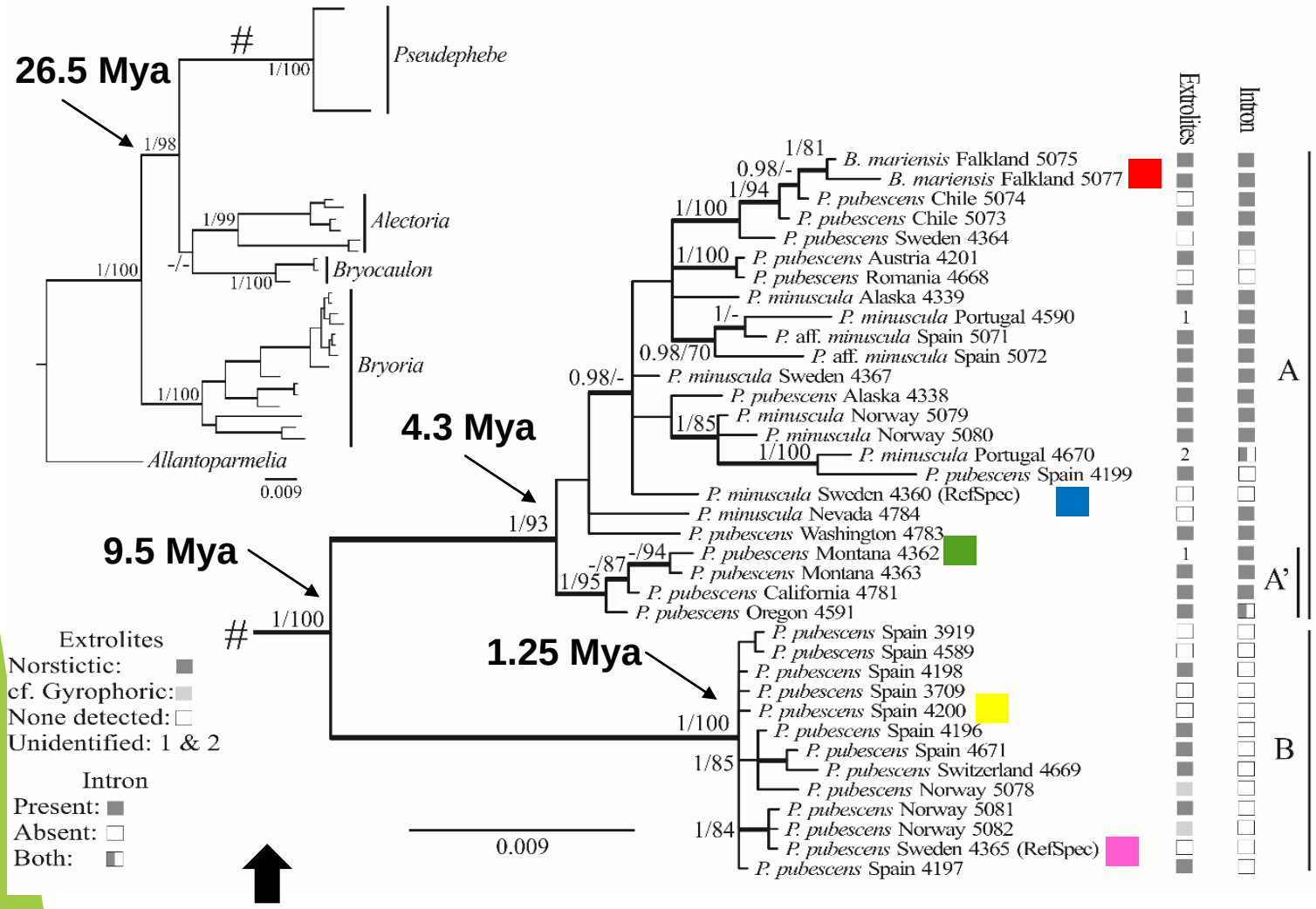
Species	DNA code	Extrolites	SSU intron	Internodes length (mm)	Branches width (mm)	Pseudo-cyphellae	Profusely branched tips	Old branches appressed	Flattened branches
Bryoria mariensis	5077	Norstictic	+	< 1–7	0·26 (0·1–1)	+	–	–	–
B. mariensis	5075	Norstictic	+	< 1–7	0·20 (0·1–0·4)	+	–	±	±
Pseudephebe minuscula	4339	Norstictic	+	< 1	0·18 (0·1–0·3)	–	+	+	+
P. pubescens	4338	Norstictic	+	< 1	0·18 (0·1–0·2)	+	+	–	±
P. minuscula	4784	Absent	+	< 1	0·32 (0·2–0·5; crusty)	+	+	+	+
P. pubescens	4781	Norstictic	+	> 1	0·10 (0·1–0·2)	perforated	+	–	–
P. pubescens	4363	Norstictic	+	< 1–2	0·17 (0·1–0·3)	±	+	±	+
P. pubescens	4362	Greyish pale spot	+	< 1	0·17 (0·1–0·2)	–	+	±	±

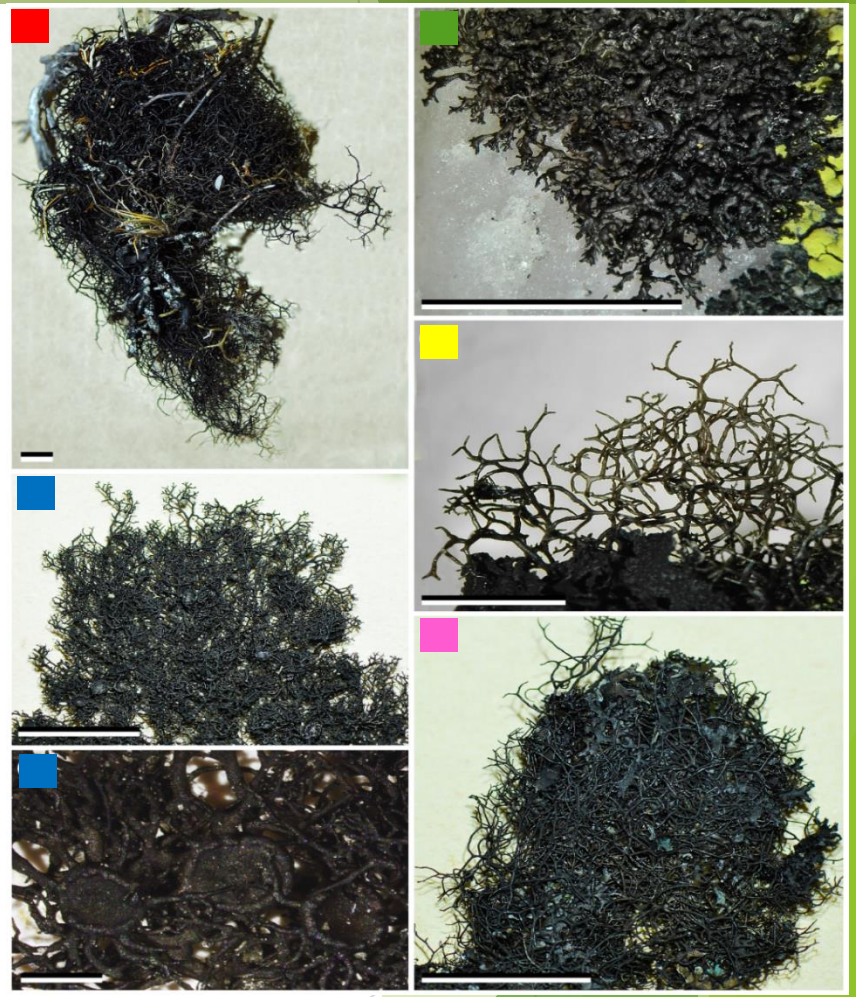
Phylogenetic tree using: nuITS, RPB1 & MCM7

Characters not reported in bibliography

Not three phenotipic groups can be obtained

Chapter 1 *Pseudephebe* species concept

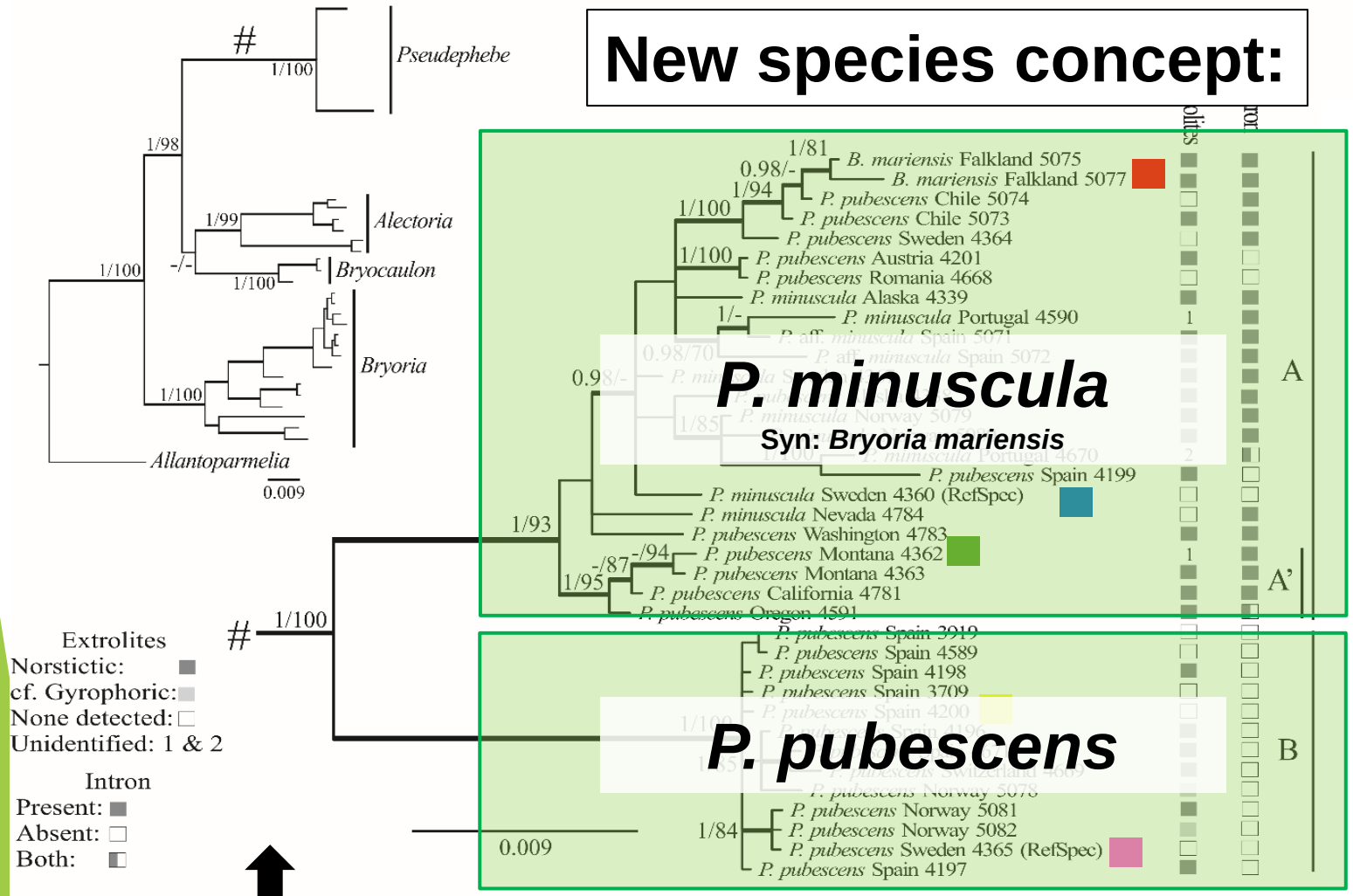


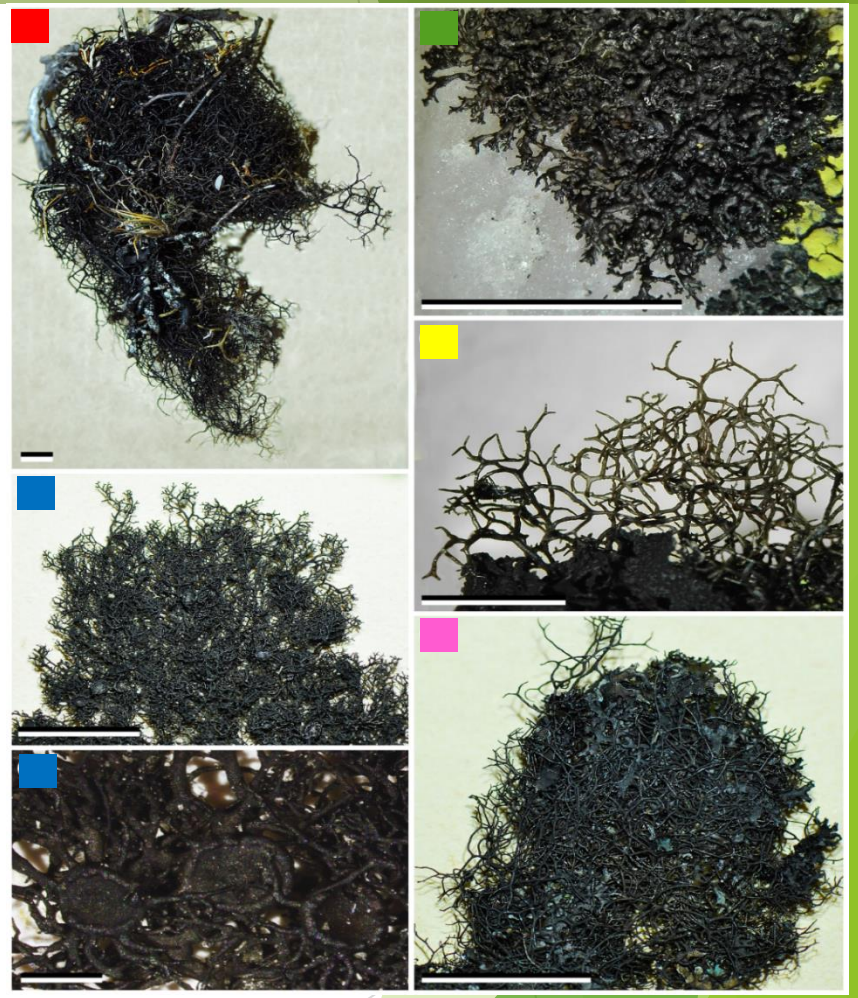


ML and bayesian phylogenetic tree from the concatenated matrix of ITS, RPB1 and MCM7.

Chapter 1 *Pseudephebe* species concept

New species concept:





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

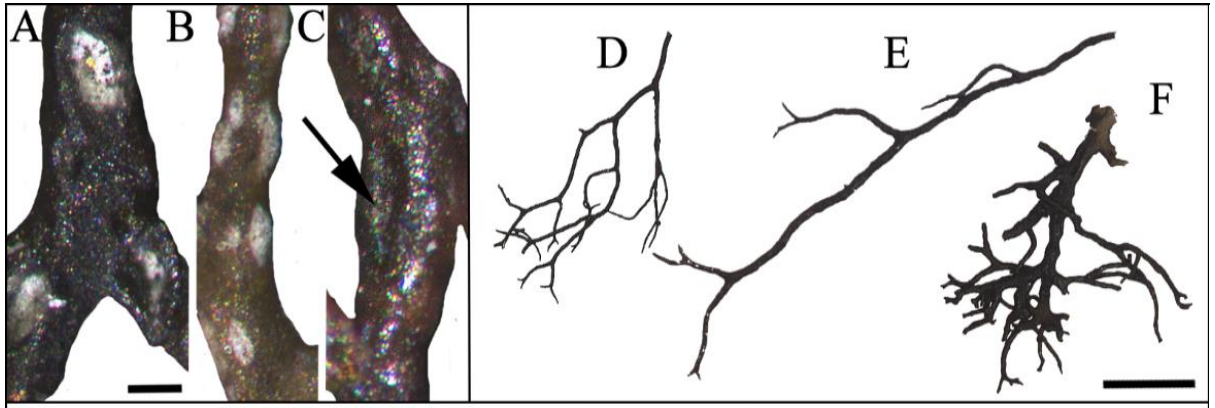
Species delimitation programs

Method/Marker	ITS	MCM7	RPB1	Concatenated
ABGD	A, B	A, B	A, B	A, B
PTP	A, A', B	A, B	A, B	A, B
BP&P	–	–	–	A, B

Chapter 1 *Pseudephebe* species concept

Pseudocyphellae

P. pubescens



P. minuscula

No discriminatory characters between species

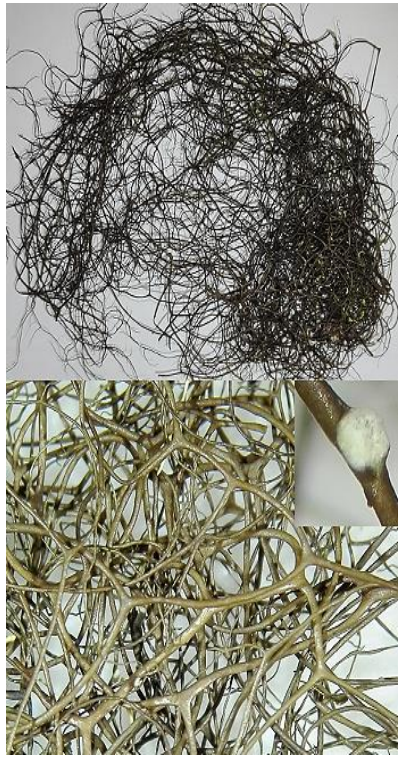
Character	*Pseudephebe minuscula*	*Pseudephebe pubescens*
Habit	Rarely subcrustose, small to large fruticose	Never subcrustose, small to medium fruticose
Thallus size (diameter)	Usually less than 3 cm, but reaching more than 8 cm	Less than 5 cm
Internode length	Usually less than 1 mm, but reaching 7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

***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.



B. glabra / fuscescens

Bryoria aff. trichodes* or *B.* sect. *Implexae

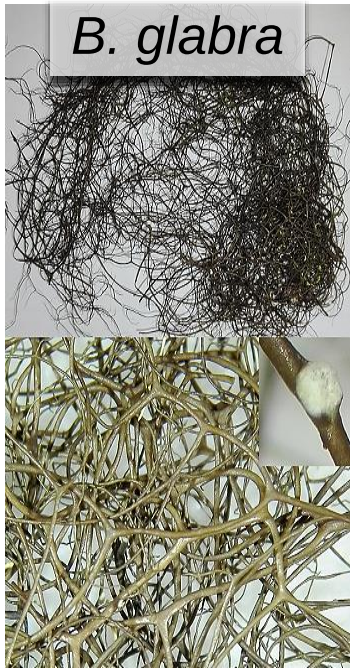
- Morphological study

- Thin layer chromatography

- nuITS, mtSSU, MCM7
  - Maximum likelihood tree
  - Bayesian tree

Chapter 2 *Bryoria araucana* sp. nov.

Fig. 2. .*Bryoria araucana*, holotype. **A.** habitat; **B.** habit; **C.** detail of branching pattern; **D & E.** detail of pseudocyphellae. Scales: B = 1 cm; C = 1 mm; D = 0.15 mm; E = 0.25 mm.



Bryoria sect. *Bryoria* is polyphyletic

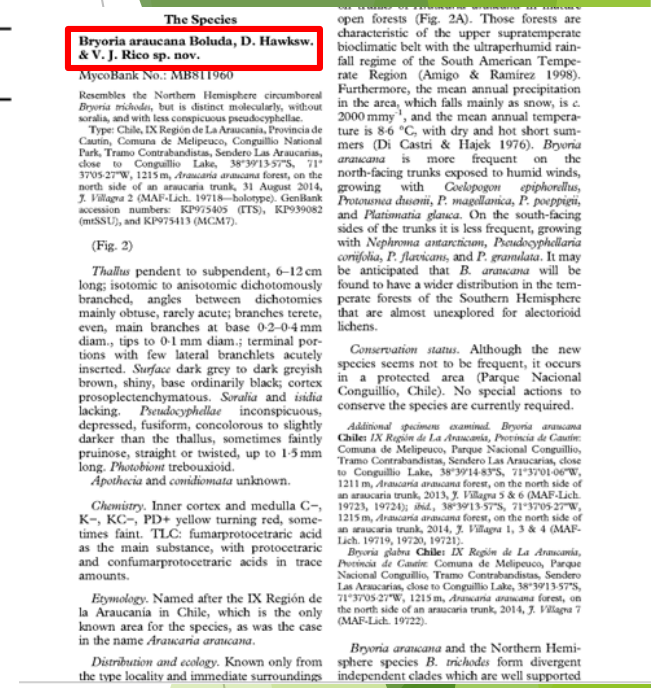
Fig. 1. Phylogenetic relationships of *Bryoria* species used in this study, 38 samples representing 19 species, based on ITS, mtSSU, and MCM7 markers analyzed in a concatenated data matrix. Tree topology depicts the results of the Bayesian Markov chain Monte Carlo (B/MCMC) analysis. Posterior probabilities and bootstrap values, when coincident with the Bayesian tree, are given on the node branches. Sections according to Myllys *et al.* (2011b). *B. glabra* (bold) = Chilean specimen. *B.araucana* (bold) = new species. *Peudephebe pubescens* used as outgroup.

Chapter 2 *Bryoria araucana* sp. nov.

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

Character/Species	*Bryoria araucana*	*B. nadvornikiana*	*B. poeltii*	*B. trichodes*	*B. furcellata*
Main chemistry	Fum	Bar, Ale, ± Atr, Fum in soralia	Fum	Fum, Chlor	Fum
Thallus	Pendent	Caespitose (base) to pendent	Caespitose (base) to pendent	Pendent	Caespitose
Pseudocyphellae	Inconspicuous, dark grey-brown	Inconspicuous, white	Conspicuous, dark brown-black	Conspicuous, white to brownish	Absent
Soralia	Absent	Tuberculate to fissural, white	Tuberculate to fissural, dark, spinulose	Rare, fissural, white	Fissural, white, spinulose (tufts)
Spinules or spinulose branches	On terminal portions, sparse	Lateral, sparse to frequent	Sparse, also on soralia	Lateral, sparse	Sparse to frequent
Colour	Dark grey-brown, base usually darker	Pale to dark brown-violet, base generally black	Dark brown to black	Pale to dark brown	Pale to dark brown, base often darker
Distribution	Chile, South America	Europe, Africa, Asia, Hawaii, North America	Himalayas	Asia, North America	Europe, Macaronesia, Asia, Oceania, North and Central America

Ale = Alectorialic acid, Atr = Atranorin, Bar = Barbatolic acid, Chlor = Chloratranorin, Fum = Fumarprotocetraric acid.



The Species

Bryoria araucana Boluda, D. Hawksw. & V. J. Rico sp. nov.

MycoBank No.: MB811960

Resembles the Northern Hemisphere circumboreal *Bryoria trichodes*, but is distinct molecularly, without soralia, and with less conspicuous pseudocyphellae.

Type: Chile, IX Región de La Araucanía, Provincia de Cautín, Comuna de Melipeuco, Conguillío National Park, Tramo Contrabandistas, Sendero Las Araucarias, close to Conguillío Lake, 38°39'13-57"S, 71° 37'05-27"W, 1215 m, *Araucaria araucana* forest, on the north side of an araucaria trunk, 31 August 2014, *J. Villagra* 2 (MAF-Lich. 19718—holotype). GenBank accession numbers: KP975405 (ITS), KP939082 (mtSSU), and KP975413 (MCM7).

(Fig. 2)

Thallus pendent to subpendent, 6–12 cm long; isotomic to anisotomic dichotomously branched, angles between dichotomies mainly obtuse, rarely acute; branches terete, even, main branches at base 0·2–0·4 mm diam., tips to 0·1 mm diam.; terminal portions with few lateral branchlets acutely inserted. *Surface* dark grey to dark greyish brown, shiny, base ordinarily black; cortex prosoplectenchymatous. *Soralia* and *isidia* lacking. *Pseudocyphellae* inconspicuous, depressed, fusiform, concolorous to slightly darker than the thallus, sometimes faintly pruinose, straight or twisted, up to 1·5 mm long. *Photobiont* trebouxioid.

Apothecia and *conidiomata* unknown.

Chemistry. Inner cortex and medulla C–, K–, KC–, PD+ yellow turning red, sometimes faint. TLC: fumarprotocetraric acid as the main substance, with protocetraric and confumarprotocetraric acids in trace amounts.

Etymology. Named after the IX Región de la Araucanía in Chile, which is the only known area for the species, as was the case in the name *Araucaria araucana*.

Distribution and ecology. Known only from the type locality and immediate surroundings open forests (Fig. 2A). Those forests are characteristic of the upper supratemperate bioclimatic belt with the ultraperhumid rainfall regime of the South American Temperate Region (Amigo & Ramírez 1998). Furthermore, the mean annual precipitation in the area, which falls mainly as snow, is *c.* 2000 mm y^{-1}, and the mean annual temperature is 8·6 °C, with dry and hot short summers (Di Castri & Hajek 1976). *Bryoria araucana* is more frequent on the north-facing trunks exposed to humid winds, growing with *Coelopogon epiphorellus*, *Protousnea diusenii*, *P. magellanica*, *P. poeppigii*, and *Platismatia glauca*. On the south-facing sides of the trunks it is less frequent, growing with *Nephroma antarcticum*, *Pseudocyphellaria coriifolia*, *P. flavicans*, and *P. granulata*. It may be anticipated that *B. araucana* will be found to have a wider distribution in the temperate forests of the Southern Hemisphere that are almost unexplored for alectorioid lichens.

Conservation status. Although the new species seems not to be frequent, it occurs in a protected area (Parque Nacional Conguillío, Chile). No special actions to conserve the species are currently required.

Additional specimens examined. Bryoria araucana **Chile:** *IX Región de La Araucanía, Provincia de Cautín:* Comuna de Melipeuco, Parque Nacional Conguillío, Tramo Contrabandistas, Sendero Las Araucarias, close to Conguillío Lake, 38°39'14-83"S, 71°37'01-06"W, 1211 m, *Araucaria araucana* forest, on the north side of an araucaria trunk, 2013, *J. Villagra* 5 & 6 (MAF-Lich. 19723, 19724); *ibid.*, 38°39'13-57"S, 71°37'05-27"W, 1215 m, *Araucaria araucana* forest, on the north side of an araucaria trunk, 2014, *J. Villagra* 1, 3 & 4 (MAF-Lich. 19719, 19720, 19721).

Bryoria glabra **Chile:** *IX Región de La Araucanía, Provincia de Cautín:* Comuna de Melipeuco, Parque Nacional Conguillío, Tramo Contrabandistas, Sendero Las Araucarias, close to Conguillío Lake, 38°39'13-57"S, 71°37'05-27"W, 1215 m, *Araucaria araucana* forest, on the north side of an araucaria trunk, 2014, *J. Villagra* 7 (MAF-Lich. 19722).

Bryoria araucana and the Northern Hemisphere species *B. trichodes* form divergent independent clades which are well supported

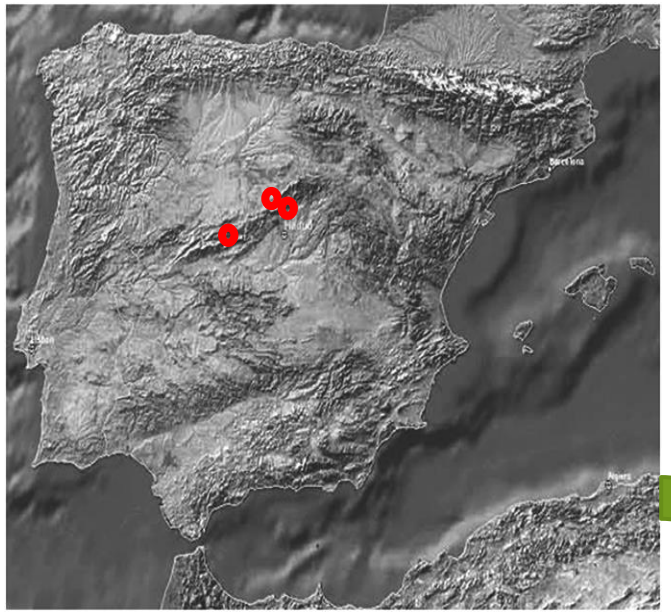
Boluda *et al.* 2015

Chapter 3

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

Typical lichen community of *B. fuscescens s.l.* in Spain

Chapter 3 *Bryoria fuscescens* mismatch

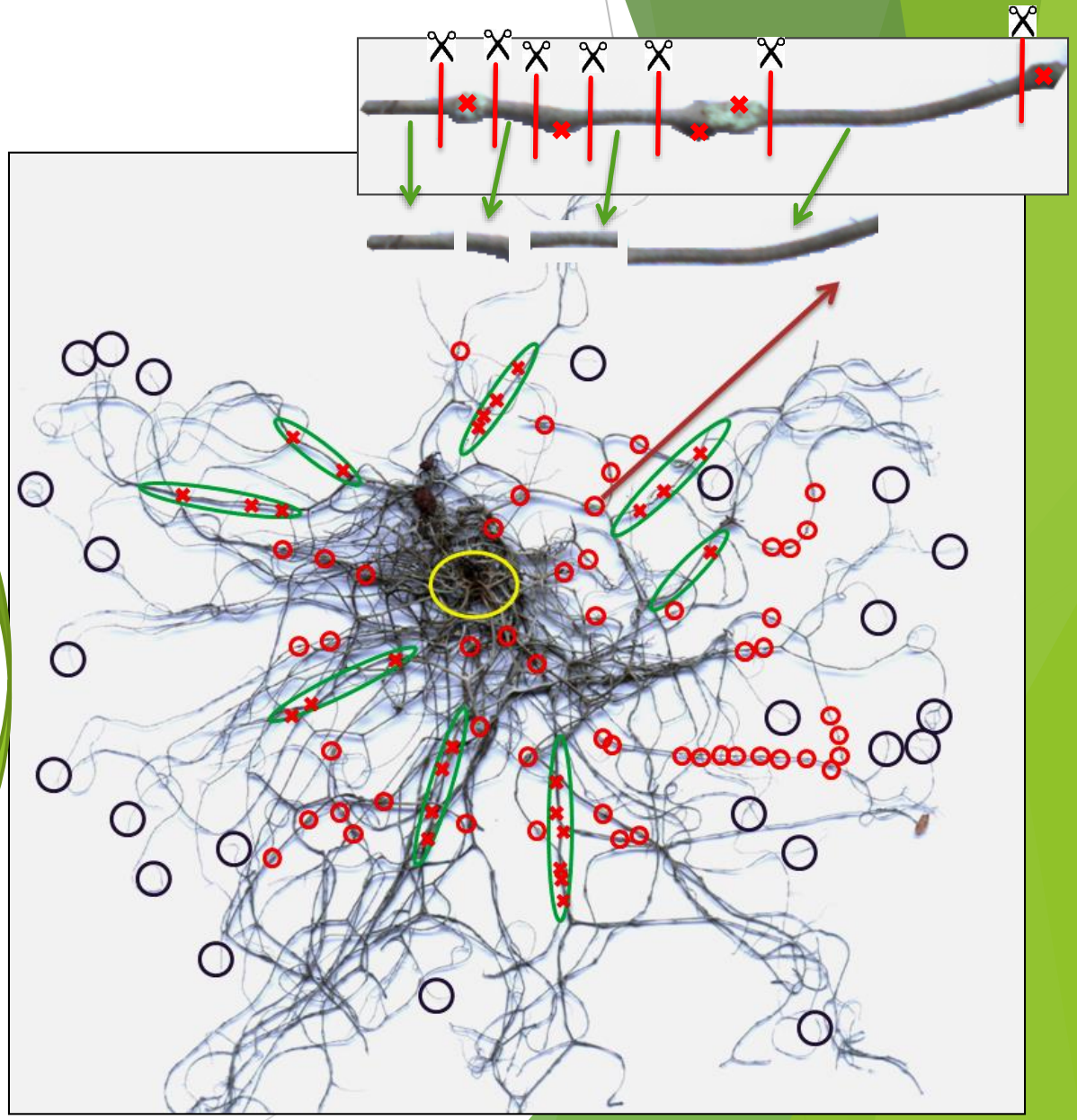


45 morphologically invariable *B. fuscescens* specimens from three close localities

TLC & nuITS

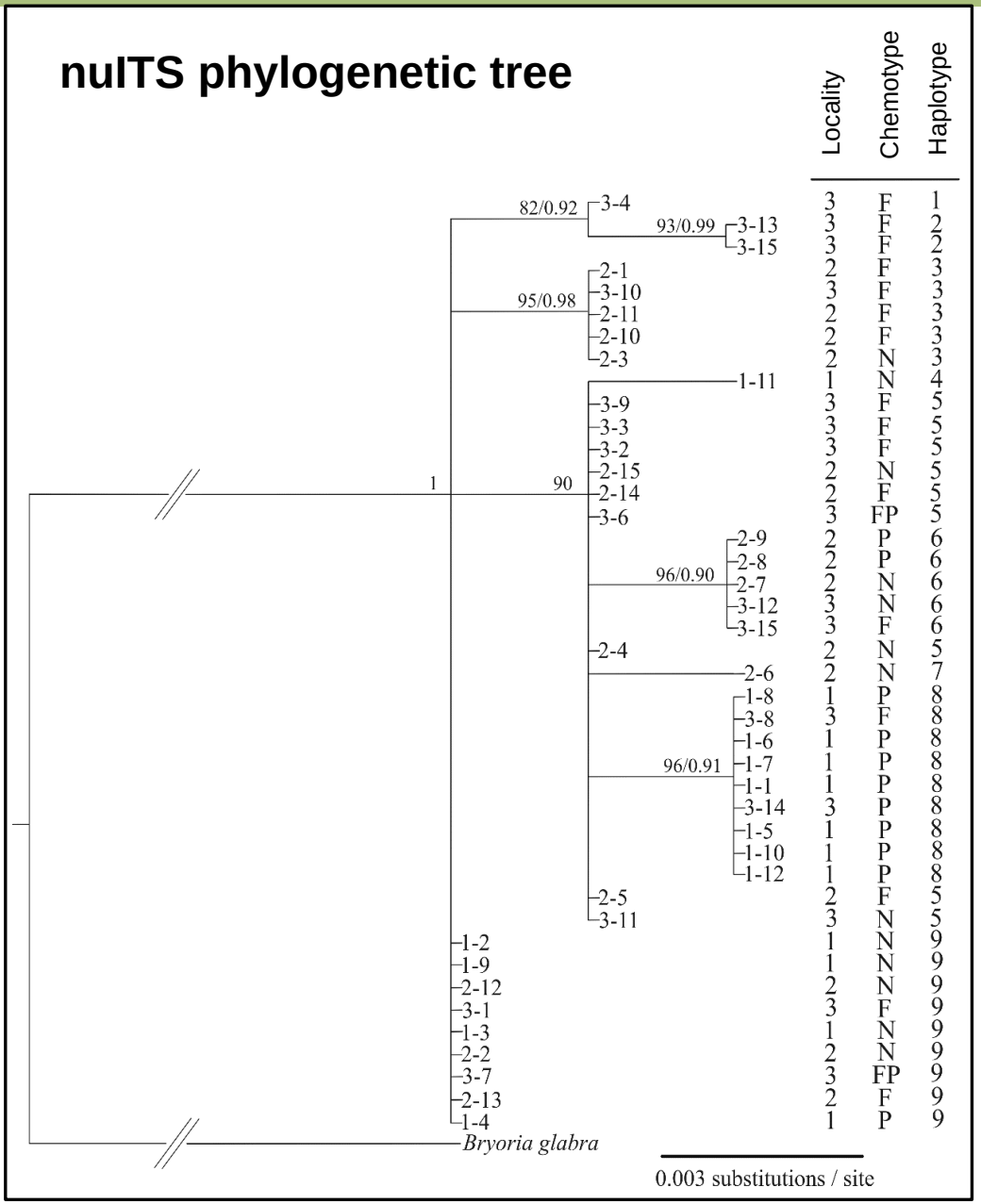
Each specimen was divided into four regions:

