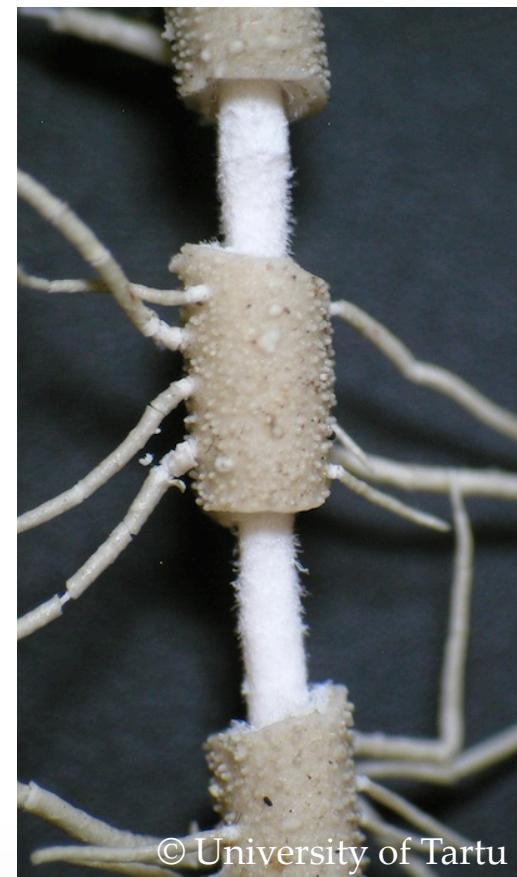


Untangling *Usnea*: Multi-locus analyses reveal recent diversification history and clusters of mixed morphospecies in the section *Usnea*

Kristiina Mark, Lauri Saag, Steven D. Leavitt, Susan
Will-Wolf, Matthew P. Nelsen, Tiiu Tõrra, Andres
Saag, Tiina Randlane, H. Thorsten Lumbsch

Genus *Usnea*

- ◆ Well-known and easily recognized by the yellowish beard-like thallus with central cord

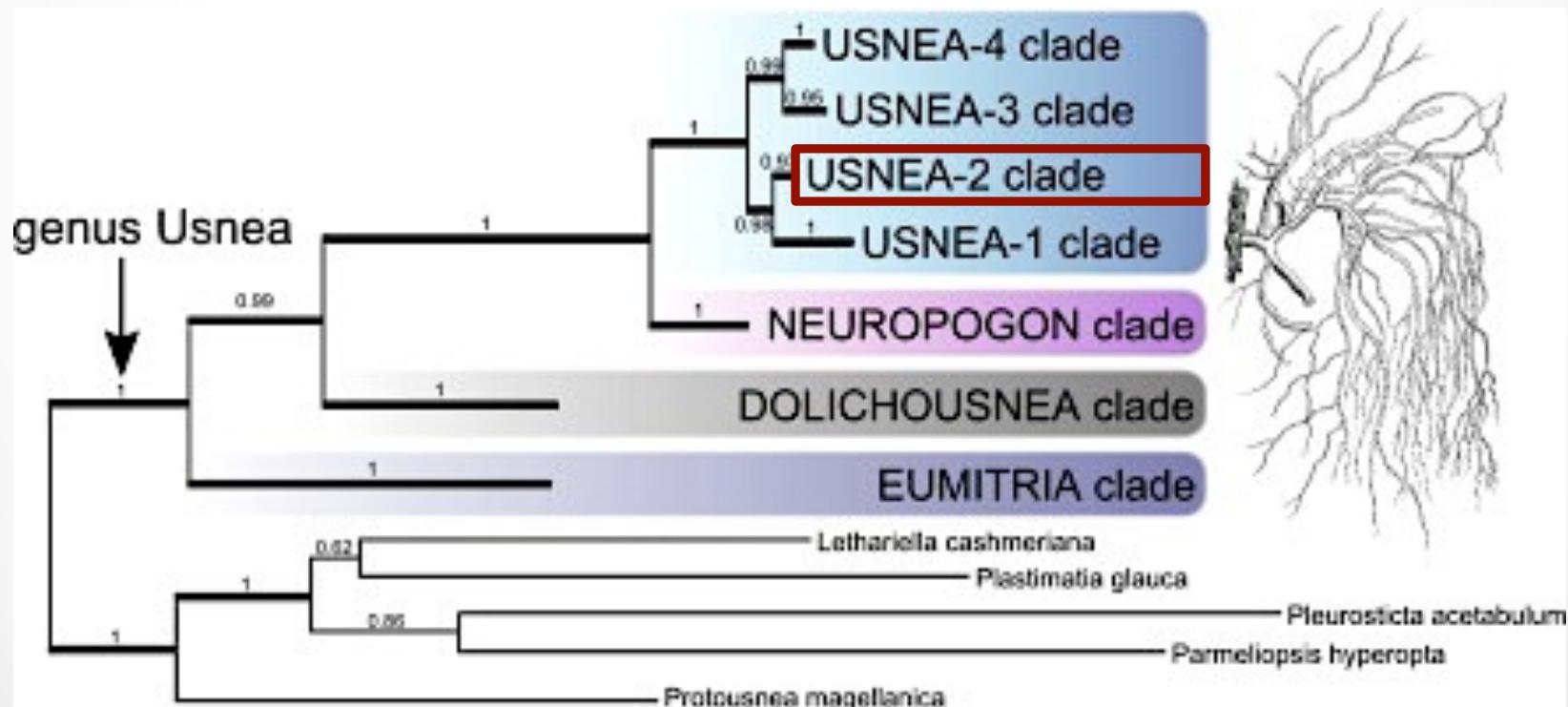


Genus *Usnea*

- ◆ One of the largest genera in the family Parmeliaceae (ca. 350 species)
- ◆ Many species non-monophyletic
- ◆ Taxonomy complicated due to a lack of easily recognisable characters and the use of homoplasic characters
- ◆ Categorised into four groups (or subgenera):
Dolichousnea, *Eumitria*, *Neuropogon*, and *Usnea*

Section *Usnea*

- ◆ Described by Ohmura (2001, 2002) and corresponds to the *Usnea*-2 clade in Truong et al. (2013)



