



# Phylogeny and species delimitation in the lichen genus *Cetrelia*

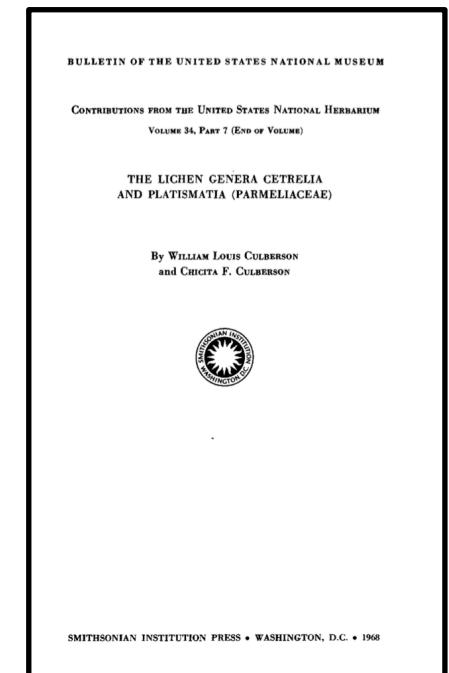
**Kristiina Mark, Tiina Randlane &  
Andres Saag**

Institute of Ecology & Earth Sciences,  
University of Tartu

The 8th IAL Symposium  
Helsinki, 1 August 2016

# ***Cetrelia* W.L. Culb. & C.F. Culb.**

- Described by the Culbersons in 1968
- Some species from *Cetraria* and *Parmelia* were combined into 2 new genera, *Cetrelia* and *Platismatia*
- Based mainly on chemical characters, correlated with some visible morphological and anatomical traits



# Characters of the genus

- Foliose, greenish-grey thallus with broad rounded lobes
- Pseudocyphellae on the upper surface
- Some species with structures of vegetative reproduction (soredia, isidia & lobules), others reproduce sexually
- Atranorin in the cortex, various orcinol depsides and depsidones in the medulla
- Distribution centre in the eastern and southeastern Asia