- **Base**
- **Branches (without soralia)**
- **Soralia**
- **Tips**

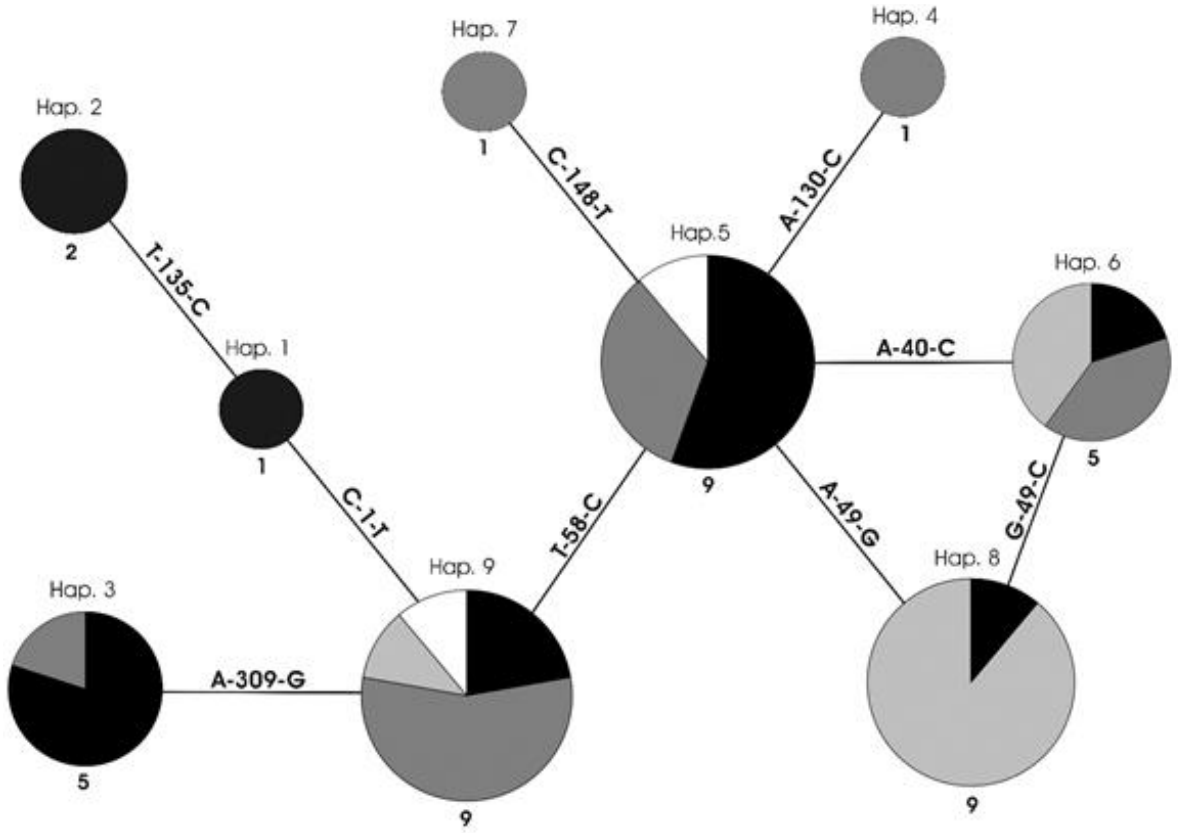


Chapter 3 *Bryoria fuscescens* mismatch

nuITS phylogenetic tree



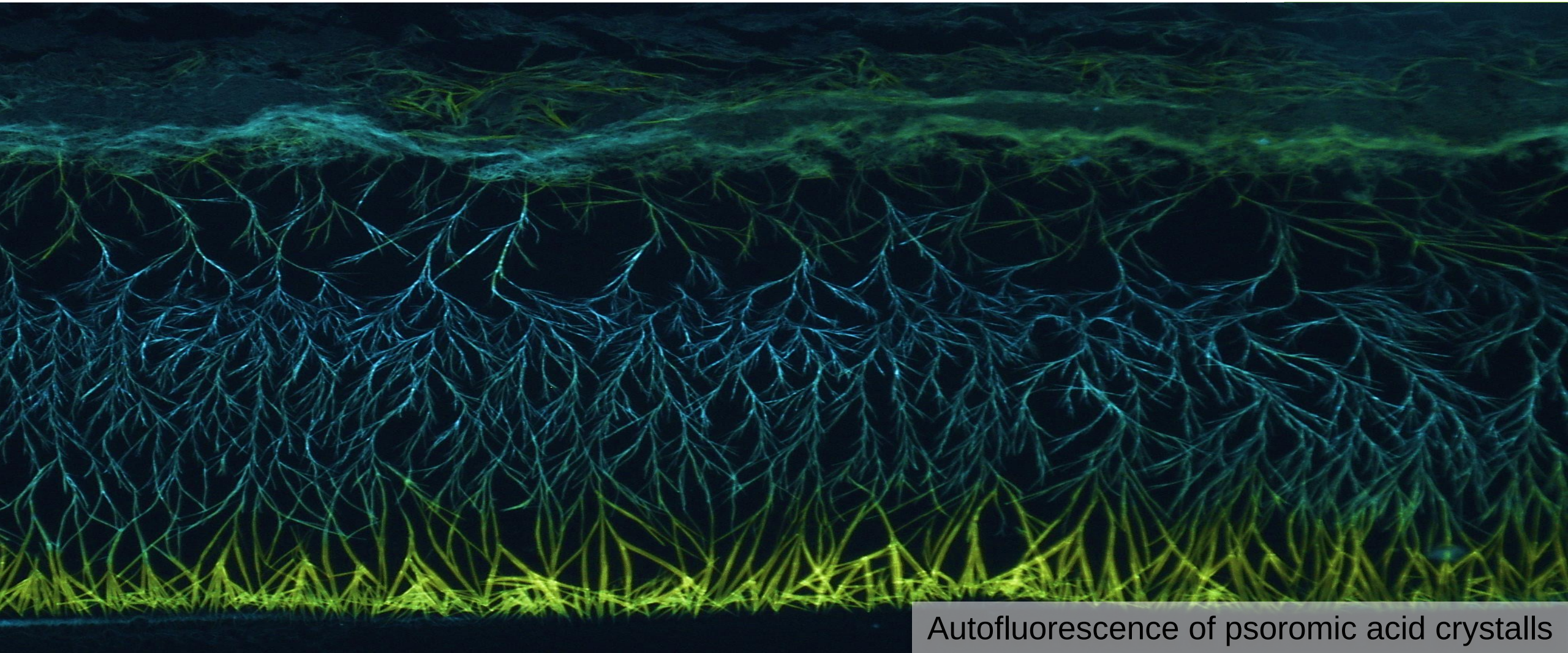
nuITS Haplotype network showing the chemotypes



	Specimen1	Specimen2
Base	Fum	Fum
Branch	Fum	Fum-Pso
Tips	Fum	Fum-Pso
Soralia	Fum-Pso	Fum-Pso

Chapter 4

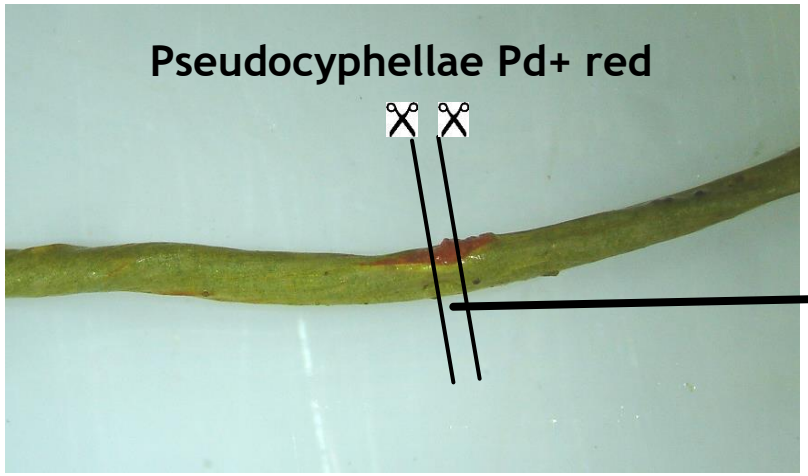
Fluorescence microscopy as a tool for the visualization of lichen substances within *Bryoria* thalli

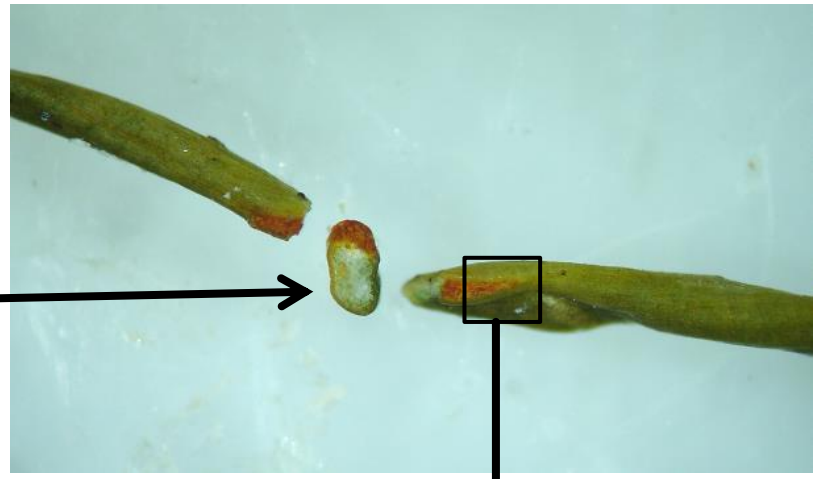


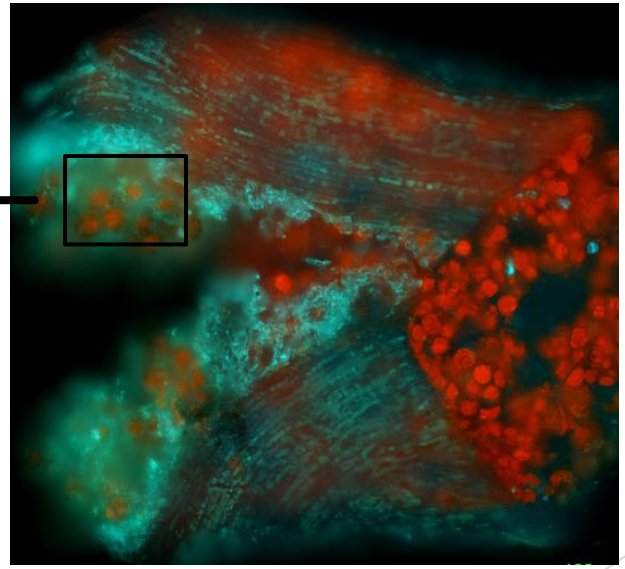
Autofluorescence of psoromic acid crystalls

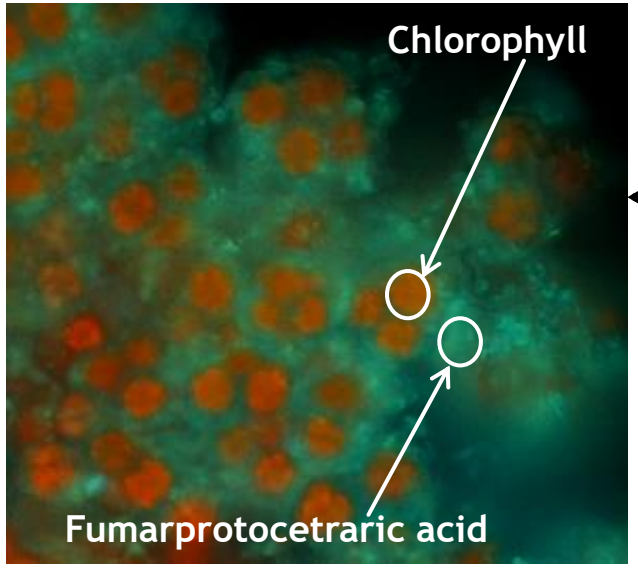
Chapter 4 Fluorescence microscopy

Fumarprotocetraric acid

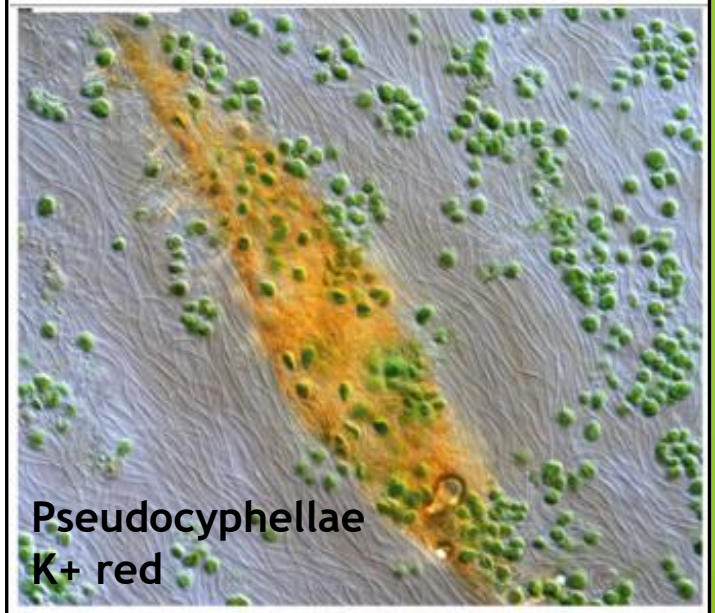


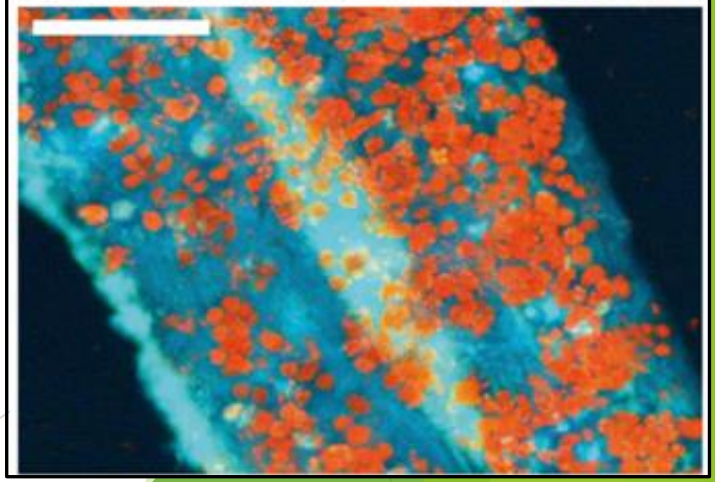






Norstictic acid



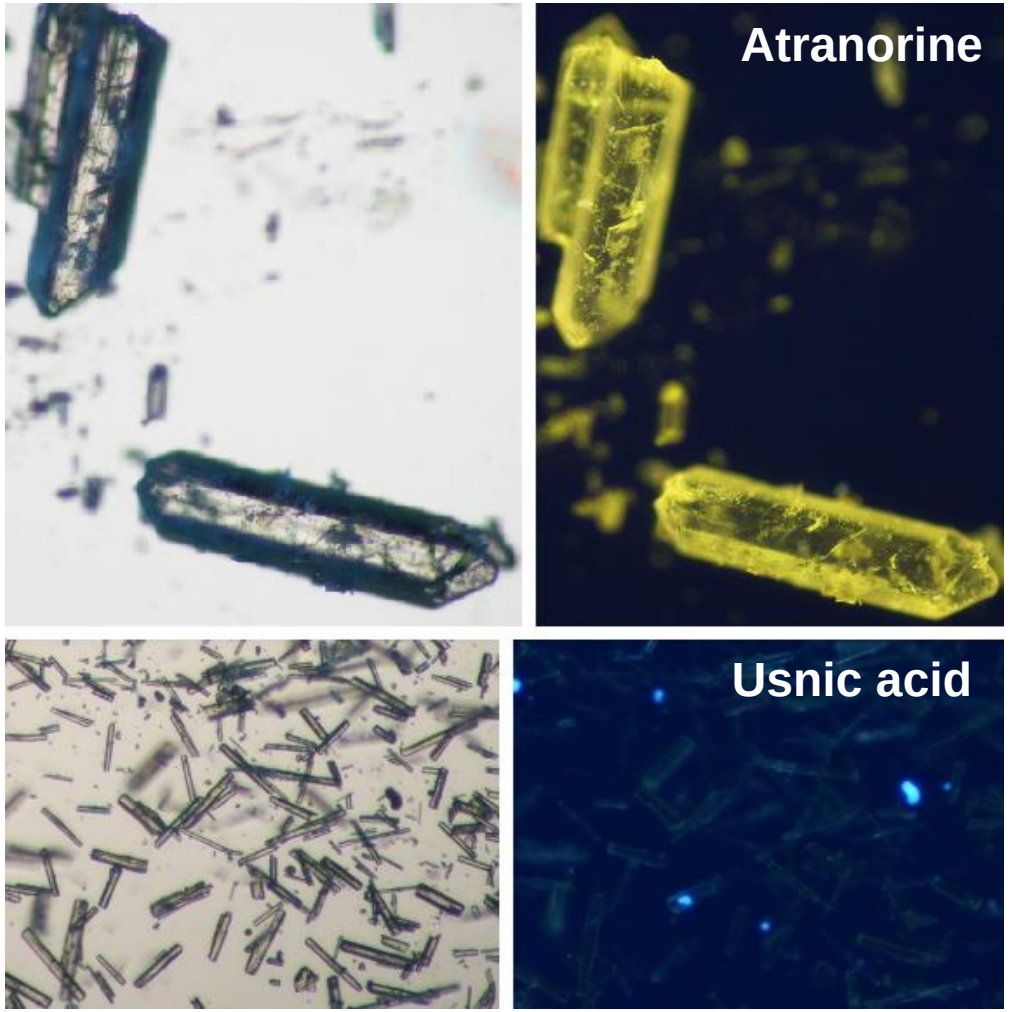


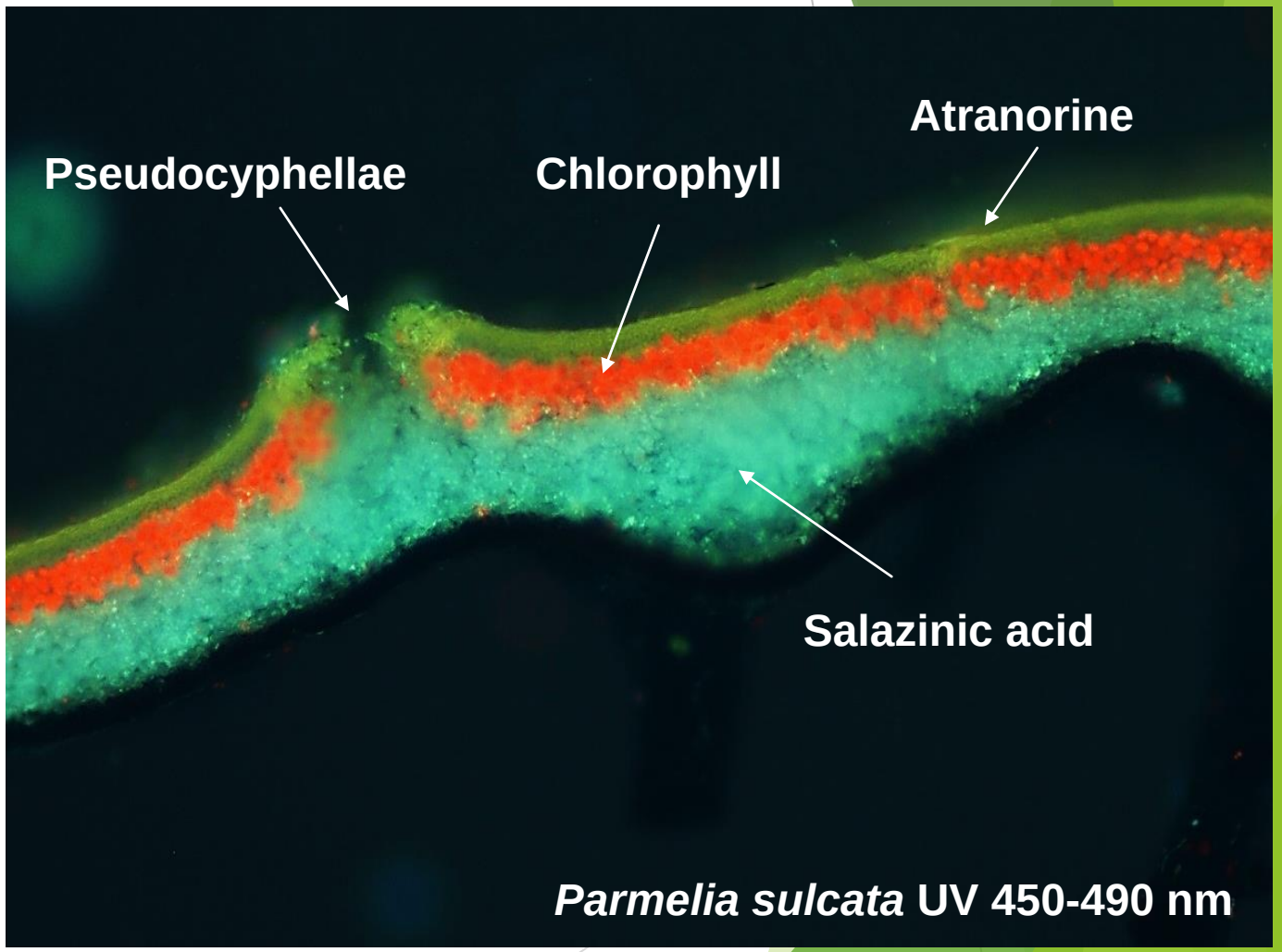
UV 450-490 nm

Chapter 4 Fluorescence microscopy

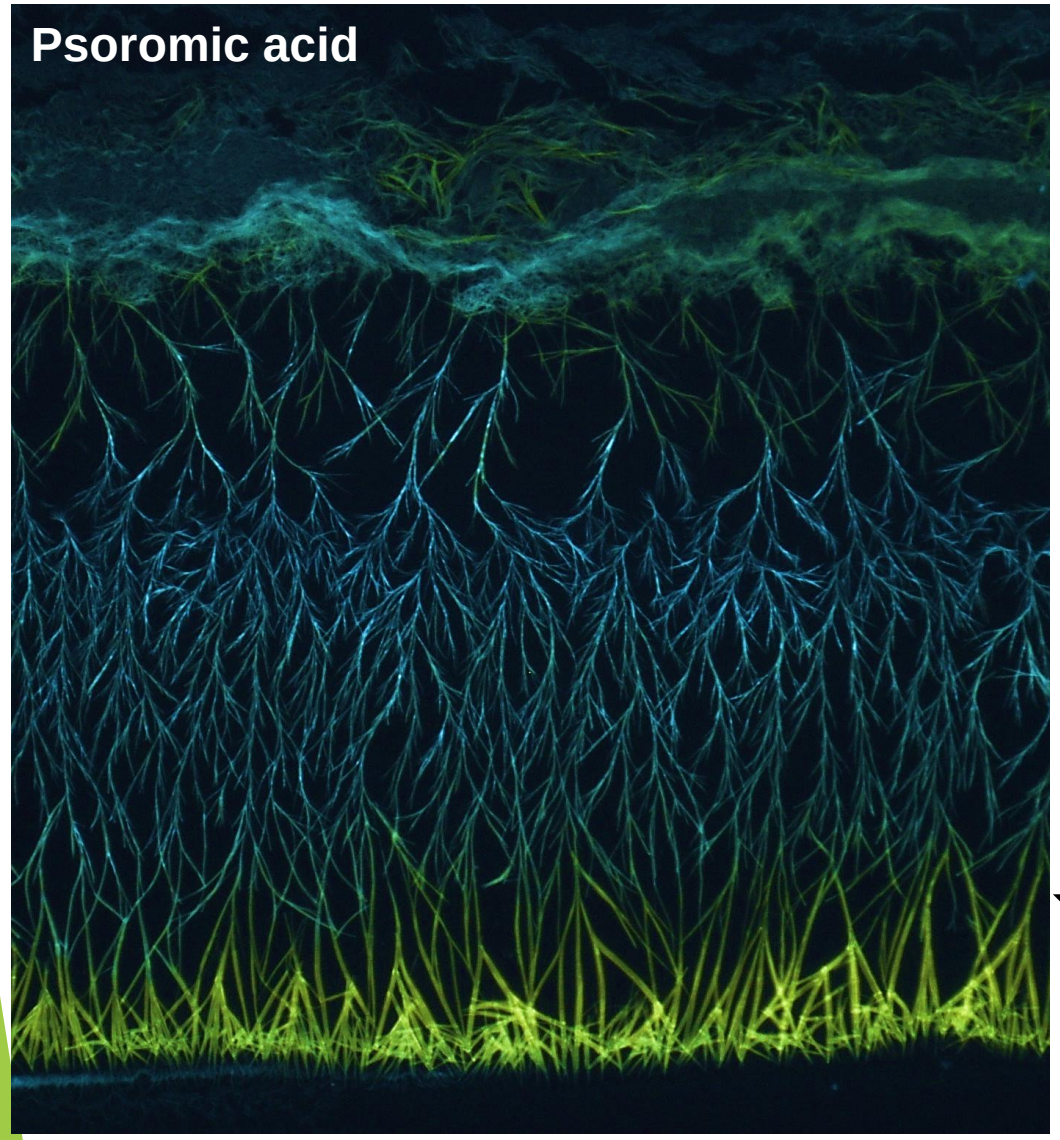
White light

UV 450-490 nm





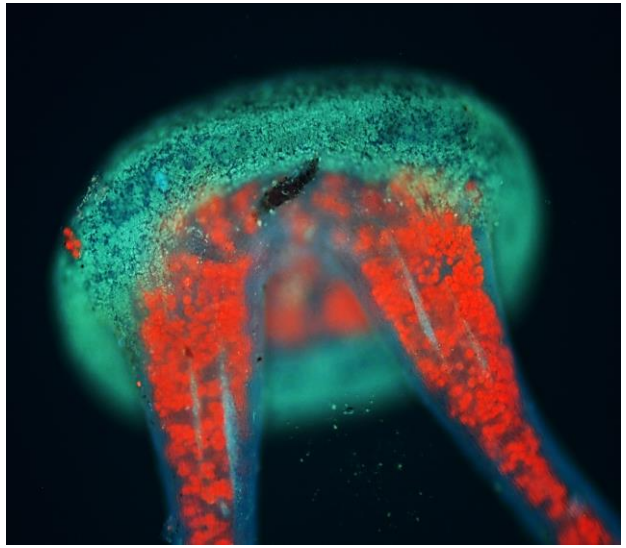
Chapter 4 Fluorescence microscopy



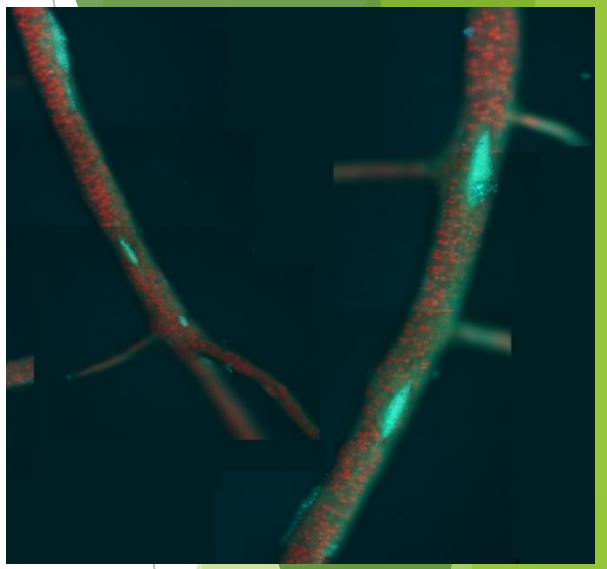
Fast crystallization

Crystallization direction

Slow crystallization



Apothecia of *Bryoria capillaris*

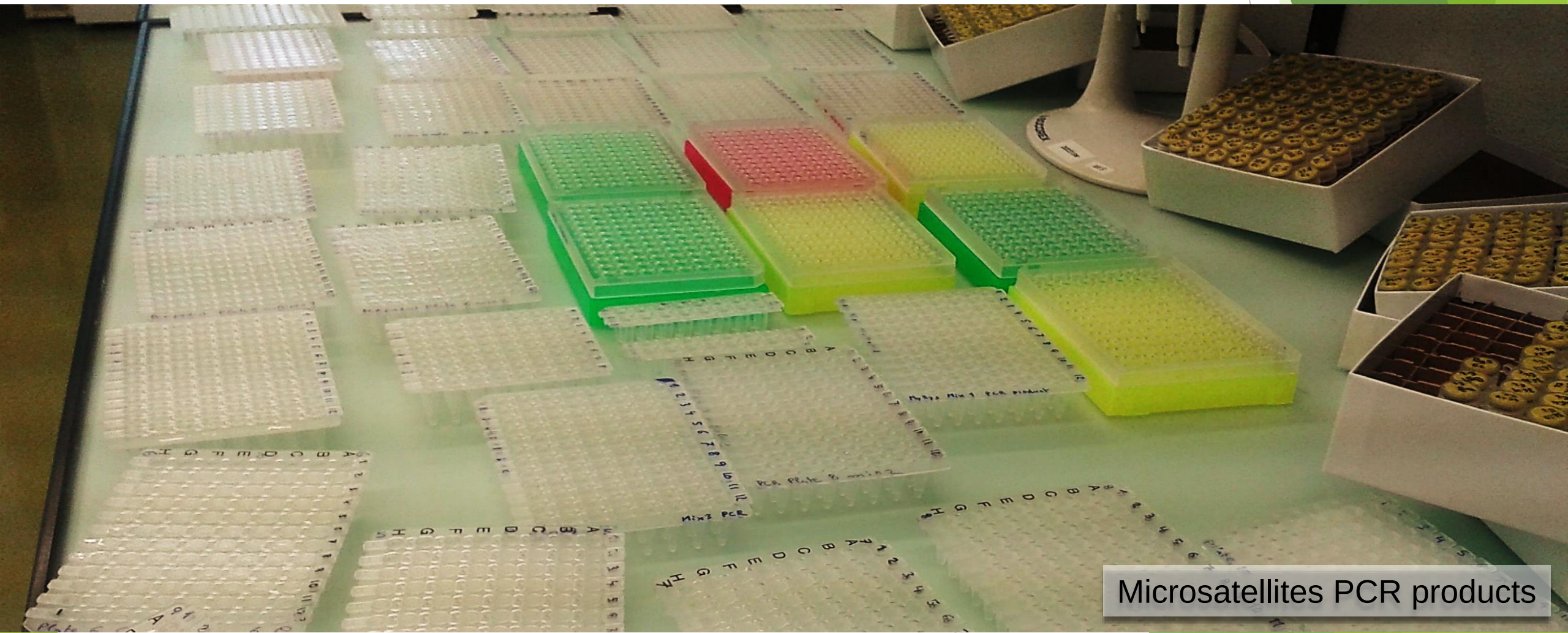


Pseudocyphellae of *Bryoria bicolor* (no extrolites)

Fluorescence microscopy is a good tool to locate and sometimes identify extrolites inside the lichen thallus