© Truong et al. 2013

Section *Usnea*

- ◆ Includes the type species of the genus – *Usnea florida* – and its relatives (e.g. *U. barbata*, *U. dasopoga* (=*U. dasypoga*=*U. filipendula*), *U. subfloridana*, *U. wasmuthii*)
- ◆ Distributed across the Northern Hemisphere



Section *Usnea*

- ◆ Relatively short branch lengths and unresolved relationships between species
- ◆ A hypothesis of post-glacial rapid radiation in *Usnea*
- ◆ Incomplete lineage sorting (ILS) is common in young diverging groups, can result in conflicting genes, and thus, impede accurate estimation of phylogenetic relationships

Aims

- ◆ Identify evolutionarily independent lineages and elucidate relationships in sect. *Usnea* using genetic, morphological, and chemical data
- ◆ Investigate the utility of some traditionally used characters in *Usnea* species identification

Material and methods

- ◆ 144 specimens of 17 species from the section *Usnea*
- ◆ Collected from North America and Europe
- ◆ Six nuclear markers (ITS, IGS, *beta*-tubulin, MCM7, RPB1, and RPB2)
- ◆ Thin layer chromatography (TLC)
- ◆ Morphological and anatomical characters

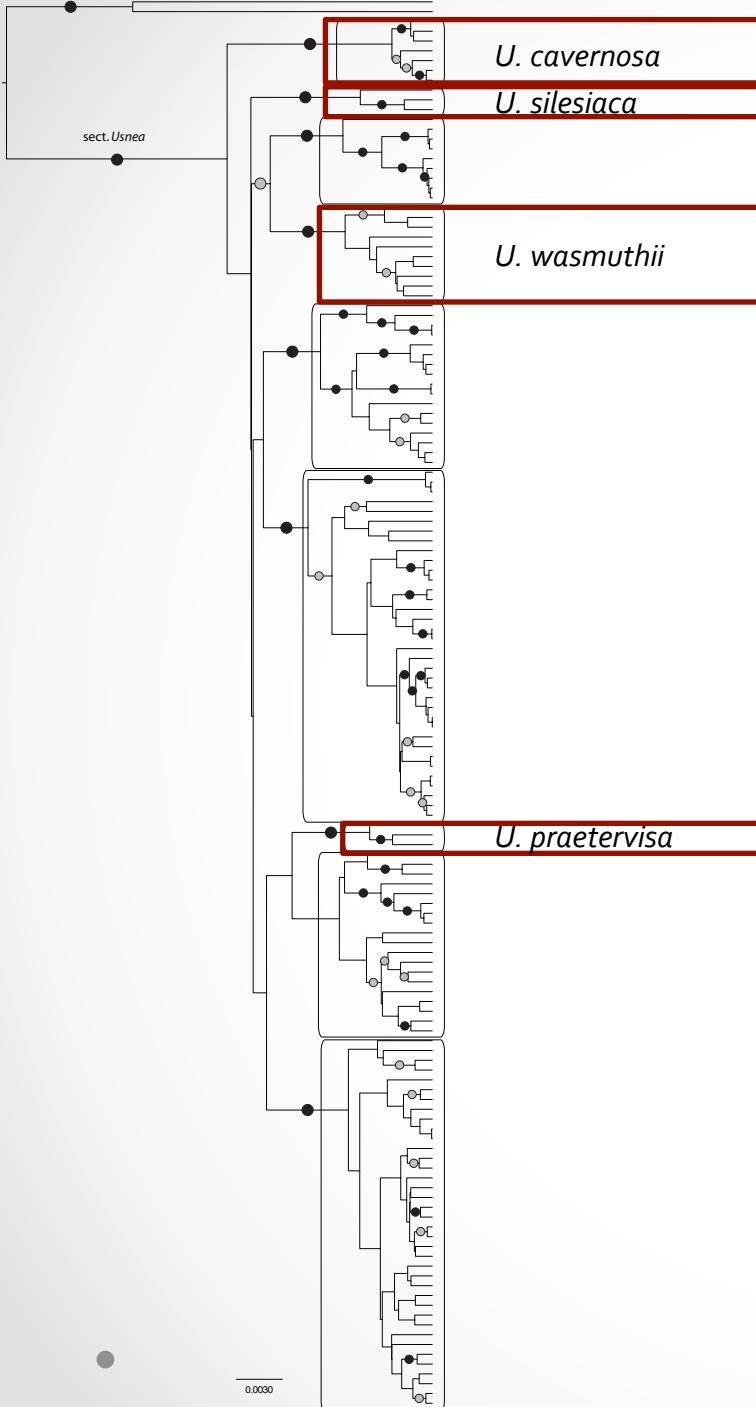
Material and methods

Phylogenetic analyses:

- ◆ **Tree reconstruction:** single-locus and concatenated analyses in Bayesian and Maximum likelihood (ML) frameworks (BEAST & RAxML)
- ◆ **Bayesian species delimitation** and species or minimal cluster tree (SMC-tree) analyses (STACEY and *BEAST)
- ◆ **Bayesian species validation** analyses (BP&P)

Results

- ◆ Weak topological structure and short branch lengths in single-locus trees
- ◆ Several distinct clades in concatenated and coalescent-based multi-locus analyses
- ◆ Four clades represented traditional morphology-based species:
 - *Usnea cavernosa*
 - *U. praetervisa*
 - *U. silesiaca*
 - *U. wasmuthii*



Monophyletic species

Usnea cavernosa

- Pendulous
- Base pale
- Foveolate brances
- Fibrils, papillae, soralia, isidiomorphs absent
- Cortex shiny, thin, medulla thick
- Salazinic acid



Usnea silesiaca

- Shrubby to pendulous
- Base black
- Soralia transversely elliptical when mature
- Cortex very thick, medulla very thin and dense
- Salazinic acid



Usnea wasmuthii

- Shrubby
- Base black with cracks
- Soralia oblong-cylindrical Cortex thick, medulla thin and dense
- Barbatic acid (!)



