

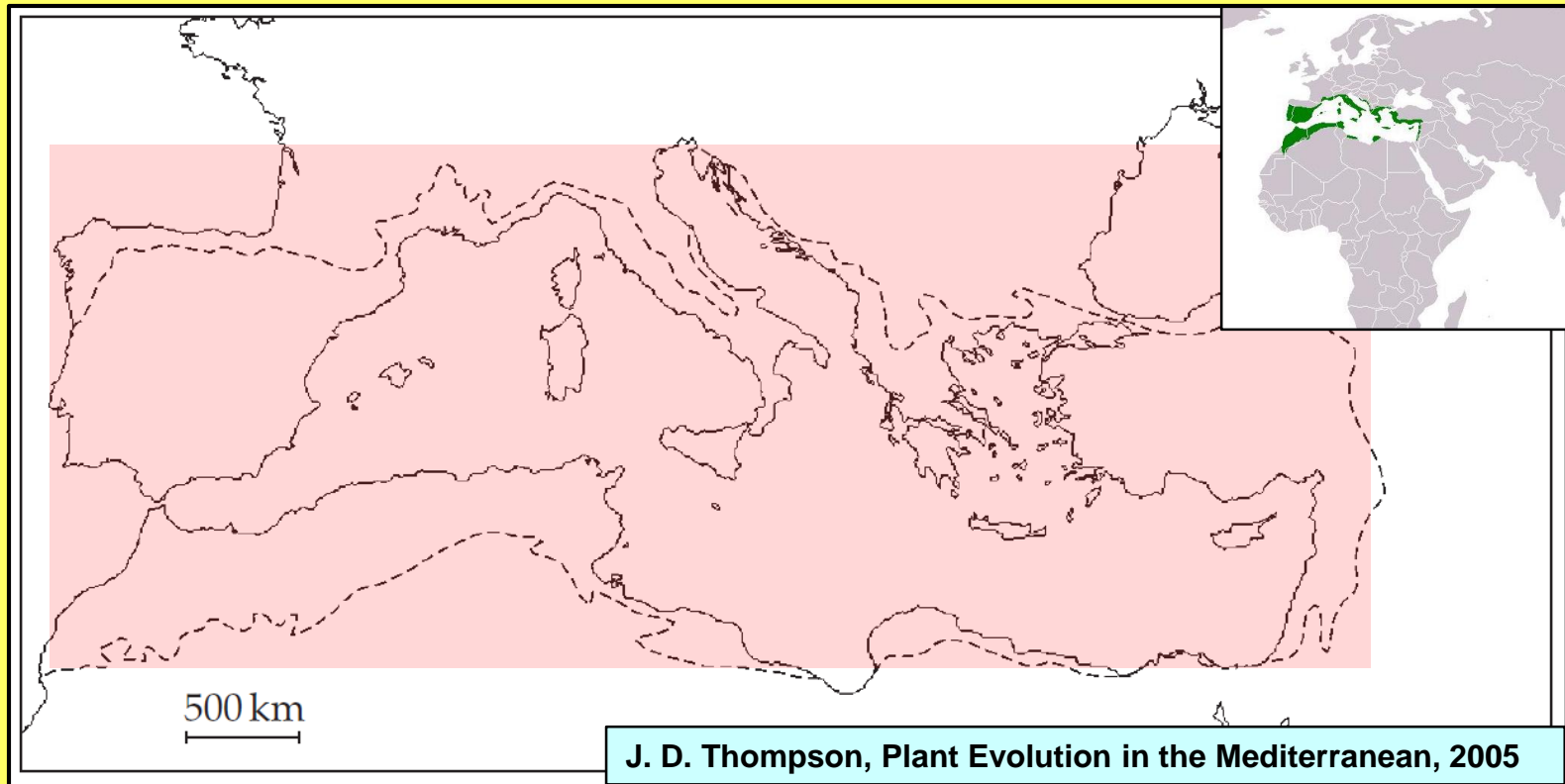
Spatio-temporal formation of the genetic diversity in the Mediterranean dwelling lichen during the Neogene and Quaternary epochs



Marek Slovák

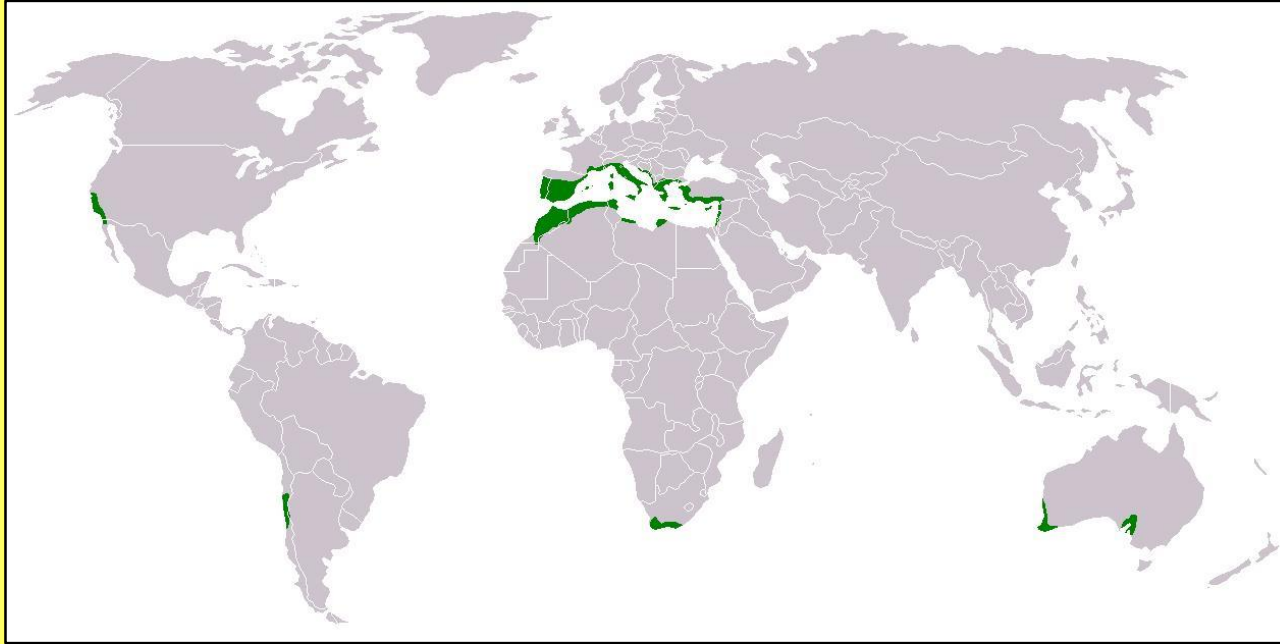
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Institute of Botany, Slovak Academy of Sciences, Bratislava, Slovakia**

The Mediterranean



- highly seasonal climate – alternation of dry and wet periods
- delimitation - olive growth (*Olea europaea*), up to ca 800 m s.n.m., no long term toleration of temperatures under 3 °C
- defined based on effective drought during the warmest period (summer)
- long term climatic stability especially during the Tertiary and Quaternary climatic oscillations

Mediterranean like regions - diversity 'hotspots'

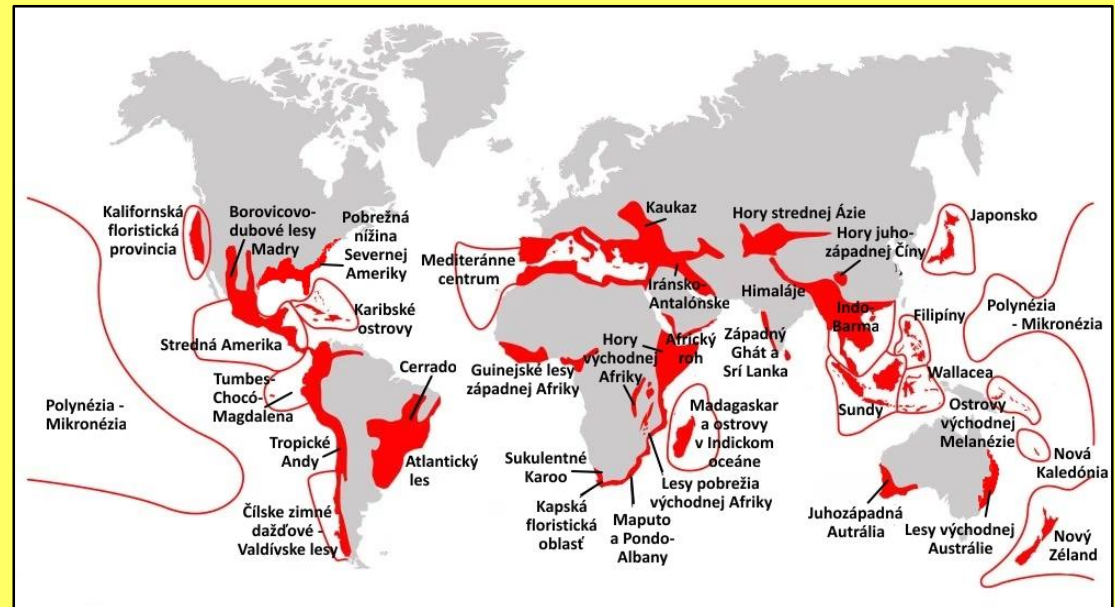


Mediterranean like regions on the Globe

J. D. Thompson, Plant Evolution in the Mediterranean, 2005

Biodiversity 'hotspots' on the Globe

Meyers et al. 2000, Nature



Mediterranean climate

Climate

- annual temperature amplitude 15-20 °C
- dry summer with lack of precipitation
- mild and wet winters, rich precipitation
- overall annual precipitation 450–1200 mm
- average summer temperatures 21–23 °C
- average winter temperatures 5–7 °C