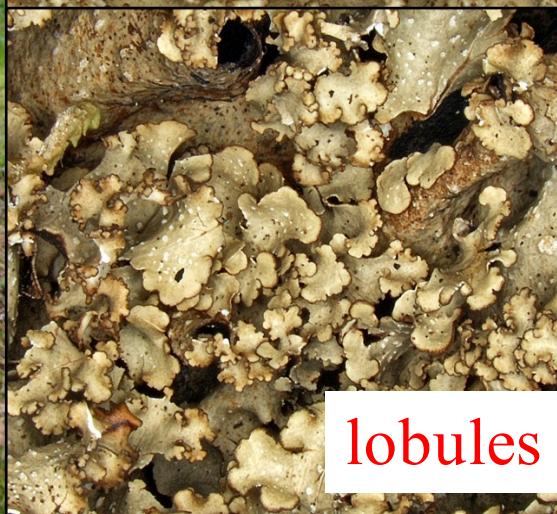
soredia



pseudocyphellae



isidia



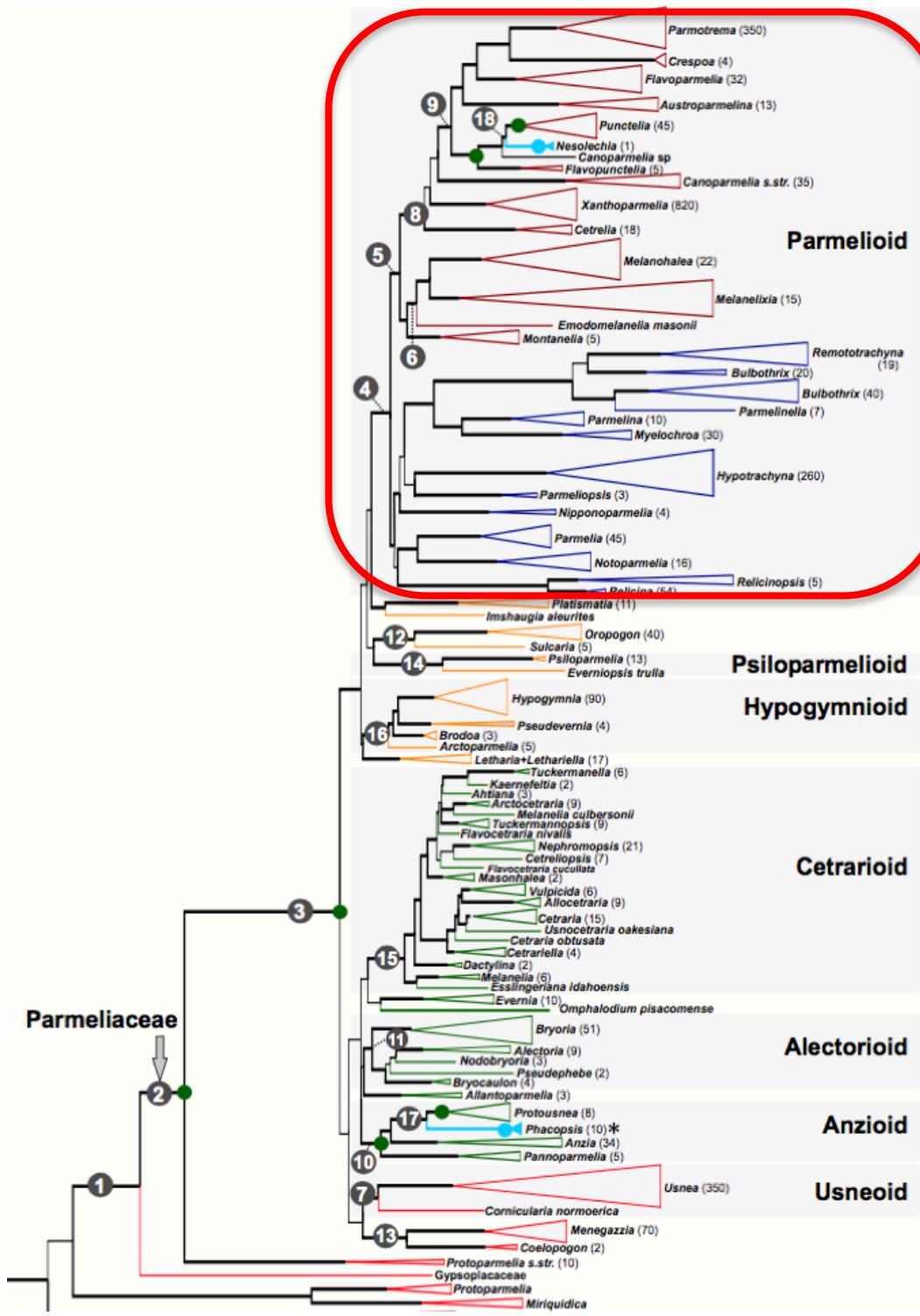
lobules



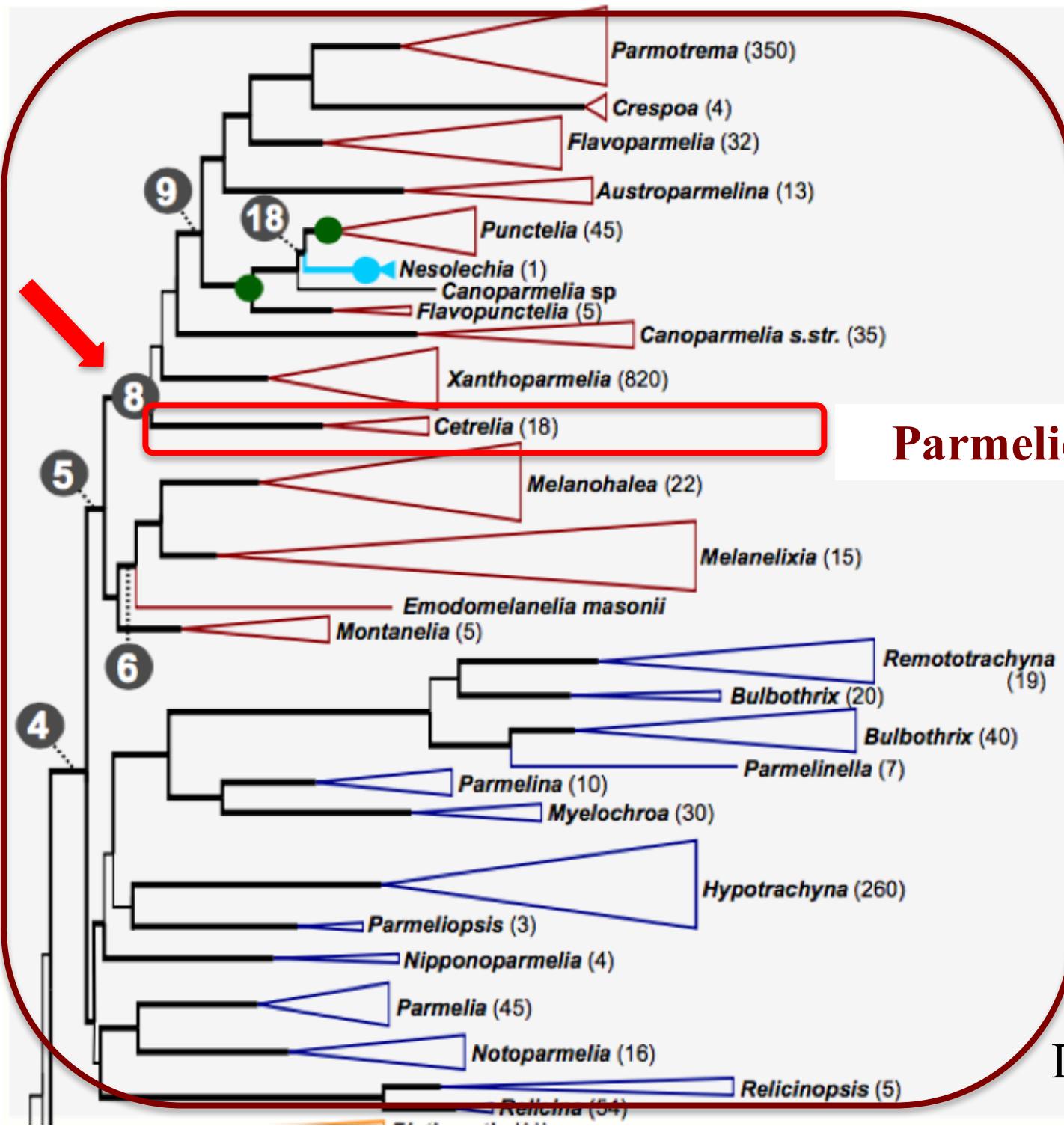
apothecia

# Position within the family

## Parmeliaceae



Divakar et al. 2015



## Parmelioid clade

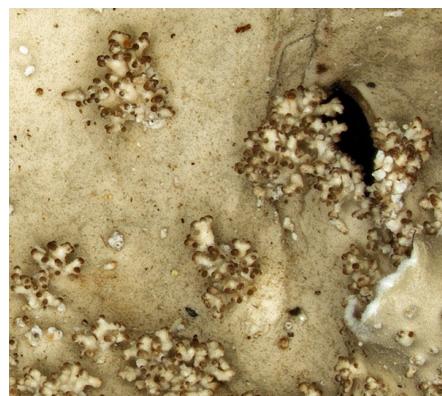
Divakar et al. 2015

# Delimitation of species

- Includes 18 species – IF chemical species concept is accepted
- Species are delimited as combinations of morpho- and chemotypes
- Samples of different chemotypes, although morphologically almost uniform, are considered as distinct species

# Morphotypes (3)

- With vegetative propagules:
  - sorediate (1)
  - isidiate/lobulate (2)
- Without vegetative propagules, often with apothecia (3)