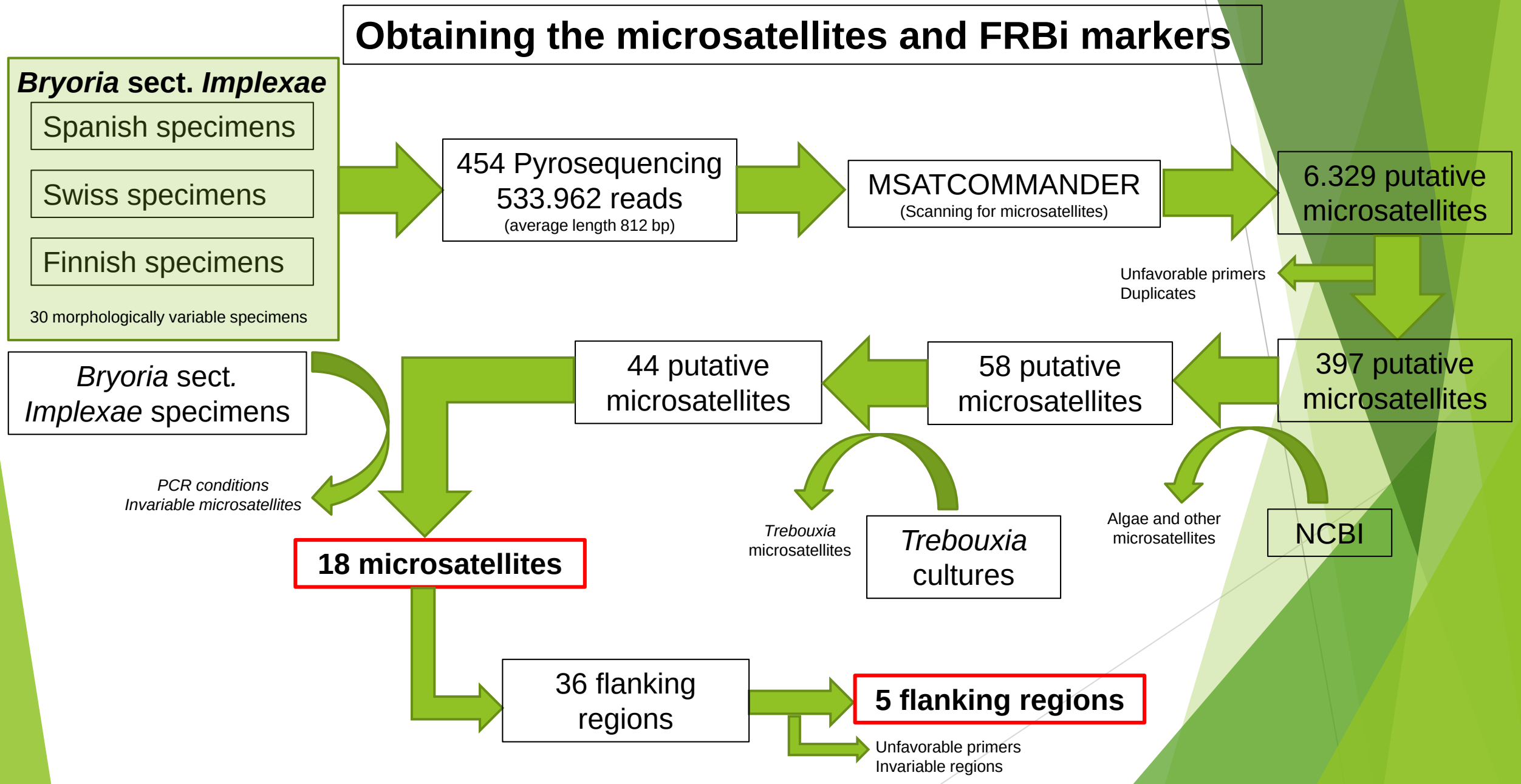
Chapter 5

Characterization of microsatellite loci in lichen-forming fungi of *Bryoria* section *Implexae*



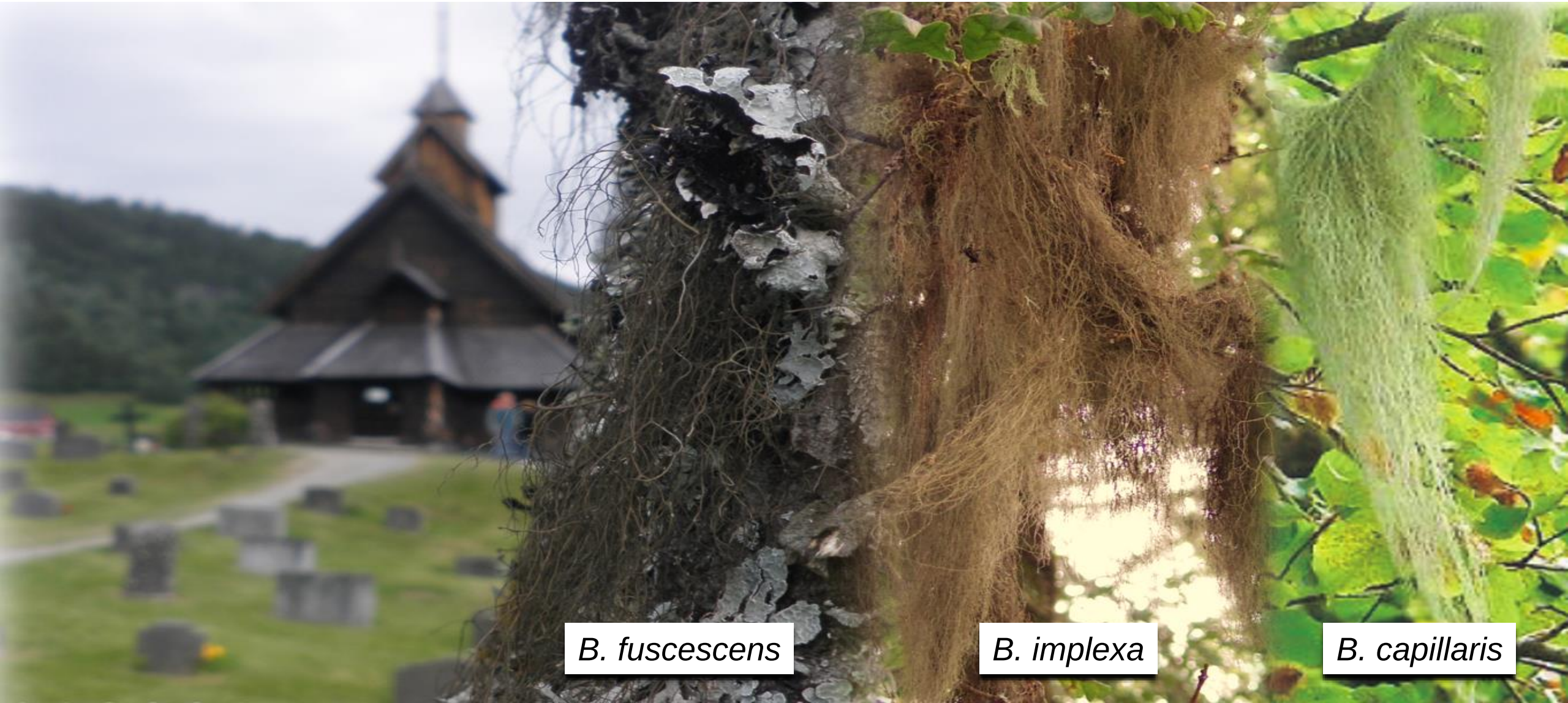
Microsatellites PCR products

Chapter 5 Mirosatellites development

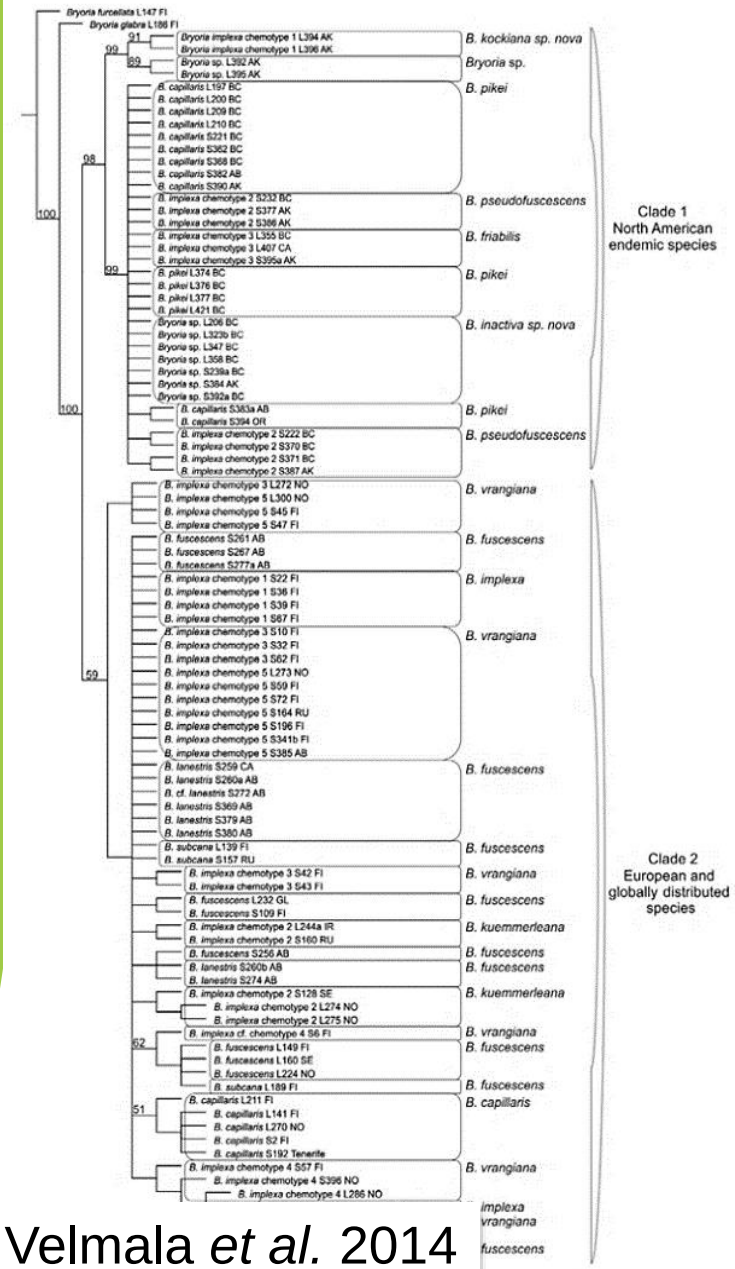


Chapter 6

Towards an integrative taxonomy of *Bryoria* sect. *Implexae*



Chapter 6 *Bryoria* sect. *Implexae* taxonomy



Velmala *et al.* 2014

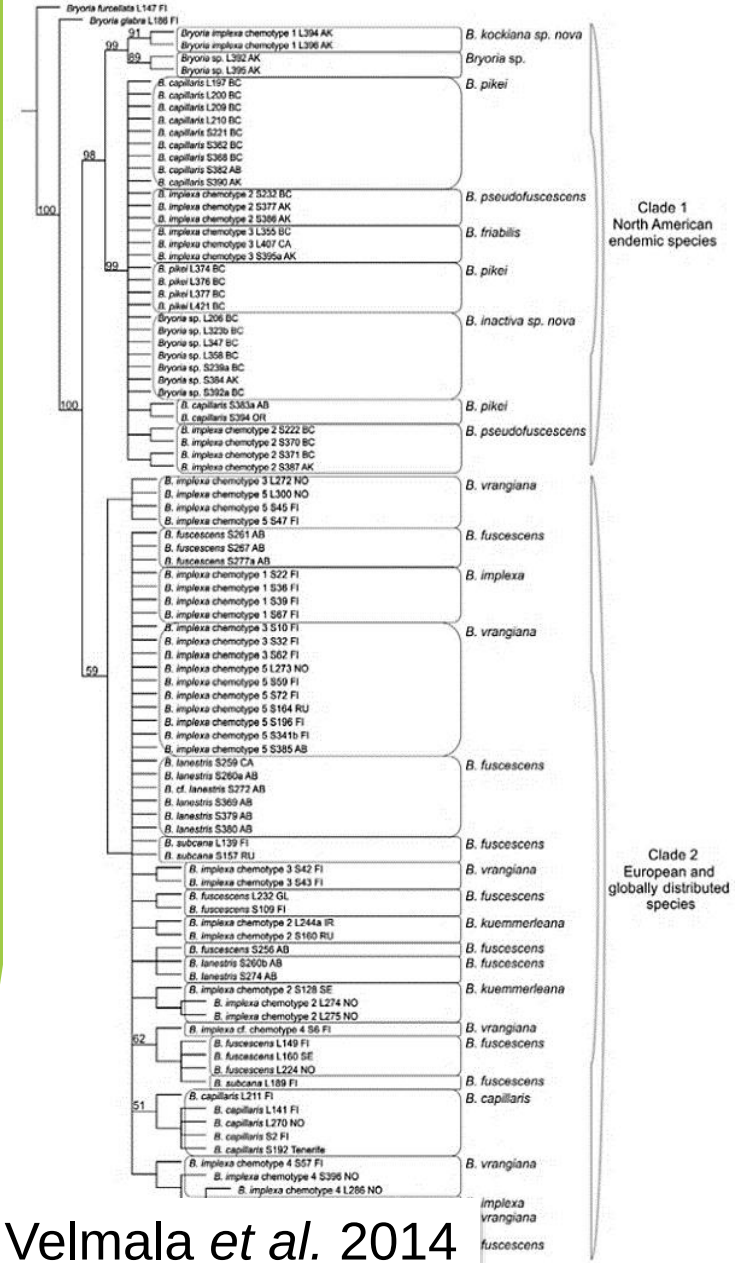
Main key characters:

- Extrolites
- Soralia
- Pseudocyphellae
- Thallus colour
- Branching angles

Tabla 2. Sustancias químicas diagnóstico que junto con carácteres adicionales permiten distinguir las especies de *Bryoria* sect. *Implexae*. Los carácteres adicionales incluyen la coloración del talo, los ángulos de ramificación, las características de los soralios y pseudocifelas y la distribución.

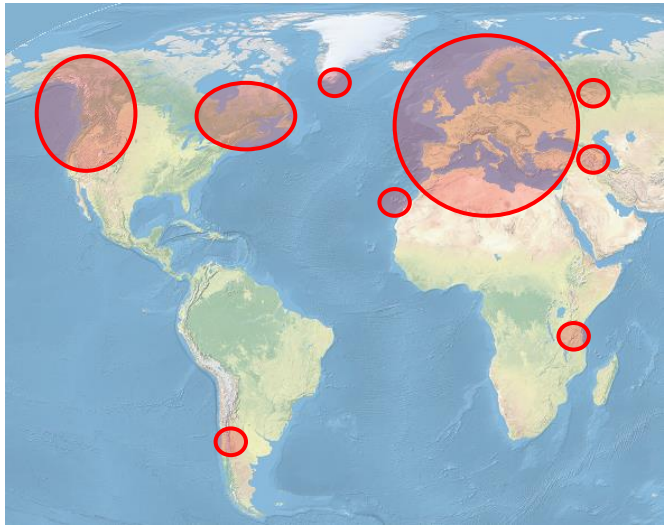
Especie	Sustancia diagnóstico
~~***Bryoria austromontana***~~	Ácido fumarprotocetrárico
B. capillaris	Ácido barbatólico
B. friabilis	Ácido girofórico
B. fuscescens	Ácido fumarprotocetrárico
B. glabra	Ácido fumarprotocetrárico
B. implexa	Ácido psorómico
B. inactiva	Sin sustancias
B. kockiana	Ácido psorómico
B. pikei	Ácido barbatólico
B. pseudofuscescens	Ácido norestíctico
~~***B. salazinica***~~	Ácido salazínico
B. vrangiana	Ácido fumarprotocetrárico

Chapter 6 *Bryoria* sect. *Implexae* taxonomy



Velmala *et al.* 2014

142 specimens from:



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

Tabla 2. Sustancias químicas diagnóstico que junto con carácteres adicionales permiten distinguir las especies de *Bryoria* sect. *Implexae*. Los carácteres adicionales incluyen la coloración del talo, los ángulos de ramificación, las características de los soralios y pseudocifelas y la distribución.

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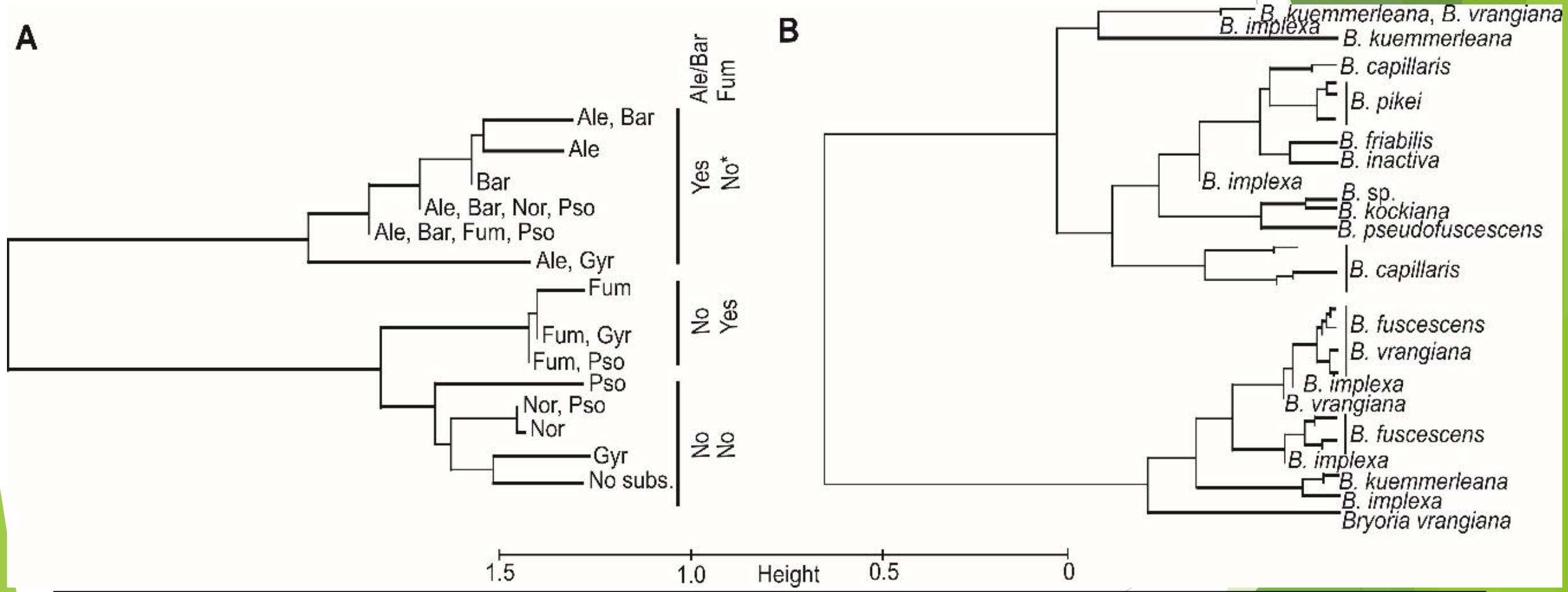
B. inactiva	Sin sustancias
B. kockiana	Ácido psorómico
B. pikei	Ácido barbatólico
B. pseudofuscescens	Ácido norestíctico
~~***B. salazinica***~~	Ácido salazínico
B. vrangiana	Ácido fumarprotocetrárico

Chapter 6 *Bryoria* sect. *Implexae* taxonomy

Phenetic analyses

Extrolites

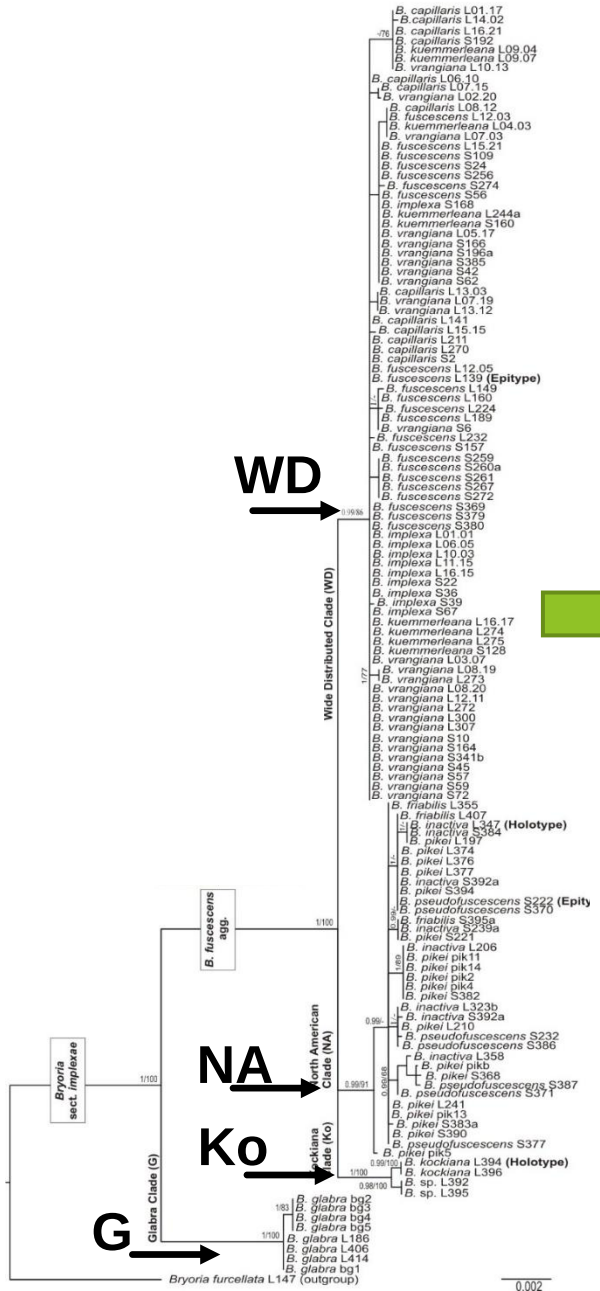
Extrolites + morphology

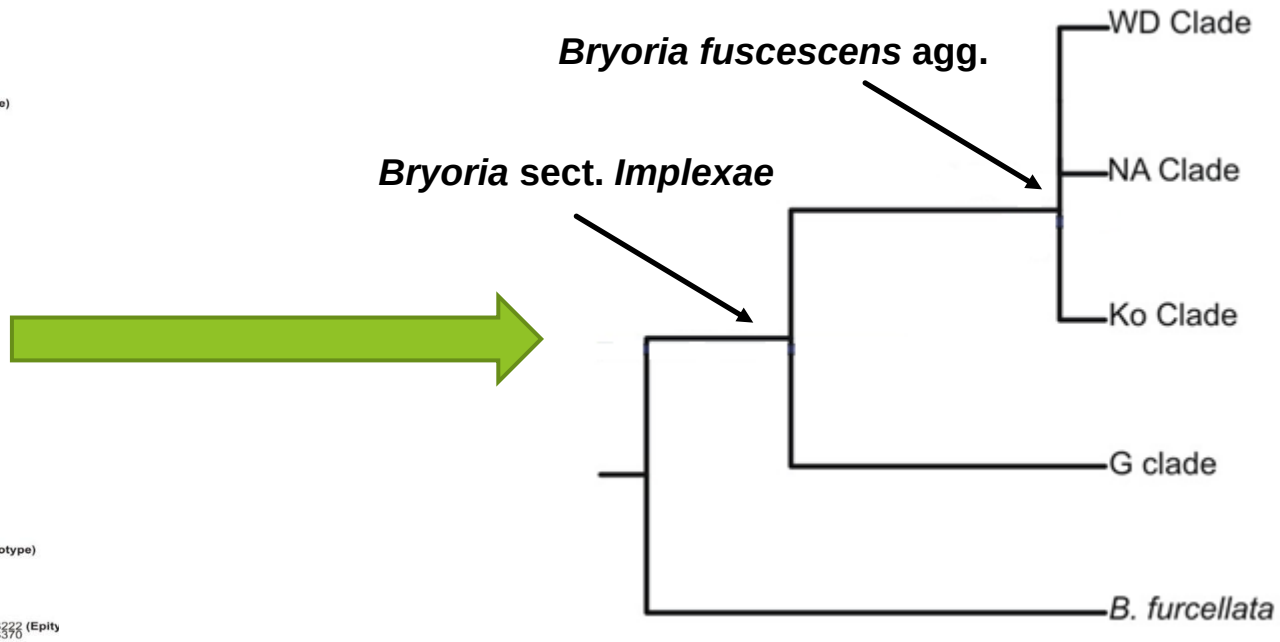


The species concept in *Bryoria* sect. *Implexae*, established mainly using septentrional specimens, break down when meridional specimens are studied.

Chapter 6 *Bryoria* sect. *Implexae* taxonomy

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

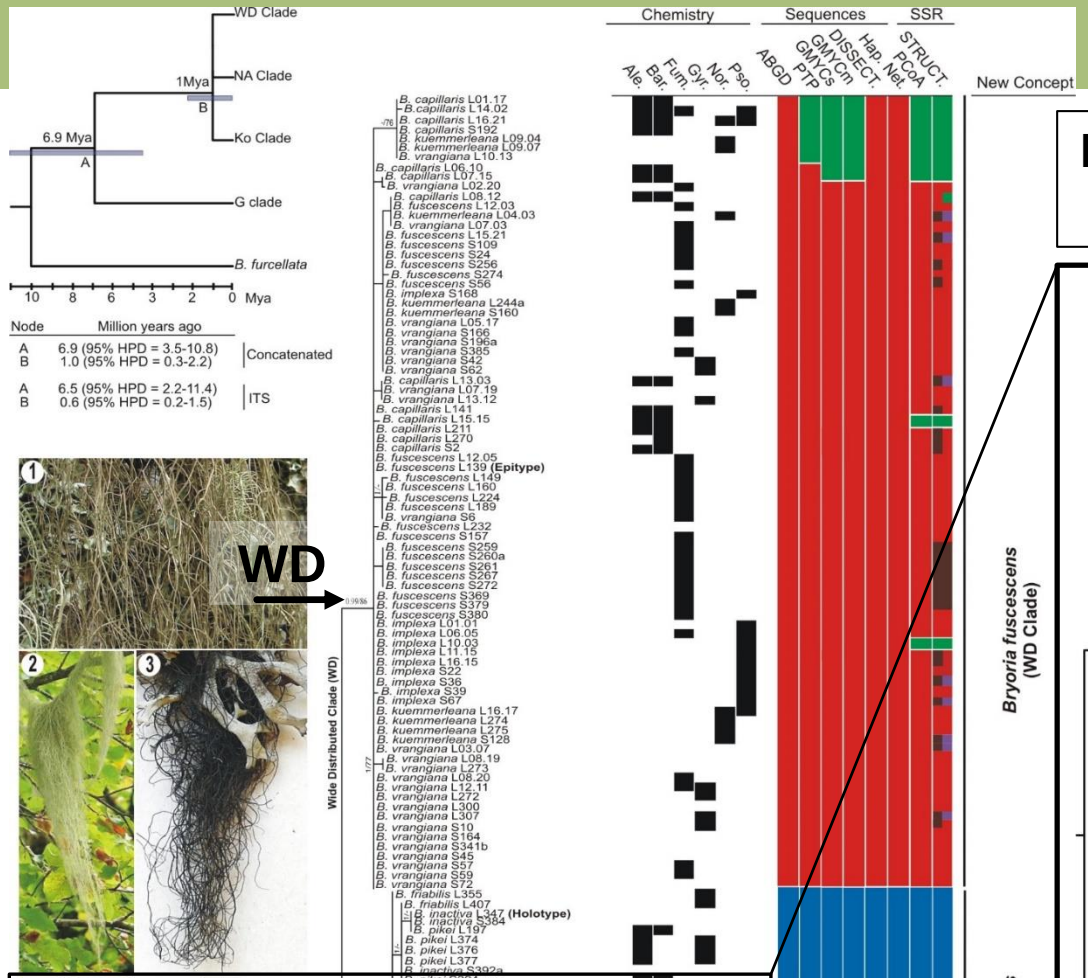


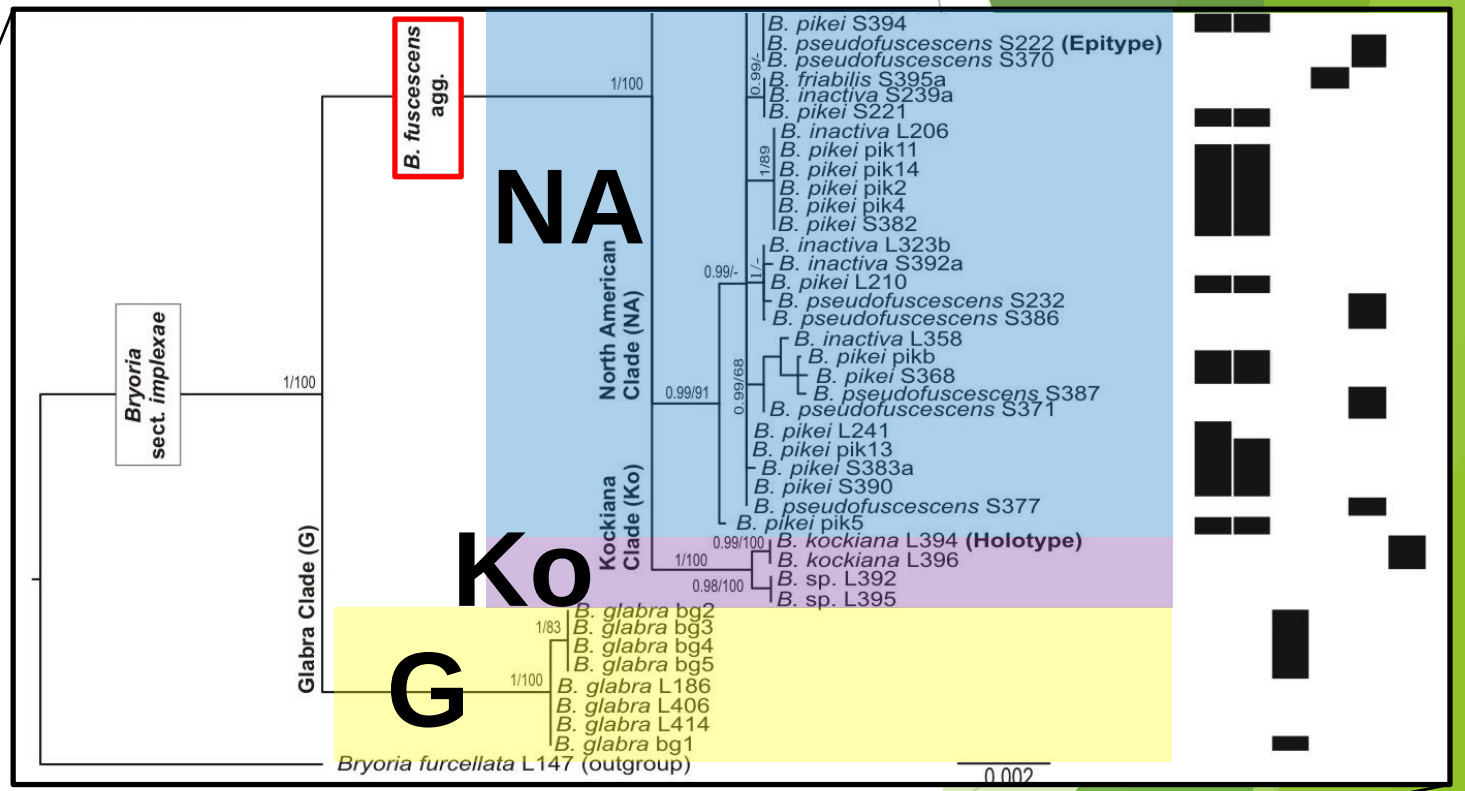


Tree backbone well supported

Chapter 6

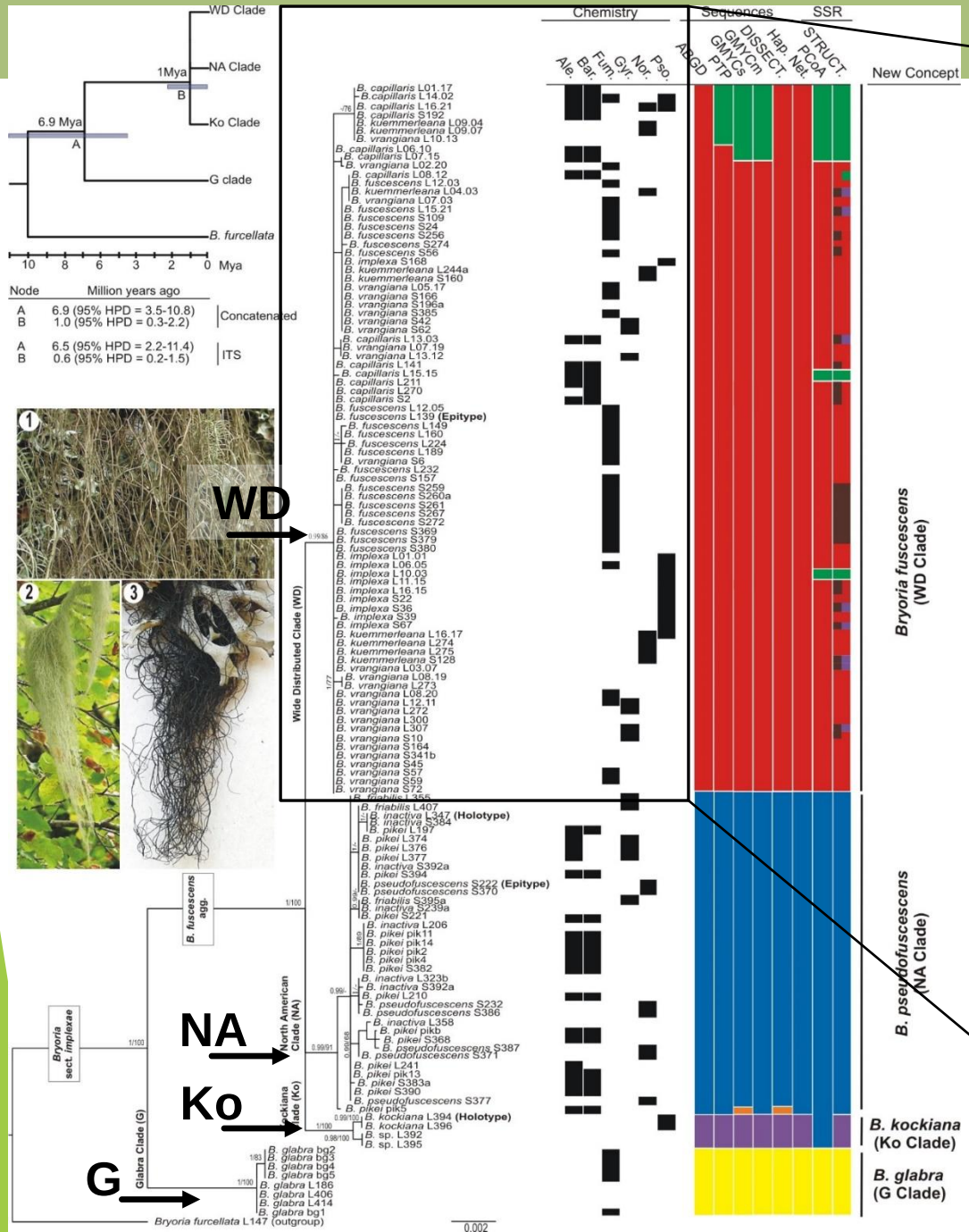
ML and Bayesian tree form the concatenated matrix (ITS, IGS & GAPDH)