Spring
February to May

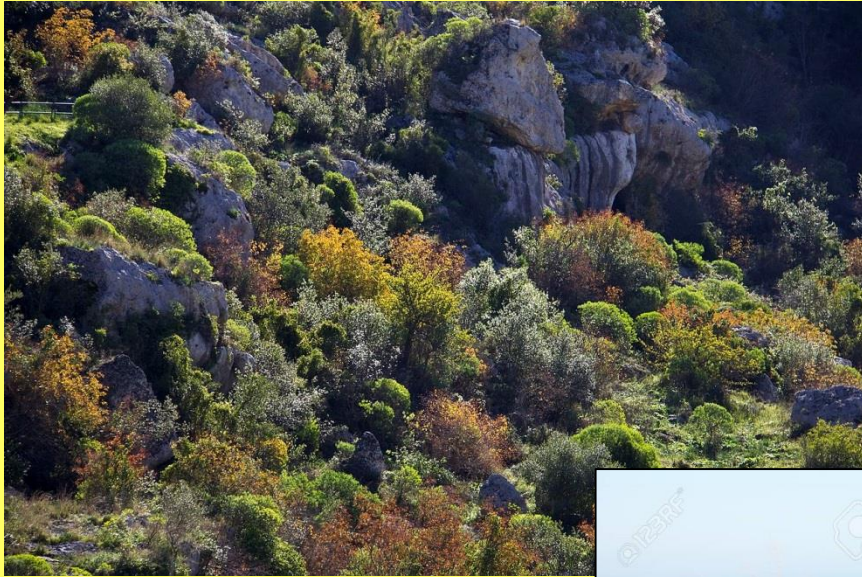
Summer
May to October



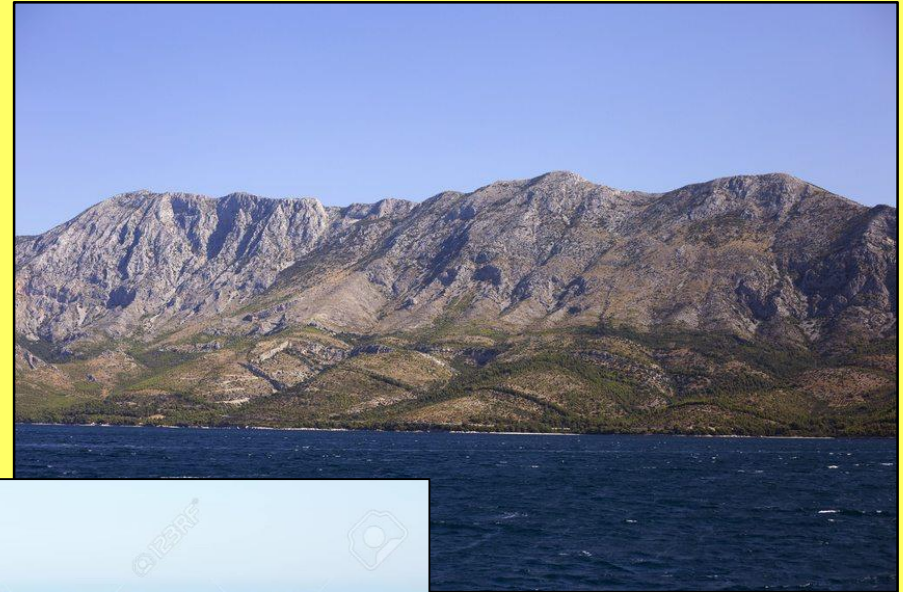
Autumn
„Second spring“
October till January



Typical habitats of the Mediterranean



Mediterranean macchia



coastal mountain ranges



coastal sand dunes



Desert mountain wadi



Evergreen xerophyllous forests

Mediterranean vegetation

Almost completely altered by **human activities** – over minimum time span of 8000 years

- Currently almost non-existent belt of coastal evergreen forests (sclerophyllous, „hard-leaved“) – *Quercus suber*, *Q. ilex*, *Q. coccifera*, *Pinus pinea*; *P. halepensis*; *Olea*, shrubs (*Arbutus unedo*, *Phyllirea latifolia*, *Pistacia lentiscus*, *Erica arborea*), lianas - creepers (*Smilax aspera*, *Tamnus communis*)



- original forests were significantly damaged by human activities and fires
- replaced by secondary plant associations
- reforestation of karstic coastal regions is fairly long term process

Mediterranean vegetation types

macchia – shrubby assemblages on more humid habitats, 3–5 m tall (*Erica*, *Ilex aquifolium*)



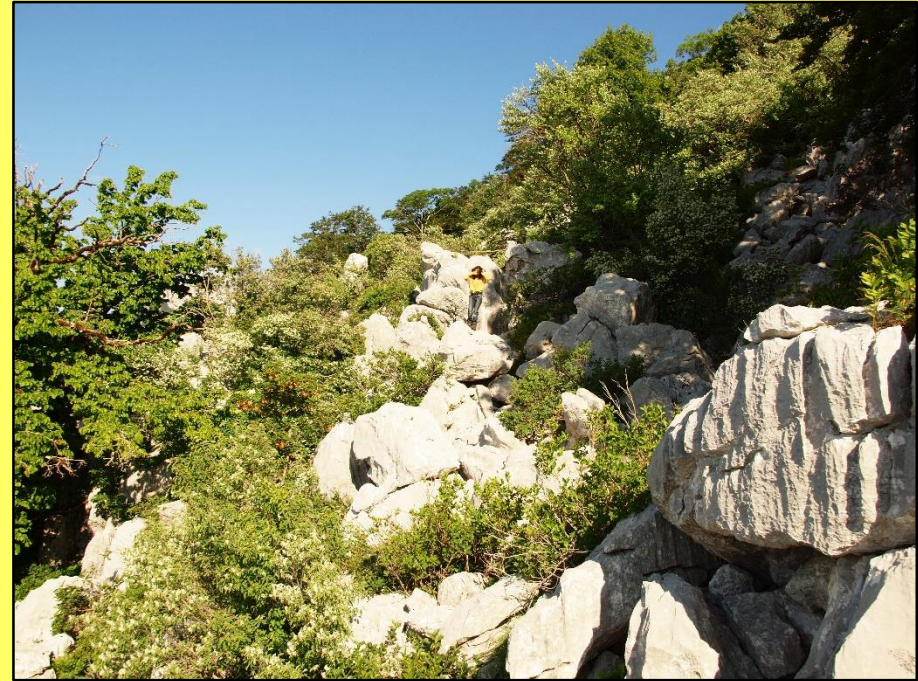
garrigue (tomillar) – lower herbal associations on dry habitats up to 1 m tall (*Thymus vulgaris*, *Rosmarinus officinalis*, *Lavandula angustifolia*, *Salvia*, *Origanum*)

frygana (Palestina – batha) – dry calcareous outcrops with semi shrub vegetation up to 0,5 m tall, degraded macchie (*Genista acanthoclada*, *Coridothymus capitatus*, *Globularia alypum*, *Asphodelus*)
edafic climax v Mediterranean



Mediterranean vegetation types

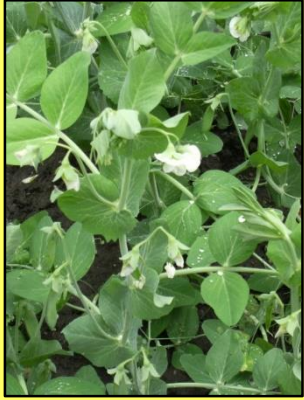
šibljak – dense macchia, previously covered by oak forests in e.g. Croatia or Bulgaria (dominants *Paliurus spinachristi*, *Cotinus coggygria*)



pseudo-steppe/cultural steppe – pastures, orchards, fields (*Aegilops geniculata*, *Stipa capensis*, *Carlina*)