Usnea praetervisa

- Shrubby to subpendent
- Base black
- Soralia minute to confluent, with isidiomorphs
- Cortex thick, medulla thin and dense
- Norstictic + stictic or connorstictic acids



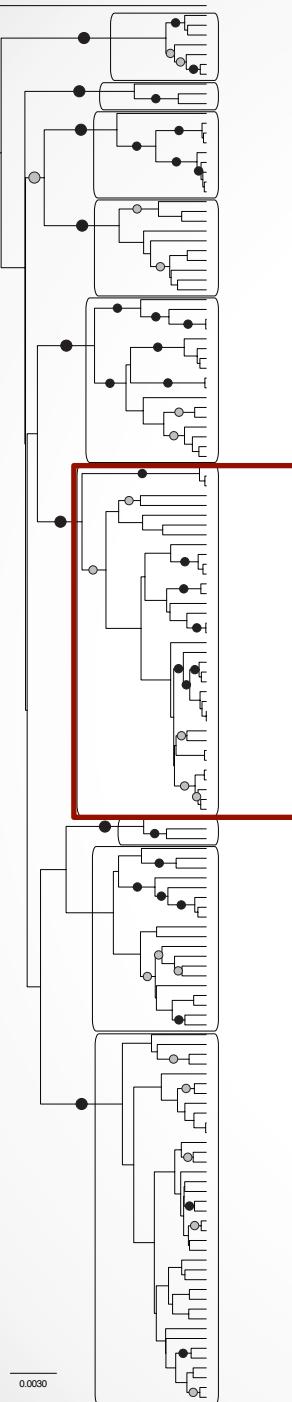
Results

Clusters of two or more species:

- ◆ *Usnea florida* – *U. subfloridana*
- ◆ *Usnea fulvoreagens* – *U. glabrescens* – *U. pacificana*
- ◆ *Usnea barbata* – *U. chaetophora* – *U. dasopoga* – *U. diplotypus*
- ◆ *Usnea barbata* – *U. intermedia* – *U. lapponica* – *U. substerilis*

Usnea florida – *U. subfloridana*

sect. *Usnea*



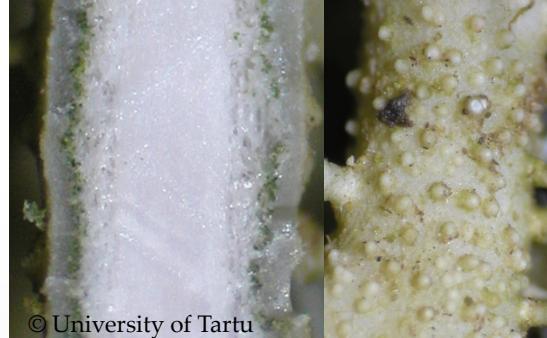
<i>U. florida</i>	<i>U. subfloridana</i>
Shrubby	Shrubby to subpendent
Base black	Base black
Sexual: apothecia	Asexual: punctiform to enlarged soralia with isidiomorphs
Cortex thick, medulla thin and dense	Cortex thick, medulla thin and dense
Thamnolic or squamatic acid	Thamnolic or squamatic acid
Distribution restricted to old forests, threatened	Common over the Northern Hemisphere



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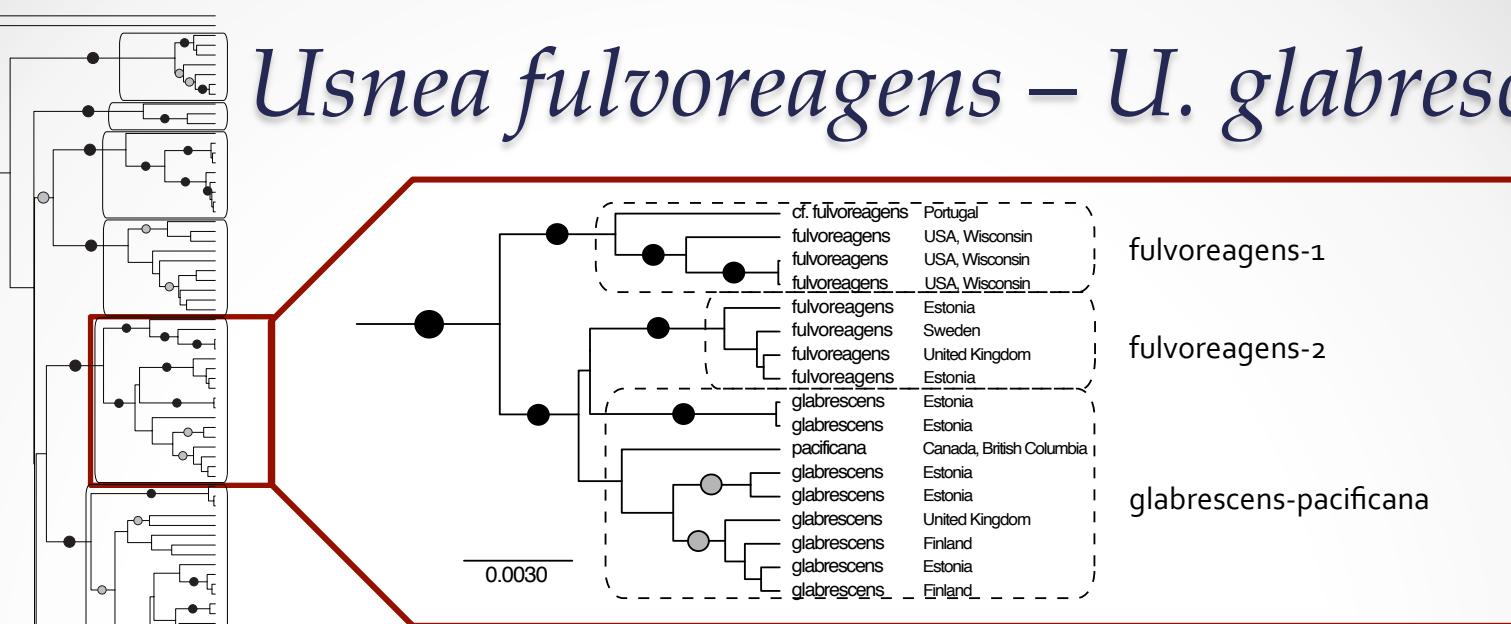
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Usnea fulvoreagens – *U. glabrescens*