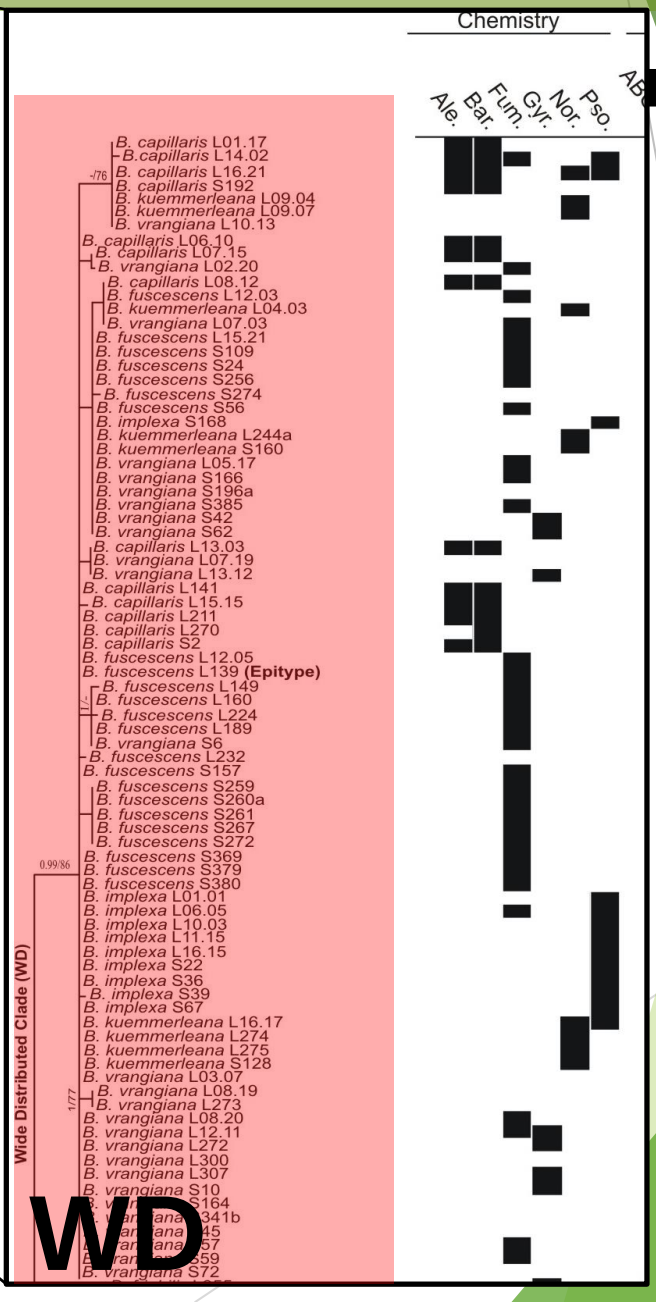




Tree backbone well supported

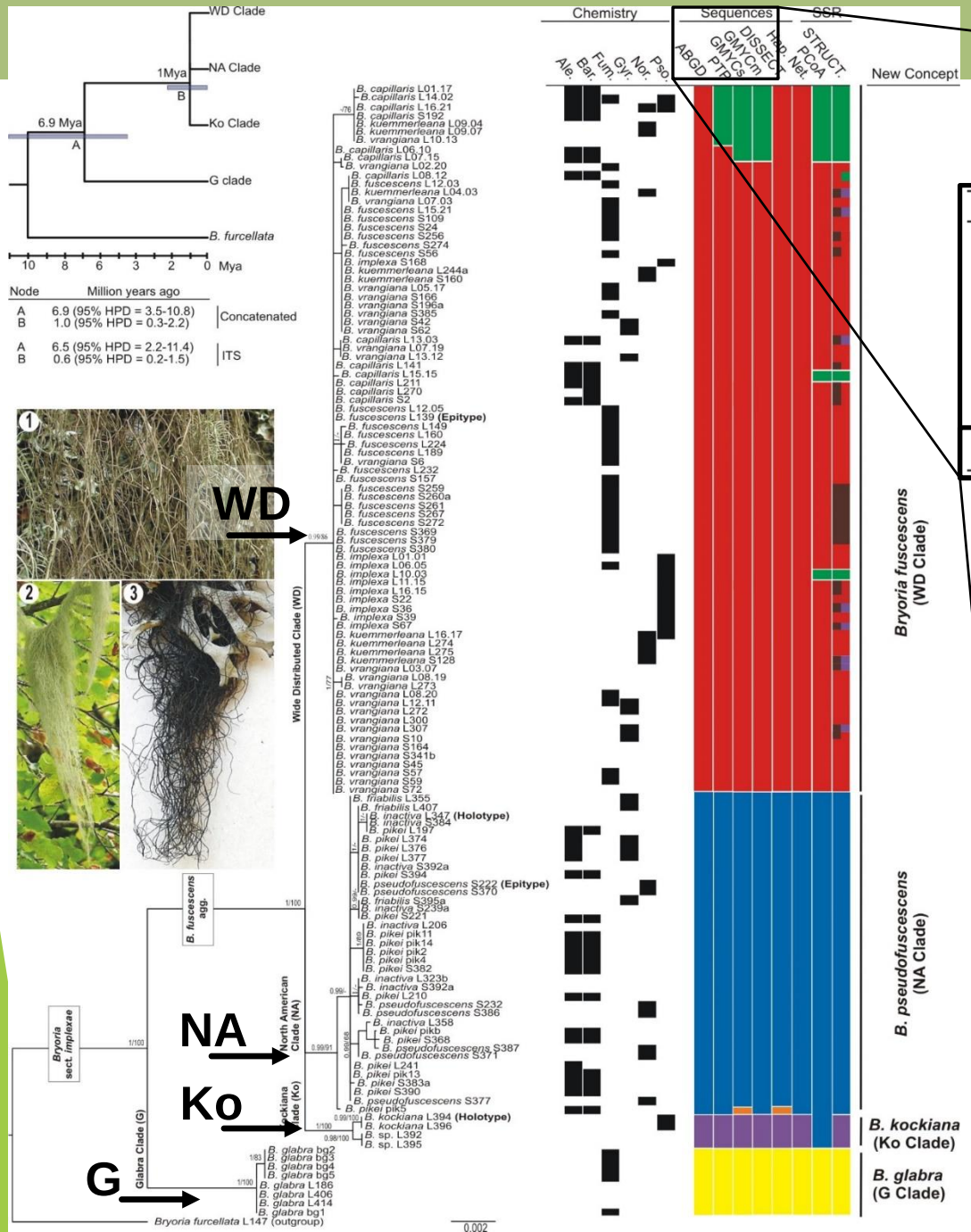
Chapter 6



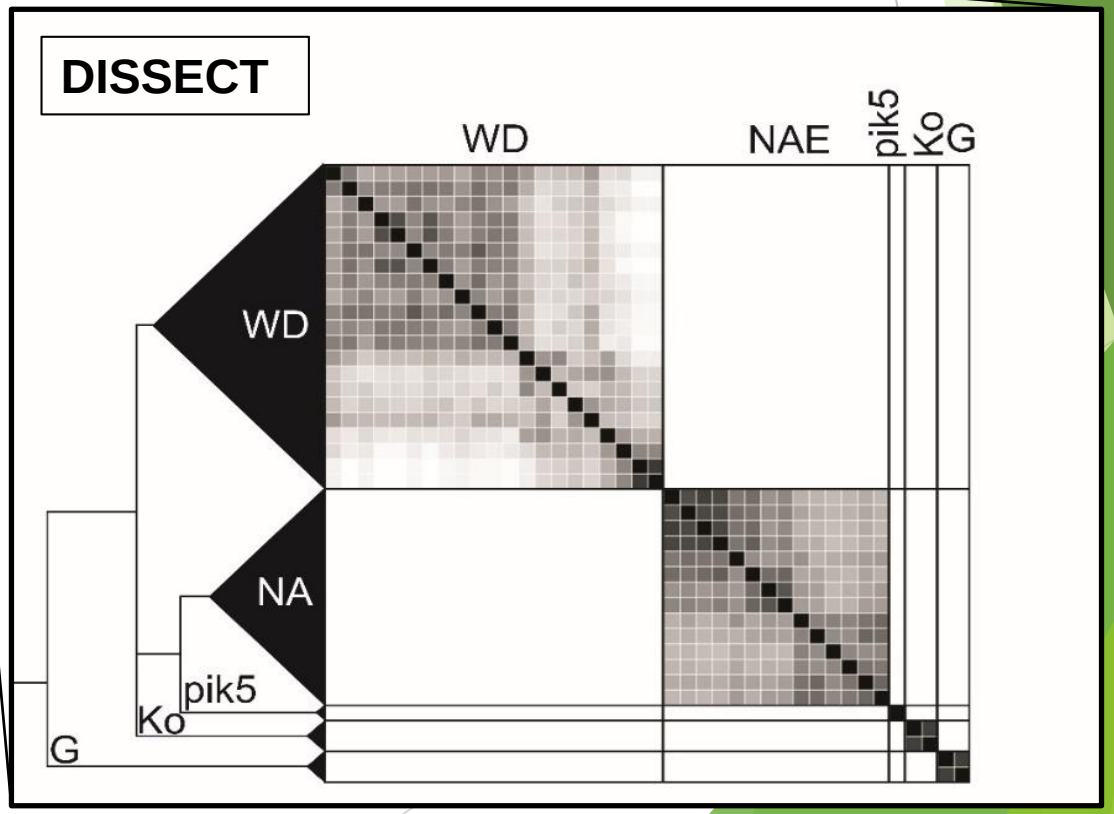


Extrolite composition

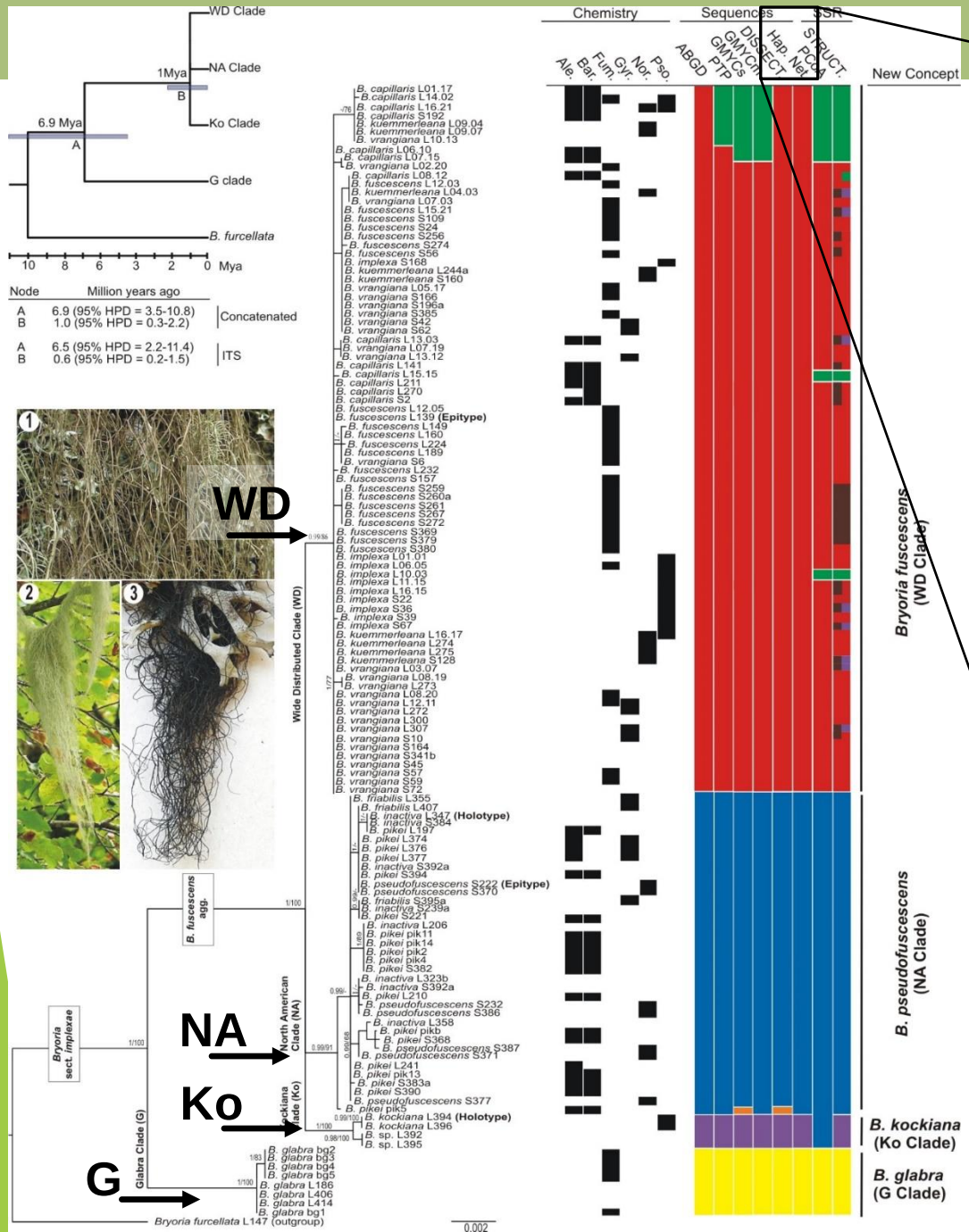
Chapter 6

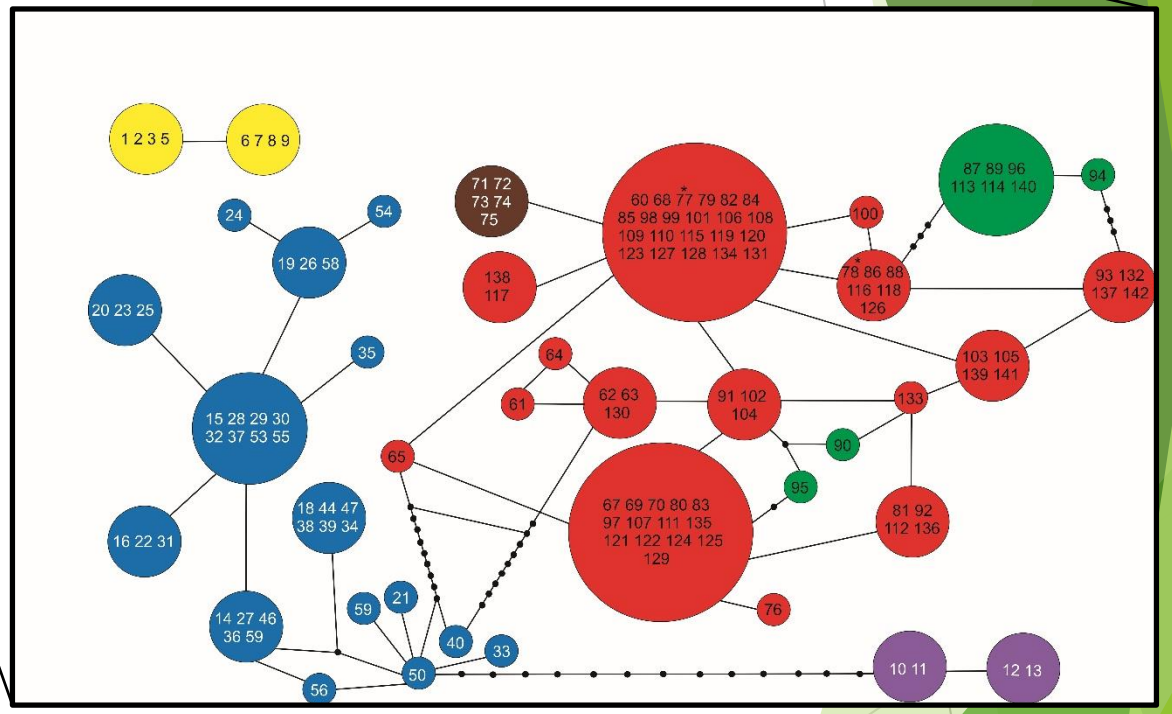


Method	ITS	IGS	GAPDH	Concatenated
ABGD	2 = G + (Ko, NA, WD)	2 = G + (Ko, NA, WD)	4 = G + Ko + NA + WD	4 = G + Ko + NA + WD
PTP	2 = G + (Ko, NA, WD)	2 = G + (Ko, NA, WD)	4 = G + Ko + NA + WD	5 = G + Ko + NA + WDr + WDg
GMYCs	4 = G + (Ko, NA, WDg) + WDr + WDr	3 = G + (Ko, WD) + NA	4 = G + Ko + NA + WD	6 = G + Ko + NA + pik5 + WDr + WDg
GMYCm	4 = G + (Ko, NA, WDg) + WDr + WDr	4 = G + (Ko, WD) + NA1 + NA2	4 = G + Ko + NA + WD	5 = G + Ko + NA + WDr + WDg
DISSECT	_	_	_	5 = G + Ko + NA + pik5 + WD