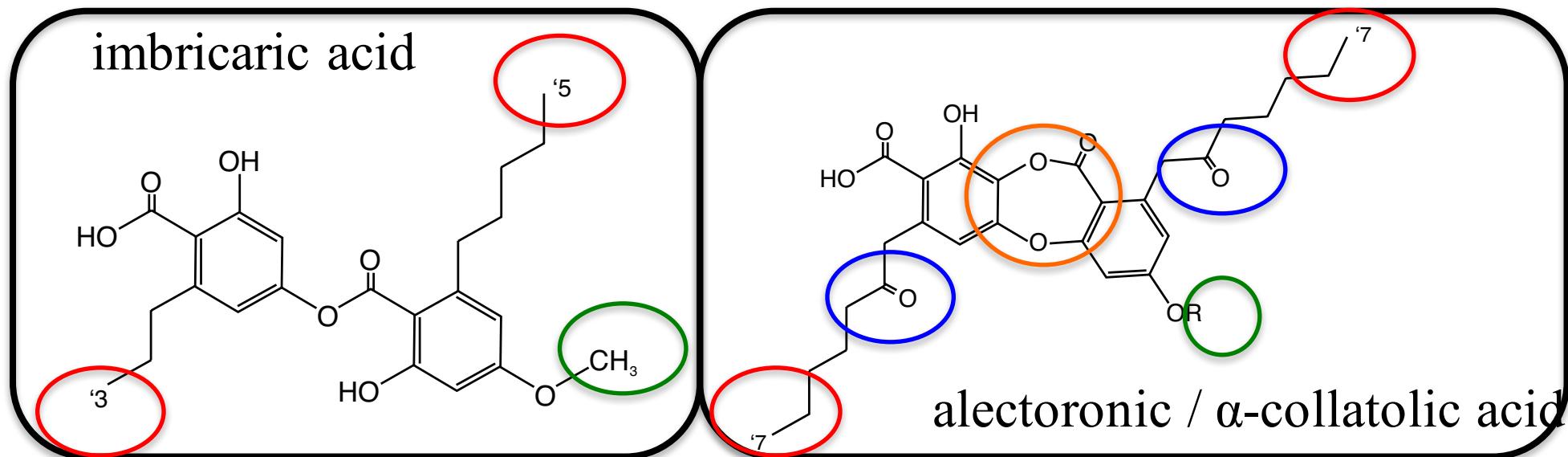


# **Checosyndromic variation**

- ... is a situation where species produce not a few medullary compounds but a set of biochemically related major and minor products
- Chemotypes are identified according to 1-2 major medullary substances
- In addition, up to 7 minor compounds may be present in one *Cetrelia* species
- The same compound can be a major product in one, and minor product in another taxon

# Chemotypes (6)

Major substances	Imbricaric	Olivetoric	Anziaic	Perlatolic	Microphyllinic	Alectornic + $\alpha$ -collatolic
Side chain lengths	3+5	5+7	5+5	5+5	7+7	7+7
Side chain oxydation	no	1 chain	no	no	both chains	both chains
Oxydative cyclization	no – depside	no – depside	no – depside	no – depside	no – depside	yes – depsidones
O-methyl.	CH <sub>3</sub>	H	H	CH <sub>3</sub>	CH <sub>3</sub>	R= H – alect. R= CH <sub>3</sub> – $\alpha$ -coll.



# The *Cetrelia olivetorum* complex

- ... is best known, most widely distributed, and taxonomically the most discussed
- Represents sorediate morphotype
- 5 species are known in the complex:
  - *Cetrelia cetrariooides*
  - *C. chicitae*
  - *C. monachorum*
  - *C. olivetorum*
  - *C. sayanensis*



# The *Cetrelia olivetorum* complex

- Morphology and chemistry of this complex was investigated in detail by Obermayer and Mayrhofer (2007) based on material from Alps
- Minute morphological differences concerning pseudocystellae and soredia were noticed between the samples from different chemotypes
- **What about the phylogenetic relationships?**