Mediterranean – the cradle of crops



Pisum sativum



Lens culinaris



Faba vulgaris



Cynara scolymus



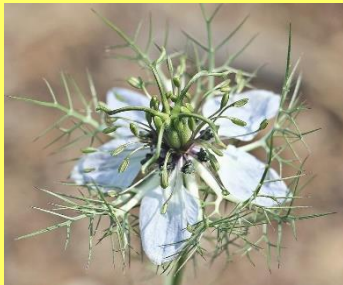
Beta vulgaris subsp. *maritima*



Leucosinapis alba



Hordeum distichon



Nigella sativa



Triticum dicoccoides

Paleoenvironmental milestones in the Mediterranean history



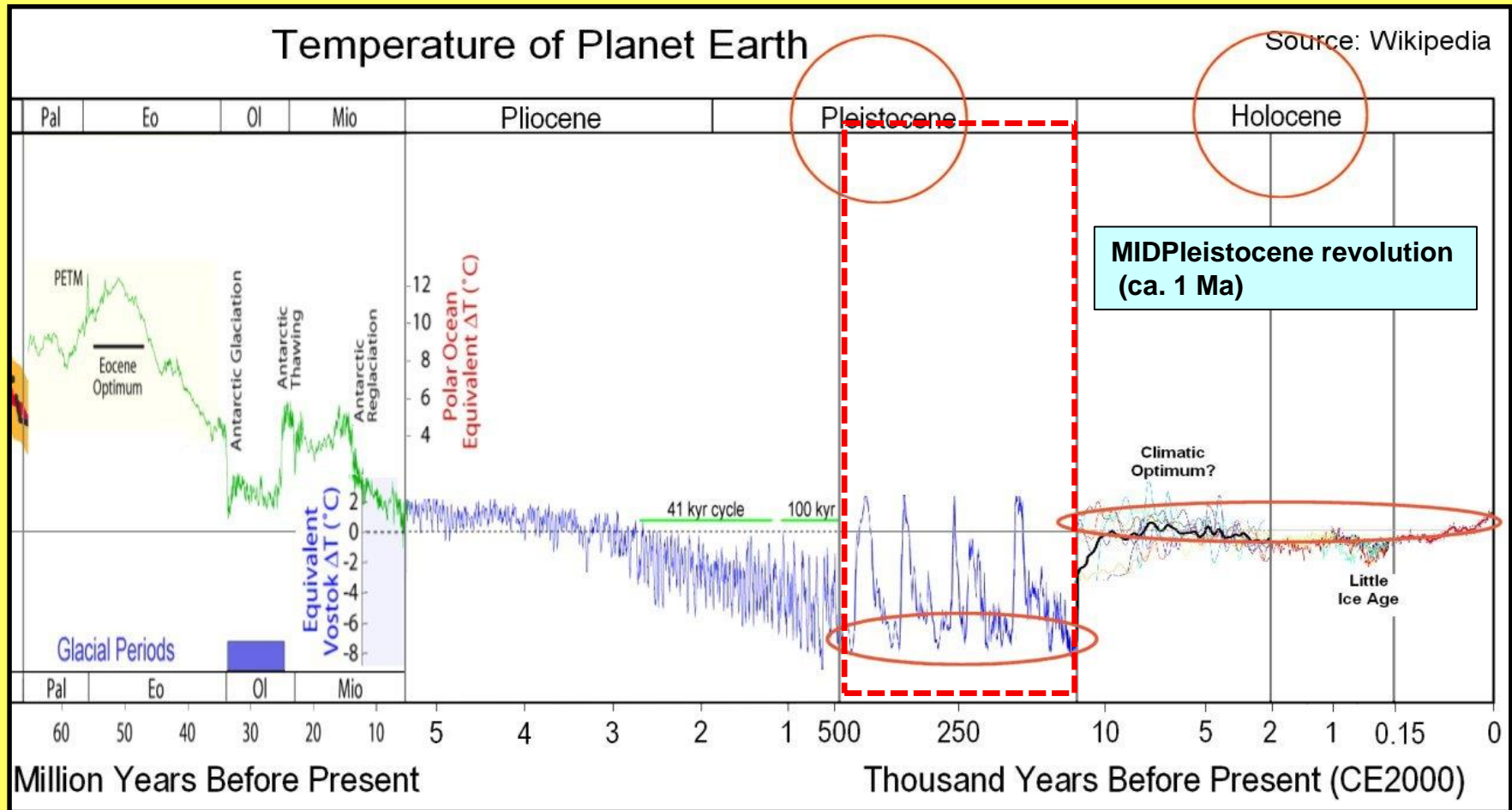
History of the Mediterranean

Messinian Salinity Crisis (5.96–5.33 Ma)



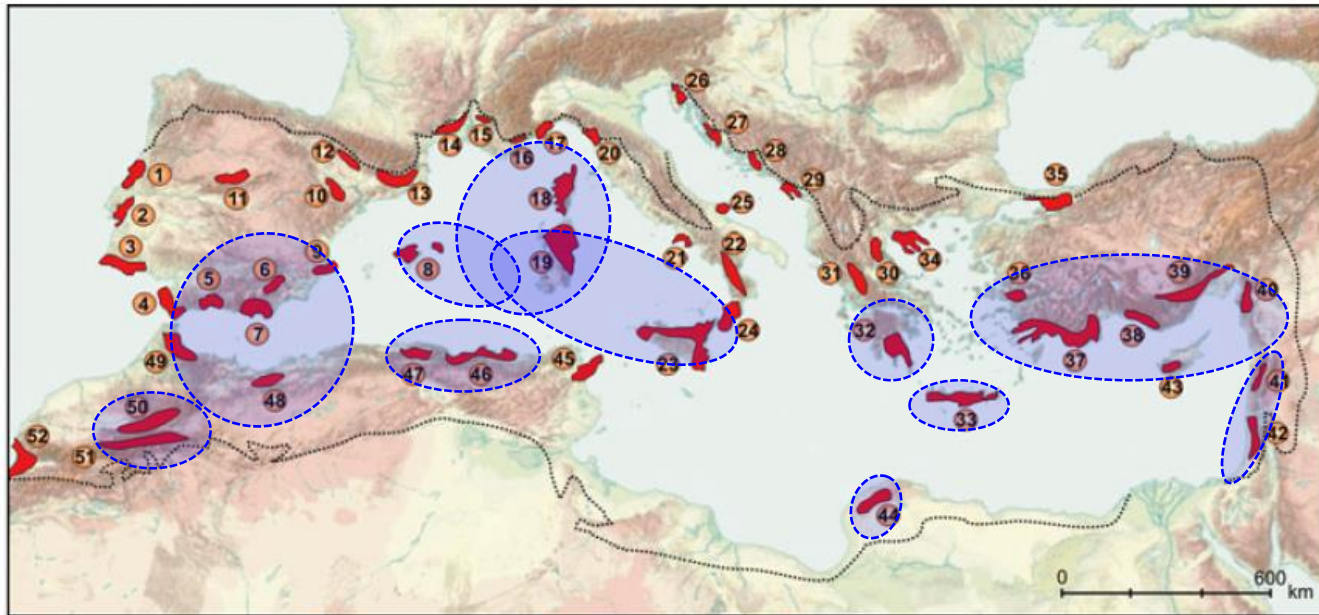
- closure of Gibraltar gulf, extensive desiccation of the Mediterranean Sea
- aridification of the climate, evolution of desert region – Sahara desert and Syrian Arab desert
- Bi-directional migration of organisms between Africa and Euro-Asia

Pleistocene glacial – interglacial cycles



- alternation of glacial and interglacial cycles, each 41 000 and 100 000 years,
- southwards migration from the temperate and boreal zone to Mediterranean
- severe of forests development of open grasslands, steppes and forest steppes regions

Diversity, endemism and glacial refugia in the Mediterranean



- | | | | | |
|--------------------------|--------------------|----------------------|-------------------------|----------------------------|
| 1 Beira litoral | 11 Sistema central | 21 Campania | 31 C. Greece (Pindos) | 42 Israel/Palestine |
| 2 Estramadura | 12 S. Pyrenees | 22 S. Apennines | 32 Peloponnese | 43 Cyprus |
| 3 Algarve | 13 S.E. Pyrenees | 23 Sicilia | 33 Crete | 44 Cyrenaic (Lybia) |
| 4 Cadiz/Algeciras region | 14 S. Cévennes | 24 S. Calabria | 34 Chalkidiki peninsula | 45 J. Zaghouan/Cap Bon |
| 5 Serrania de Ronda | 15 Mont Ventoux | 25 Gargano | 35 Izmit region | 46 Petite Kabylie/de Collo |
| 6 Sierra Cazorla/Segura | 16 E. Provence | 26 N. Istria | 36 Boz/Aydin dag | 47 Grande Kabylie |
| 7 Sierra Nevada/Gata | 17 Maritime Alps | 27 Velebit Mountains | 37 S.W. Anatolia | 48 Tlemcen Mountains |
| 8 Balearic Islands | 18 Corsica | 28 S. Bosnia/Biokovo | 38 C. Taurus | 49 Rif Mountains |
| 9 Valencia region | 19 Sardinia | 29 Montenegro | 39 E. Taurus | 50 Middle Atlas |
| 10 Ebro Valley | 20 Alpi Apuani | 30 Olympe/Katalympos | 40 Amanus | 51 High Atlas |
| | | | 41 Lebanon range | 52 Souss/W. Anti Atlas |

Fine-scale geographical distribution of the 52 putative refugia within the Mediterranean obtained from the analysis of the phylogeographical patterns of 82 plant species (41 trees and 41 herbs).

Médail and Diadema 2009, Journal of Biogeography

Diversity, endemism and glacial refugia in the Mediterranean

- ca. 60 % of flora native to Mediterranean is endemic
- Out of them ca 60% are stenoendemics
- in some regions, but especially in islands and high mountain ranges endemism reaches from 10 to 20 % of local flora