Chapter 6

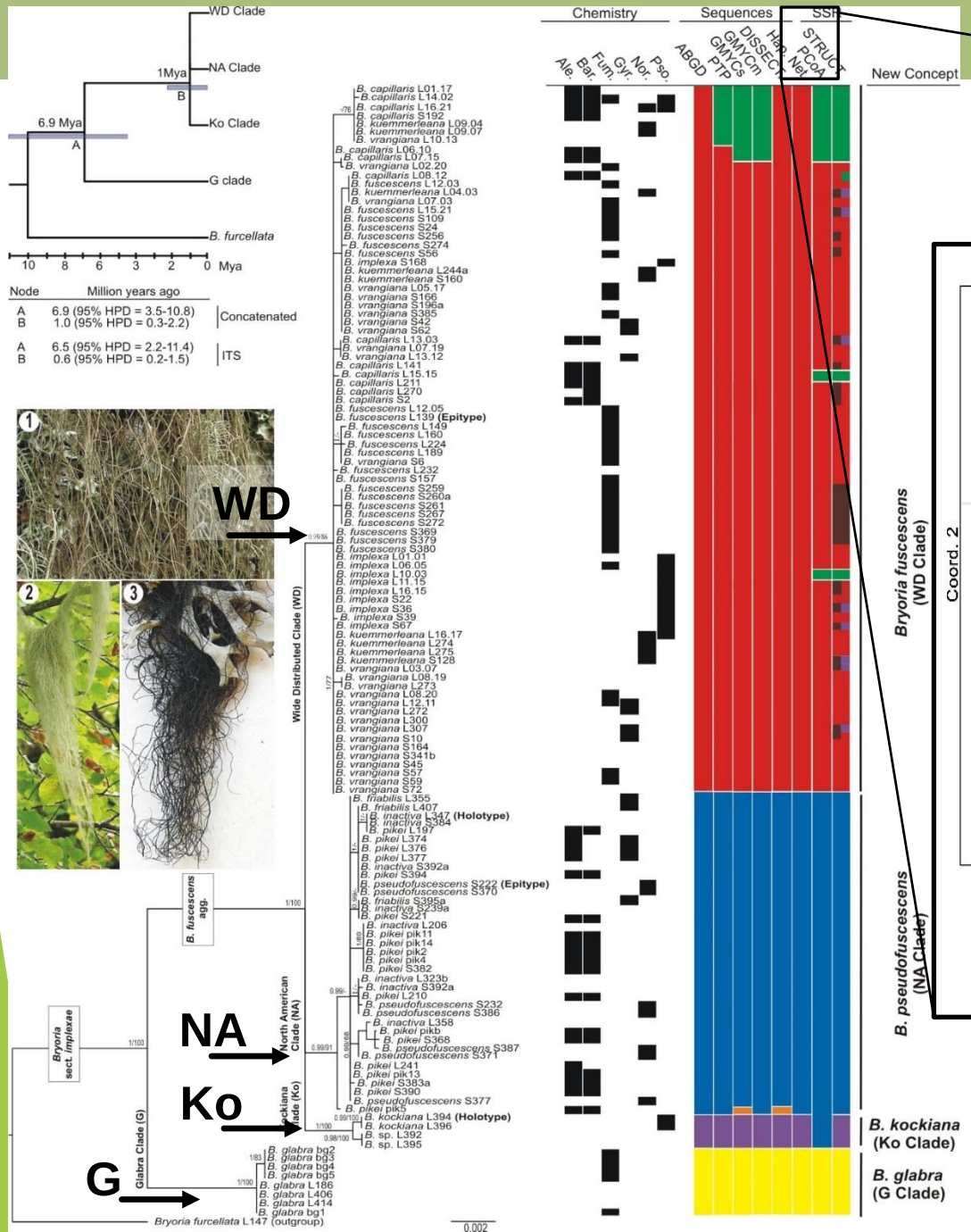


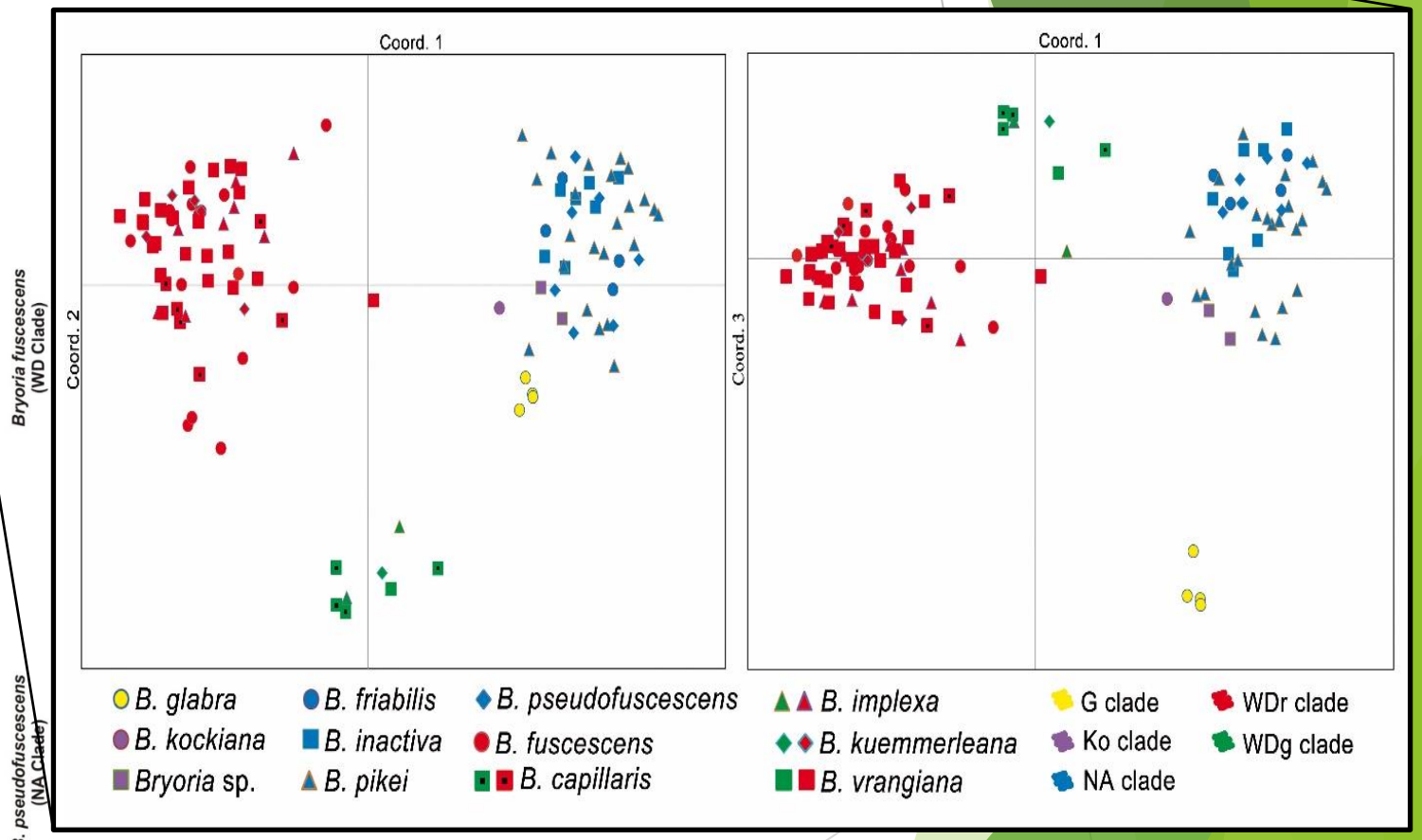


Haplotype network

gaps missing
95% connection limit

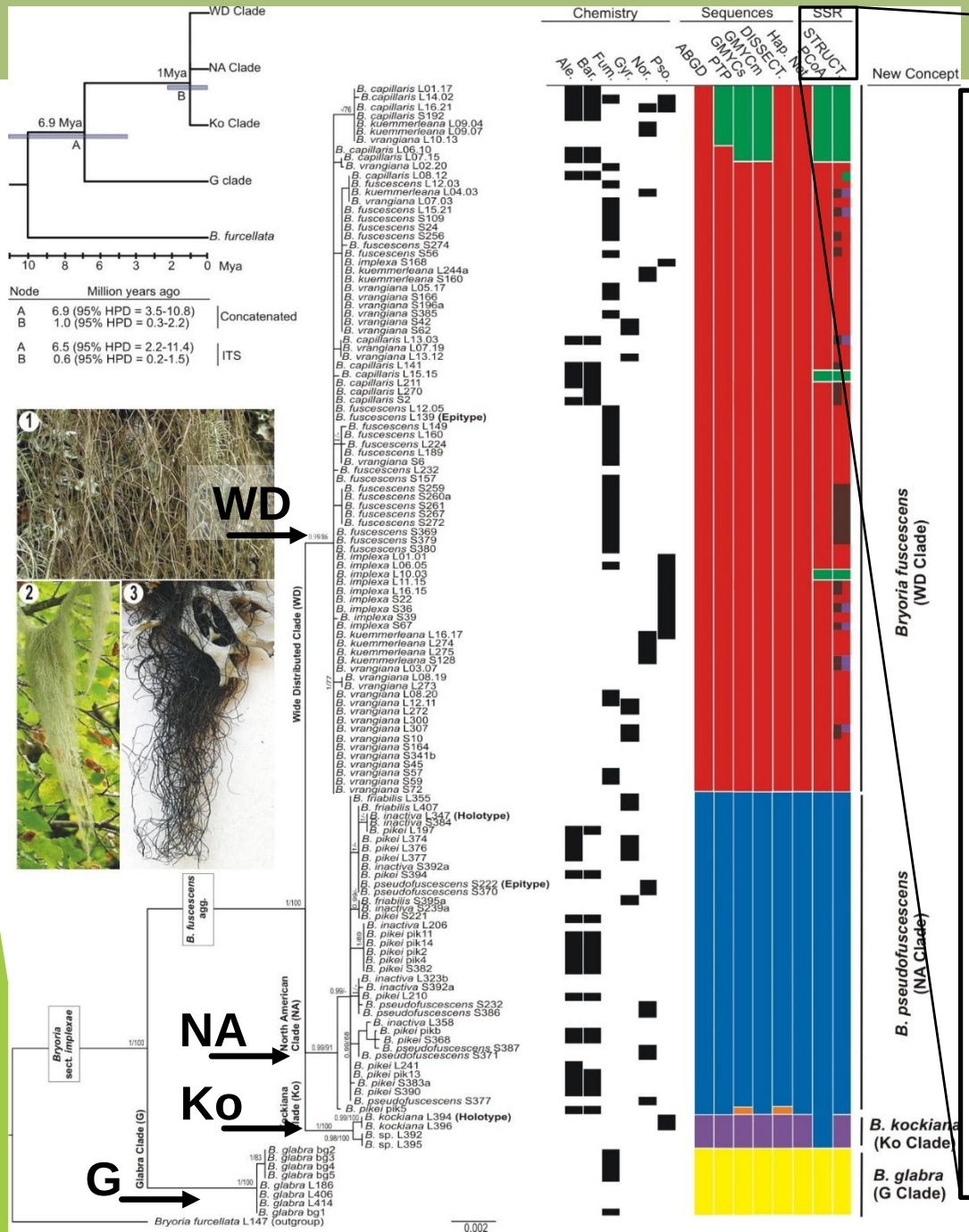
Chapter 6



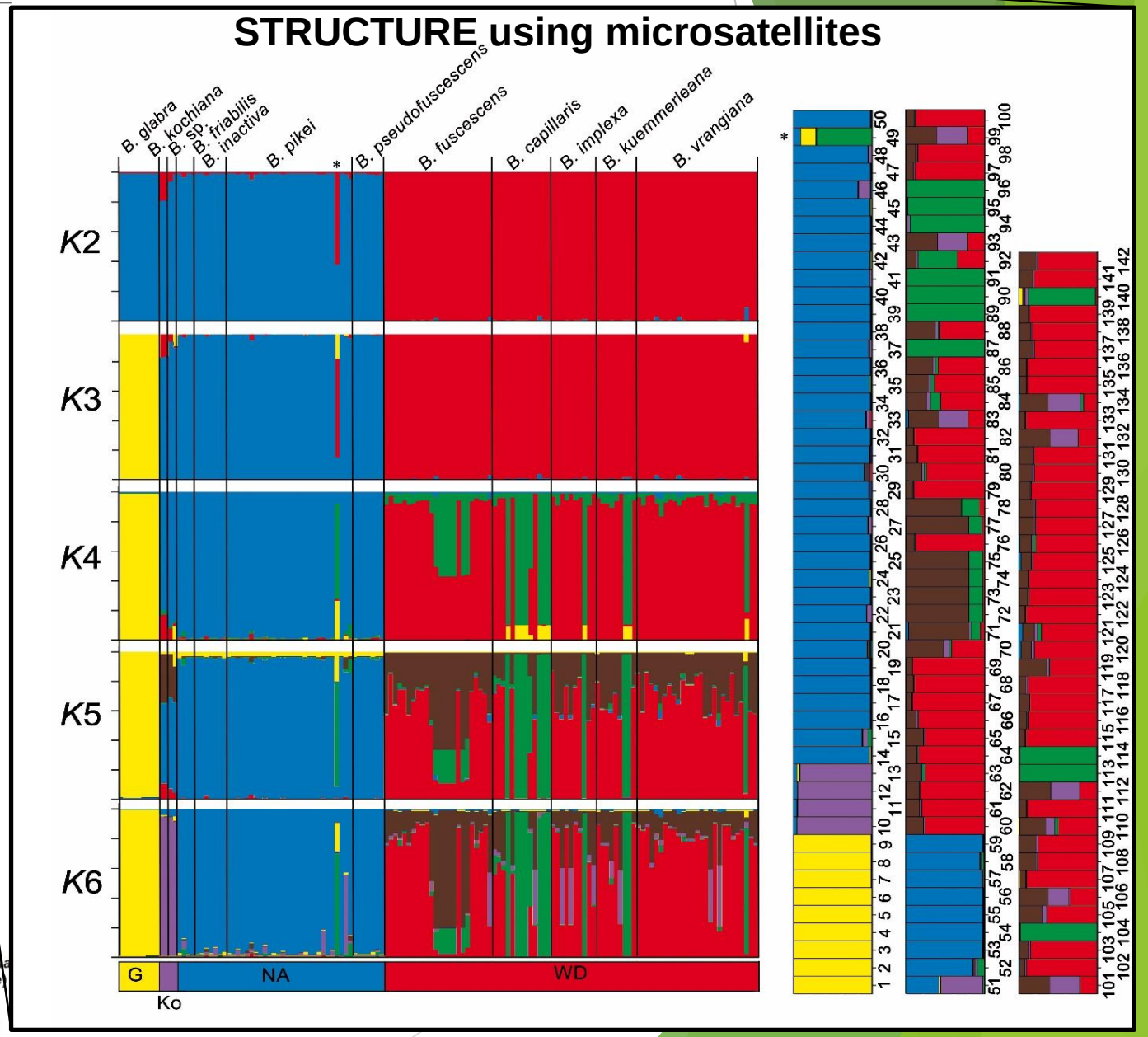


Principal coordinate analyses using microsatellites

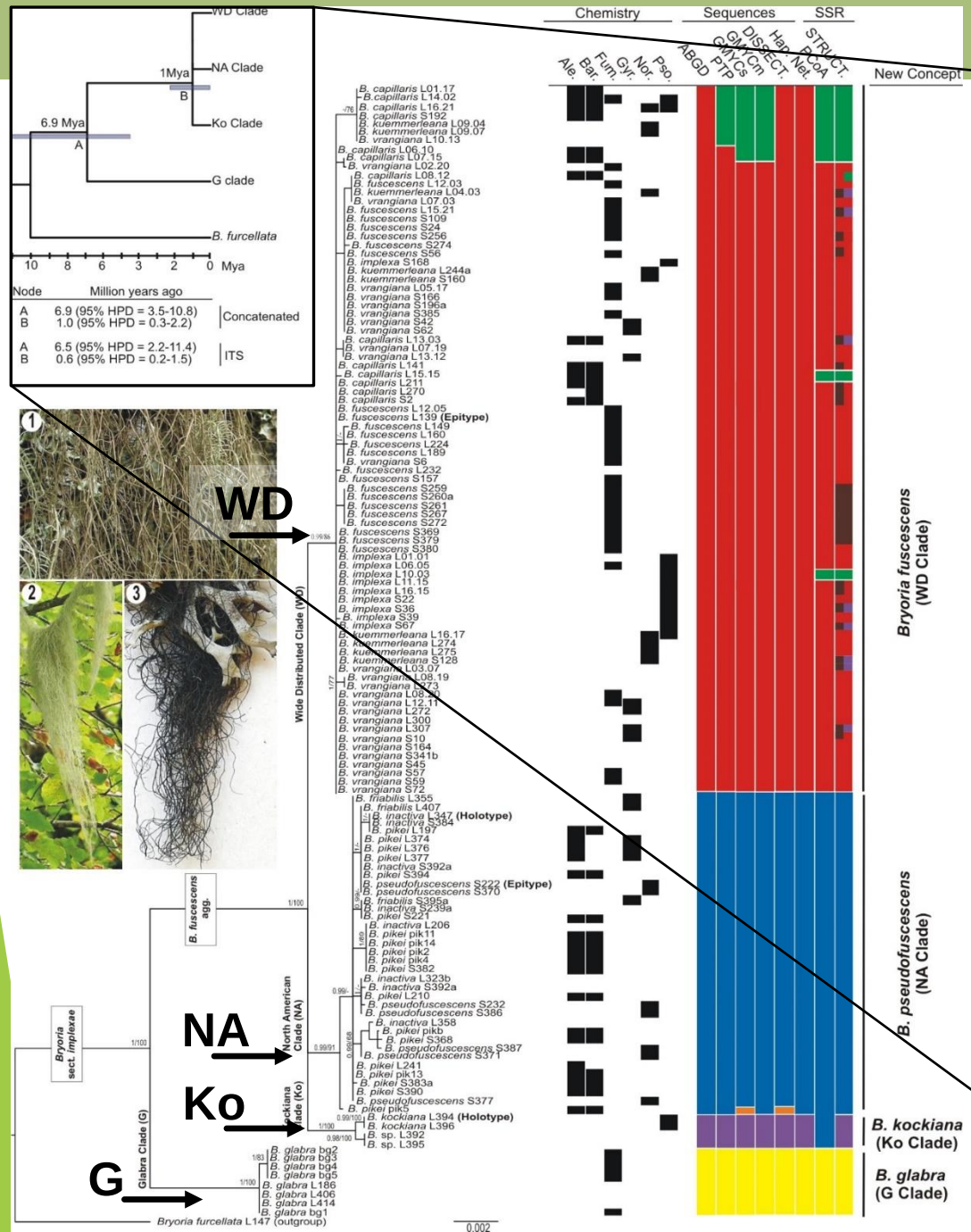
Chapter 6

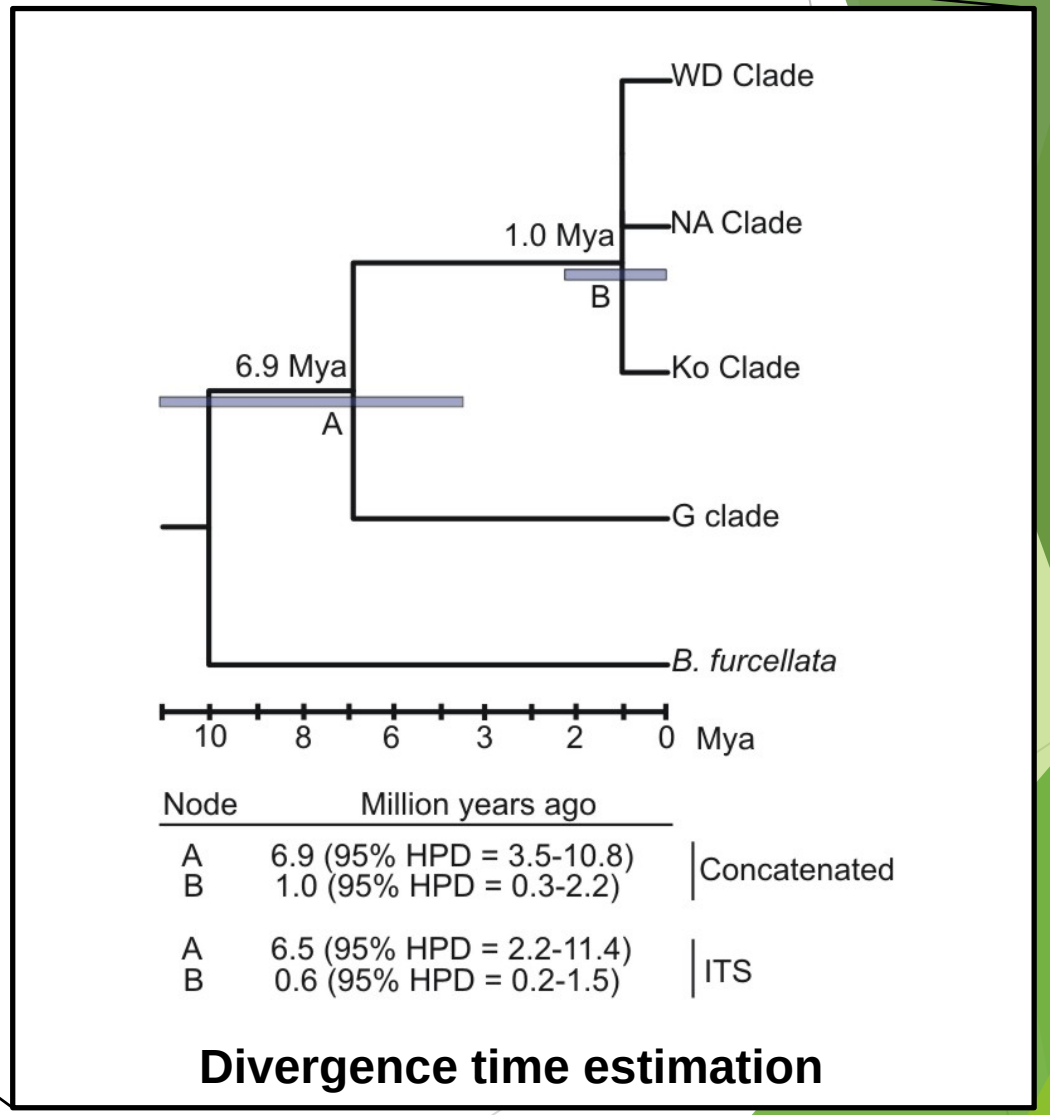

WD Clade
1Mya
NA Clade
B
6.9 Mya
Ko Clade
A
G clade
B. furcellata
10 8 6 3 2 0 Mya
Node
Million years ago
A 6.9 (95% HPD = 3.5-10.8)
B 1.0 (95% HPD = 0.3-2.2)
Concatenated
A 6.5 (95% HPD = 2.2-11.4)
B 0.6 (95% HPD = 0.2-1.5)
ITS
WD
NA
Ko
G
Wide Distributed Clade (WD)
B. fuscescens agg.
Bryoria sect. implexae
North American Clade (NA)
Kockiana Clade (Ko)
Glabra Clade (G)
Bryoria furcellata L147 (outgroup)
0.002

Chemistry
Sequences
SSR
Ale.
Bar.
Fum.
Gyr.
Nor.
Pso.
ABGD
PTP
GMYCs
GMYCm
DISSECT.
Hap.
STRUCT
PCoA
New Concept
Bryoria fuscescens (WD Clade)
B. pseudofuscescens (NA Clade)
B. kockiana (Ko Clade)
B. glabra (G Clade)

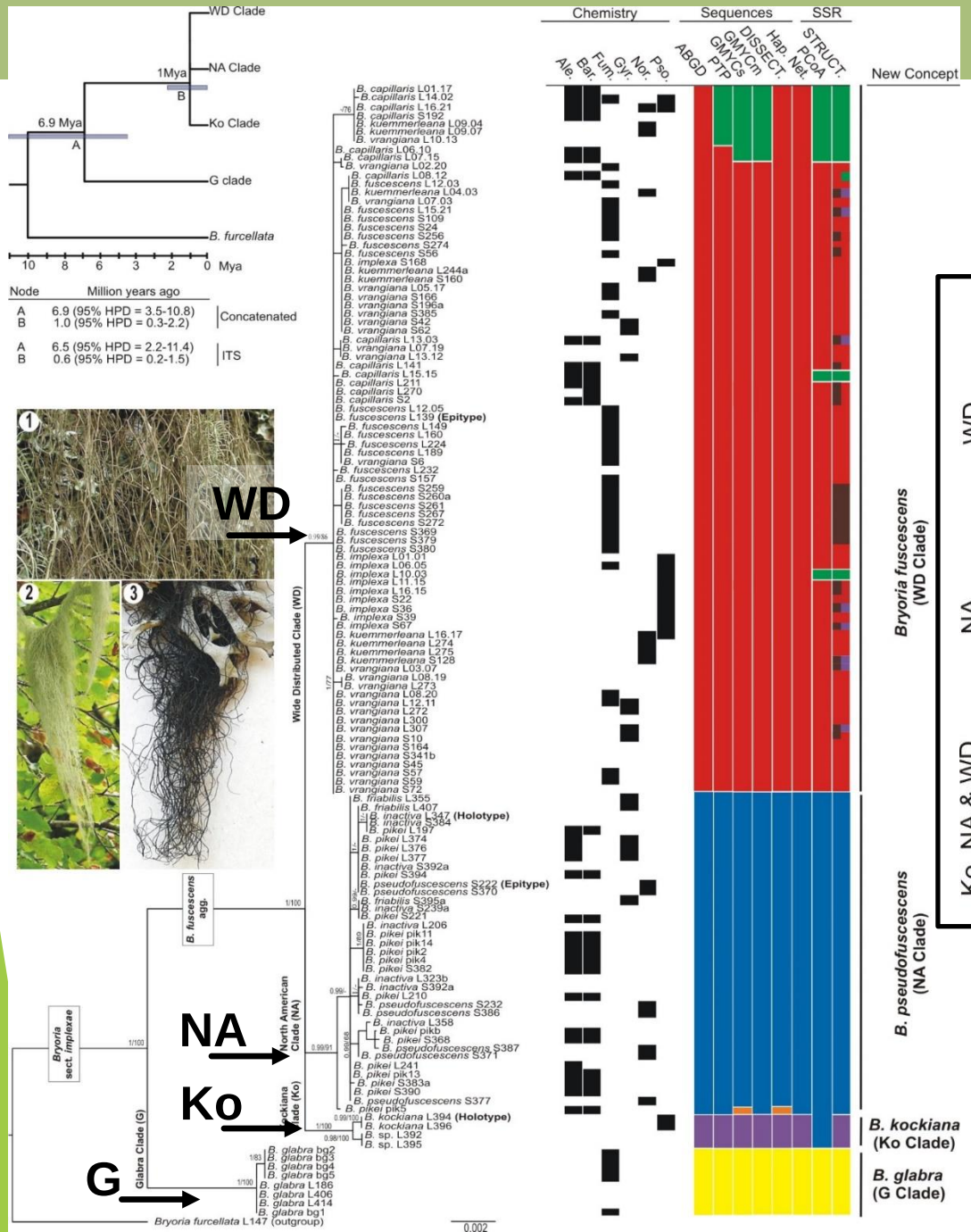

STRUCTURE using microsatellites
B. glabra
B. kochiana
B. sp.
B. friabilis
B. inactiva
B. pikei
B. pseudofuscescens
B. fuscescens
B. capillaris
B. implexa
B. kuemmerleana
B. vrangiana
K2
K3
K4
K5
K6
G
Ko
NA
WD

Chapter 6

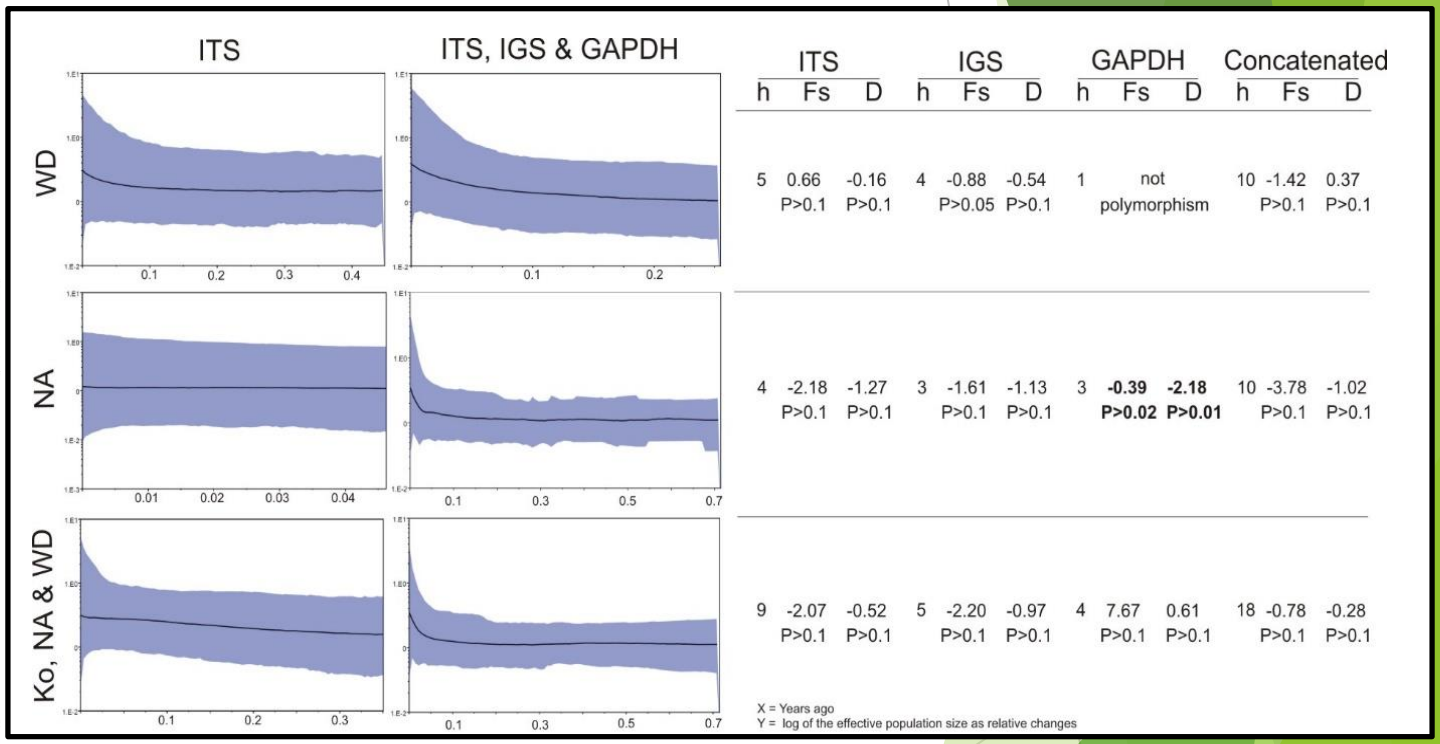

WD Clade
1Mya
NA Clade
B
Ko Clade
6.9 Mya
A
G clade
B. furcellata
10 8 6 3 2 0 Mya
Node Million years ago
A 6.9 (95% HPD = 3.5-10.8) Concatenated
B 1.0 (95% HPD = 0.3-2.2)
A 6.5 (95% HPD = 2.2-11.4) ITS
B 0.6 (95% HPD = 0.2-1.5)
Chemistry
Sequences
SSR
Ale. Bar. Fum. Gyr. Nor. Pso. ABGD PTP GMYCs GMYCm DISSECT. Haps Net. STRUCT. PCoA New Concept
WD
NA
Ko
G
Wide Distributed Clade (WD)
North American Clade (NA)
Kockiana Clade (Ko)
Glabra Clade (G)
B. fuscescens agg.
Bryoria sect. Implexae
Bryoria furcellata L147 (outgroup)
0.002
Bryoria fuscescens (WD Clade)
B. pseudofuscescens (NA Clade)
B. kockiana (Ko Clade)
B. glabra (G Clade)


WD Clade
1.0 Mya
NA Clade
B
6.9 Mya
A
Ko Clade
G clade
B. furcellata
10 8 6 3 2 0 Mya
Node Million years ago
A 6.9 (95% HPD = 3.5-10.8) Concatenated
B 1.0 (95% HPD = 0.3-2.2)
A 6.5 (95% HPD = 2.2-11.4) ITS
B 0.6 (95% HPD = 0.2-1.5)
Divergence time estimation

Chapter 6



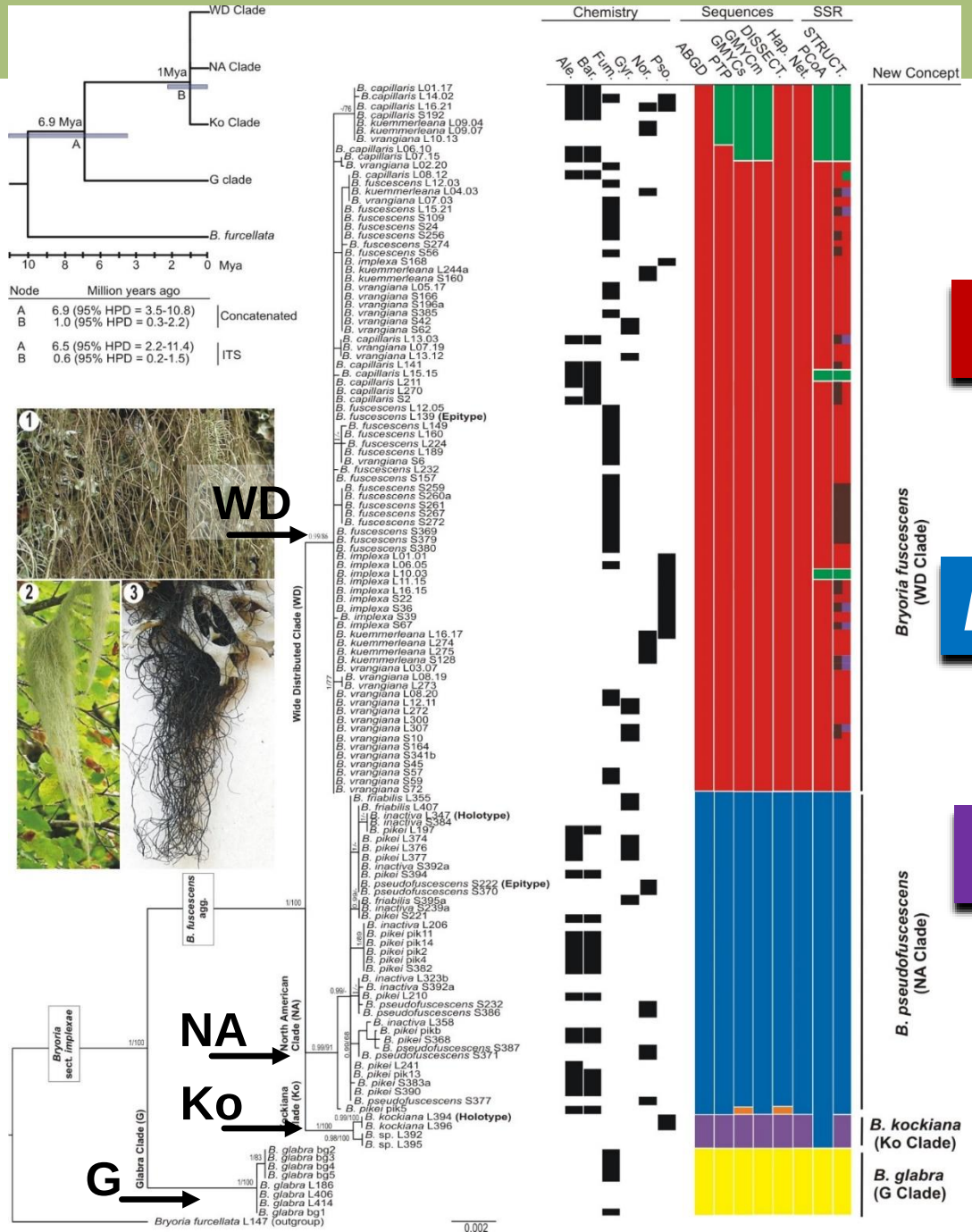
Past population dynamics



	ITS			IGS			GAPDH			Concatenated		
	h	Fs	D	h	Fs	D	h	Fs	D	h	Fs	D
WD	5	0.66 P>0.1	-0.16 P>0.1	4	-0.88 P>0.05	-0.54 P>0.1	1	not polymorphism		10	-1.42 P>0.1	0.37 P>0.1
NA	4	-2.18 P>0.1	-1.27 P>0.1	3	-1.61 P>0.1	-1.13 P>0.1	3	**-0.39 P>0.02**	**-2.18 P>0.01**	10	-3.78 P>0.1	-1.02 P>0.1
Ko, NA & WD	9	-2.07 P>0.1	-0.52 P>0.1	5	-2.20 P>0.1	-0.97 P>0.1	4	7.67 P>0.1	0.61 P>0.1	18	-0.78 P>0.1	-0.28 P>0.1

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

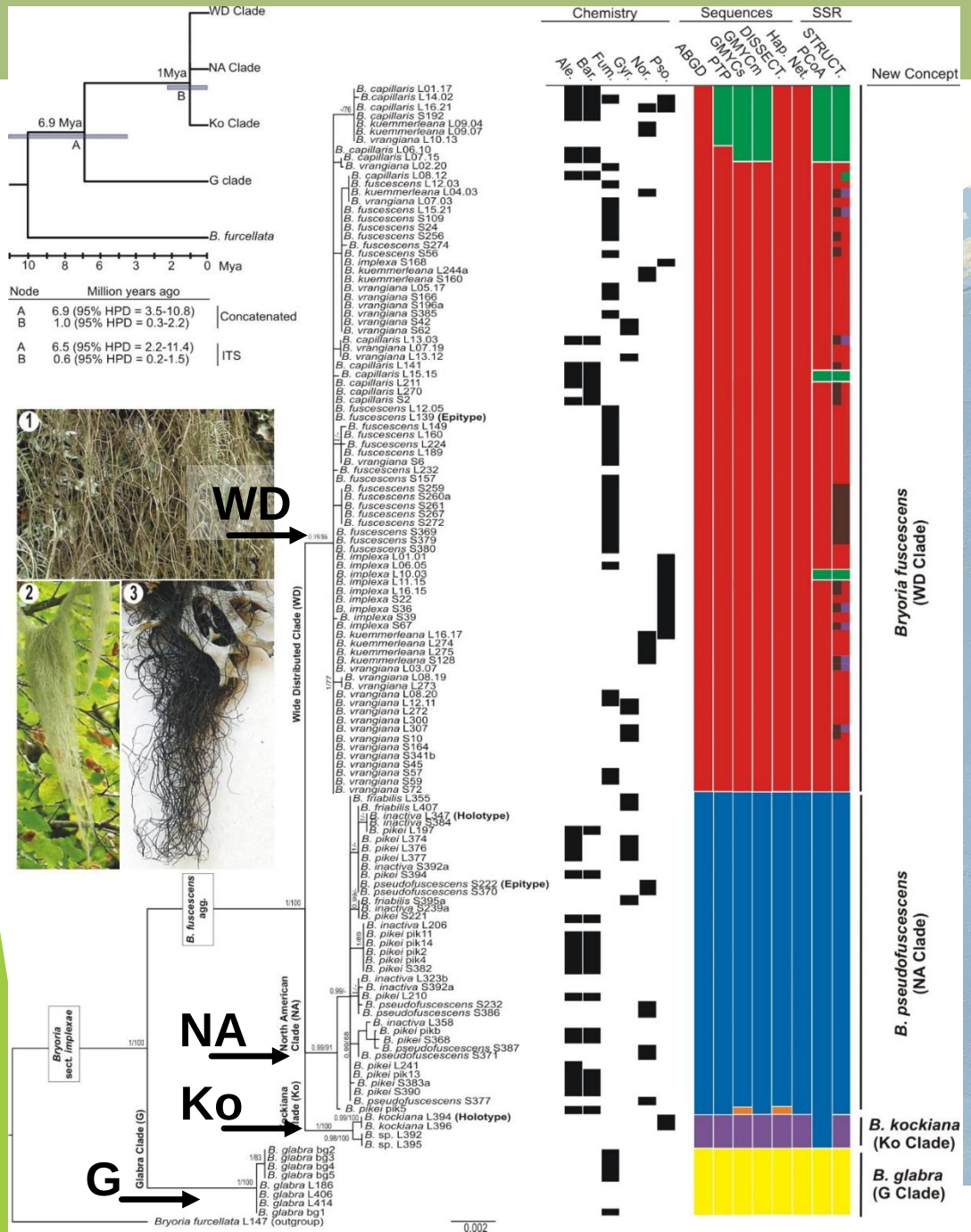
Syn: ***B. sp.***

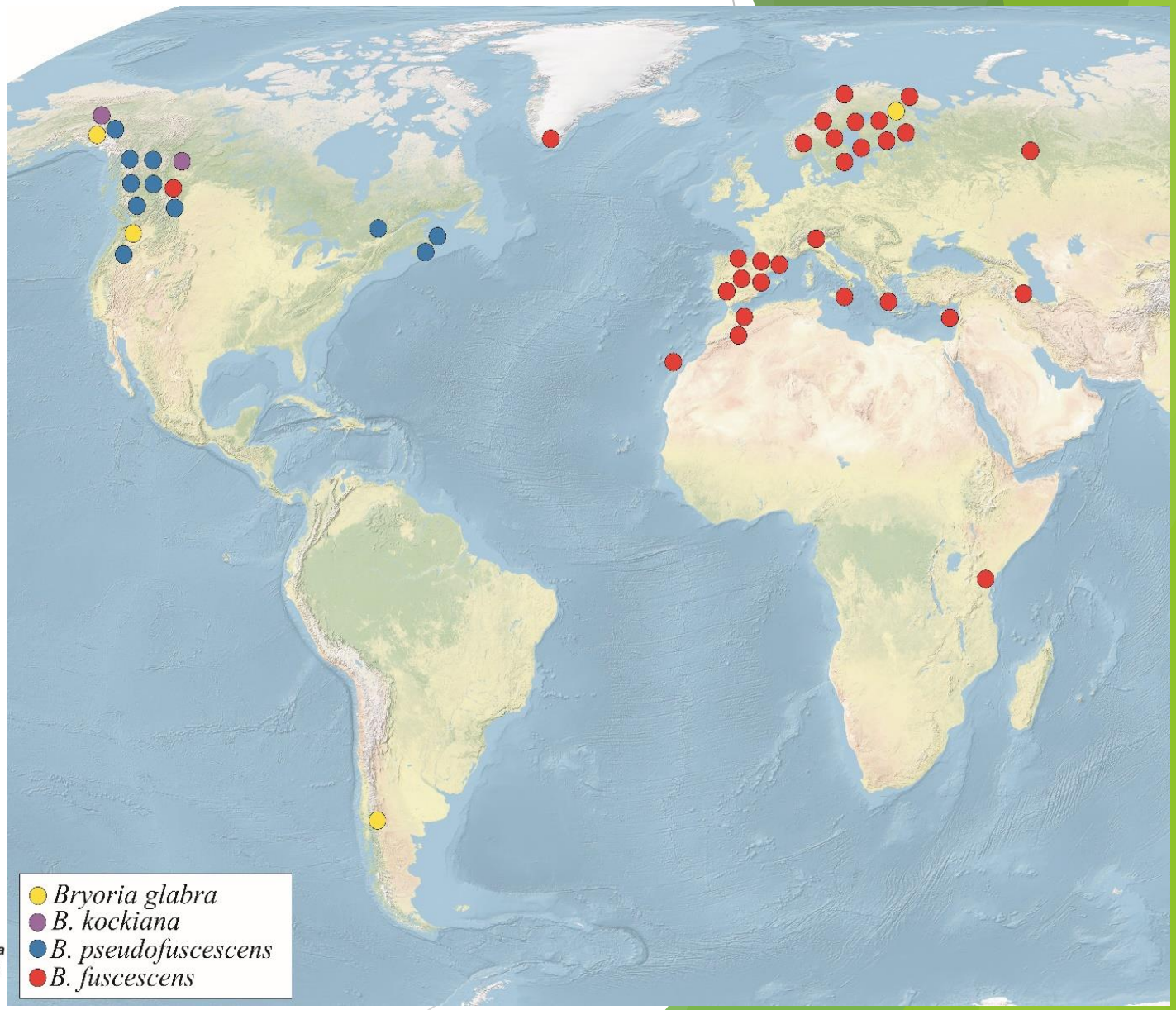
Bryoria glabra

Cryptic

1 Mya of divergence

Chapter 6

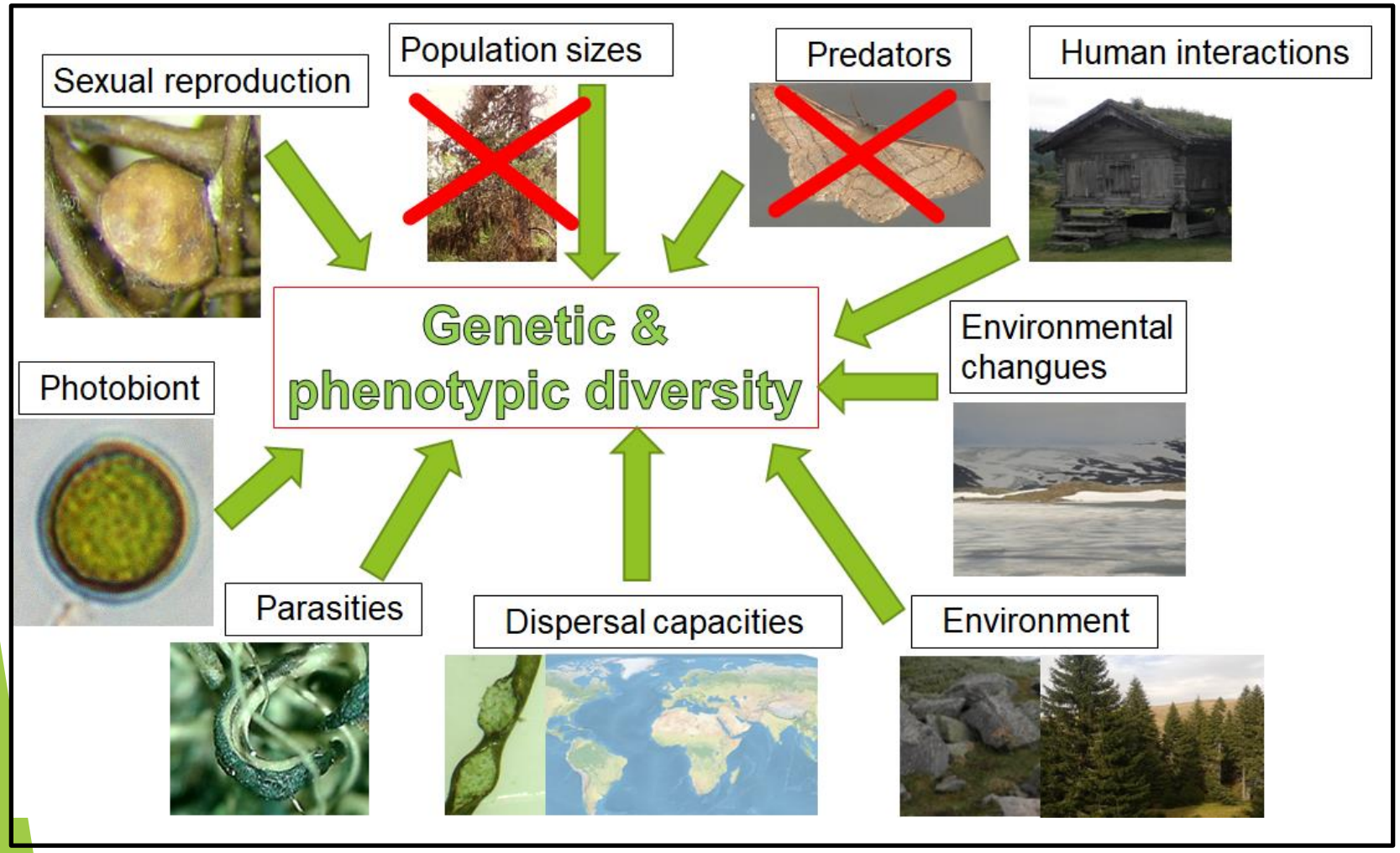

WD Clade
1Mya
NA Clade
B
6.9 Mya
Ko Clade
A
G clade
B. furcellata
10 8 6 3 2 0 Mya
Node
Million years ago
A 6.9 (95% HPD = 3.5-10.8)
B 1.0 (95% HPD = 0.3-2.2)
Concatenated
A 6.5 (95% HPD = 2.2-11.4)
B 0.6 (95% HPD = 0.2-1.5)
ITS
Chemistry
Sequences
SSR
Ale.
Bar.
Fum.
Gyr.
Nor.
Pso.
ABGD
PTP
GMYCs
GMYCm
DISSECT.
Hap. Net.
STRUCT.
PCoA
New Concept
1
2
3
WD
NA
Ko
G
Wide Distributed Clade (WD)
B. fuscescens agg.
North American Clade (NA)
Kockiana Clade (Ko)
Glabra Clade (G)
Bryoria sect. Implexae
Bryoria fuscescens (WD Clade)
B. pseudofuscescens (NA Clade)
B. kockiana (Ko Clade)
B. glabra (G Clade)
Bryoria furcellata L147 (outgroup)
0.002


Bryoria glabra
B. kockiana
B. pseudofuscescens
B. fuscescens

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

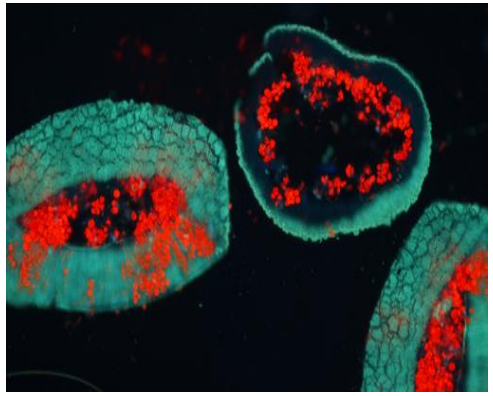
Phenotype-*capillaris*

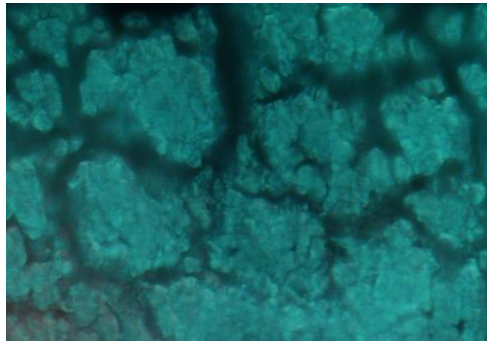
- Usually pale
- With barbatolic acid
- Soralia rare
- Angles usually acute

Bryoria fuscescens s. str.