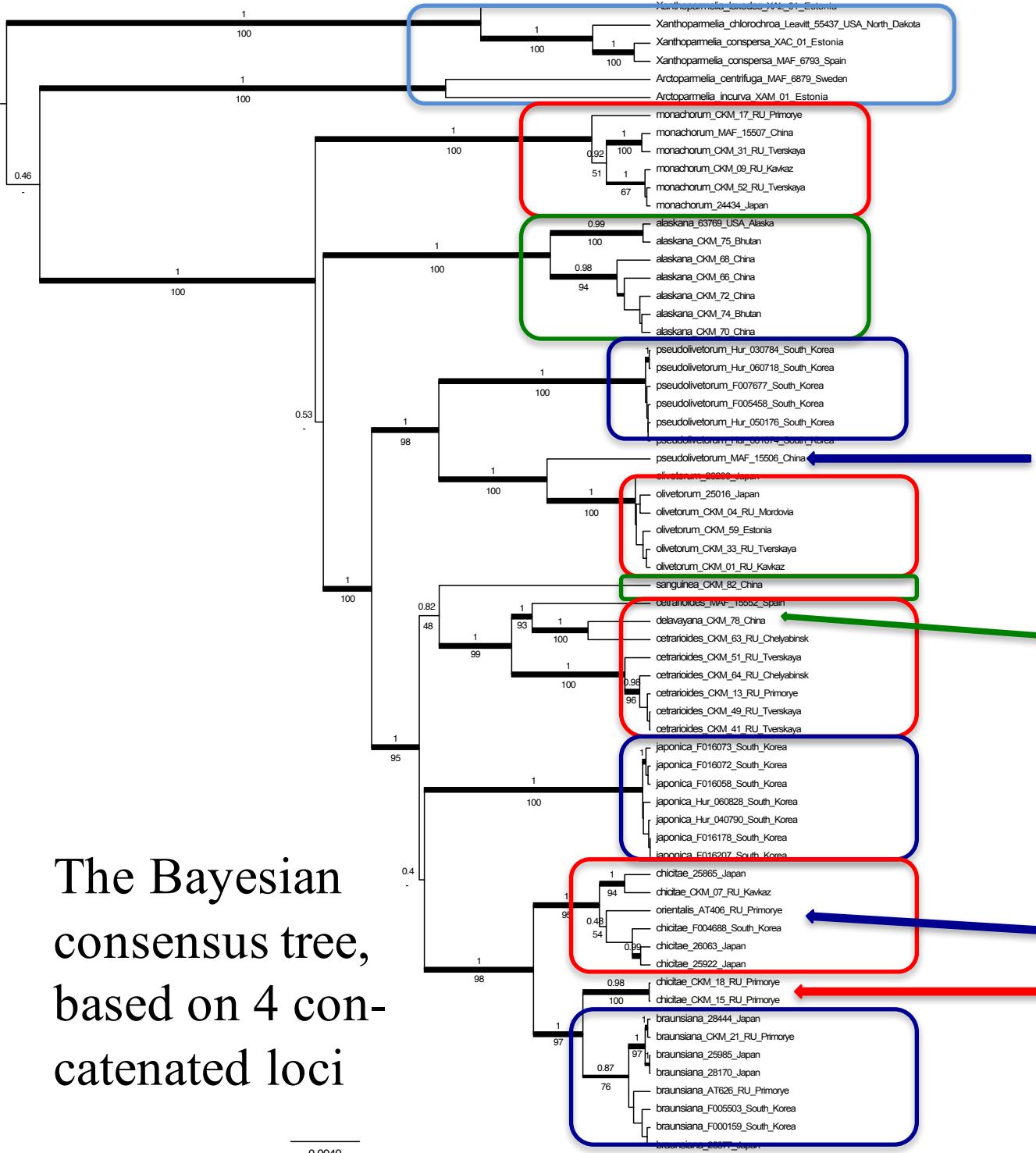
# Aims of our study

- To clarify the species concept in *Cetrelia* through molecular phylogenetic analyses:
  - Is the treatment of taxa of the same morphotype but different chemotypes as one species phylogenetically acceptable?
  - Is the theory by Culbersons (1976) that chemical evolution in *Cetrelia* was directed towards shorter side chains of orcinol compounds reliable?

# Material & Methods

- 58 samples representing 11 species, all 6 chemotypes and all reproductive structures
- 4 molecular markers – ITS, IGS, MCM7, RPB1
- Maximum likelihood (ML) single-locus trees using the RAxML
- ML and Bayesian concatenated analyses using RAxML and BEAST