Cyclamen repandum



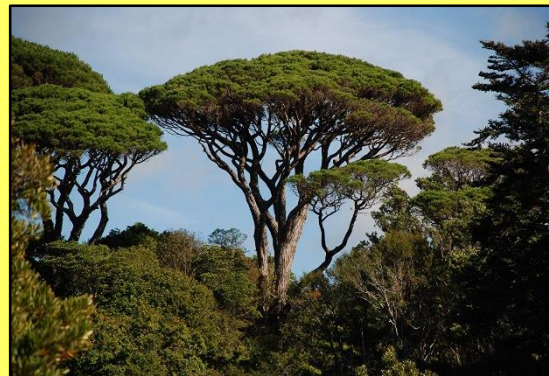
Cedrus atlantica



Iris marie



Glebionis coronaria



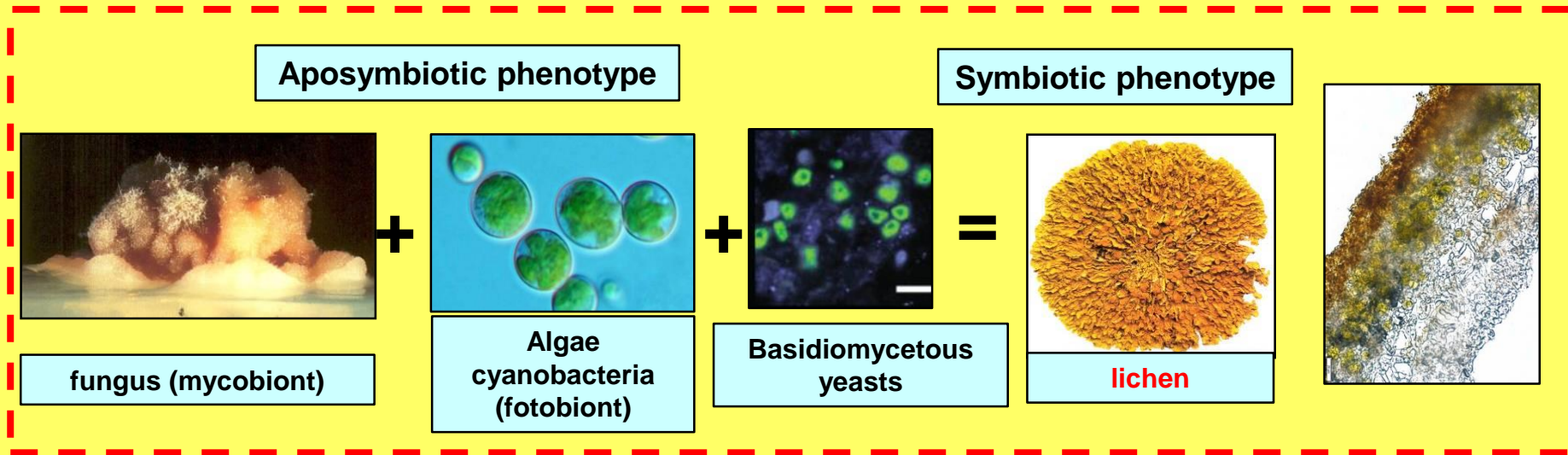
Pinus pinea



Lavandula officinalis

Lichens

- Lichenization one of the fundamental life strategies on the Earth
- Large portion of knowledge on the evolution, biology and systematics of lichens is still unknown



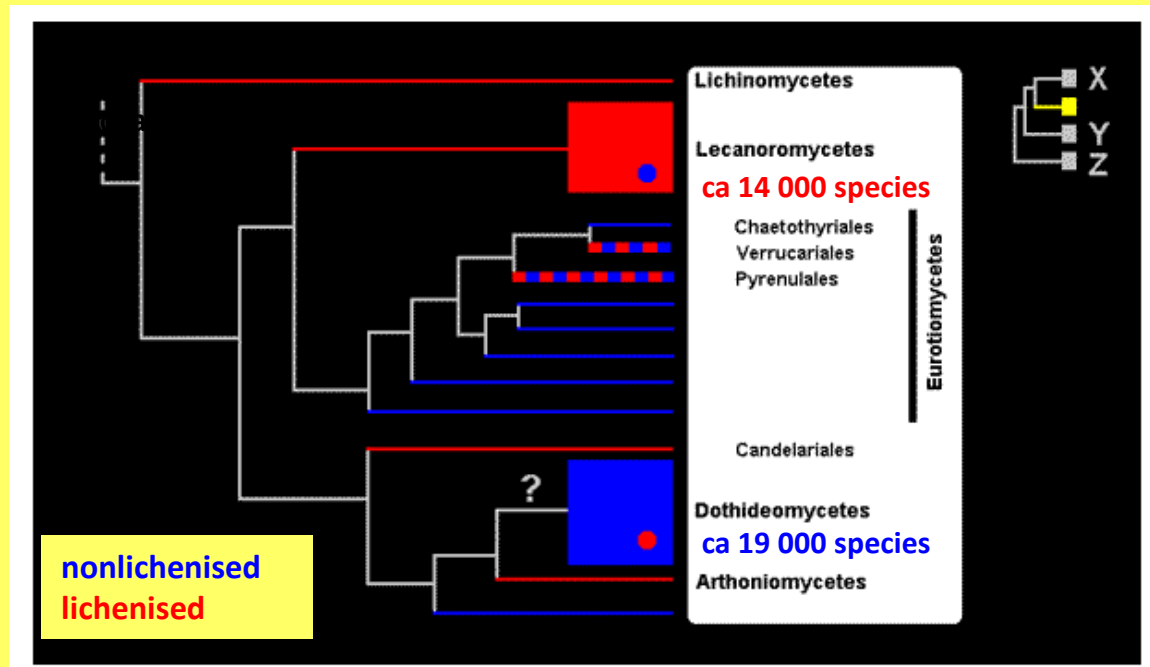
- Stable mutualistic association + presence of another bionts (lichenicolous fungi, bacteria, viruses)
- Lichens are polyphyletic = lichenization evolved during the evolutionary history of fungi independently in several different lineages



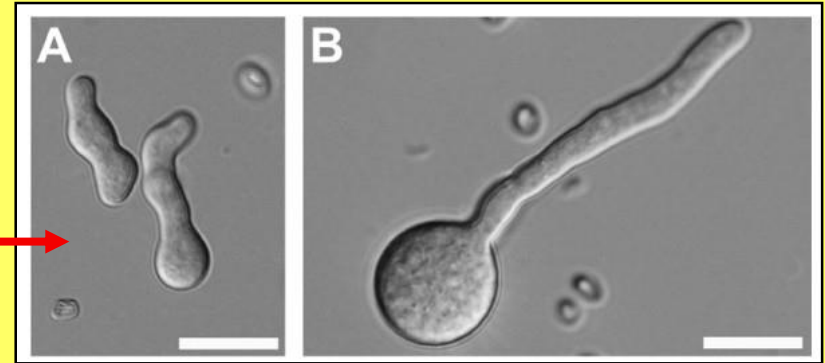
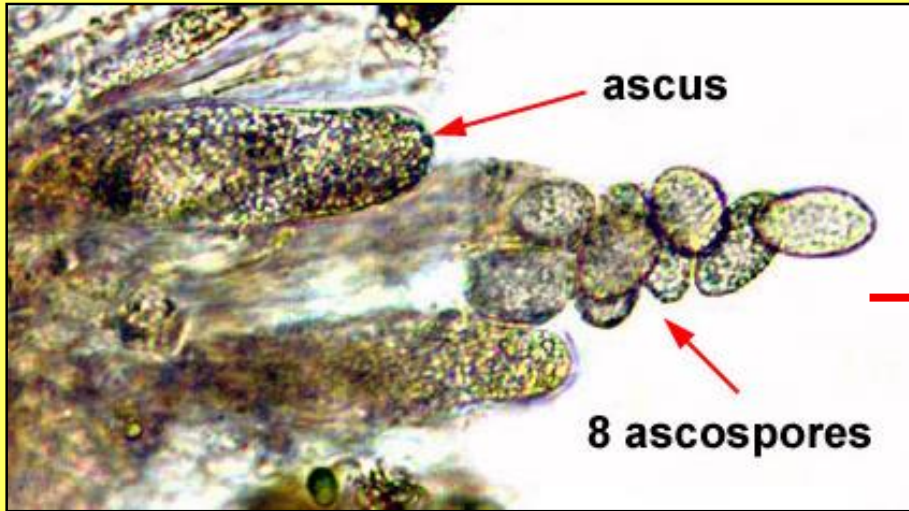
Lichenization – the successful life strategy



- ca 20% of fungal species are lichenised (Kirk et al. 2008)
- lichens are dominant organismal group in more than 12% of terrestrial biomes
- Lichens are not monophyletic, lichenization evolved several times independently