sect. *Usnea*



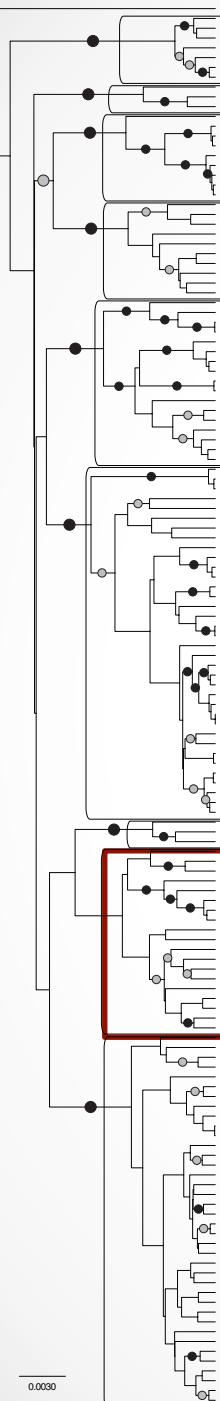
<i>U. fulvoreagens</i>	<i>U. glabrescens</i>	<i>U. pacifica</i>
Shrubby	Shrubby	Shrubby to subpendent
Base black	Base black	Base black
Soralia irregular, excavate when mature	Soralia punctiform at young, larger and round when mature	Soralia minute and with isidiomorphs
Cortex thick, medulla thin and dense	Cortex thick, medulla thin and dense	Cortex thick, medulla thin and dense
Norstictic acid	Norstictic +/- salazinic acids	Baeomycesic, barbatic, and squamatic acids



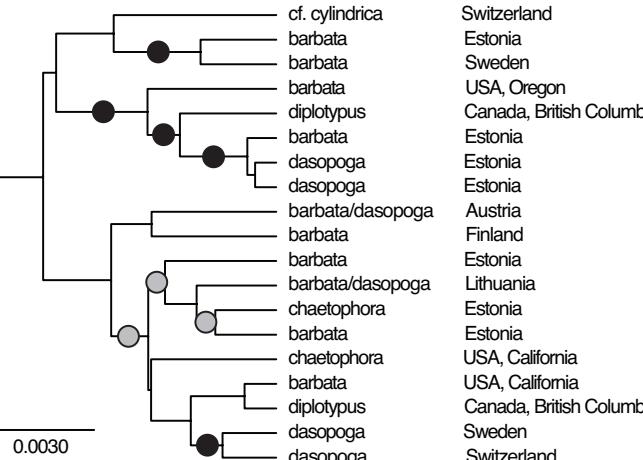
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Usnea dasopoga clade

sect. *Usnea*



<i>U. barbata</i>	<i>U. chaetophora</i>	<i>U. dasopoga</i>	<i>U. diplotypus</i>
Pendent	Pendent	Pendent	Shrubby to subpendent
Base mainly black	Base black	Base black	Base mainly black
Branches with depressions and ridges	Branches divided into segments by annular cracks	Branches gradually tapering	Branches with depressions and ridges
Soralia punctiform to irregular with isidiomorphs	Soralia punctiform, few	Soralia punctiform at young, larger and round when mature	Soralia punctiform with isidiomorphs
Cortex thick to very thick, medulla thin and dense to compact	Cortex thick, medulla thin and dense to compact	Cortex thick, medulla thin and dense to compact	Cortex thick, medulla thin with variable density
Salazinic acid	Salazinic acid	Salazinic acid	Salazinic acid