# The Bayesian consensus tree, based on 4 concatenated loci



## outgroup

# sorediate

## without veget. struct.

**lobulate**  
**lobulate**  
**sorediate**

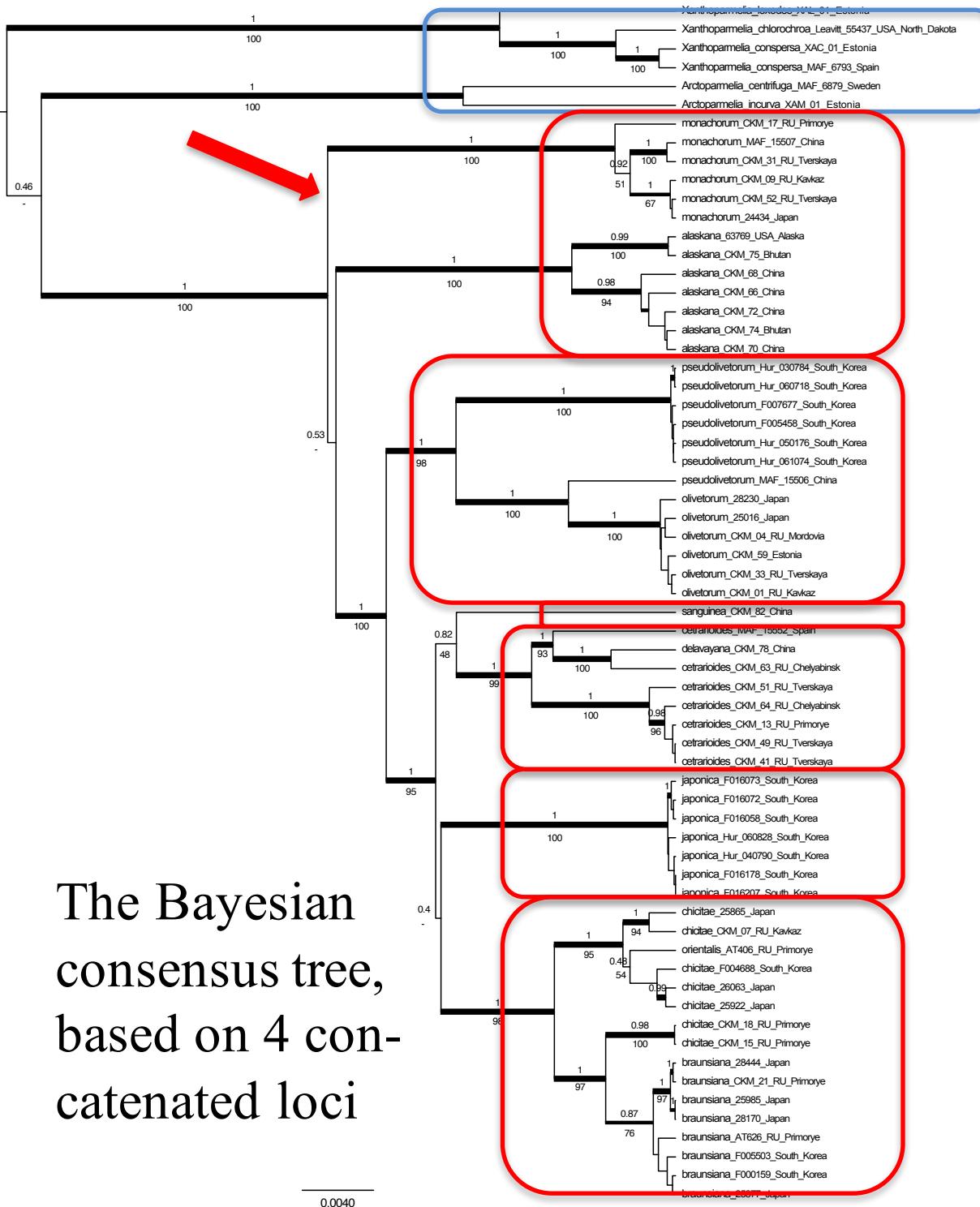
without veget. struct.  
without veget. struct.  
**sorediate**