Sexual reproduction of lichens



spore germination

Formation of asci with ascospores



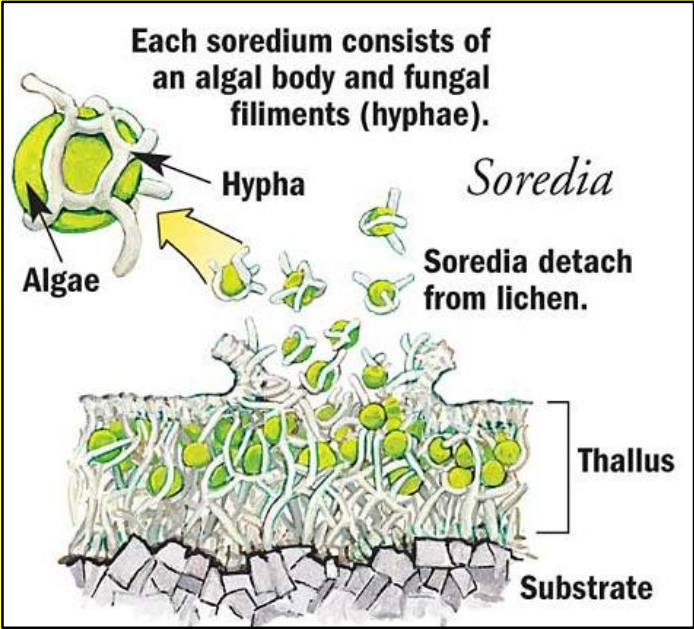
Formation of novel thallus



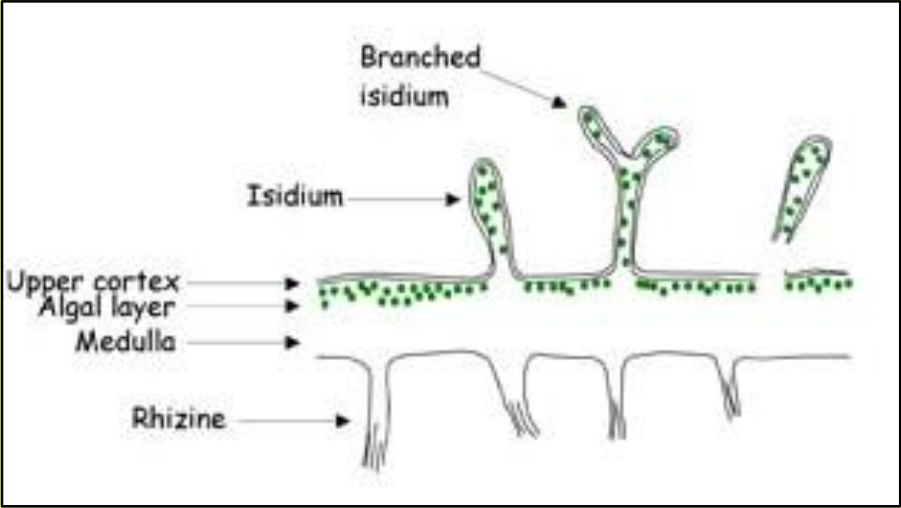
Lichenization of the algal photobiont

Asexual reproduction of lichens

2. soredia



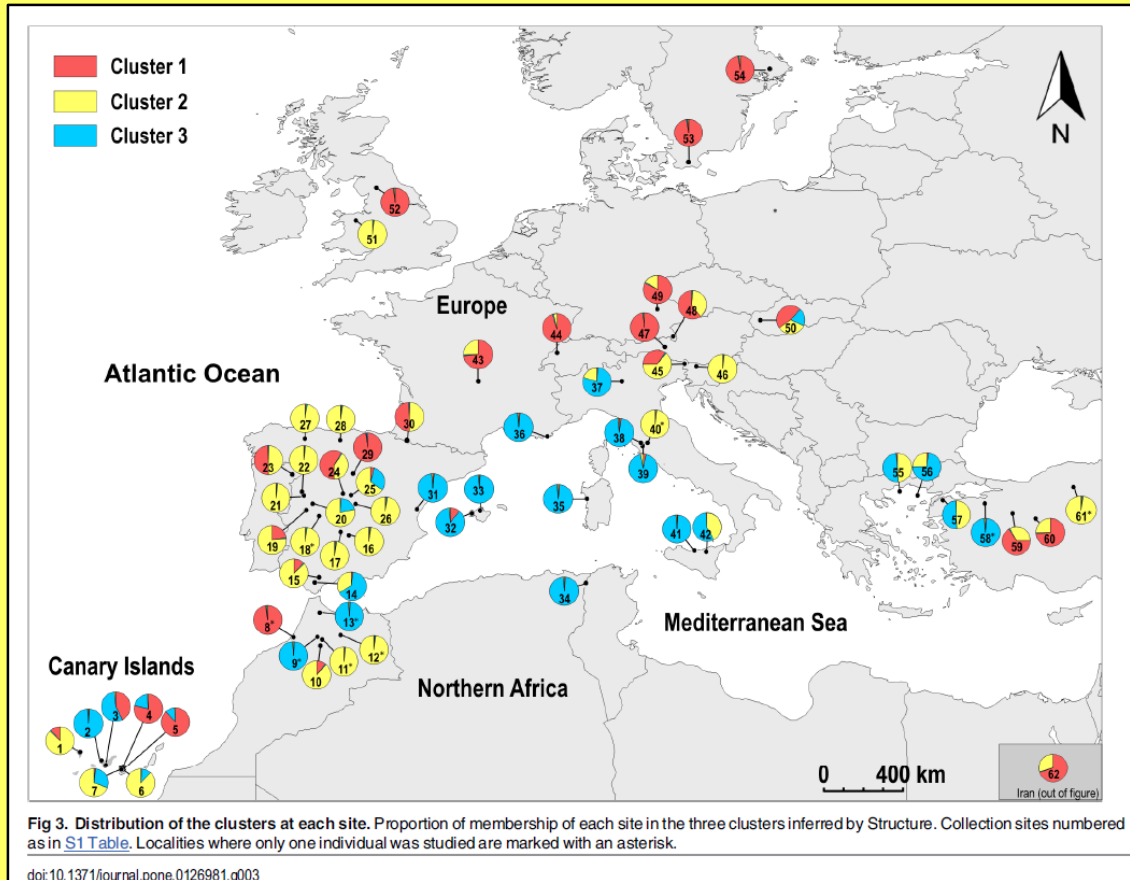
1. thallus fragmentation



3. isidia

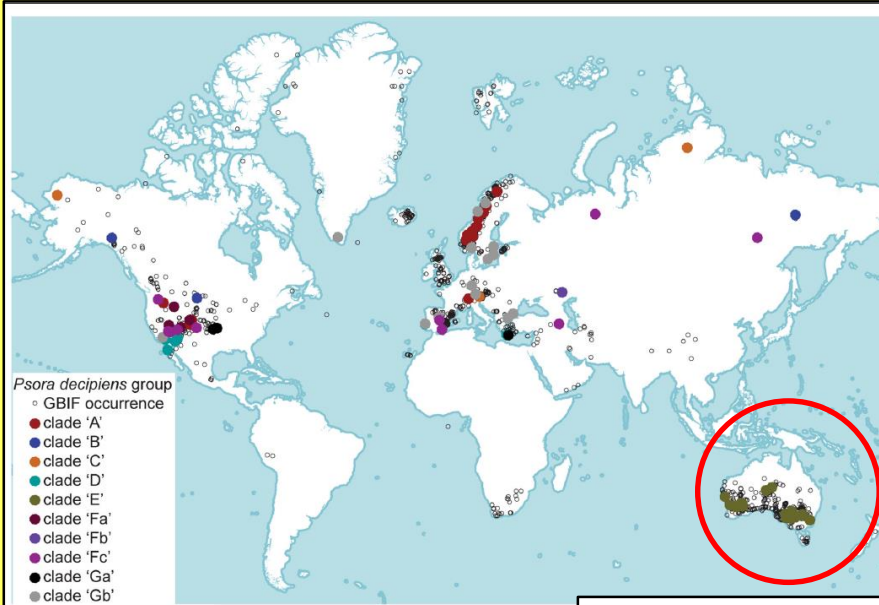
Biogeographic patterns in lichens

Genetic patterns of lichens often display a mosaic-like and difficult to interpret structure, reflecting their complex bio-ecological characteristics rather than their evolutionary history

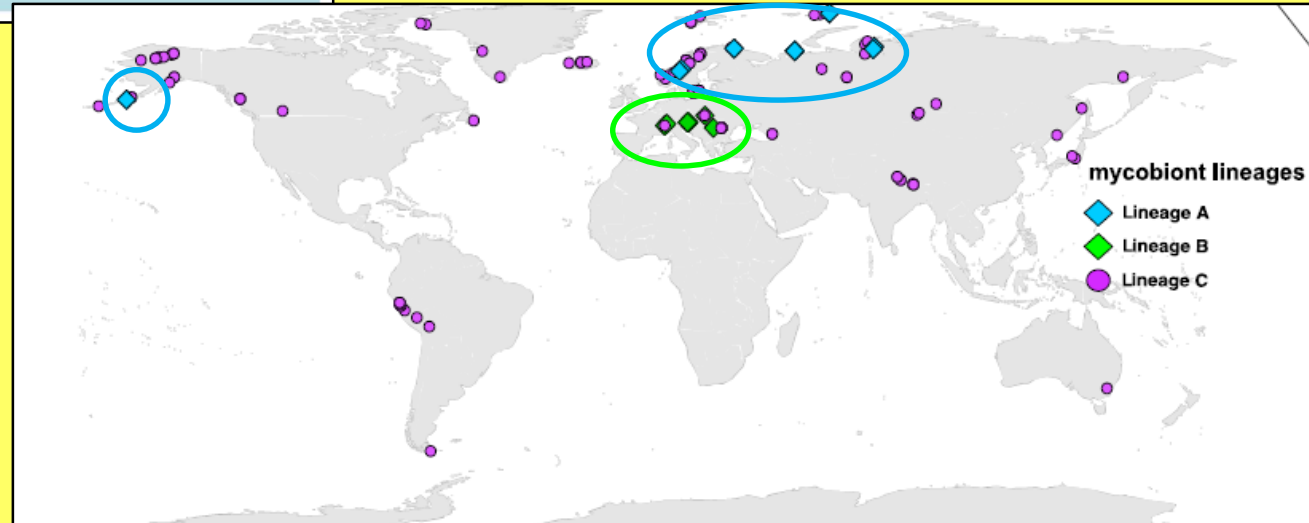


Biogeographic patterns in lichens

Species with intercontinental distributions may comprise a number of species-level, biogeographically and ecologically structured genetic lineages



Psora decipiens, genetic lineage - **geography**
Leavitt et al. 2018 - *Frontiers in Microbiology*