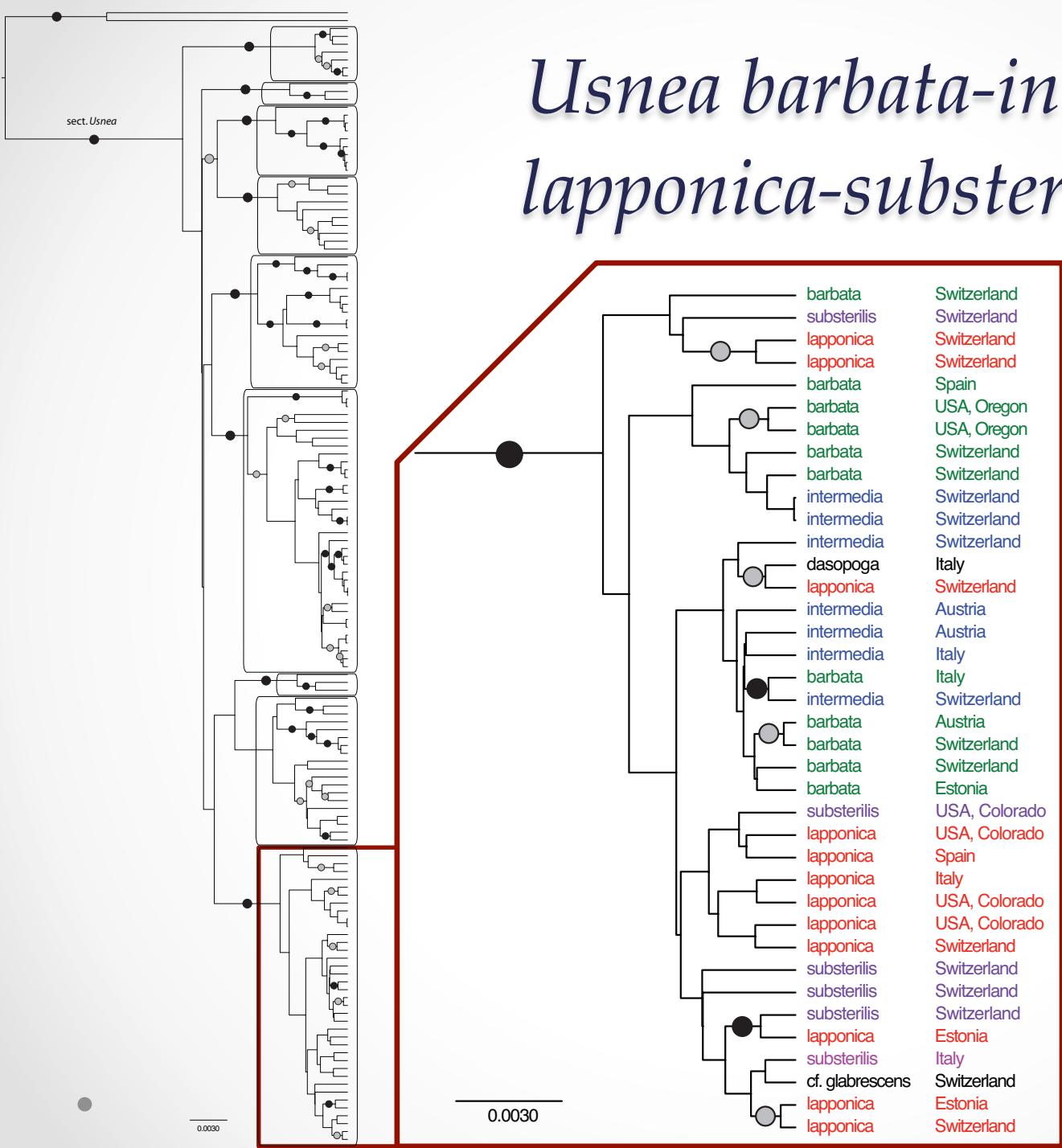


◆ Clade with DIVERSE MORPHOLOGY: shrubby to pendent, isidia and papillae absent to abundant, few to many minute to enlarged soralia, usually with isidiomorphs