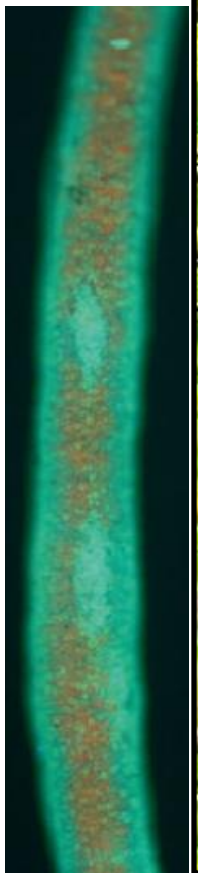
Phenotype-*fuscescens*

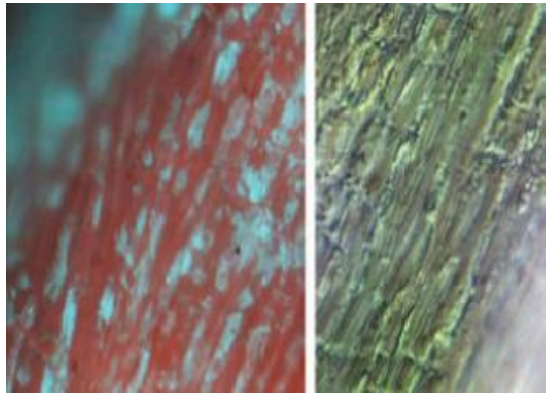
- Usually dark
- Without barbatolic acid
- Soralia frequent
- Angles variable

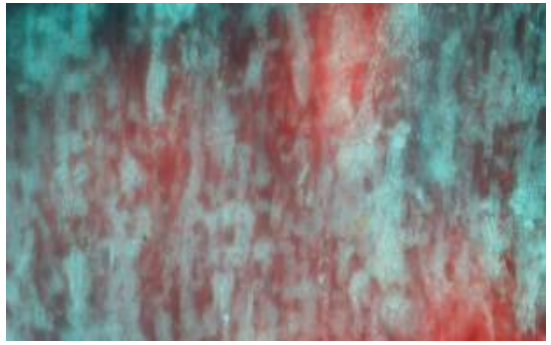




Cortical UV autofluorescence







Cortical UV autofluorescence

Chapter 7 *Bryoria* phylogeography

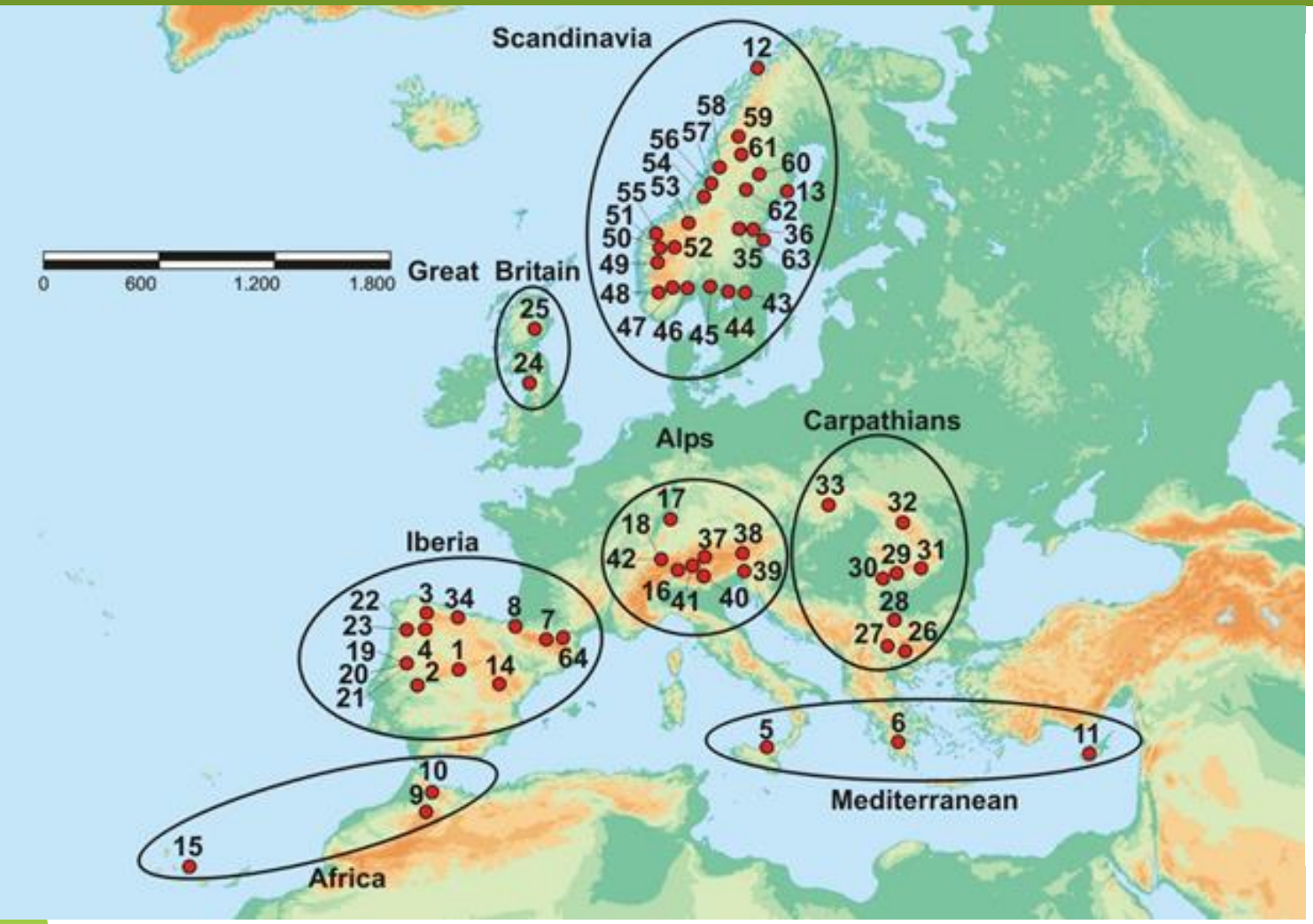


Table 1. Amplified and analysed SSRs. Left: Number of specimens with successful amplification for each locus, and its respective number of alleles. Right: Selected loci and specimens for the analyses after remove unexpected alleles and specimens with missing data.

	Amplified SSRs		SSRs used for the analyses	
Locus	Specimens	Alleles	Specimens	Alleles
Bi01	1384	22	Not used	Not used
Bi02	1123	6	Not used	Not used
Bi03	1391	5	1359	5
Bi04	1388	8	1359	7
Bi05	1359	14	1359	10
Bi06	1366	22	1359	21
Bi07	1368	6	1359	6
Bi08	1385	5	1359	5
Bi09	597	3	Not used	Not used
Bi10	1393	5	1359	3
Bi11	1391	12	1359	10
Bi12	1399	22	1359	21
Bi13	1359	18	1359	18
Bi14	1391	4	1359	3
Bi15	1071	3	Not used	Not used
Bi16	1360	6	1359	6
Bi18	1359	9	1359	9
Bi19	1388	8	1359	6

1.400 specimens, 64 populations, 18 microsatellites

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

1.359 specimens
14 microsatellites
No missing data

Chapter 7 *Bryoria* phylogeography

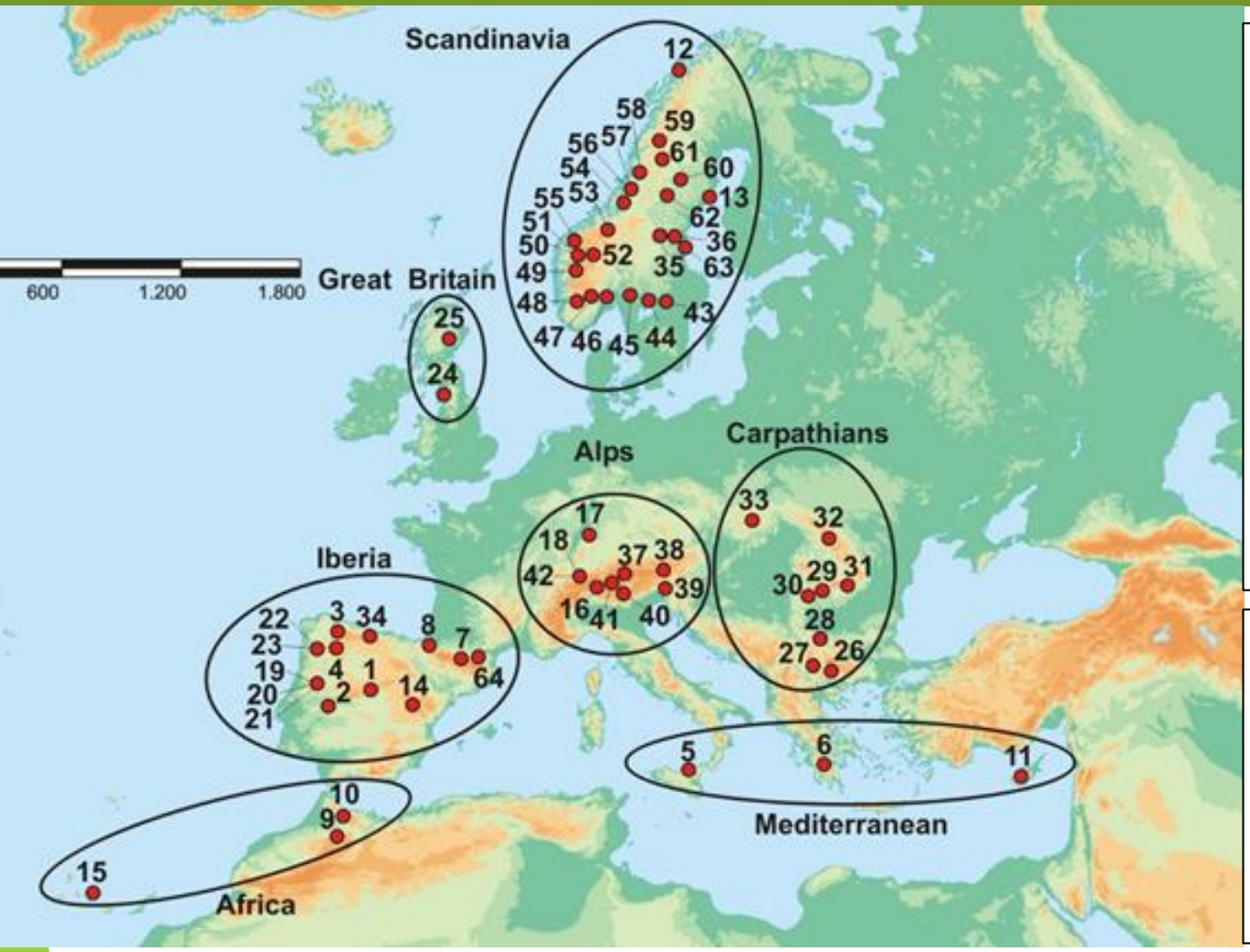


Table S6. Global Analysis of Molecular Variance (AMOVA) using 14 loci, 1359 individuals, 64 populations and the 7 geographical regions from Fig. 1. F_{SC} = 0.21381, F_{ST} = 0.23187 and F_{CT} = 0.02297, statistically significant with $P \leq 0.035$.

Source of variation	df	Sum of squares	Variance components	% of variation
Among regions	6	211.80	0.089	2.30
Among populations within regions	57	1146.23	0.808	20.89
Within populations	1295	3846.72	2.970	76.81
Total	1358	5204.75	3.867	

AMOVA

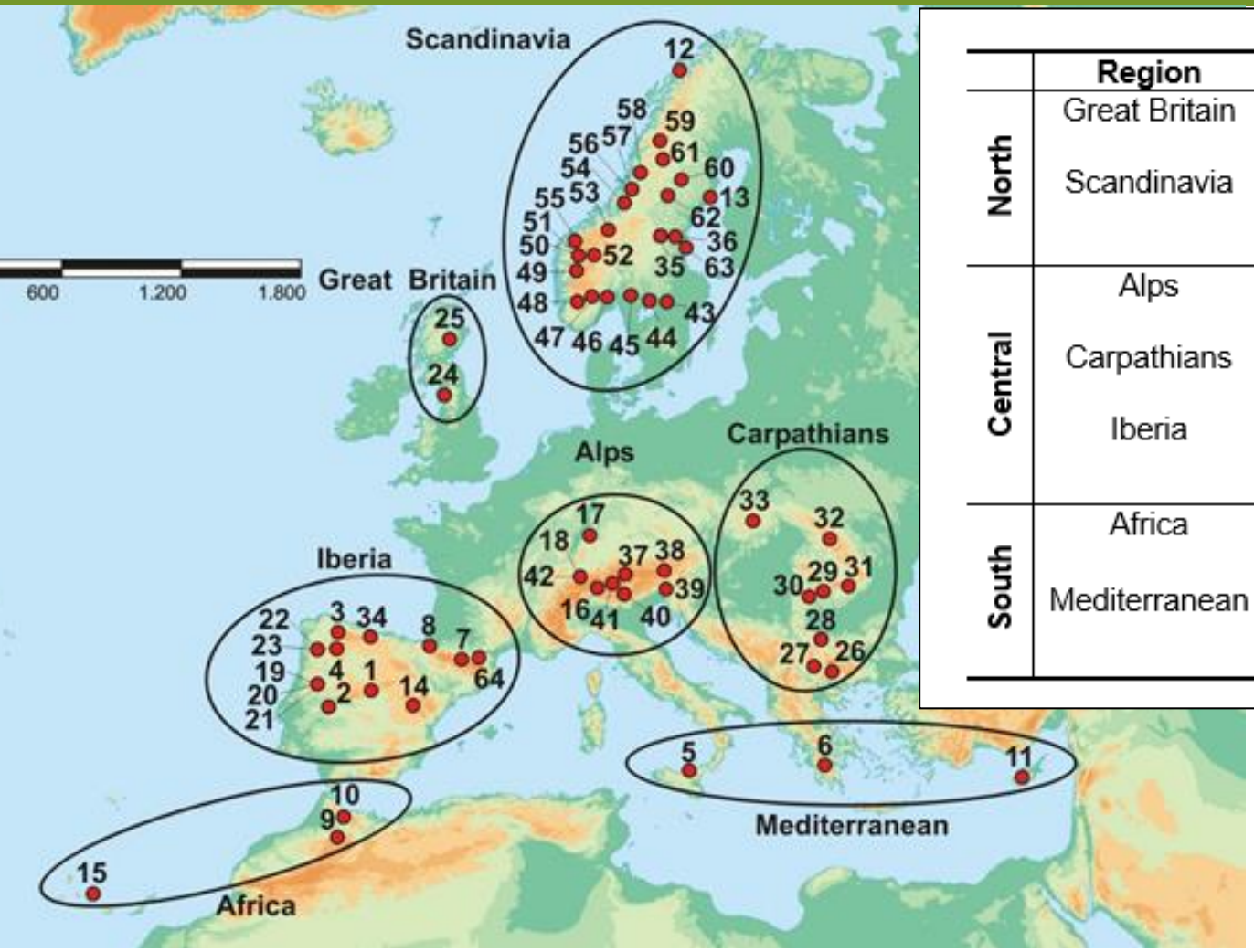
Table S5. Allelic richness (AR) and private allelic richness (PAR) detected in each type of substrate. Standard deviations are showed in brackets.

	Twigs			Branches			Trunks			Rock	
	AR	PAR		AR	PAR		AR	PAR		AR	PAR
n:203	5.93 (1.15)	0.57 (0.21)	n: 30	3.57 (0.63)	0.07 (0.07)	n:256	6.07 (1.15)	0.68 (0.29)	n: 37	2.79 (0.39)	0.00 (0.00)
AR/n	0.029	-	AR/n	0.119	-	AR/n	0.024	-	AR/n	0.075	-

Allelic richness

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

Chapter 7 *Bryoria* phylogeography



	Region	specimens	AR	PAR	specimens	AR	PAR
North	Great Britain	32	2.642 (0.439)	0.000 (0.000)	588	7.500 (1.207)	1.357 (0.452)
	Scandinavia	556	7.428 (1.170)	1.357 (0.452)			
Central	Alps	189	5.357 (1.014)	0.357 (0.199)	641	7.071 (1.442)	0.857 (0.274)
	Carpathians	179	5.428 (0.976)	0.000 (0.000)			
	Iberia	273	5.571 (0.976)	0.285 (0.125)			
South	Africa	65	4.571 (0.947)	0.428 (0.227)	130	5.571 (1.087)	0.500 (0.251)
	Mediterranean	65	4.214 (0.575)	0.071 (0.071)			

Scandinavia: ↑↑

Alps: ↑

Iberia: ↑

Carpathians: ↑↓

Great Britain: ↓

↑ **High diversity**

↓ **Low diversity**

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 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).

Population number	n	Non-clonal specimens	Polymorphic loci (%)	uh mean (stand. error)	rBarD	AR	PAR	K-test	Putative population disturbances
Pops. 1-13 not showed									
14	19	16	100 %	0.525 (0.038)	**0.224**	3.214 (0.350)	0.003 (0.003)	7	-
15	22	16	100 %	0.559 (0.053)	**0.128**	3.428 (0.571)	0.129 (0.088)	8	-
16	20	20	100 %	0.573 (0.033)	**0.126**	3.285	0.071 (0.071)	4	Some tourism, apothecia present
17	14	6	71 %	0.255 (0.066)	**0.221**	2.071	0.071 (0.071)	**14 expansion**	Apothecia present
18	23	16	100 %	0.519 (0.046)	**0.175**	3.285 (0.398)	0.000 (0.000)	5	Apothecia present
19	23	13	100 %	0.366 (0.053)	0.031	2.642 (0.289)	0.000 (0.000)	10	-
20	9	1	0 %	0.000 (0.000)	NA	1.000 (0.000)	0.000 (0.000)	**14 expansion**	Saxicolous
21	10	1	0 %	0.000 (0.000)	NA	1.000 (0.000)	0.000 (0.000)	**14 expansion**	Saxicolous
22	23	4	71 %	0.276 (0.059)	**0.380**	1.928	0.000 (0.000)	**11 expansion**	Village close, apothecia present
23	23	5	86 %	0.356 (0.057)	**0.183**	2.000 (0.148)	0.000 (0.000)	7	Near pastures
24	9	5	64 %	0.317 (0.079)	**0.258**	2.071 (0.286)	0.000 (0.000)	10	Artificial forest
25	23	14	71 %	0.375 (0.072)	**0.096**	2.214 (0.317)	0.002 (0.002)	7	-
26	23	21	100 %	0.505 (0.046)	**0.268**	3.357	0.000 (0.000)	7	Some tourism, apothecia present
27	23	21	93 %	0.451 (0.063)	0.023	3.071 (0.412)	0.000 (0.000)	8	Human activities and constructions
28	23	18	100 %	0.466 (0.041)	**0.336**	3.214 (0.350)	0.002 (0.002)	6	Tourism
29	23	23	100 %	0.494 (0.057)	**0.144**	3.571	0.008 (0.008)	10	Human constructions, apothecia present
30	22	20	86 %	0.337 (0.072)	0.034	2.857	0.008 (0.008)	**12 expansion**	Apothecia present
31	23	20	100 %	0.056 (0.048)	**0.184**	3.785 (0.612)	0.000 (0.000)	5	Apothecia present
32	21	19	93 %	0.380	0.044	2.642	0.000 (0.000)	7	Apothecia present
Pops. 33-64 not showed									

Chapter 7 *Bryoria* phylogeography

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Population number						PAR	K-test	Putative population disturbances
Pops. 1-								
14						3 (0.003)	7	-
15						9 (0.088)	8	-
16						1 (0.071)	4	Some tourism, apothecia present
17						1 (0.071)	**14 expansion**	Apothecia present
18						0 (0.000)	5	Apothecia present
19						0 (0.000)	10	-
20						0 (0.000)	**14 expansion**	Saxicolous
21						0 (0.000)	**14 expansion**	Saxicolous
22						0 (0.000)	**11 expansion**	Village close, apothecia present
23						0 (0.000)	7	Near pastures
24						0 (0.000)	10	Artificial forest
25						2 (0.002)	7	-
26						0 (0.000)	7	Some tourism, apothecia present
27						0 (0.000)	8	Human activities and constructions
28						2 (0.002)	6	Tourism
29						8 (0.008)	10	Human constructions, apothecia present
30						8 (0.008)	**12 expansion**	Apothecia present
31						0 (0.000)	5	Apothecia present
32	21	19	93 %	0.380 (0.068)	0.044	2.642	0.000 (0.000)	7	Apothecia present

Pops. 33-64 not showed

Chapter 7 *Bryoria* phylogeography

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20	9	1	0 %	0.000 (0.000)	NA	1.000 (0.000)	0.000 (0.000)	**14 expansion**	Saxicolous
21	10	1	0 %	0.000 (0.000)	NA	1.000 (0.000)	0.000 (0.000)	**14 expansion**	Saxicolous
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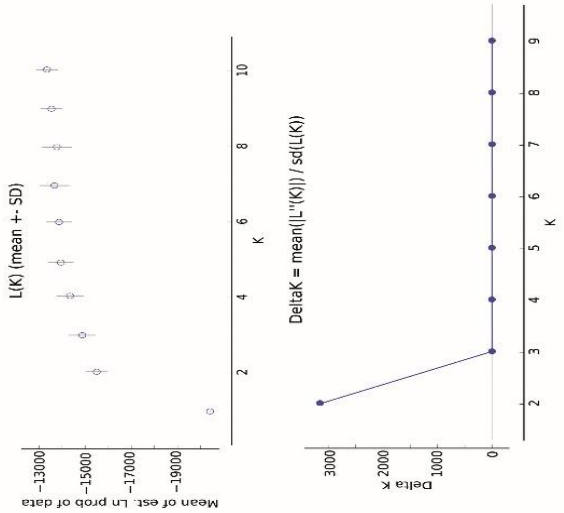
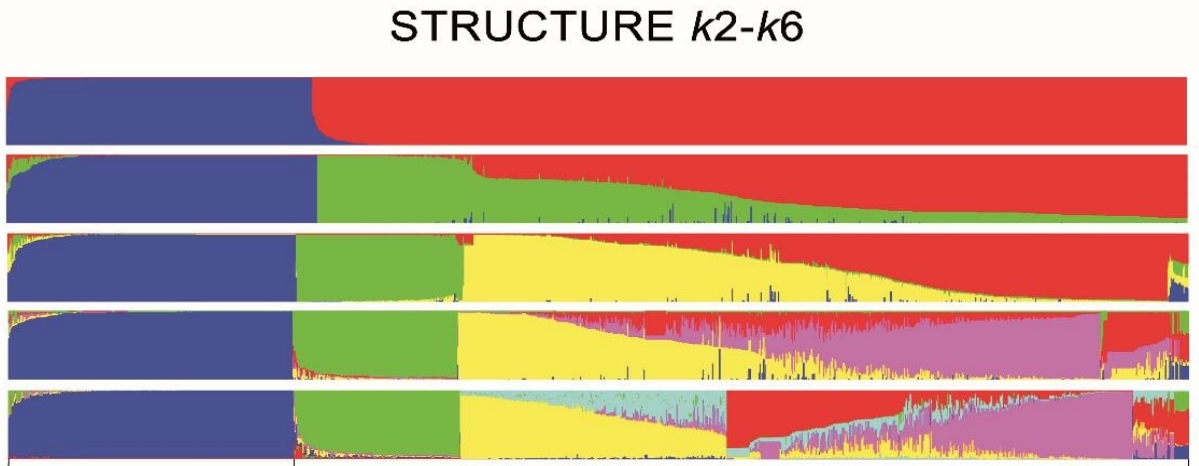
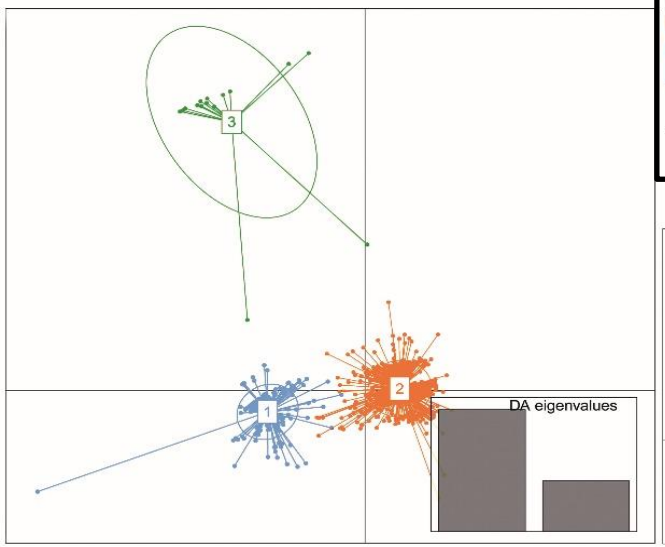
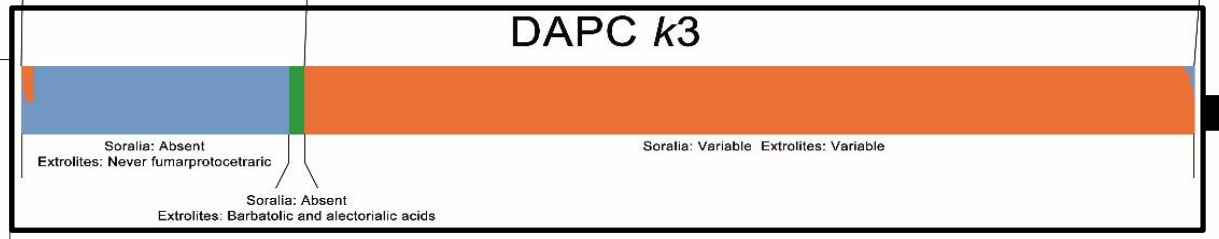
Pops. 33-64 not showed									

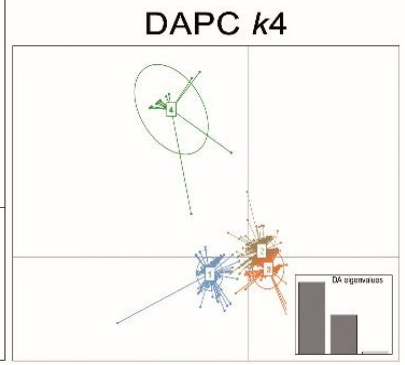
Chapter 7 *Bryoria* phylogeography

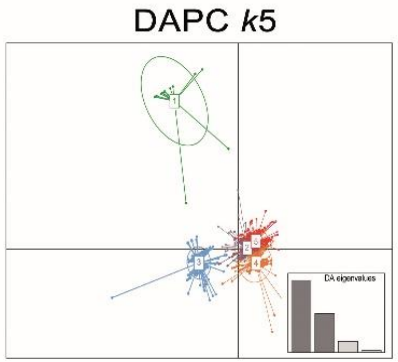


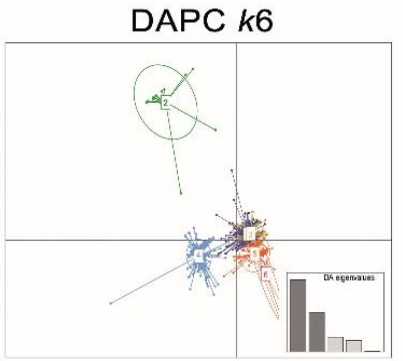












Best genepools:
DAPC *K*3

Genepool 1

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

Genepool 2

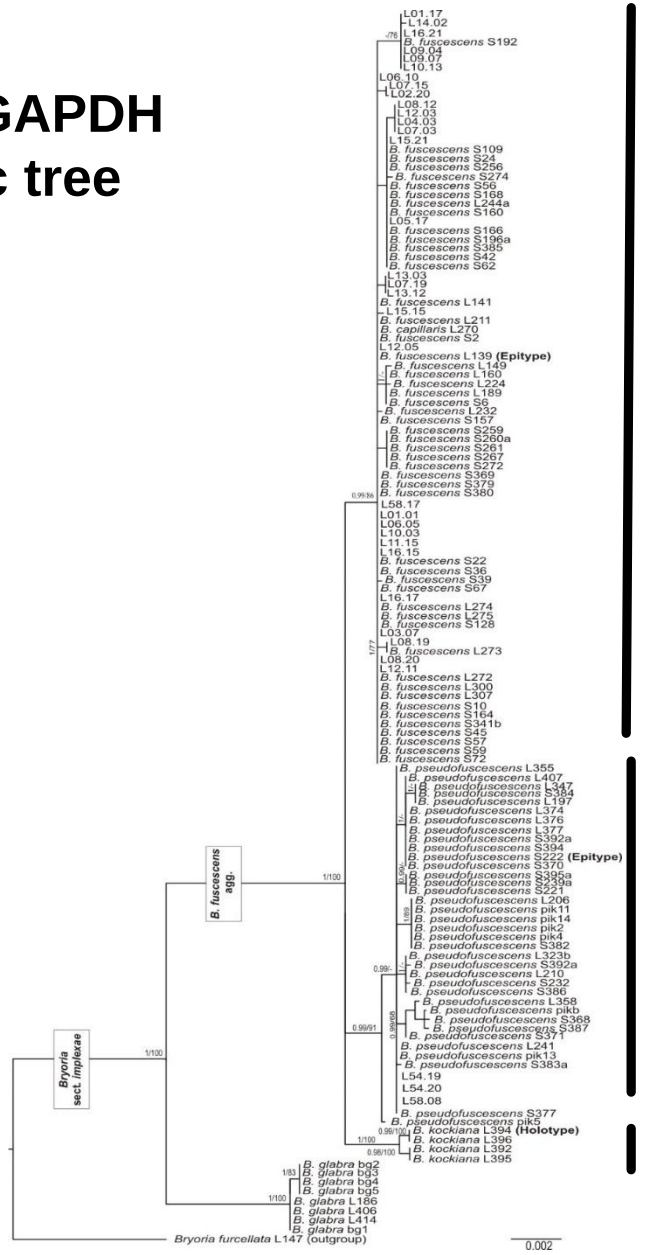
Soralia: Absent
Fum.: No
Ph. *capillaris* : 87%
Ph. *fuscescens* : 13%

Genepool 3

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

Chapter 7 *Bryoria* phylogeography

nuITS, IGS & GAPDH
Phylogenetic tree



Bryoria fuscescens

B. pseudofuscescens

B. kockiana

Best genepools:
DAPC *K*3

Genepool 1

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

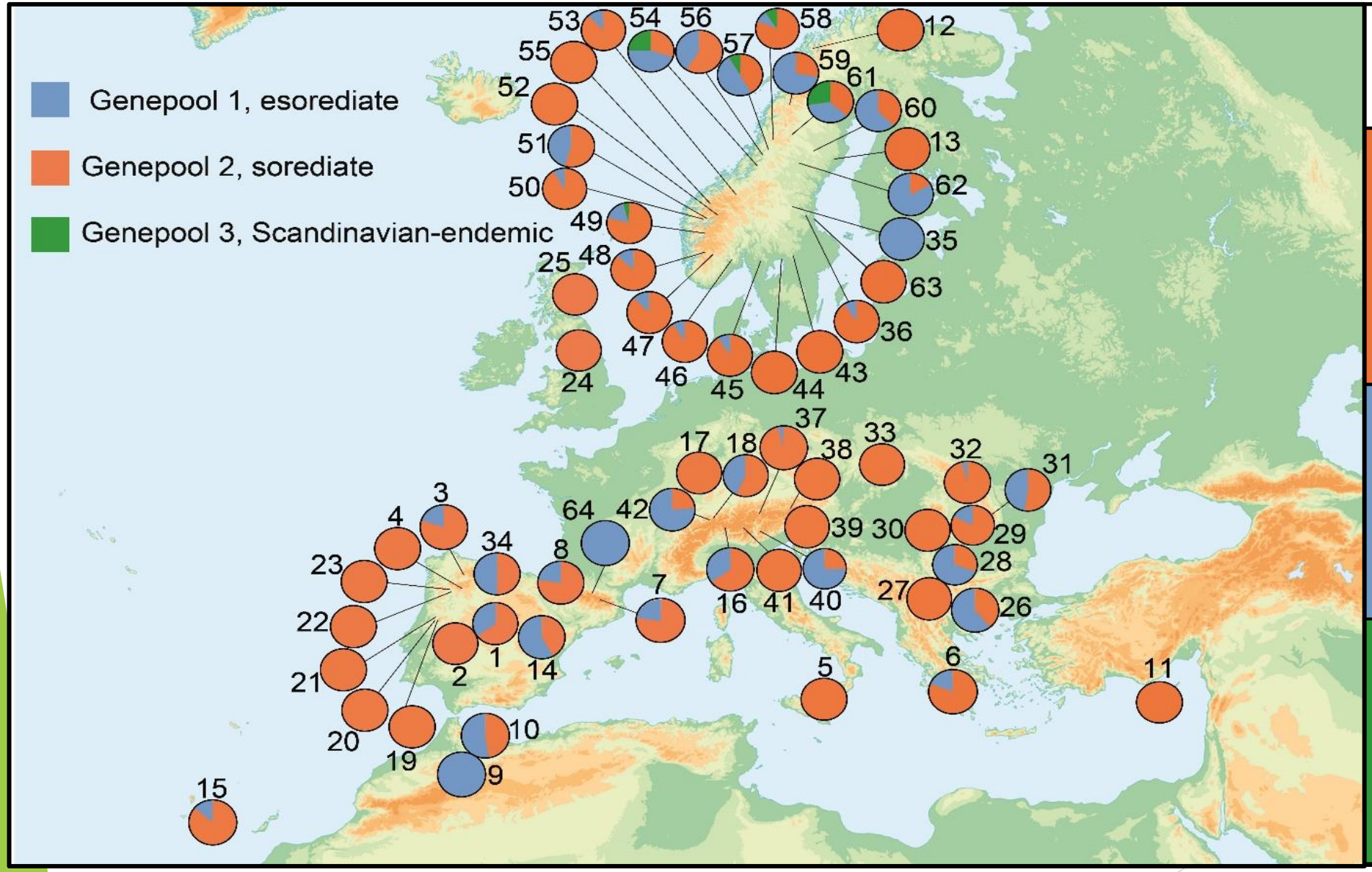
Genepool 2

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



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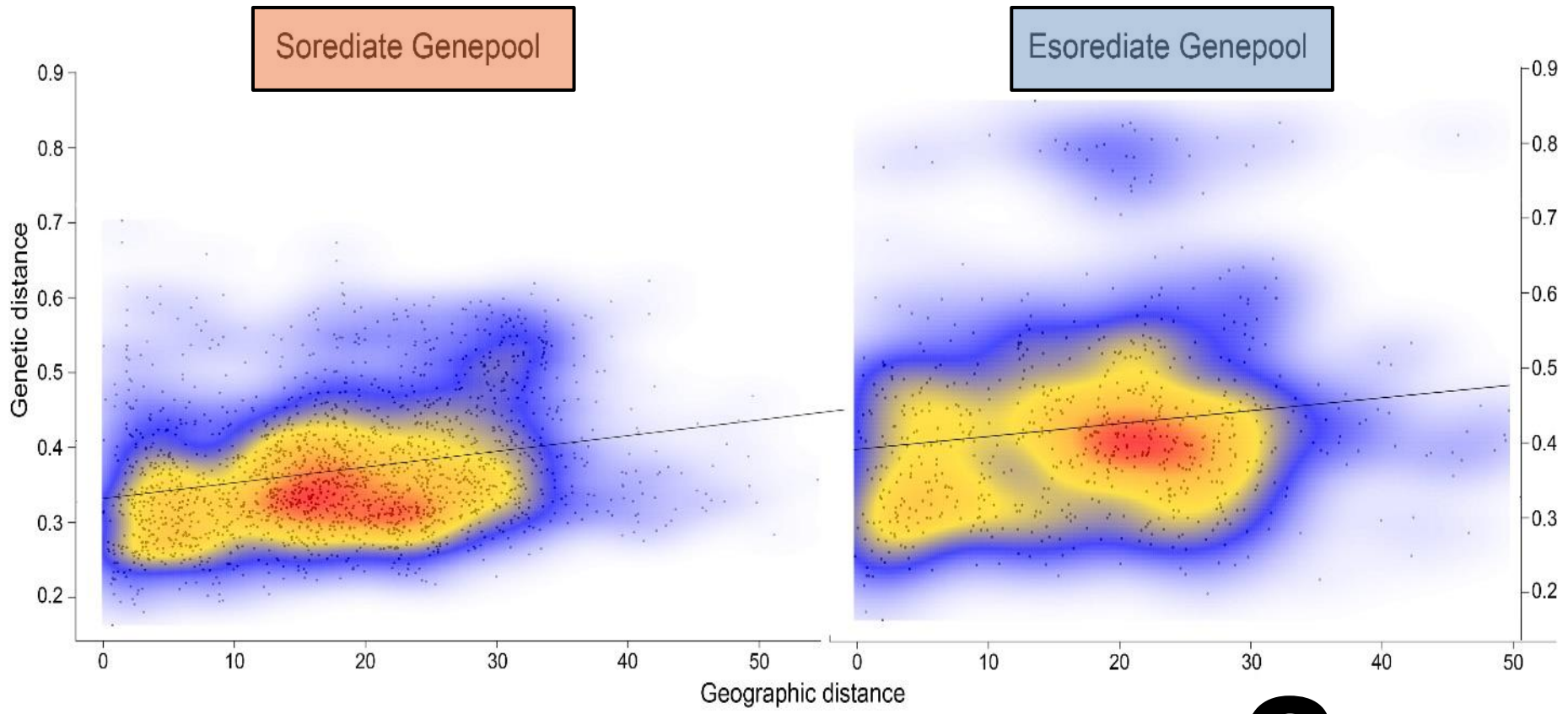
Fum.: No
Ph. *capillaris* : 87%
Ph. *fuscescens* : 13%

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



Soralia are not favouring dispersion ?