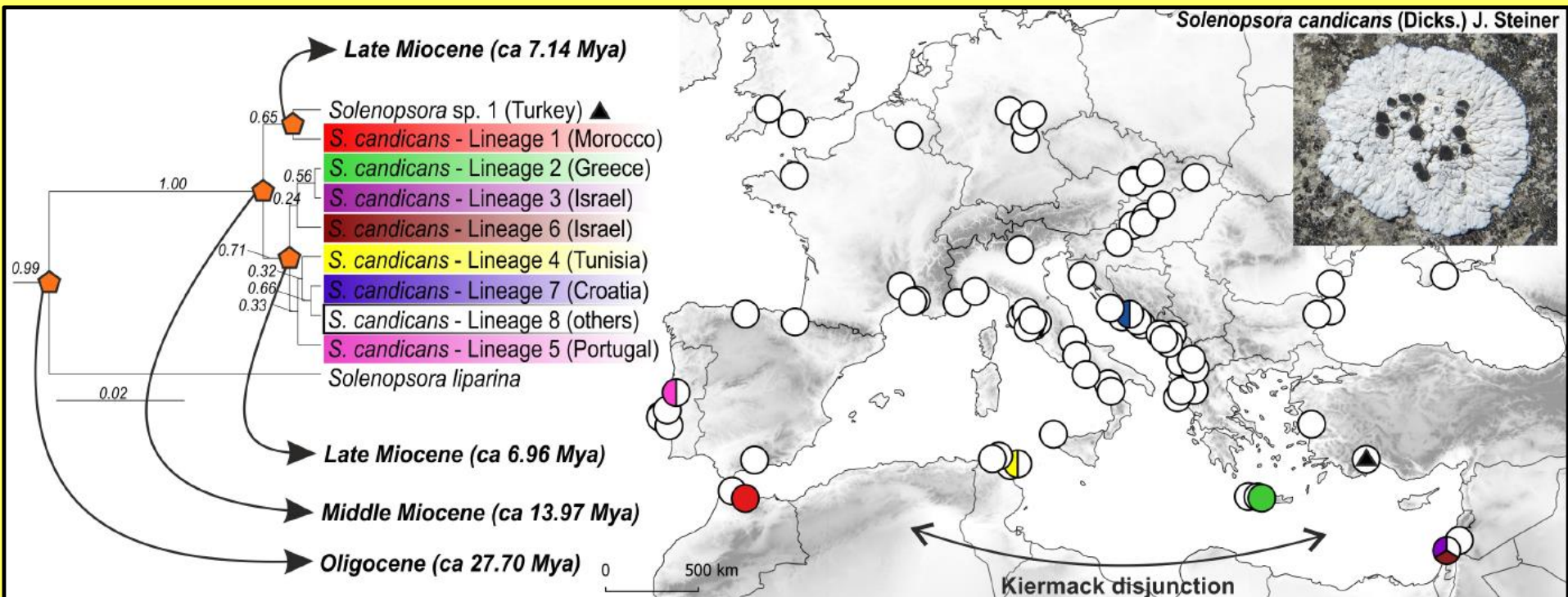
Thamnolia vermicularis, Onuț-Brännströmt al. 2017, *Ecology and Evolution*; Genetic lineages - **ecology**

Genus *Solenopsora* A. Massal. (Leprocaulaceae)

- The genus comprises ca 25 species distributed in temperate and subtropical regions
- But especially in Mediterranean-like biomes



Spatio-temporal formation of the genetic diversity in the Mediterranean dwelling lichen during the Neogene and Quaternary epochs



Authors team: Zuzana Fačková, Marek Slovák, Peter Vďačný, Andrea Melichárková, Judita Zozomová-Lihová & Anna Guttová

**European members of the genus
*Solenopsora***



S. cesatii



S. liparina



S. grisea



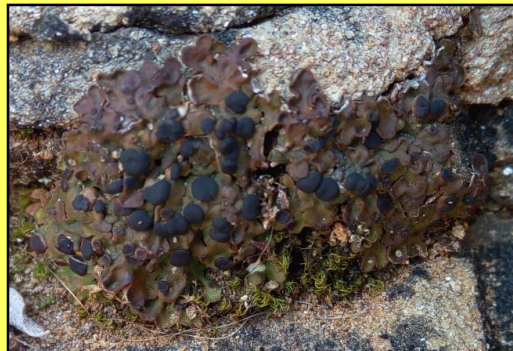
S. vulturiensis



***S. olivacea* subsp.
*olbiensis***



S. olivacea* subsp. *olivacea



S. holophaea



S. marina

European members of the genus *Solenopsora*

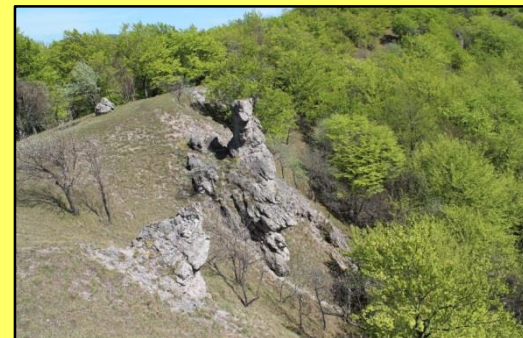


S. candicans

Solenopsora candicans (Dicks.) J. Steiner

Habitats:

- Open calcareous outcrops, xerotherms, rocky macchia, steppe and forest-steppe, Mediterranean deciduous and mixed forests with sclerophyllous vegetation in the Mediterranean and temperate zone



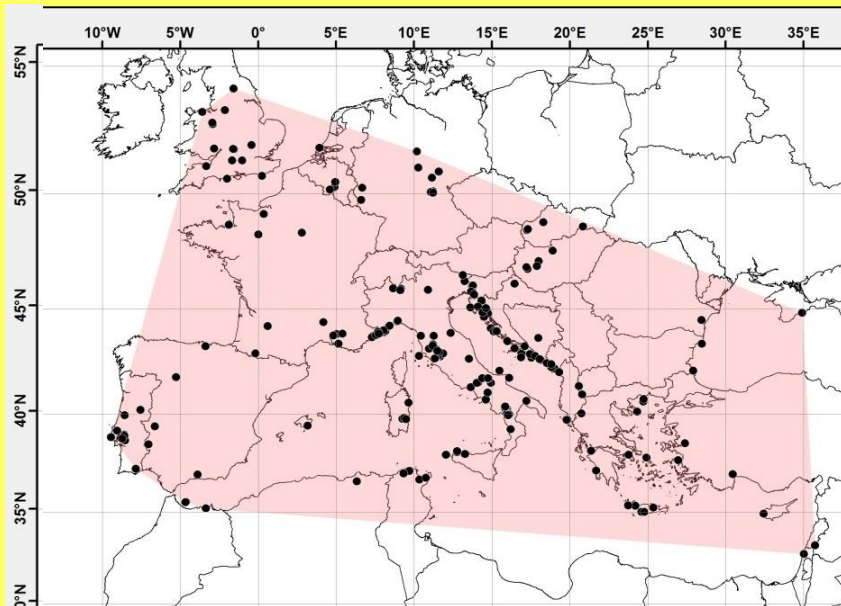
Malé Karpaty Mts.,
Slovakia



Buda Mts., Hungary



Fos do Lisandro, Portugal



Manisa Dagi, Turkey



Basilicata, Italy



Biokovo, Croatia

Aims and questions of the study

What is the genetic structure and evolutionary history of lichen-forming fungus *Solenopsora candicans* (Dicks.) J. Steiner. In the Mediterranean?

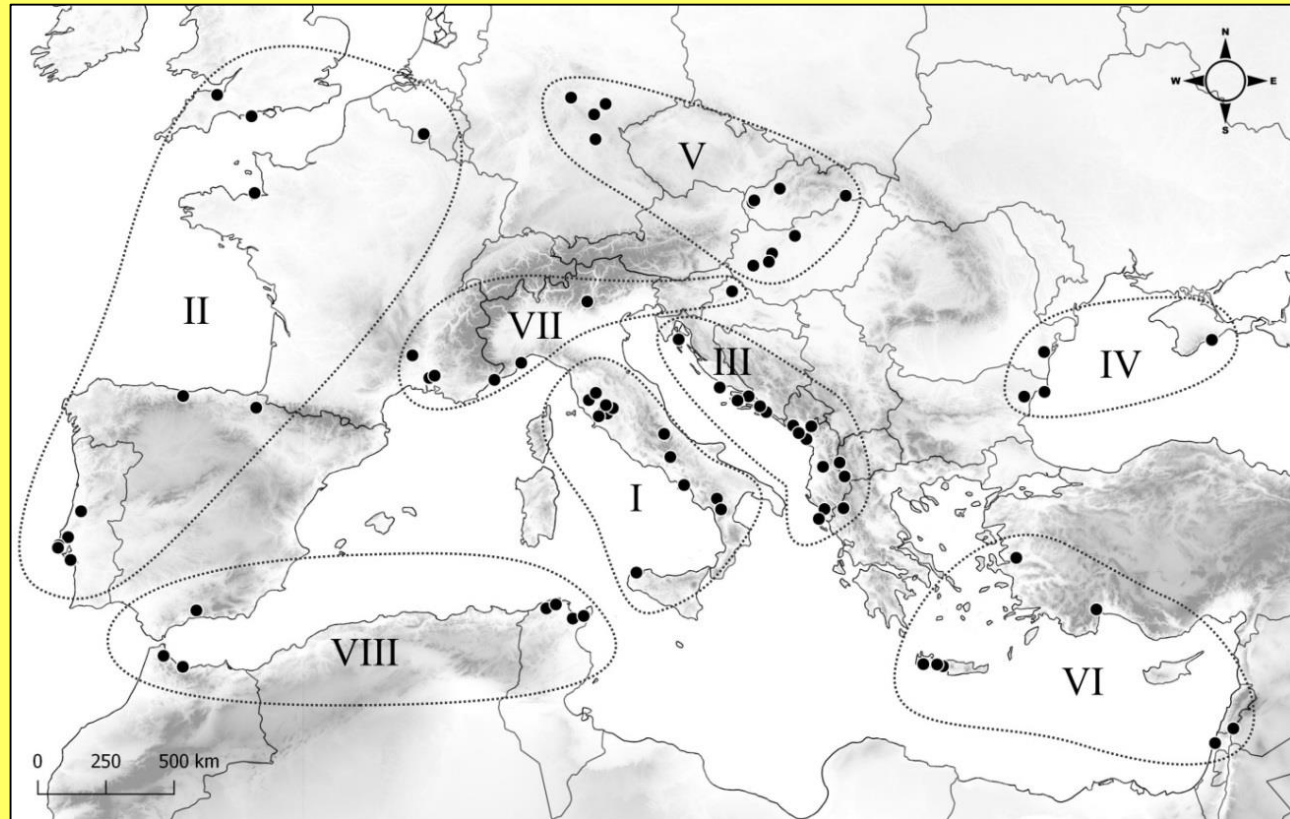
- **(1) Is the genetic pattern of *S. candicans* non-random and does it mirrors paleoclimatic and paleogeological events in the Mediterranean?**
- **(2) Could *S. candicans* have survived Pleistocene glaciations in the extra-Mediterranean refugia in Central Europe and in the central part of the European Atlantic coast.**