◆ Further studies needed

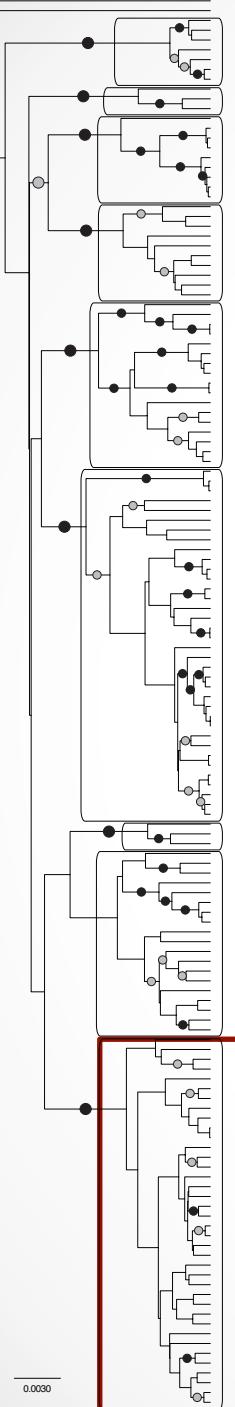


Usnea barbata-intermedia-lapponica-substerilis clade



Usnea barbata-intermedia-lapponica-substerilis clade

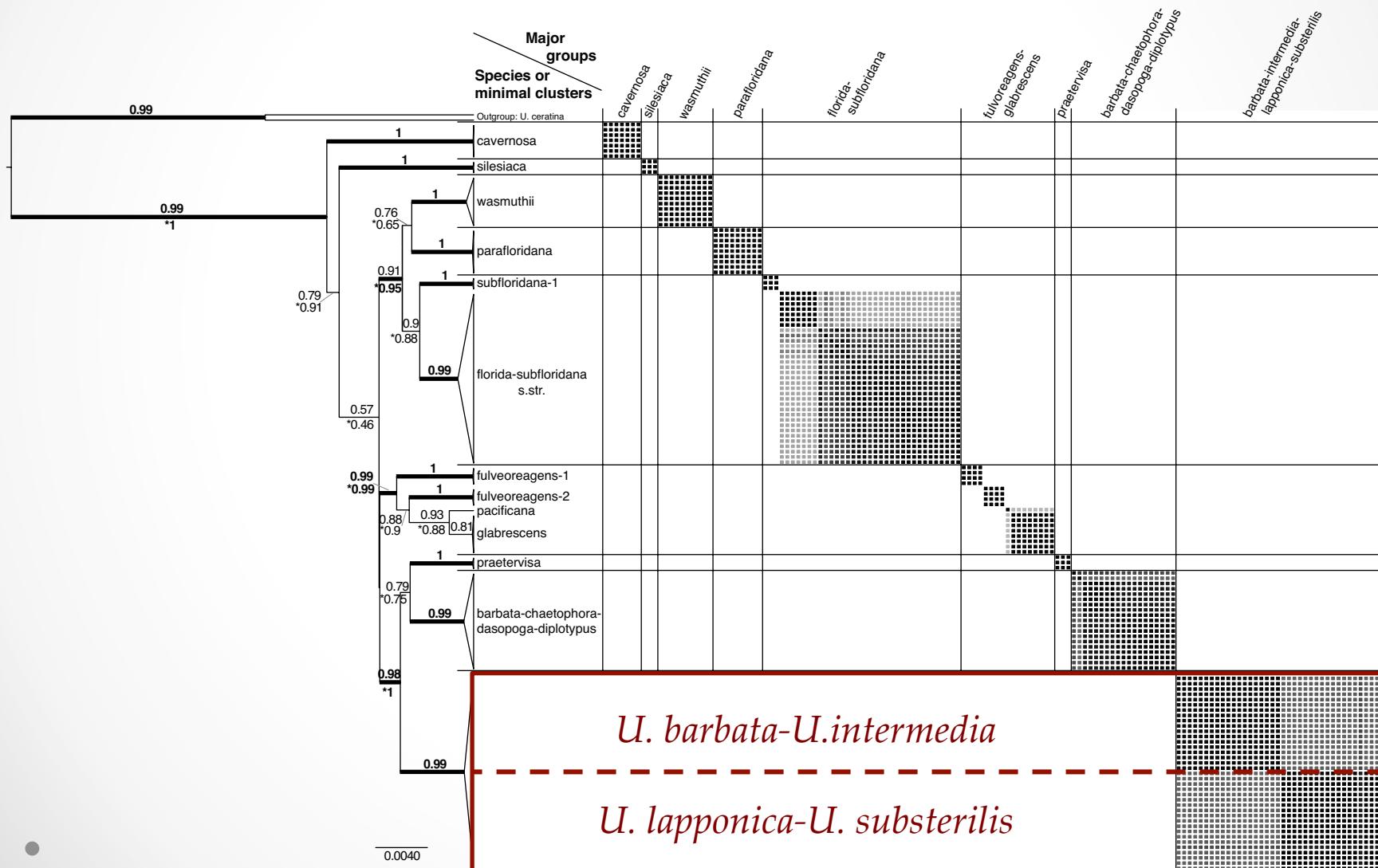
sect. *Usnea*

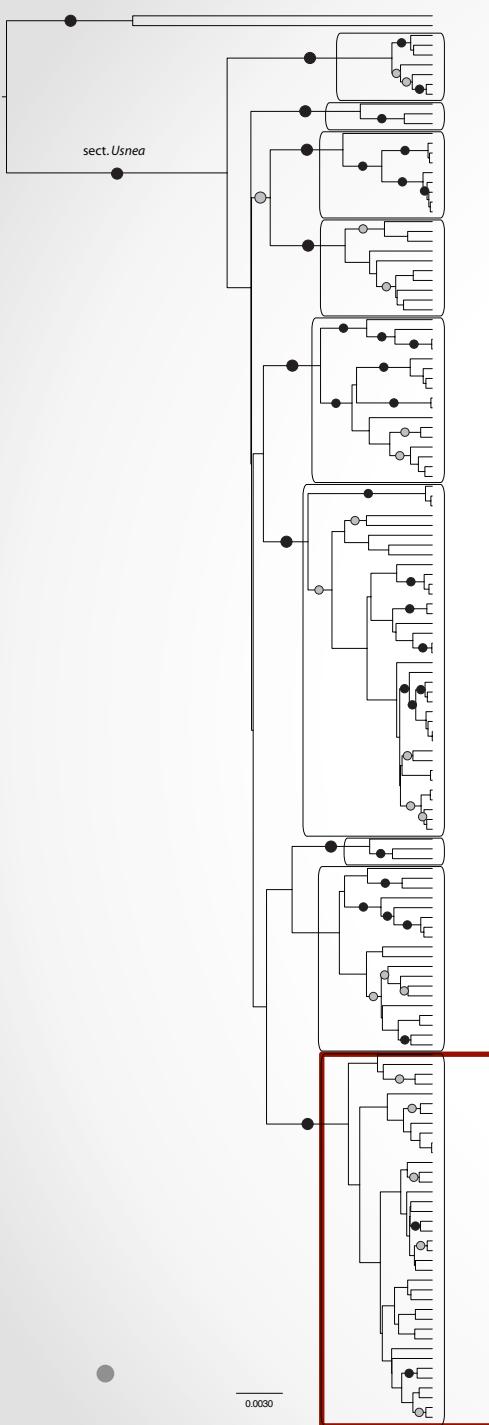


<i>U. barbata</i>	<i>U. intermedia</i>	<i>U. lapponica</i>	<i>U. substerilis</i>
Pendent	Shrubby	Shrubby	Shrubby
Base mainly black	Base mainly black	Base mainly black	Base mainly black
Branches with depressions and ridges	Branches with depressions and ridges	Branches with depressions and ridges	Branches with depressions and ridges
Soralia punctiform to irregular with isidiomorphs	Sexual: apothecia	Soralia large and irregular to bracelet-like, flat to concave	Soralia irregular, tuberculate to excavate with isidiomorphs
Cortex thin to moderately thick, medulla moderately thin and lax	Cortex thin, medulla moderately thin and lax	Cortex thin to moderately thick, medulla moderately thin and lax	Cortex thin to moderately thick, medulla moderately thin and lax
Salazinic and/or pannaric acid	Salazinic acid	Salazinic, pannaric and/or psoromic acids	Salazinic and/or pannaric acids



Species delimitation clusters barbata-intermedia-lapponica-substerilis as one species but shows a slight difference between *U. barbata*-*U. intermedia* and *U. lapponica*-*U. substerilis*





Usnea lapponica and *U. substerilis*

- ◆ Considered as closely related morphospecies; identical in studied molecular markers
- ◆ Taxa differ in the shape of soralia and presence/absence of isidiomorphs
- ◆ Differences in the soralium morphology can be explained with the development of soralia and erosion of isidiomorphs
- ◆ We propose synonymization of *U. substerilis* under *U. lapponica*

<i>U. lapponica</i>	<i>U. substerilis</i>
Shrubby	Shrubby
Base mainly black	Base mainly black
Branches with depressions and ridges	Branches with depressions and ridges
Soralia large and irregular to bracelet-like, flat to concave	Soralia irregular, tuberculate to excavate with isidiomorphs
Cortex thin to moderately thick, medulla moderately thin and lax	Cortex thin to moderately thick, medulla moderately thin and lax
Salazinic acid	Salazinic acid