**lobulate**

sorediate  
lobulate  
sorediate

# isidiate

# The Bayesian consensus tree, based on 4 concatenated loci



## outgroup

# imbricaric acid

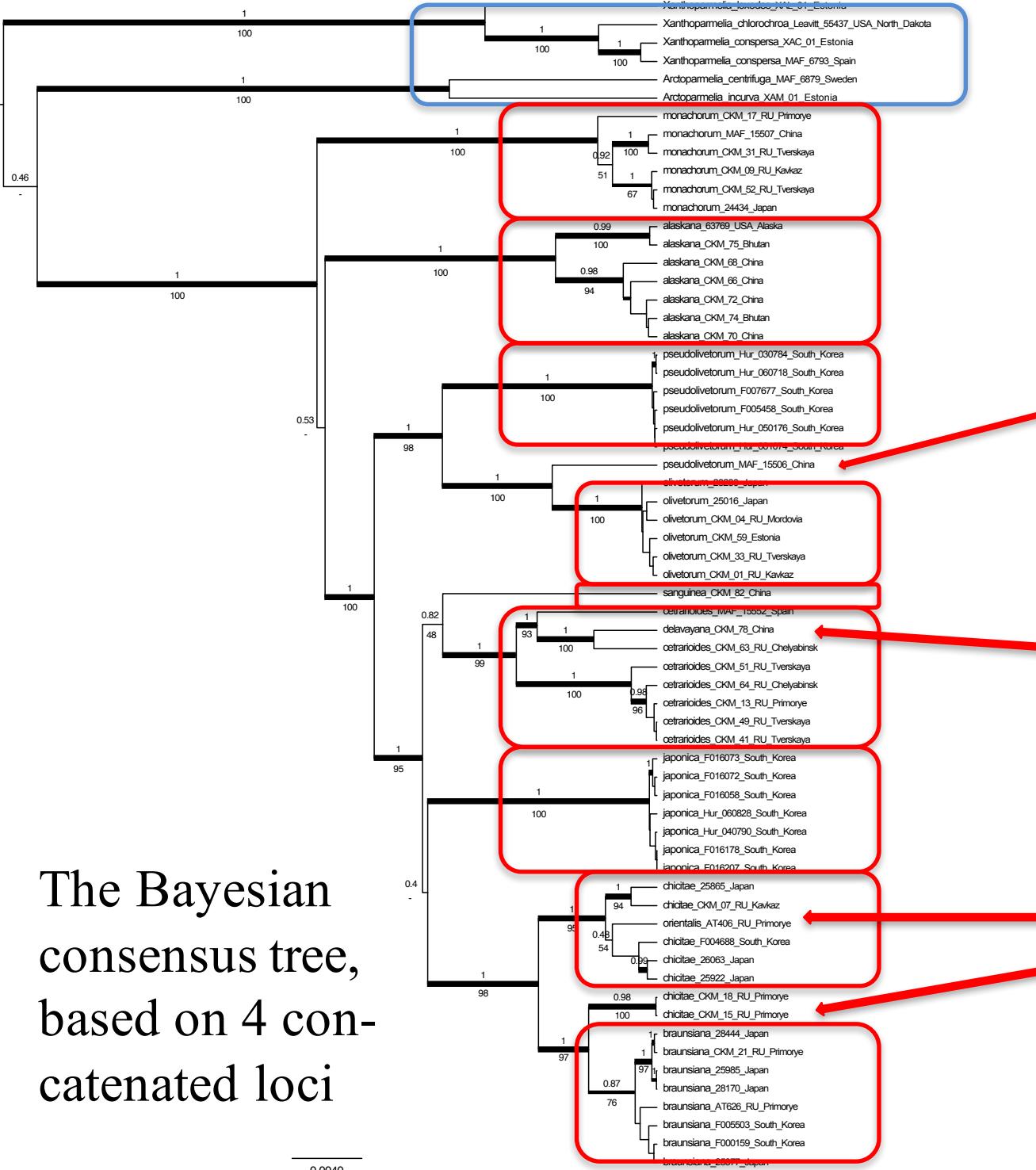
# olivetoric acid

## anziaic acid

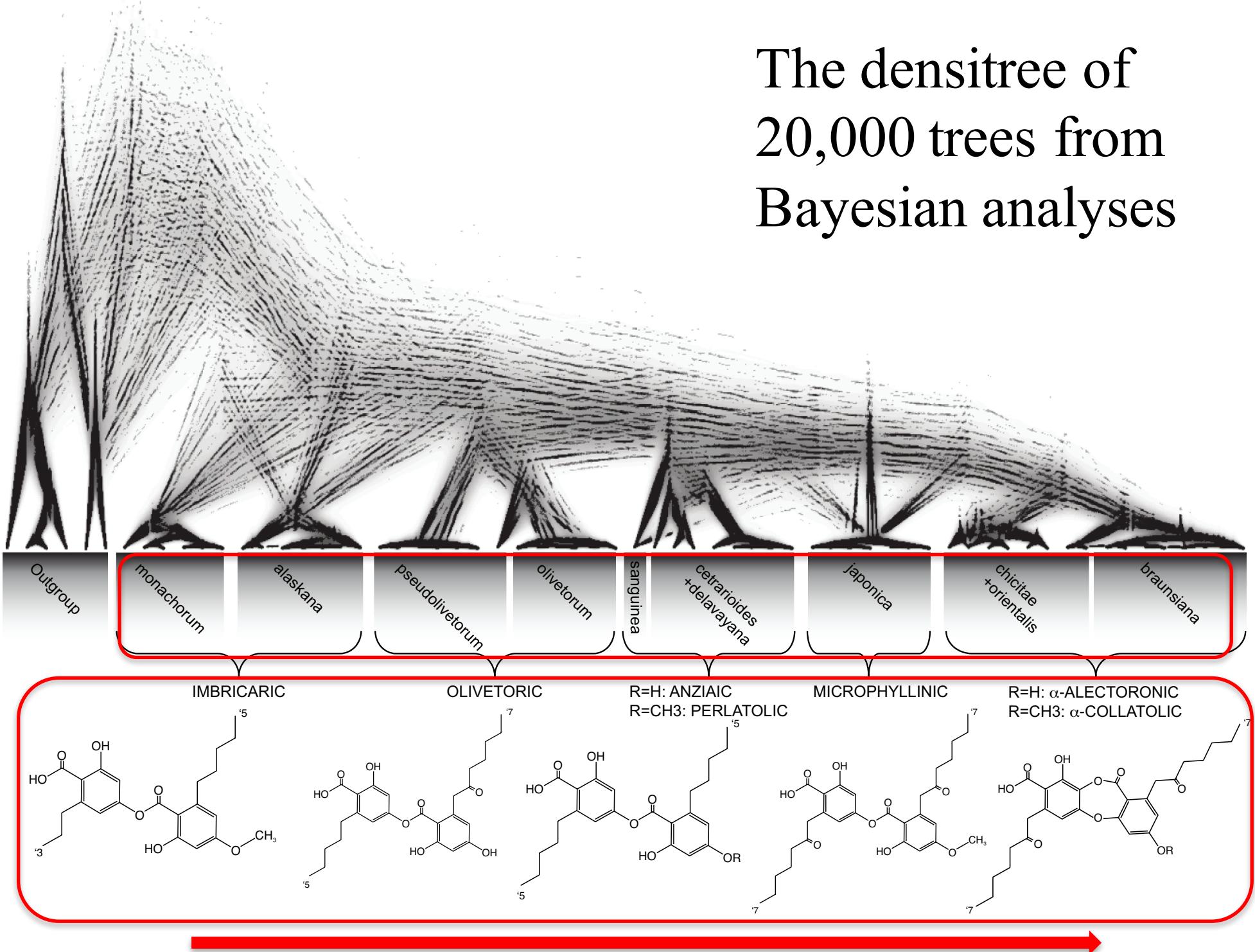
# perlatolic acid

# microphyllinic acid

alecteronic +  $\alpha$ -  
collatolic acids



# The densitree of 20,000 trees from Bayesian analyses



# Conclusions 1

- Sec. chemistry clearly correlates with molec. phylogeny in *Cetrelia*, morphology does not
- Chemotypes form monophyletic clades that include subclades generally correlating with morphotypes → chemical evolution in *Cetrelia* occurred before diversification of morphotypes
- The direction of chemical evolution in *Cetrelia* is towards more complex substances, not towards shorter side chains

# Conclusions 2

- The chemical species concept should be accepted in *Cetrelia*
- The species can be treated as combinations of a chemotype and a morphotype
- Samples of the same morphotype but different chemotype should be recognized as distinct species

# Identification key for *Cetrelia* species (18)

MORPHOTYPES (3)	CHEMOTYPES (6)					
	Imbricaric	Olivetoric	Anziaic	Perlatolic	Microphyllinic	Alectononic + α-collatolic
Sorediate	<p><i>C. monachorum</i> soralia labriform, soredia coarse</p> <p><i>C. sayanensis</i> soralia labriform to pustulate-capitate, soredia fine</p>	<p><i>C. olivetorum</i> soralia labriform, soredia fine</p>	?	<p><i>C. cetrariooides</i> soralia labriform, soredia fine</p>	?	<p><i>C. chictae</i> soralia labriform, soredia coarse</p>