Best genepools:
DAPC *K*3

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%

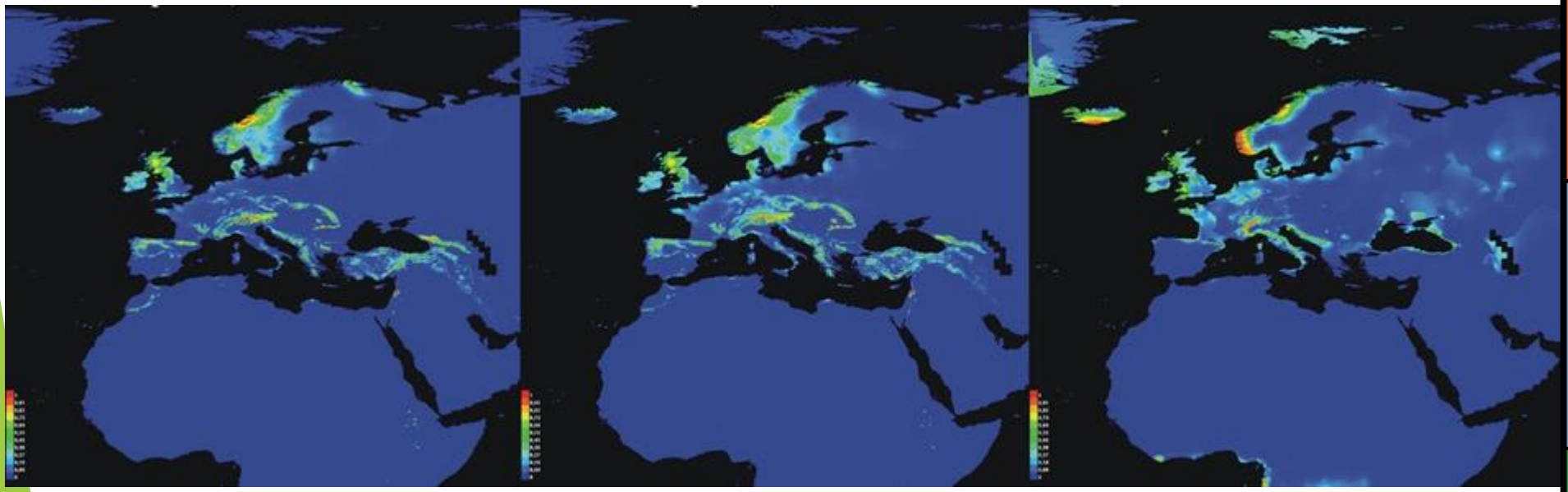
Chapter 7 *Bryoria* phylogeography

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

Genepool 1

Genepool 2

Genepool 3



Phenotype-*fuscescens*

Phenotype-*capillaris*

B. fuscescens-pseudofuscescens

Best genepools:
DAPC *K*3

Genepool 1

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

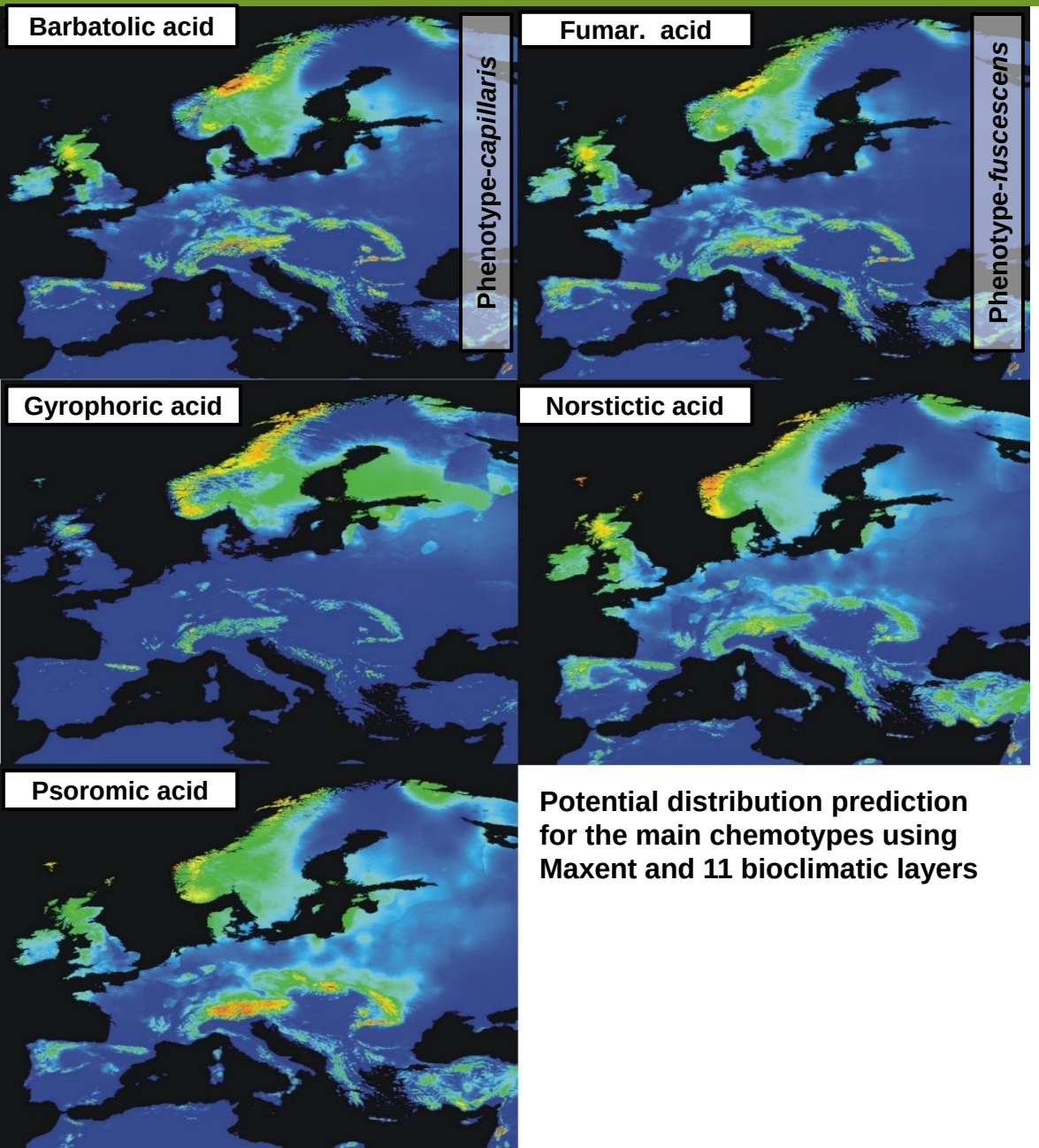
Genepool 2

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 *K*3

Genepool 1

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

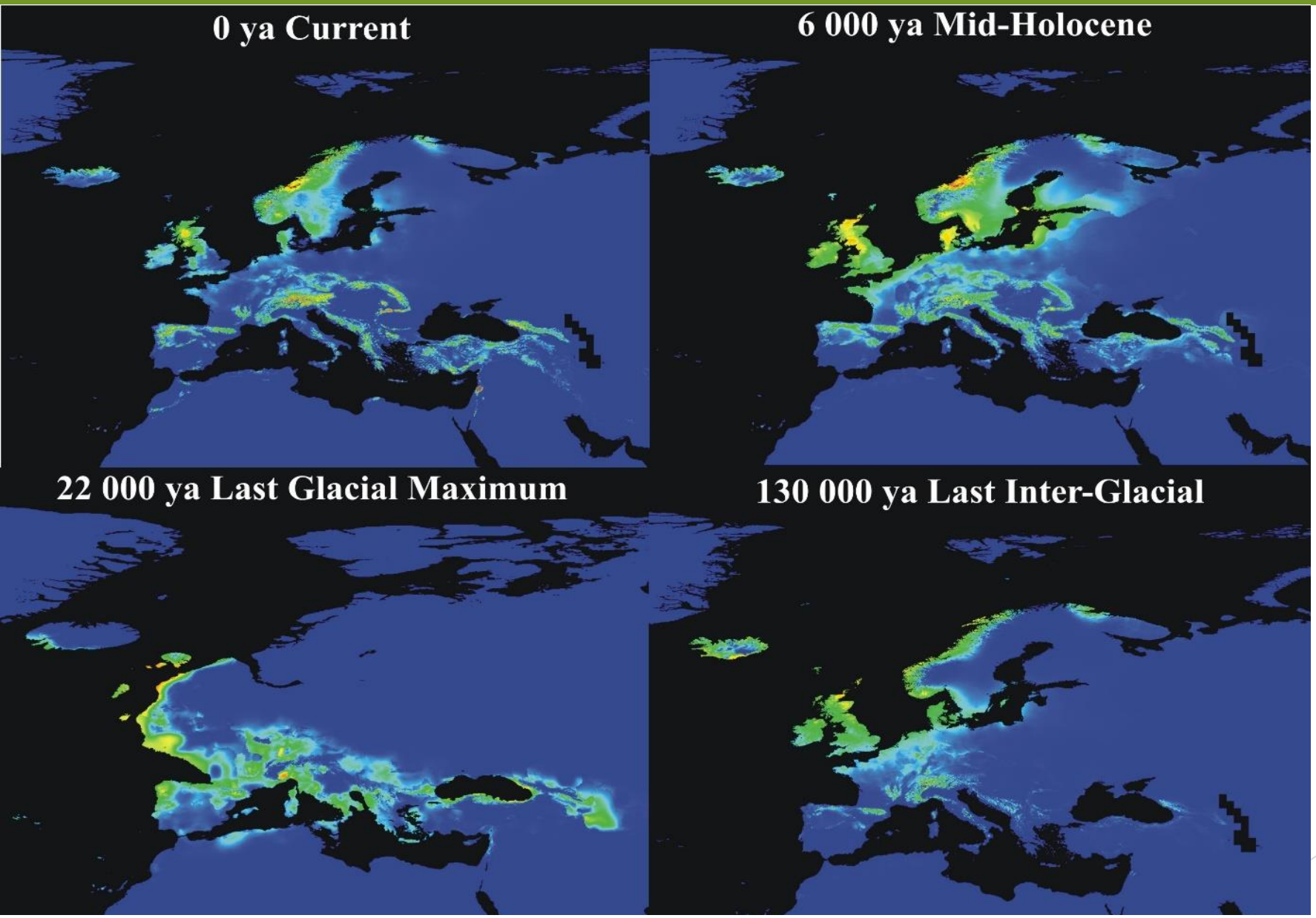
Genepool 2

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 *Bryoria fuscescens s. str*. using Maxent and 11 bioclimatic layers

Glacial refugia candidates:

East of British Isles

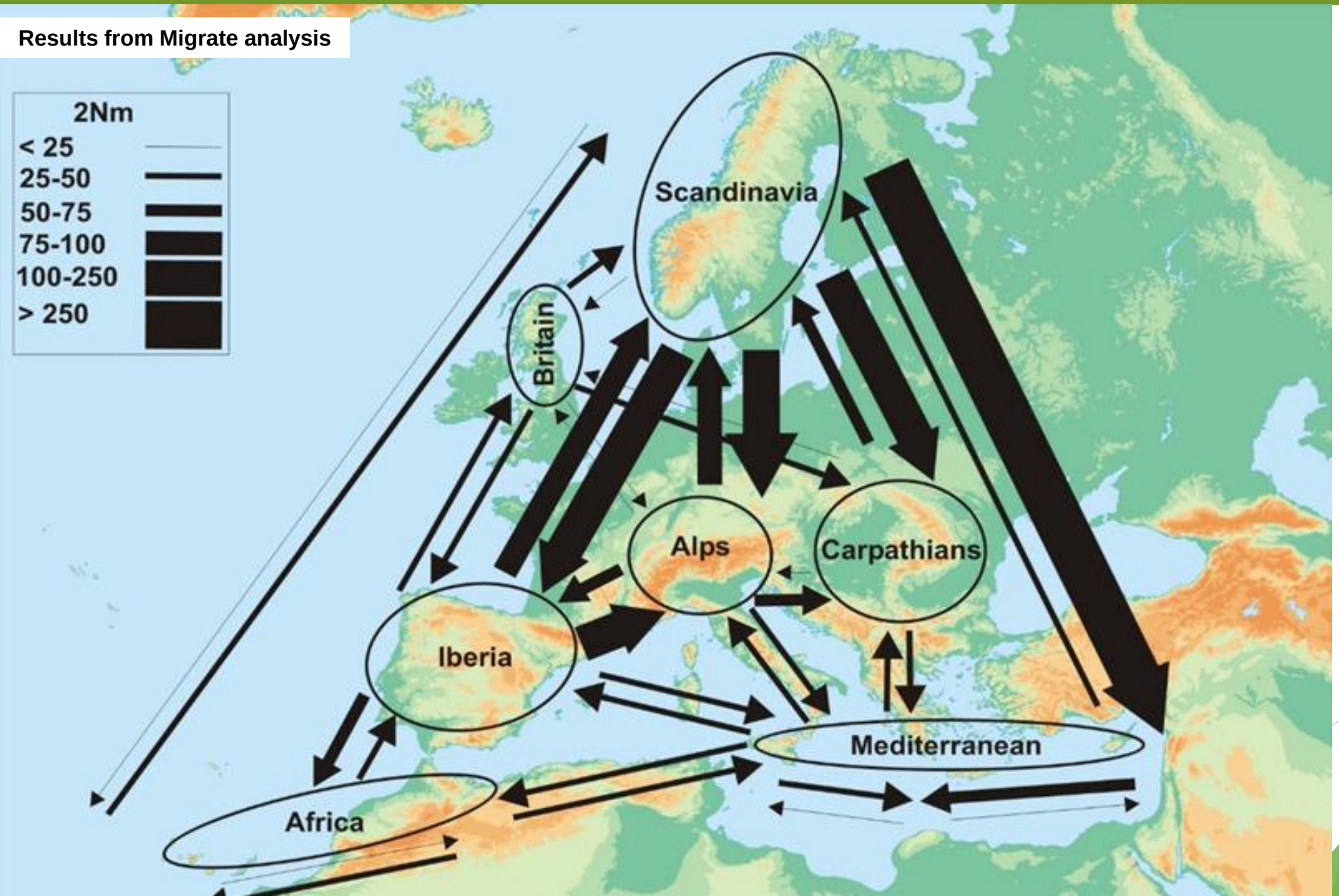
Northwest of Iberian Peninsula

Alps lowlands

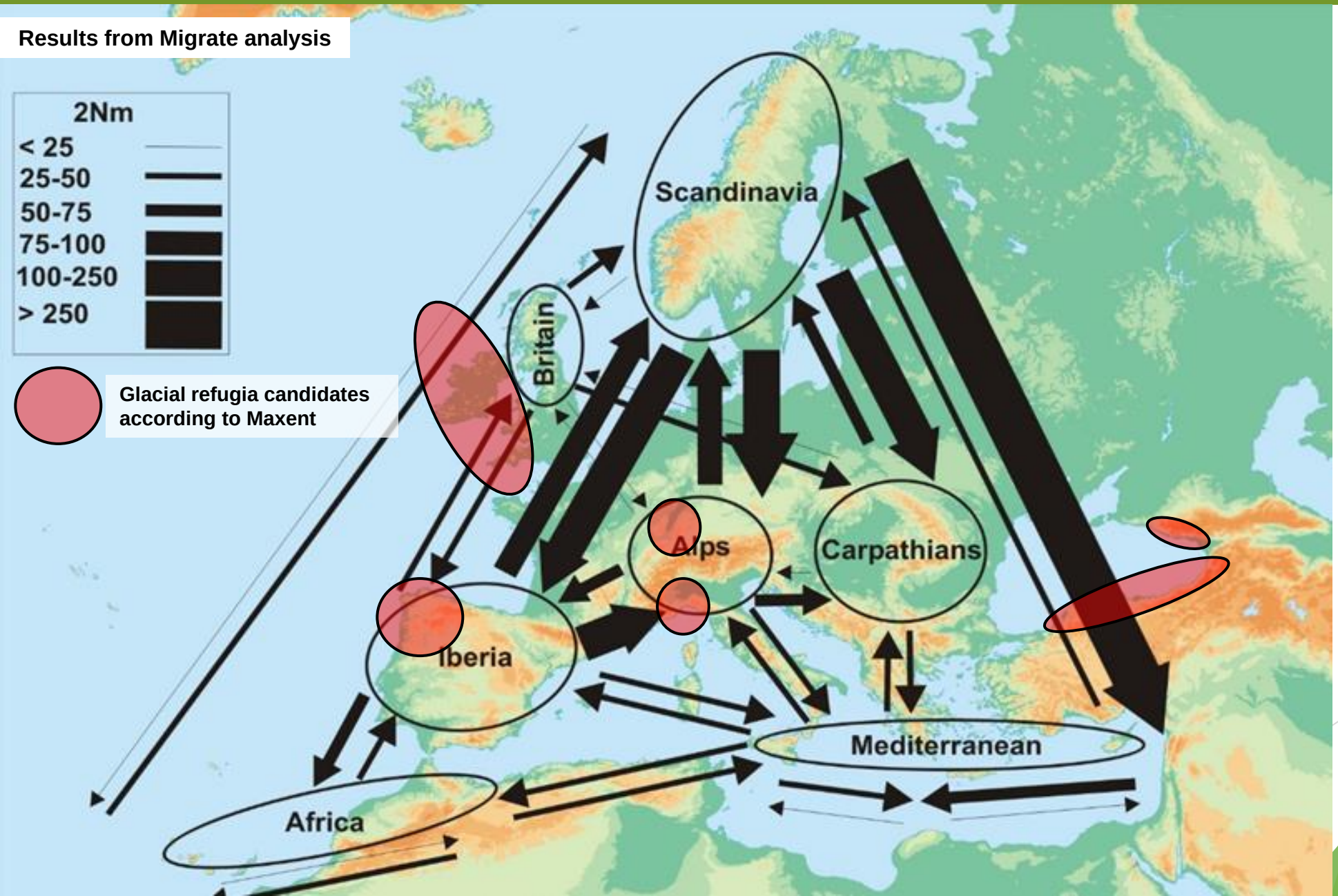
Black sea

Chapter 7 *Bryoria* phylogeography

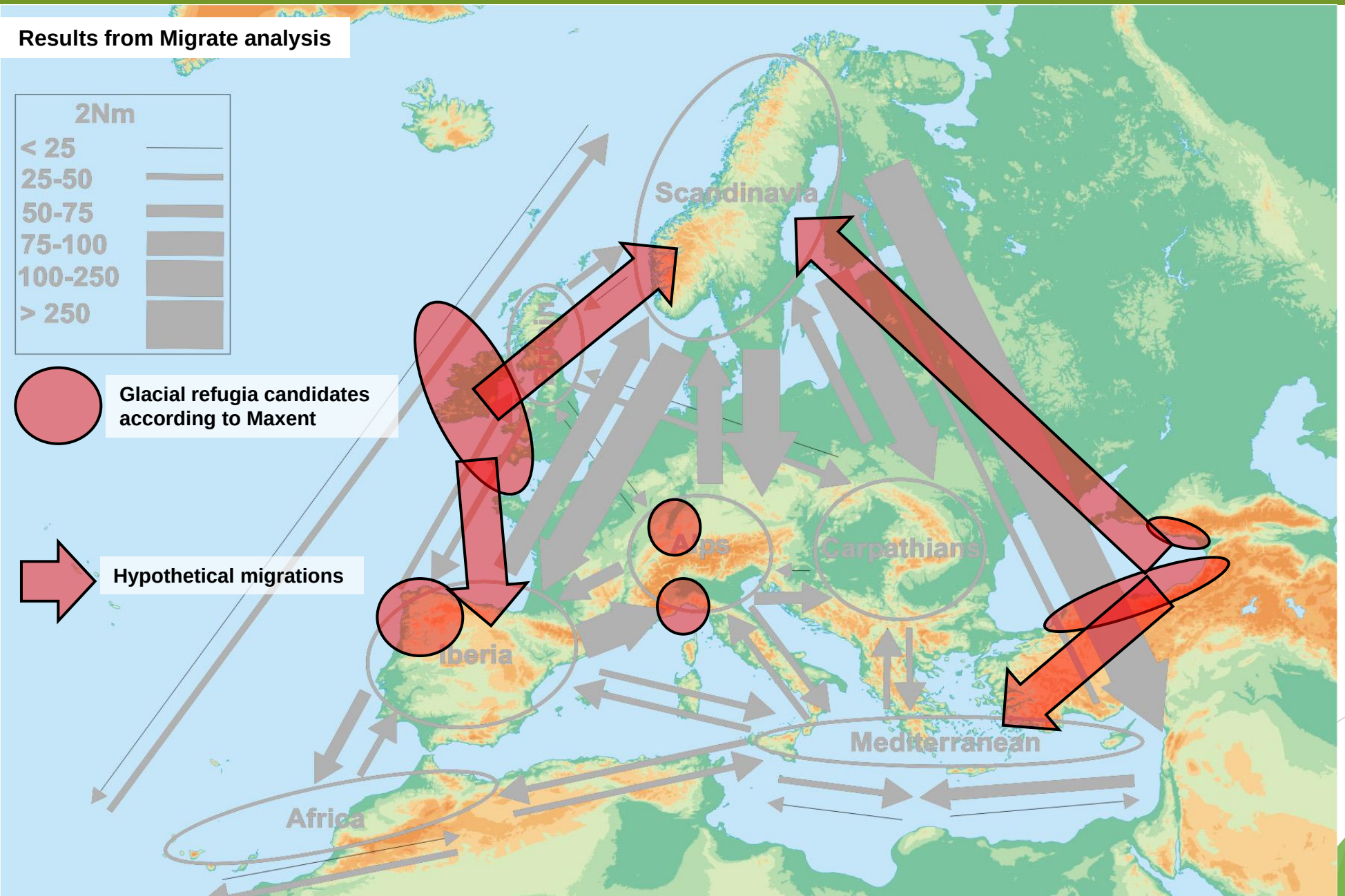
Results from Migrate analysis



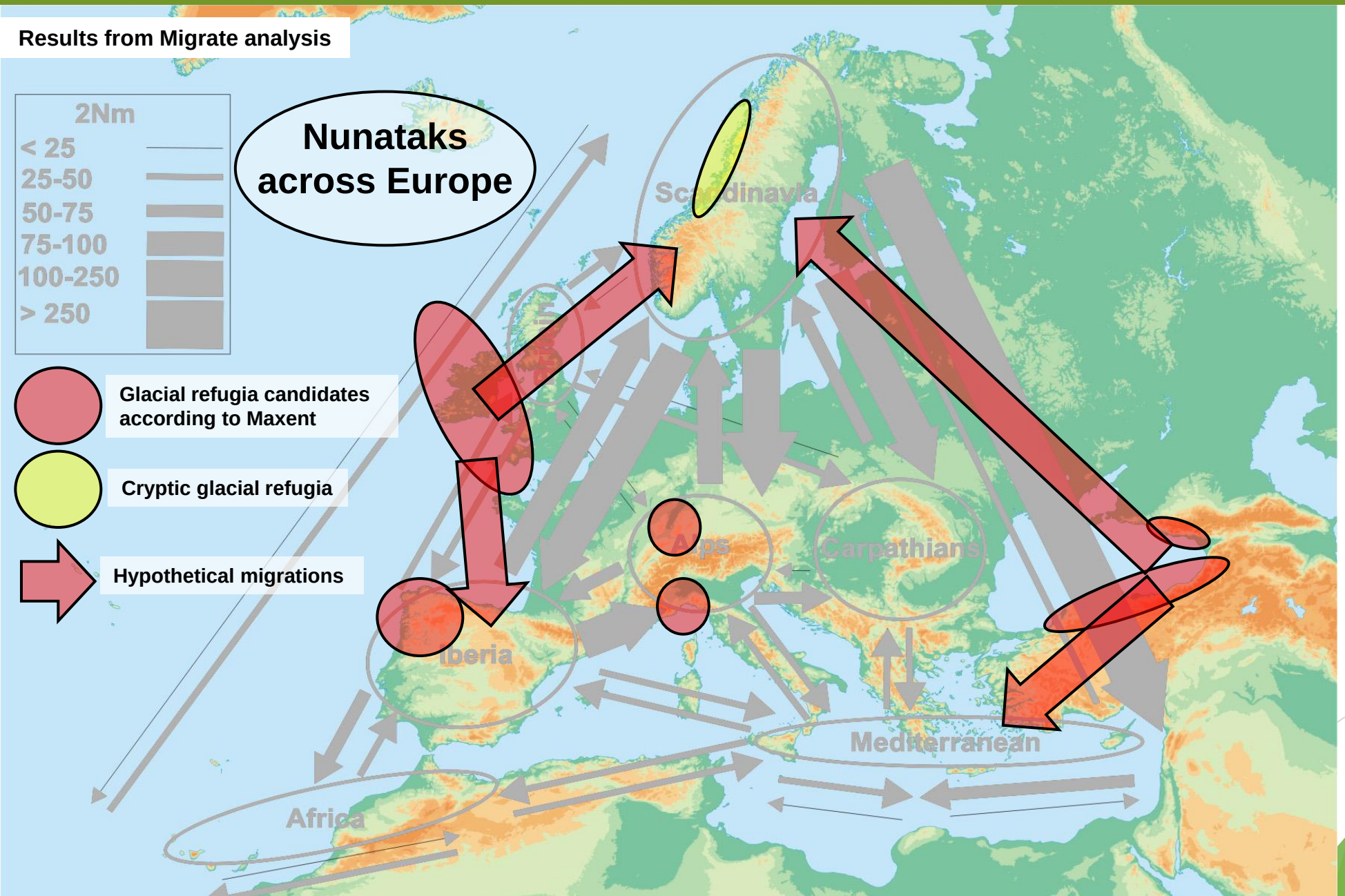
Chapter 7 *Bryoria* phylogeography



Chapter 7 *Bryoria* phylogeography

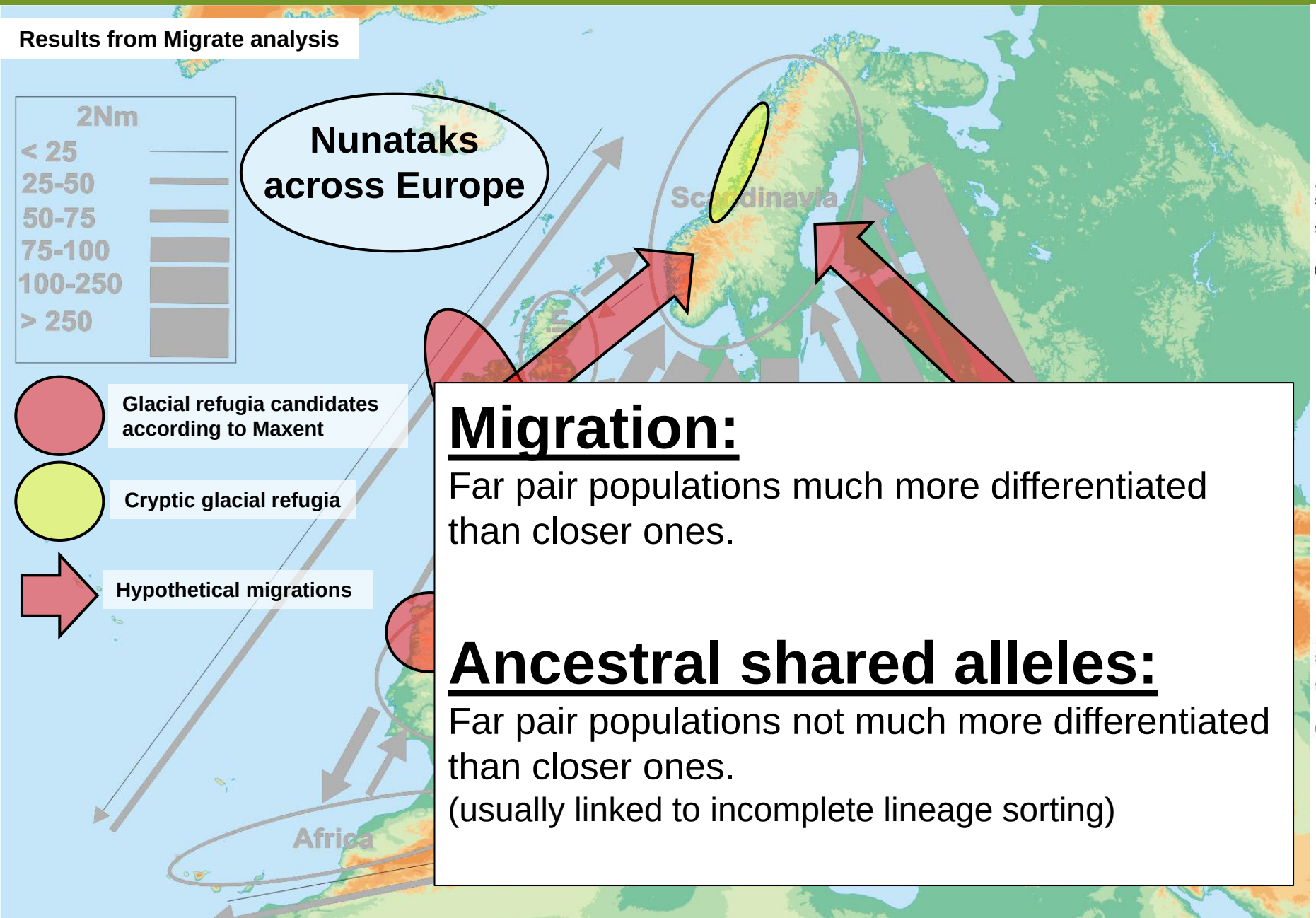


Chapter 7 *Bryoria* phylogeography



Chapter 7 *Bryoria* phylogeography

Results from Migrate analysis

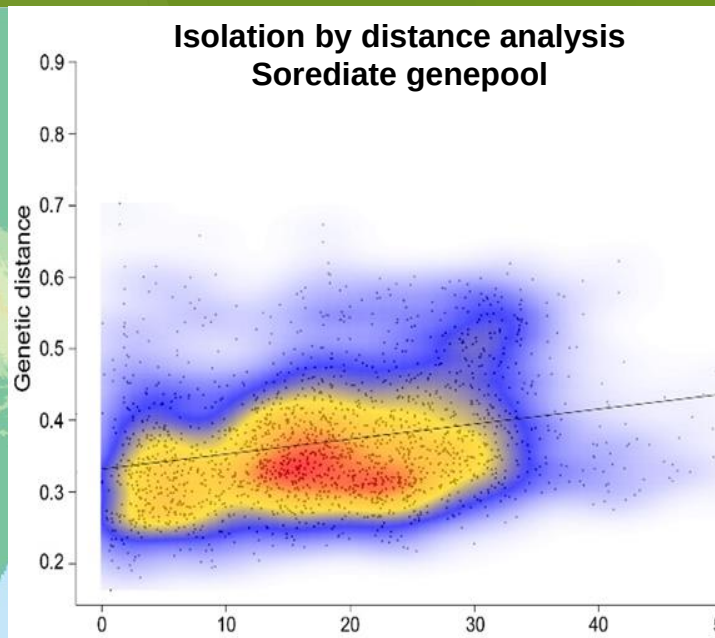


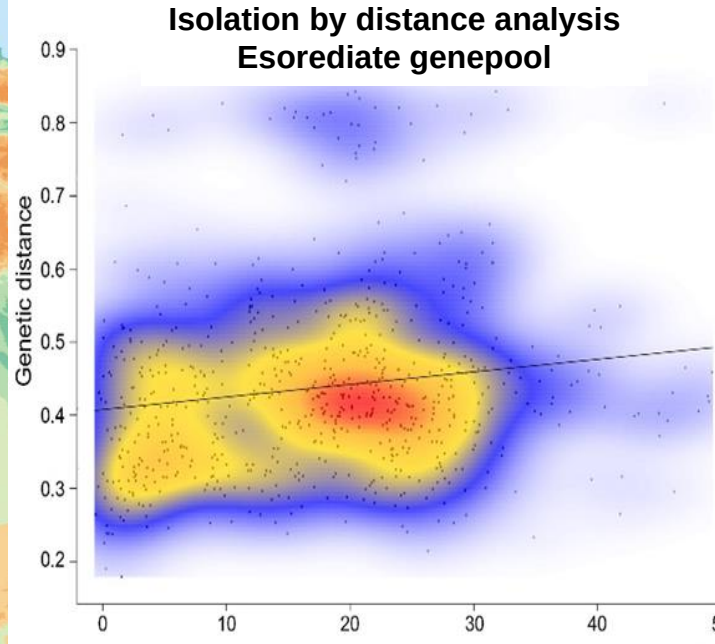
Migration:

Far pair populations much more differentiated than closer ones.