sect. Usnea

0.0030

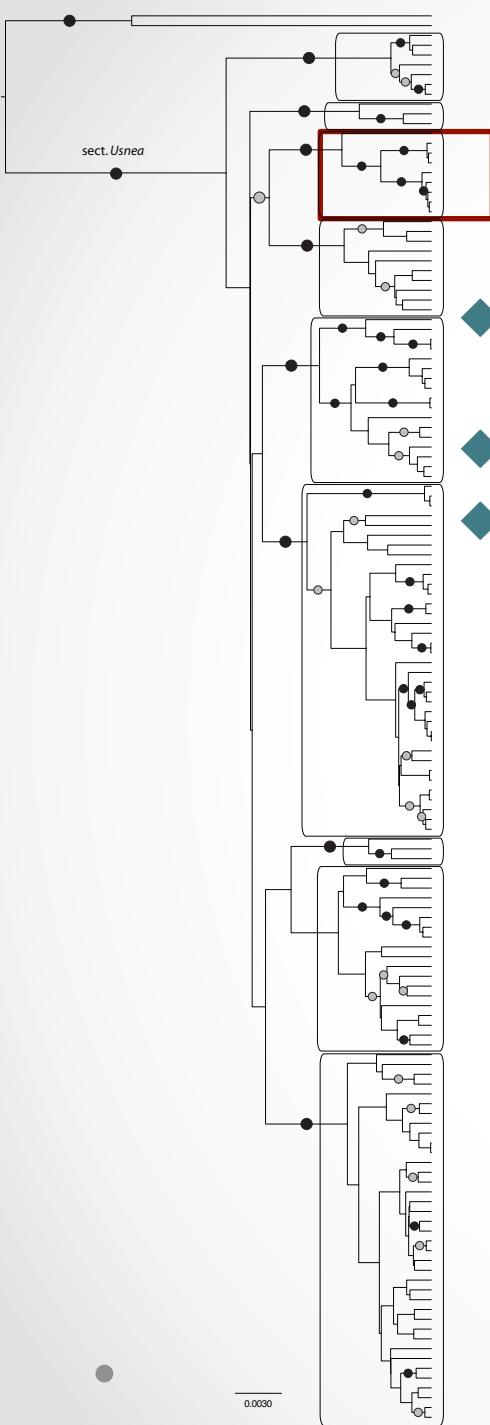
Usnea barbata and *U. intermedia*

<i>U. barbata</i>	<i>U. intermedia</i>
Pendent	Shrubby
Base mainly black	Base mainly black
Branches with depressions and ridges	Branches with depressions and ridges
Soralia punctiform to irregular with isidiomorphs	Sexual: apothecia
Cortex thin to moderately thick, medulla moderately thin and lax	Cortex thin, medulla moderately thin and lax
Salazinic acid	Salazinic acid



◆ Similar situation as in the *U. florida-U. subfloridana* clade: sexual vs. asexual species

◆ Synonymization could be proposed after further studies in the *U. barbata* complex

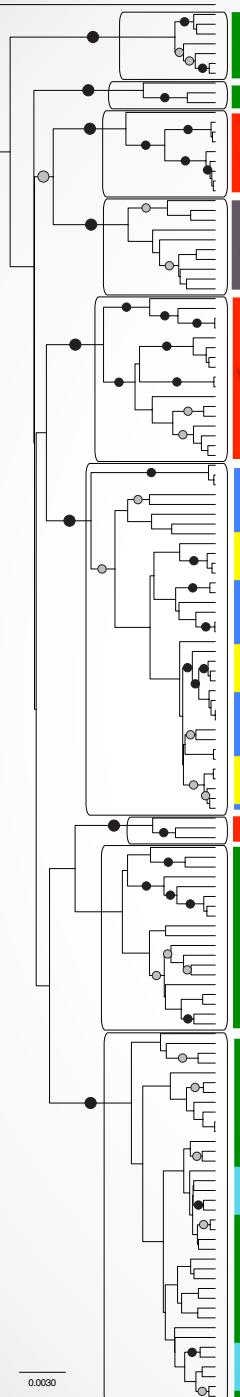


New species: *Usnea parafloridana*

- ◆ Resembles *Usnea subfloridana* or *U. praetervisa*
- ◆ Chemically *U. glabrescens*
- ◆ Genetically related to *U. wasmuthii*

- Shrubby
- Base black
- Few and thick branches
- Soralia punctiform to enlarged with isidiomorphs
- Cortex thick, medulla thin and compact
- Norstictic +/- salazinic acids
- Known from USA, Wisconsin in old, humid forests