Isidiate and/or lobulate	<p><i>C. sinensis</i> lobulae palmately divided</p>	<p><i>C. pseud- olivetorum</i> lobulae multibranched</p>	<p><i>C. isidiata</i> isidia globose to slightly branched and coralloid</p>	?	<p><i>C. japonica</i> lobulae multibranched</p>	<p><i>C. braunsiana</i> isidia granular to coralloid</p> <p><i>C. orientalis</i> lobulae palmately divided</p>
Without vegetative propagules	<p><i>C. collata</i> apothecia frequent, pseudocypellae large</p> <p><i>C. alaskana</i> apothecia rare, pseudocypellae small</p>	<p><i>C. davidiana</i> apothecia frequent, pseudocypellae small</p>	<p><i>C. sanguinea</i> apothecia frequent, pseudocypellae small</p>	<p><i>C. delavayana</i> apothecia frequent, pseudocypellae small</p>	<p><i>C. pseudo- collata</i> apothecia frequent, pseudocypellae large</p>	<p><i>C. nuda</i> apothecia frequent, pseudocypellae large</p>

## Conclusions 2

- The chemical species concept should be accepted in *Cetrelia*
- The species can be treated as combinations of a chemotype and a morphotype
- Samples of the same morphotype but different chemotype should be recognized as distinct species
- Chemically different sorediate species *C. cetrariooides*, *C. chicitae*, *C. monachorum* and *C. olivetorum* should not be considered as one!

## Co-authors:



Kristiina Mark

Andres Saag

## Acknowledgements:

- Financial support from the Estonian Science Foundation grants ETF9109 and PUT1017
- Thanks to the collectors of the samples, esp. J.-S. Hur, E. Kuznetsova, A. Notov, W. Obermayer, G. Thor, I. Urbanaviciene & G. Urbanavicus



Thank you for the attention!