Ancestral shared alleles:

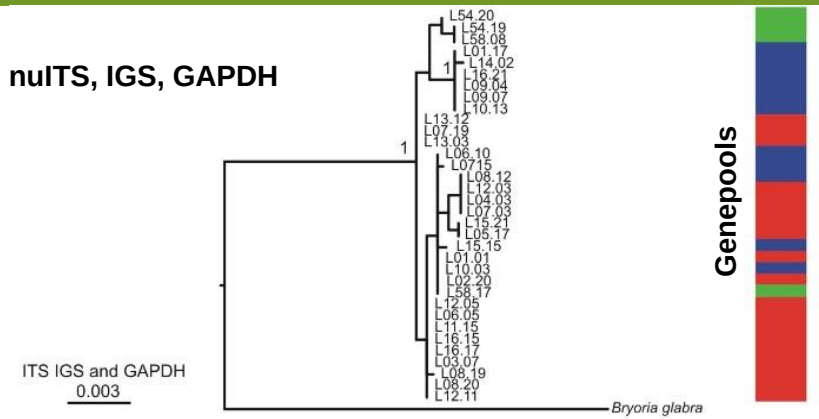
Far pair populations not much more differentiated than closer ones.
(usually linked to incomplete lineage sorting)



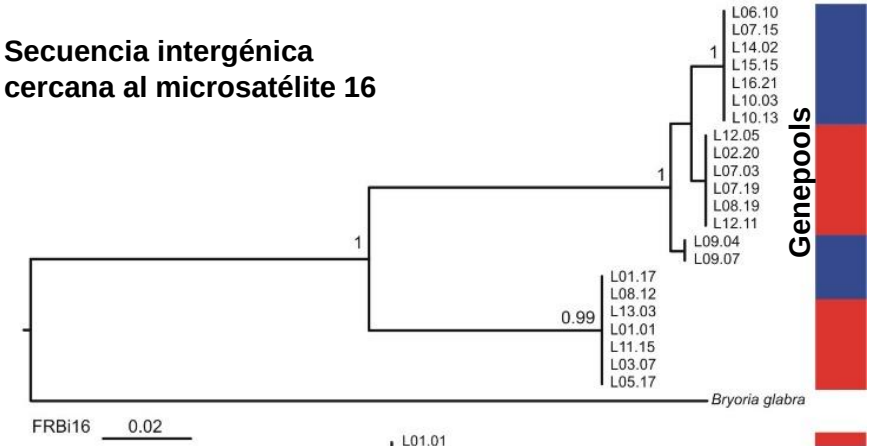


Chapter 7 *Bryoria* phylogeography

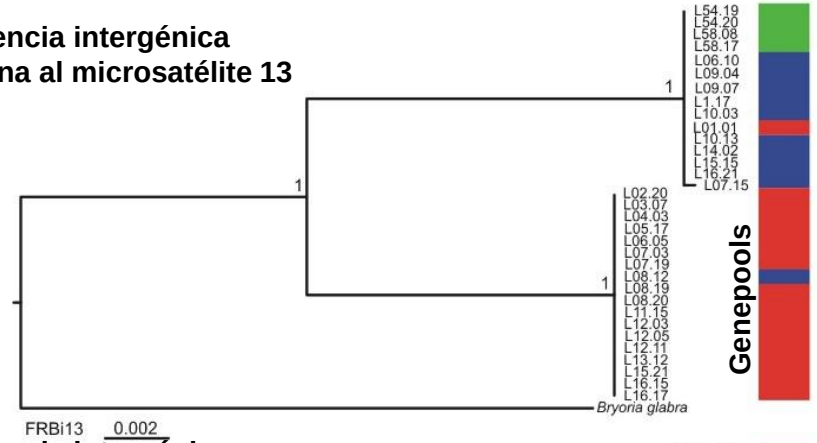
nuITS, IGS, GAPDH



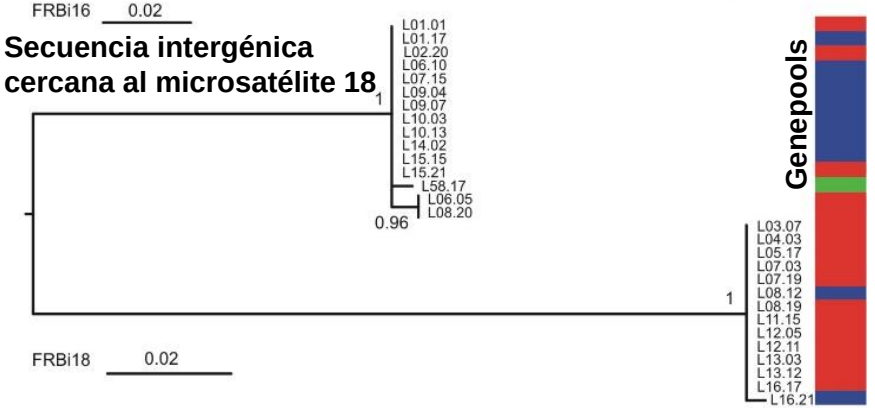
Secuencia intergénica cercana al microsatélite 16



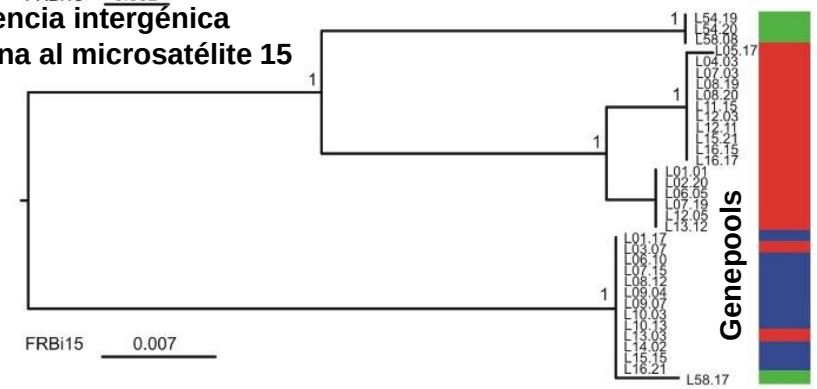
Secuencia intergénica cercana al microsatélite 13



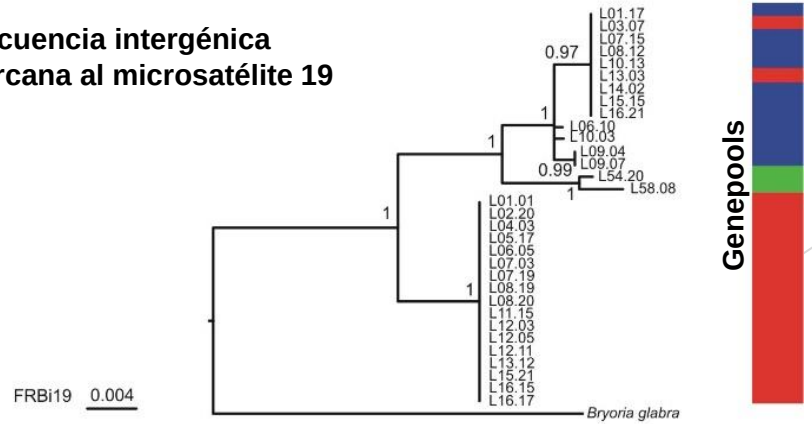
Secuencia intergénica cercana al microsatélite 18



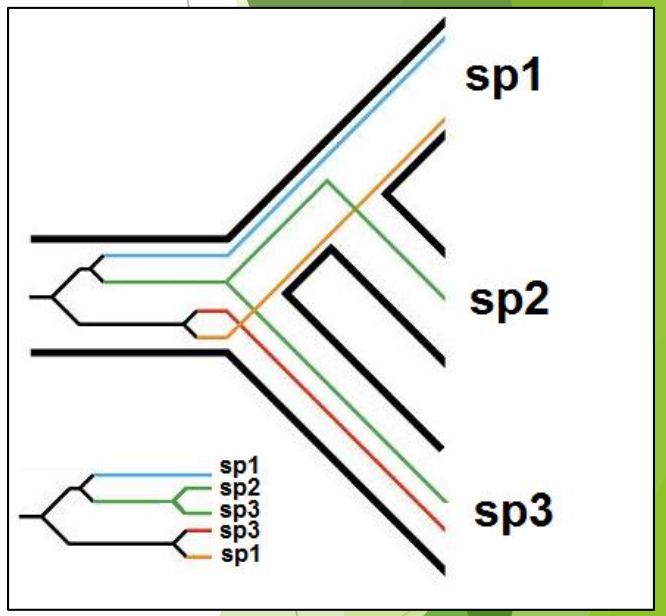
Secuencia intergénica cercana al microsatélite 15



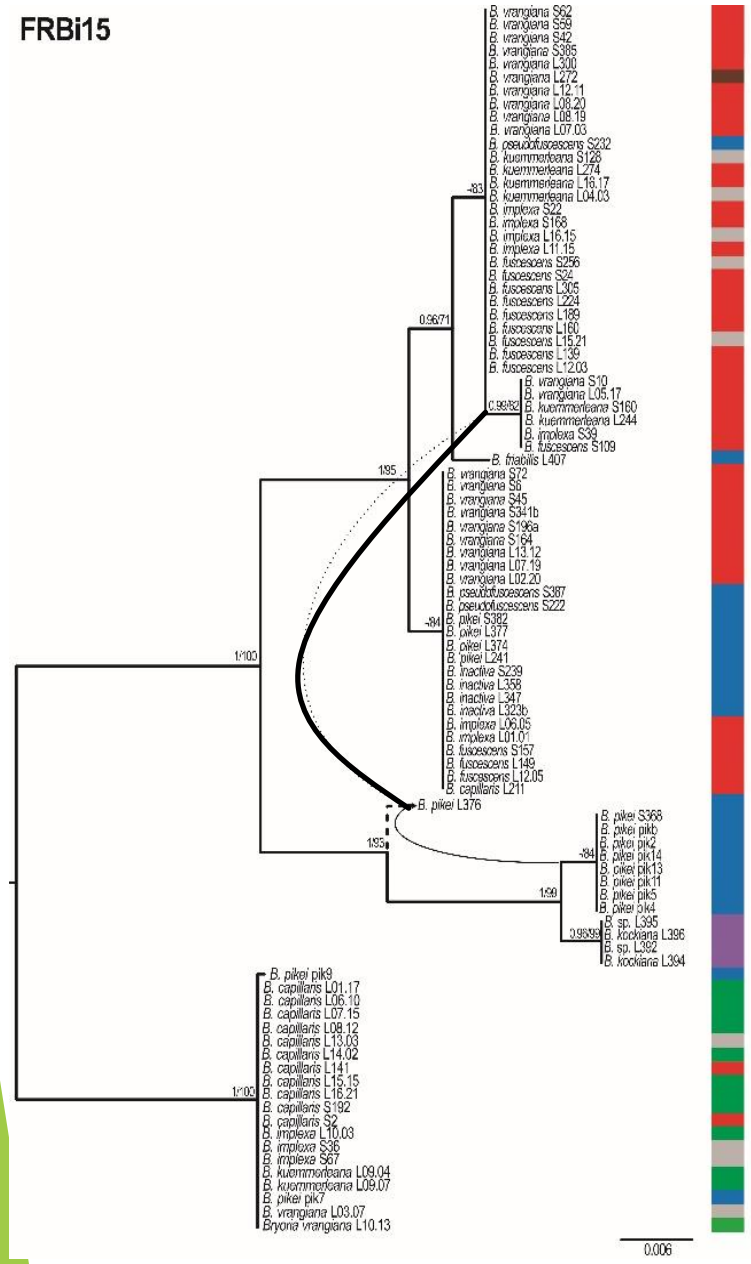
Secuencia intergénica cercana al microsatélite 19

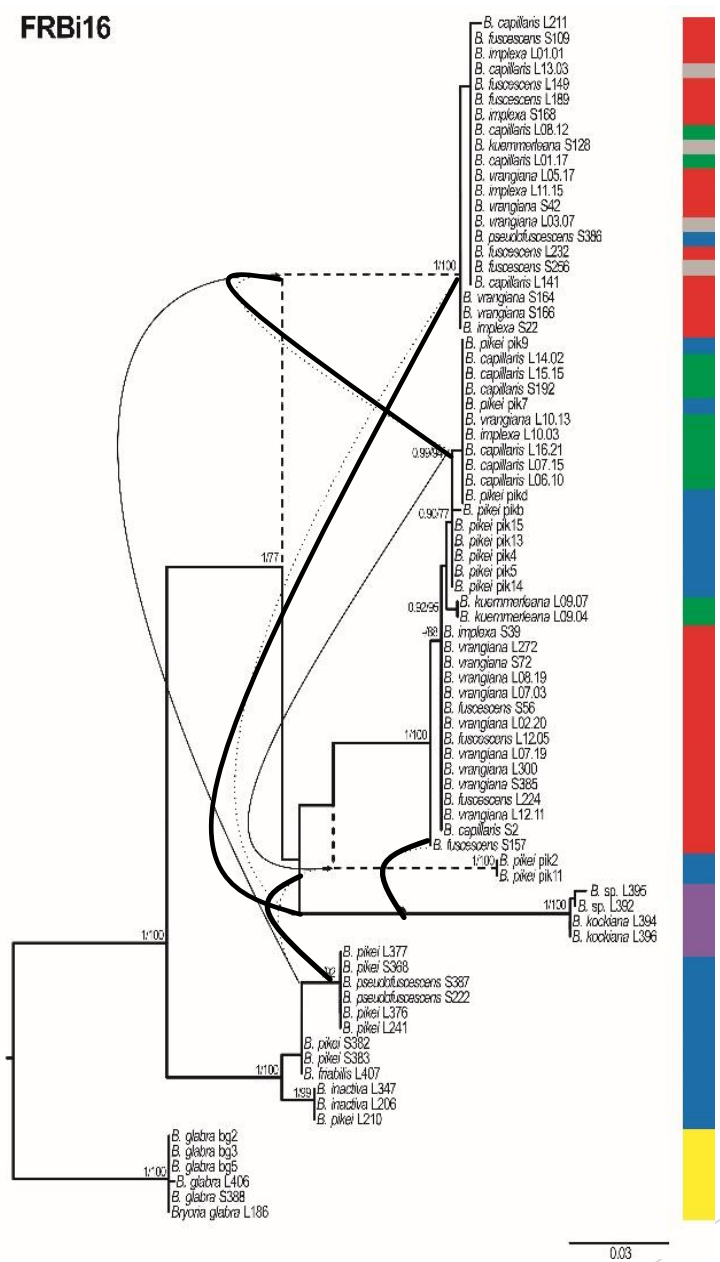


Evidence of incomplete lineage sorting



Chapter 7 *Bryoria* phylogeography



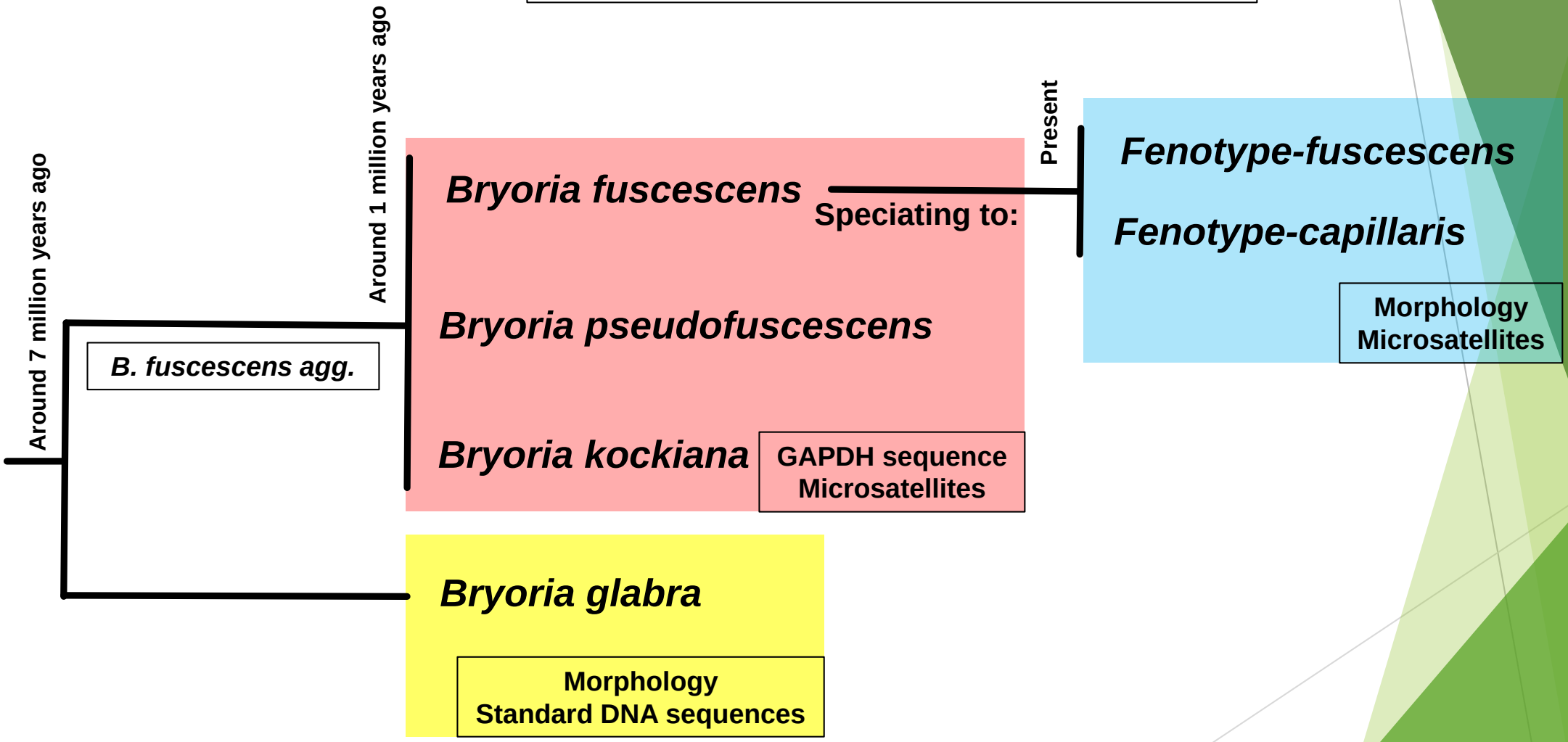


ML & Bayesian phylogenetic reconstruction of intergenic loci FRBi15 & FRBi16

Evidence of recombination

Chapter 7 *Bryoria* phylogeography

Evolutive conclusions



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.

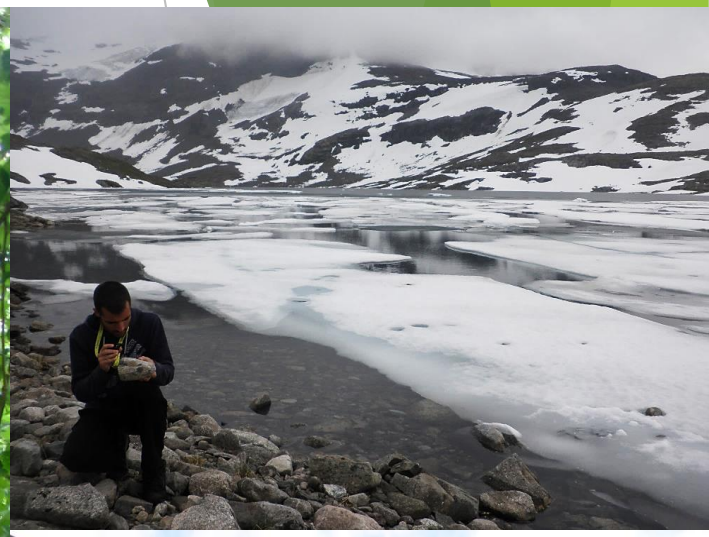
Main Conclusions

- **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:



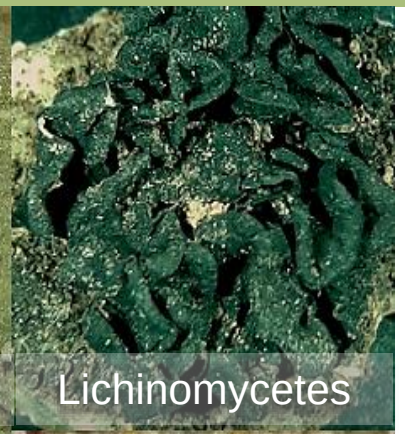



!Muchas gracias!

Introduction


Arthoniomycetes


Lecanoromycetes


Lichinomycetes


Arthoniomycetes


Lecanoromycetes


Sordariomycetes


Eurotiomycetes


Lecanoromycetes


Lecanoromycetes

Introduction

Sexual reproduction

Population sizes

Predators

Human interactions

Genetic & phenotypic diversity

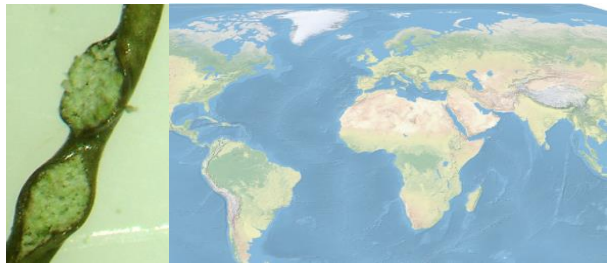
Species conservation

Basic knowledge

Photobiont

Parasities

Dispersal capacities



Environment

Environmental changues

Chapter 1 *Pseudephebe* species concept

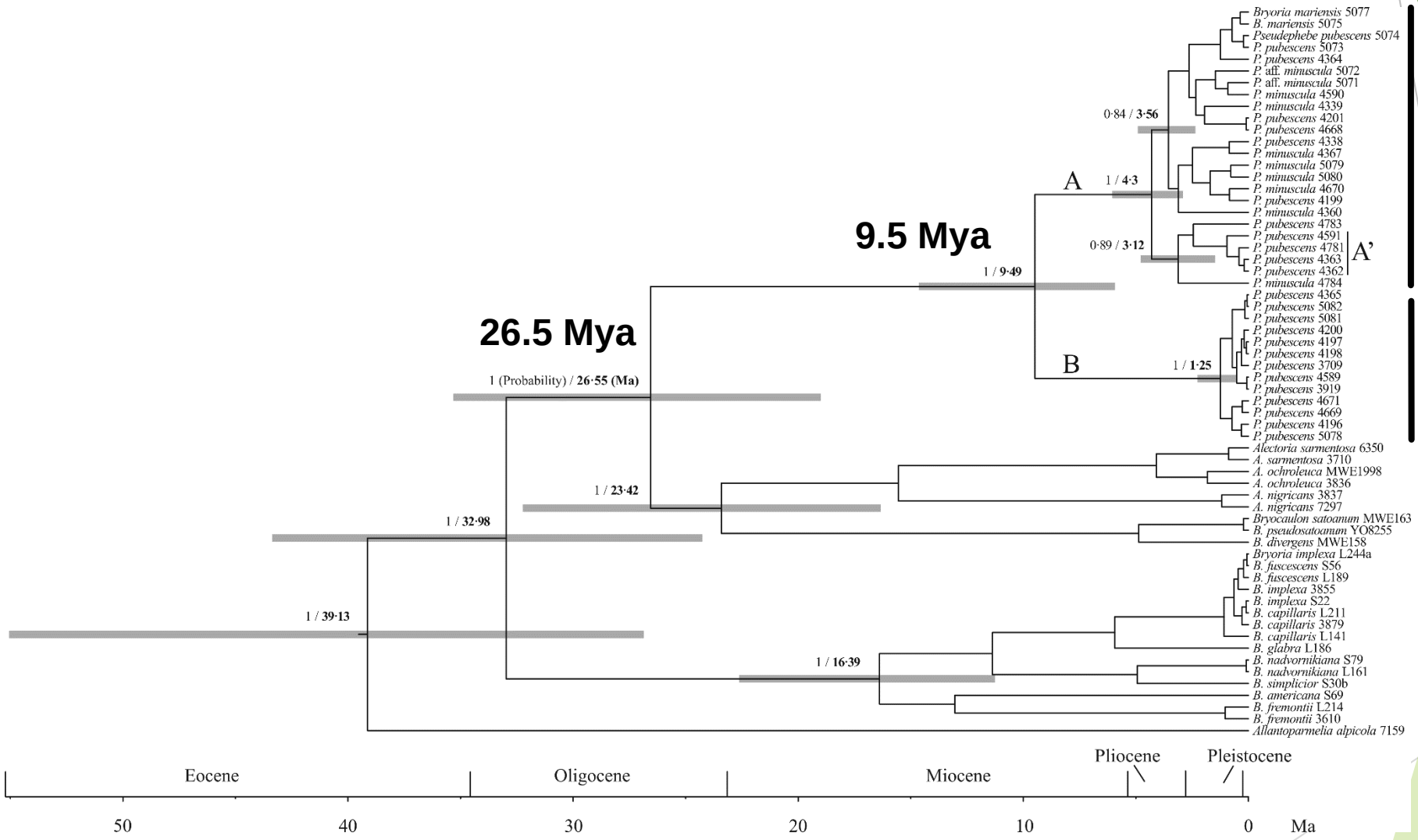
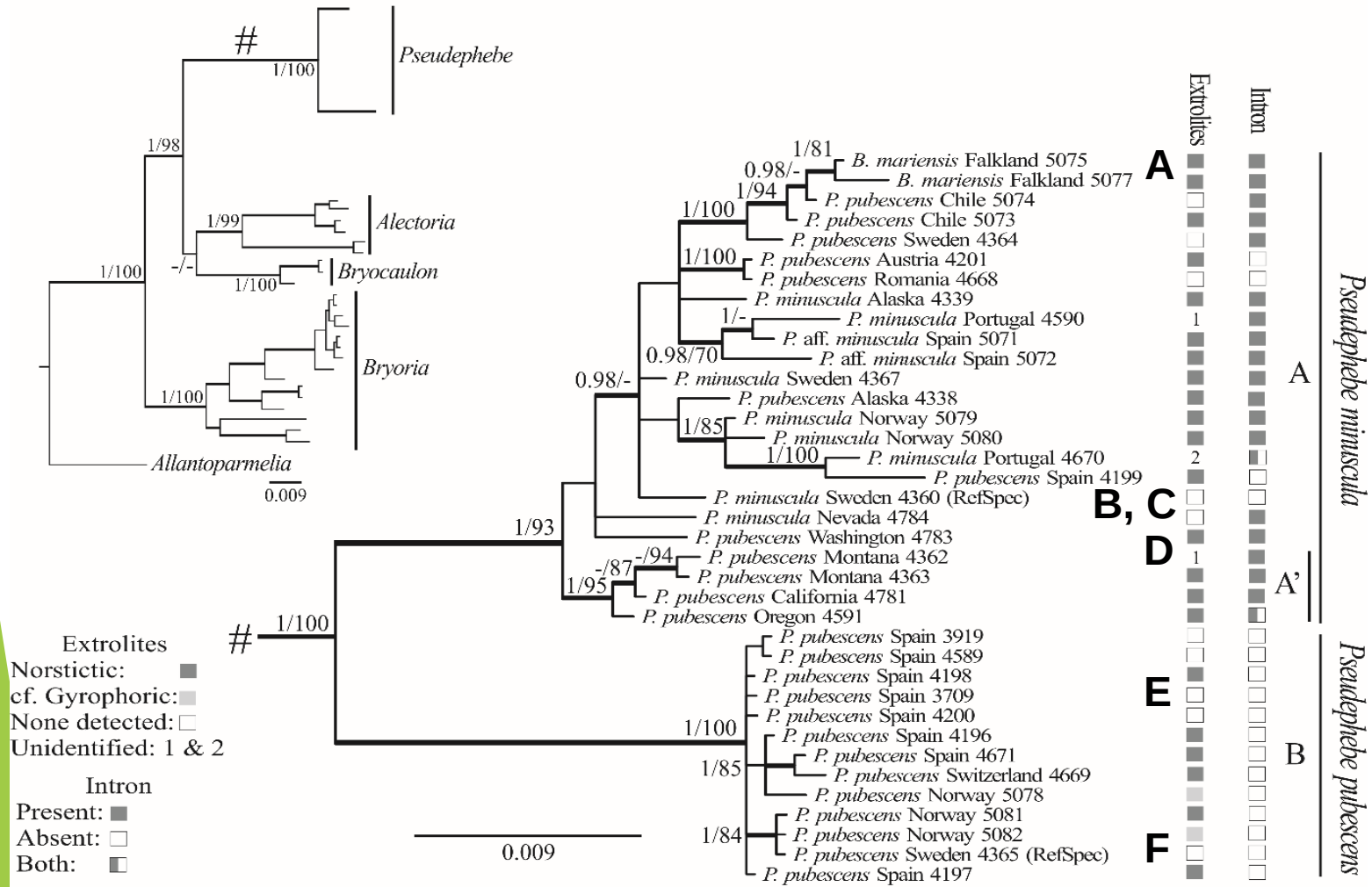


Fig. S1. Dated BEAST maximum clade credibility tree estimated from three-loci concatenated data. Grey bars indicating the 95% highest posterior density interval for the estimated divergence times. Posterior probabilities of interesting nodes and its divergence time as the mean posterior estimate of their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

Chapter 1 *Pseudephebe* species concept



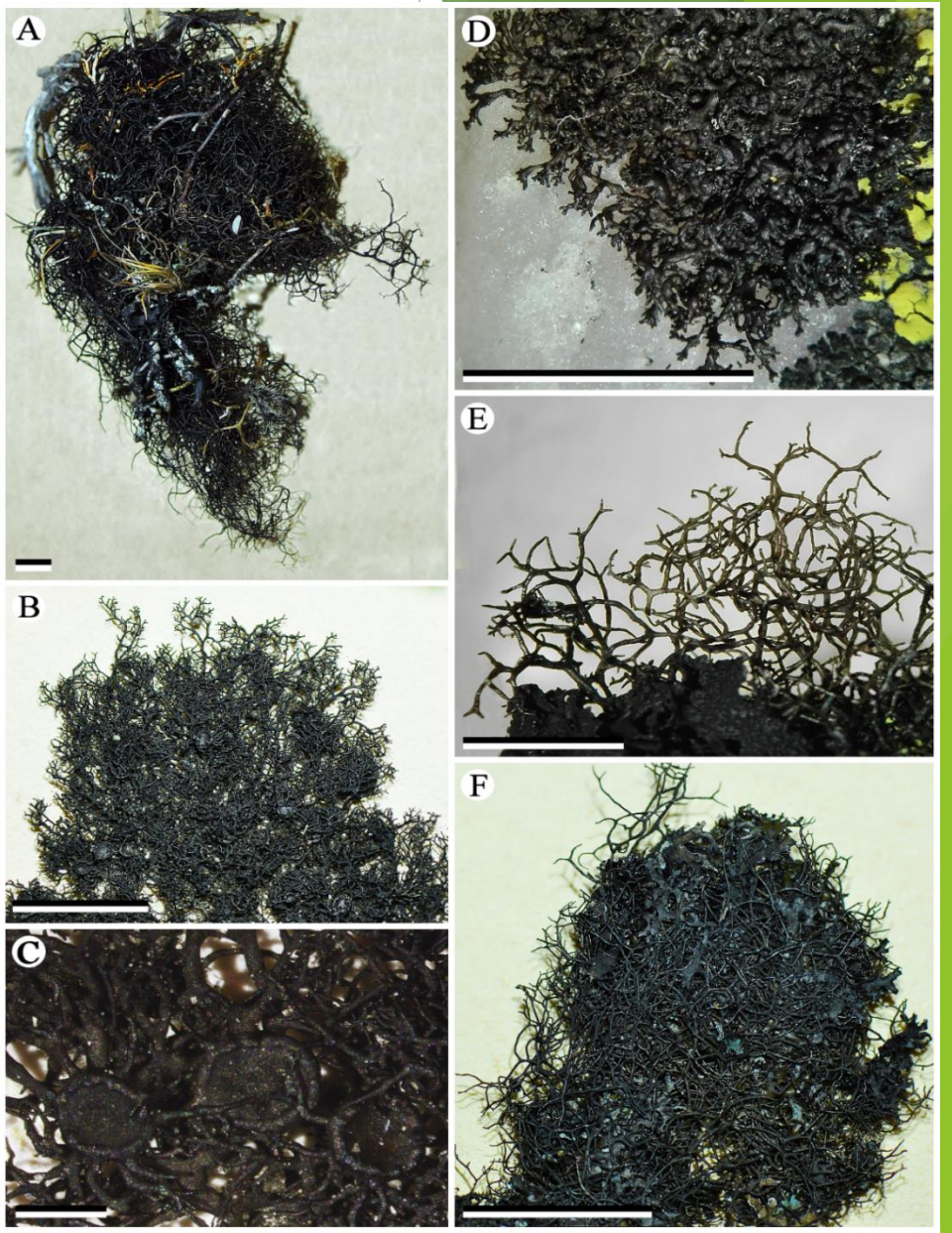


Fig. 1. Phylogenetic consensus tree based on ITS, RPB1 and MCM7 markers analyzed as a concatenated data matrix (Table1). Whole tree in the upper left corner using *Allantoparmelia* as outgroup, with the *Pseudephebe* clade shown in detail.Tree topology depicts the results of Bayesian inference, showing 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.