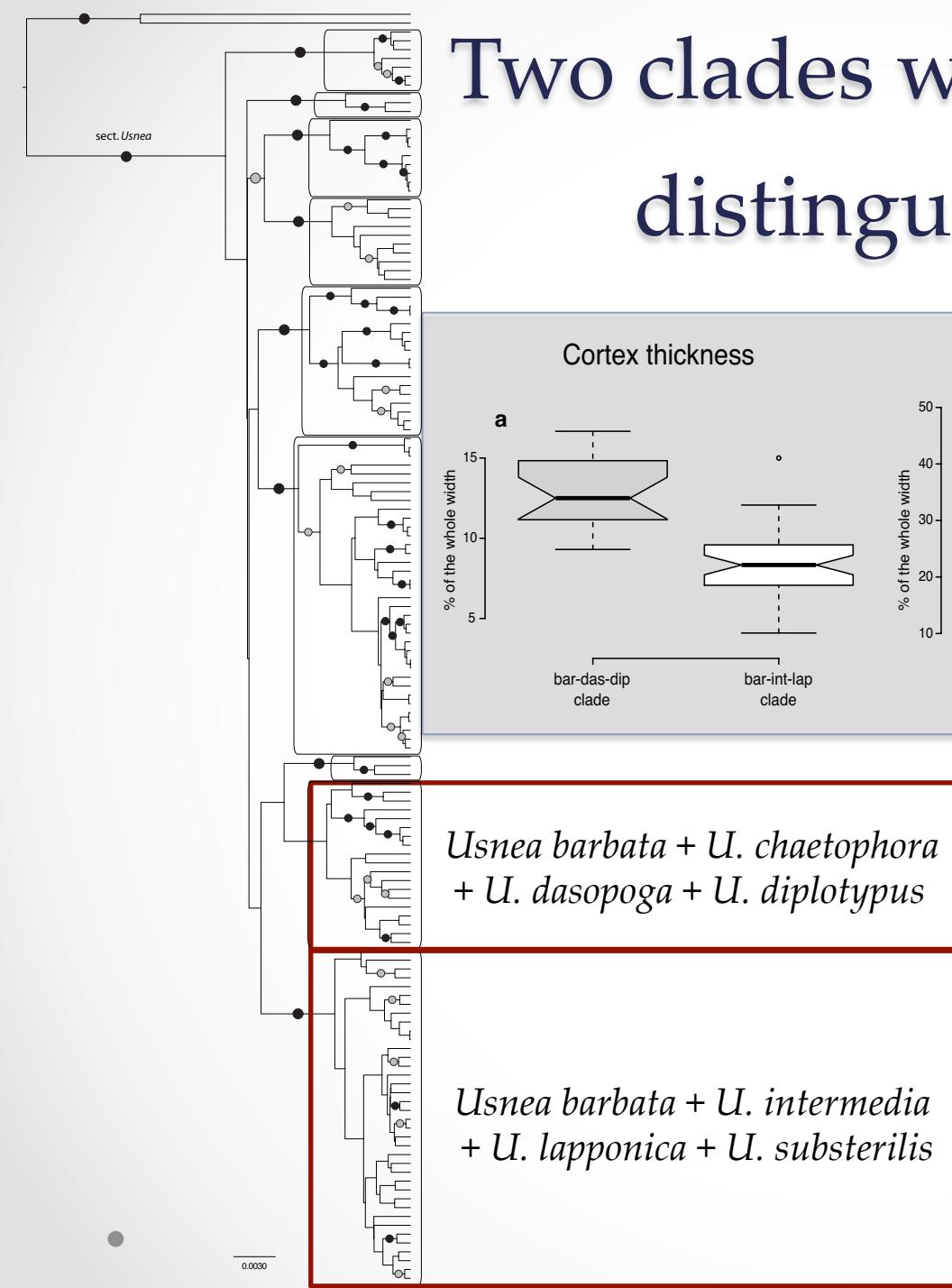


sect. *Usnea*

Taxon or group	Chemistry		
<i>U. cavernosa</i>	Salazinic acid		
<i>U. silesiaca</i>	Salazinic acid		
<i>U. parafloridana</i>	Norstictic acid	+/-	Salazinic acid
<i>U. wasmuthii</i>	Barbatic acid		
<i>U. fulvoreagens</i>	Norstictic acid	+/-	Stictic acid complex
<i>U. glabrescens</i>	Norstictic acid	And	Salazinic acid
<i>U. pacifica</i>	Baeomycesic acid	And	Barbatic and squematic acids
<i>U. florida-U. subfloridana</i>	Thamnolic acid	Or	Squematic acid
<i>U. praetervisa</i>	Norstictic acid	And	Stictic acid
<i>U. dasopoga</i> clade	Salazinic acid		
<i>U. barbata-U. intermedia-U. lapponica-U. substerilis</i>	Salazinic acid	And/Or	Pannaric or psoromic acid

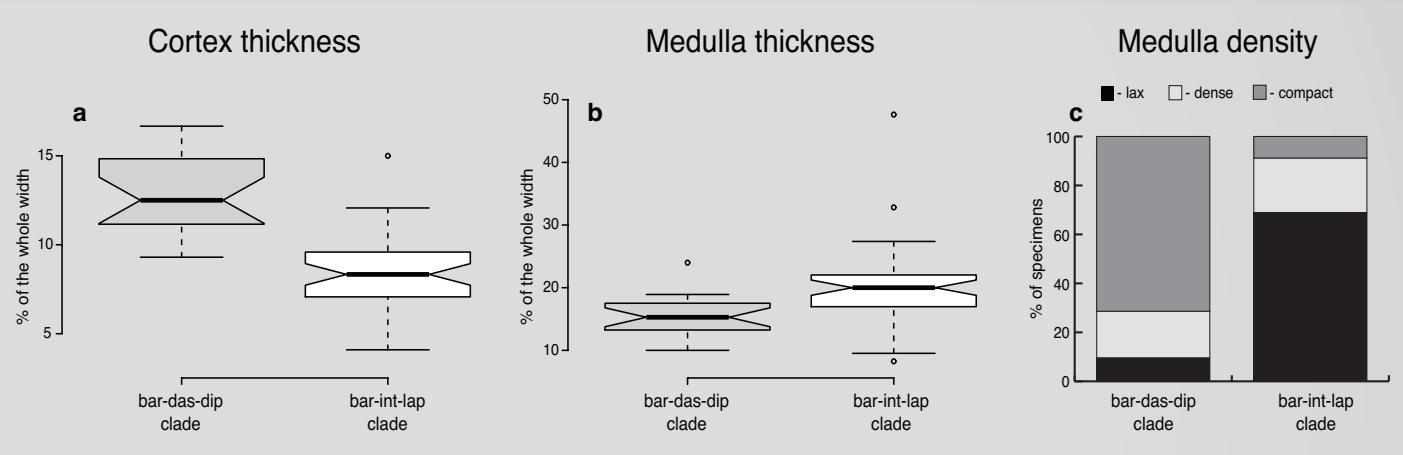
Lichen chemistry generally correlated to genetic clades in sect. *Usnea*

Two clades with cf. *Usnea barbata* distinguish in anatomy



Usnea barbata + *U. chaetophora*
+ *U. dasopoga* + *U. diplotypus*

Usnea barbata + *U. intermedia*
+ *U. lapponica* + *U. substerilis*



Cortex thicker,
medulla thin and
more dense



Cortex thinner,
medulla moderately
thin and lax



Conclusions

- ◆ Only four of 17 *Usnea* species proved monophyletic:
 - *U. cavernosa*
 - *U. praetervisa*
 - *U. silesiaca*
 - *U. wasmuthii*
- ◆ New species – *Usnea parafloridana* – currently known from northern Wisconsin (USA) but probably with wider distribution. Semi-cryptic – TLC and genotyping may be needed for correct identification

Conclusions

- ◆ Four clades where two or more morphospecies were grouped:
 - *Usnea florida* – *U. subfloridana*
 - ➔ species are conspecific but formal shift affects *U. florida* conservation
 - *Usnea fulvoreagens* – *U. glabrescens* – *U. pacificana*
 - ➔ *U. fulvoreagens* is polyphyletic with two cryptic species
 - ➔ *U. glabrescens* is paraphyletic with *U. pacificana* within
 - *Usnea dasopoga* clade
 - ➔ Morphologically very diverse, includes morphospecies *U. chaetophora*, *U. barbata* (partly), *U. dasopoga*, *U. diplotypus*, and *U. cf. cylindrica*
 - *Usnea barbata* – *U. intermedia* – *U. lapponica* – *U. substerilis*
 - ➔ Groups as one species but with some distinction between *U. barbata* – *U. intermedia* and *U. lapponica* – *U. substerilis*
 - ➔ We suggest synonymization of *U. substerilis* under *U. lapponica*, while *U. barbata* species complex needs further studies

Conclusions

- ◆ The genus *Usnea* sect. *Usnea* seems to represent a recently diverged **young species complex**
- ◆ **Short branch lengths** and **unresolved relationships** between species suggest a rapid radiation of genetic lineages
- ◆ Using most diagnostic morphological characters together with **branch anatomy** and **thallus chemistry** are useful for delimiting some genetic lineages in sect. *Usnea*,
- ◆ while other clades have wide morphological variation, and many currently accepted diagnostic characters do not prove useful for delimiting these clades



ARCHIMEDES



DoRa



Thank you!

- ◆ Co-authors: Lauri Saag, Steven D. Leavitt, Susan Will-Wolf, Matthew P. Nelsen, Tiiu Tõrra, Andres Saag, Tiina Randlane, H. Thorsten Lumbsch
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