Study design Sampling

- 5 to 8 thalli per one population
- 77 population samples with 361 thali covering entire distribution range

8 geographic regions
predefined

- I. Apennine
- II. Atlantic
- III. Balkan
- IV. Black Sea coast
- V. Central Europe
- VI. East Mediterranean
- VII. Peri-alpine
- VIII. South-Western Mediterranean



Geographical distribution of studied *S. candicans* populations within the eight geographical areas fitted with phylogeographic patterns

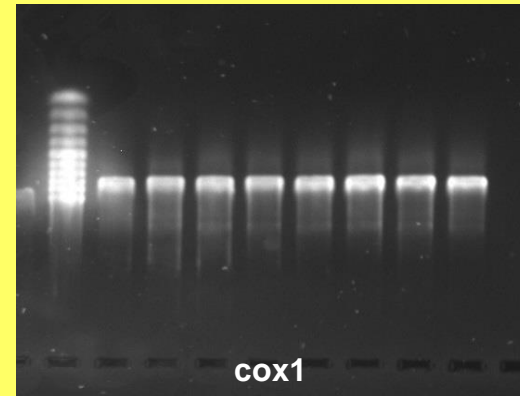
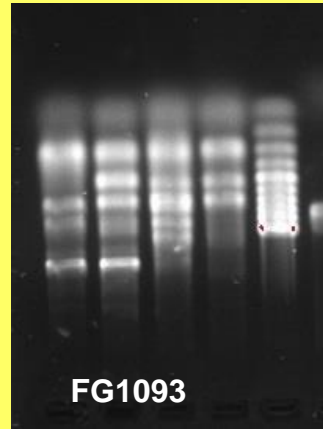
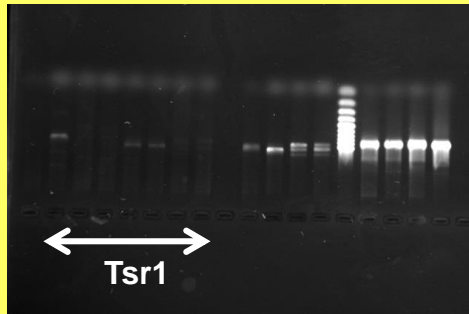
Genetic markers Mycobiont

Excluded due to amplification of
unspecific regions:

- Tsr1
- FG1093
- Gpd

Excluded due to lack of variation:

- Cox1
- MS204
- mtSSU



Regions used for population genetic analyses:

- **ITS** (ITS1 + 5.8S gene + ITS2)
- **β -tubulín** (2 introns, 2 exons)

Regions used for phylogenetic analyses:

- **nrLSU**

Analytical approaches

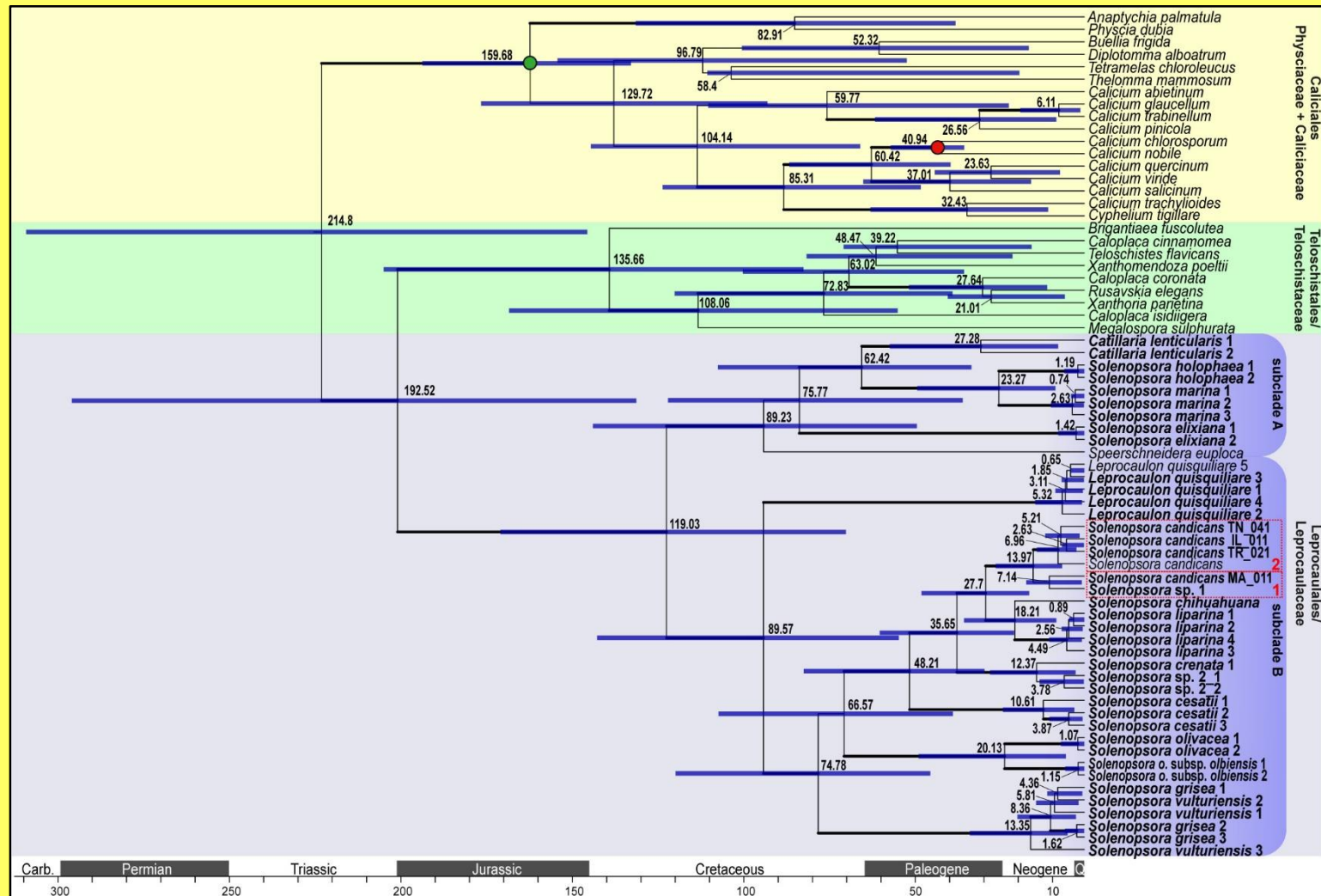
Phylogenetic inference

- maximum likelihood in the RAxML 8.2.0, Bayesian analyses in MrBayes
- coalescence-based species tree employing the Bayesian Multispecies Coalescent (MSC) model using STACEY ver. 1.2.2 in the software BEAST 2.4.4. (Bouckaert et al. 2014)
- topology and divergence times estimated using the Bayesian Markov chain Monte Carlo algorithm implemented in BEAST 2.4.4 (Drummond et al. 2012). The phylogeny calibration based on combinations of primary and secondary calibration points.

Population genetic analyses

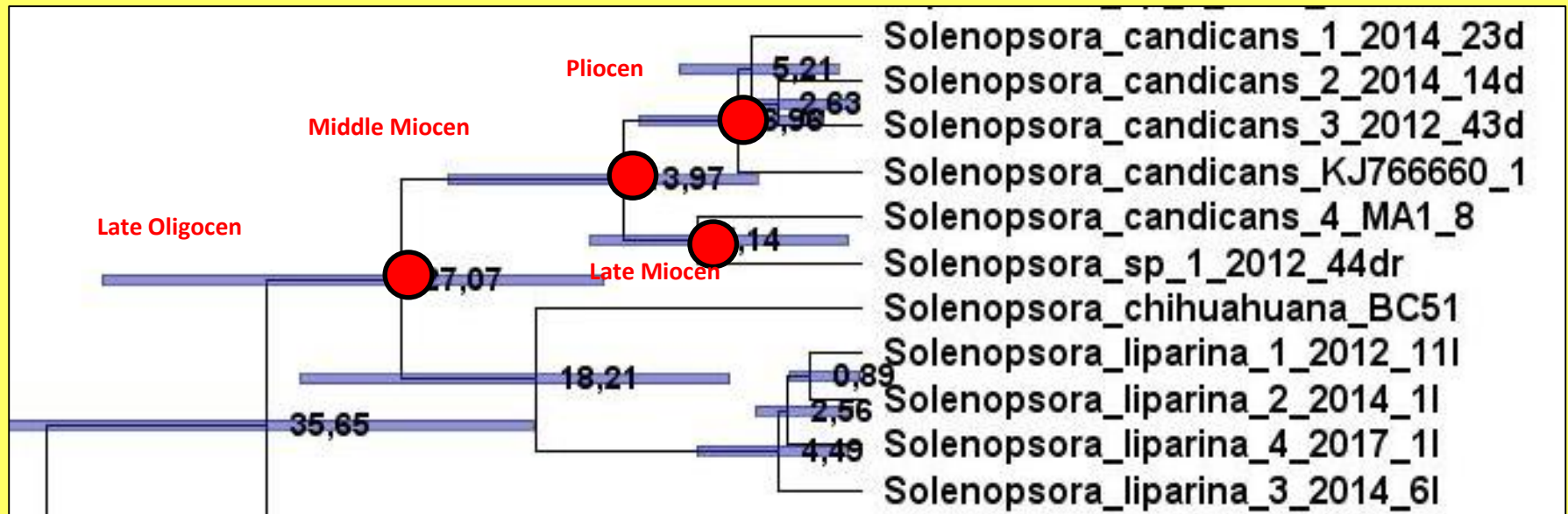
- Haplotype TCS networks in PopART (Leigh & Bryant 2015)
- Genetic diversity estimation – haplotype (H_d) and nucleotide (P_i) diversity, number of polymorphic sites (s), number of haplotypes (h) in DnaSP v5.10 (Librado & Rozas 2009)
- Tests of selective neutrality: Tajima's D (Tajima 1989) and Fu's FS (Fu 1997) tests in Arlequin v3.5.2
- Fixation indices F_{ST} and the exact test of genetic differentiation in Arlequin v3.5.2 (Excoffier & Lischer 2010)

Phylogenetic inference and divergence time estimation



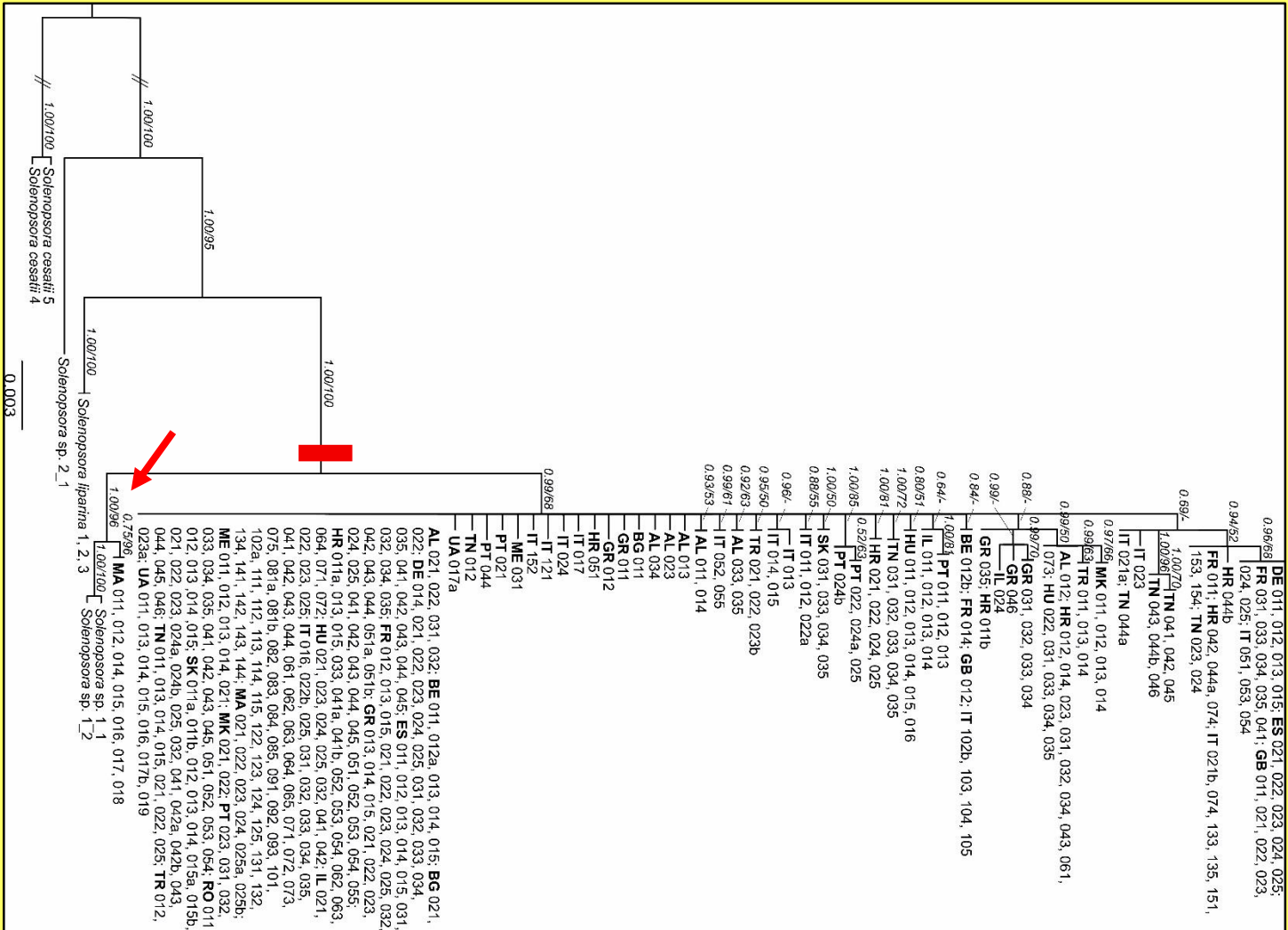
Chronogram showing times of diversifications based on the nrLSU dataset and estimated by the BM3C algorithm in BEAST. The primary calibration (red circle) was based on fossil of *Calicium* with a mean set to 35 Mya and offset to 20 Mya, combined with the secondary calibration (green circle) of age of Caliciaceae-Physciaceae clade (132 Mya and 199 Mya, min. max. bounds). Values above branches represent median diversification ages in Mya. The branches with the posterior probability values of Bayesian inference > 0.90 are indicated by the thicker line.

Phylogenetic inference and divergence time estimation



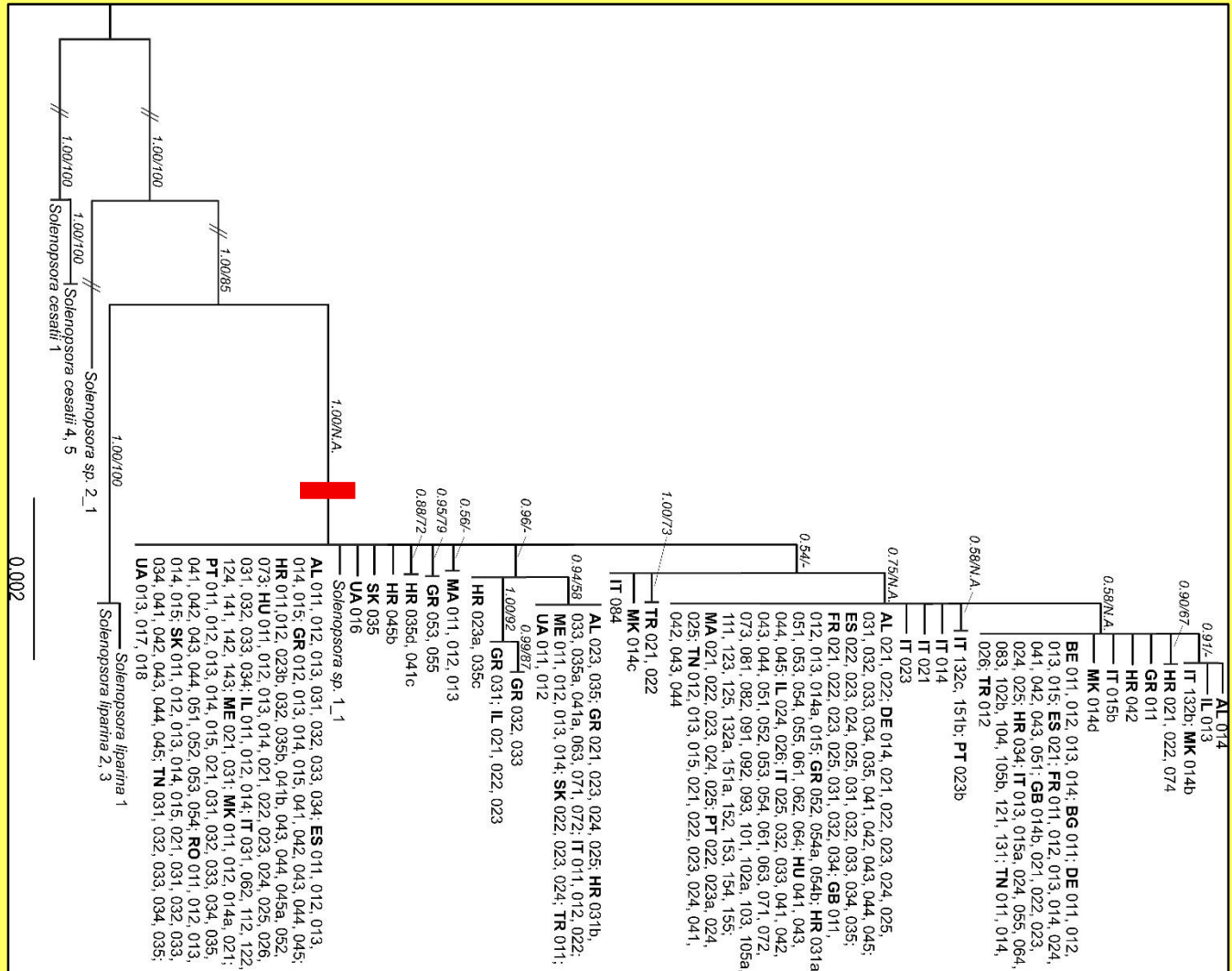
- the early Cretaceous (120–112.2–119.3 Mya) - the most recent common ancestor (MRCA) of *Solenopsora* and related genera
- the Late Cretaceous (88.1–84.9–89.2 and 91.4–85–89.6 Mya, respectively) - the crown divergence of subclades A and B
- the Late Oligocene (ca. 27.3–27.5–28.8 Mya) - the MRCA of the *S. candicans* group
- the Middle Miocene (13.9–12.7–14 Mya) - the crown divergence of the two sister subclades 1 and 2 of the *S. candicans* group
- the Late Miocene and Pliocene (7.1–6.7–7.2 and 7.0–6.4–7.0 Mya, respectively) - the diversification of intraspecific diversity within both subclades of *S. candicans*

Phylogenetic inference and genetic structure based on ITS



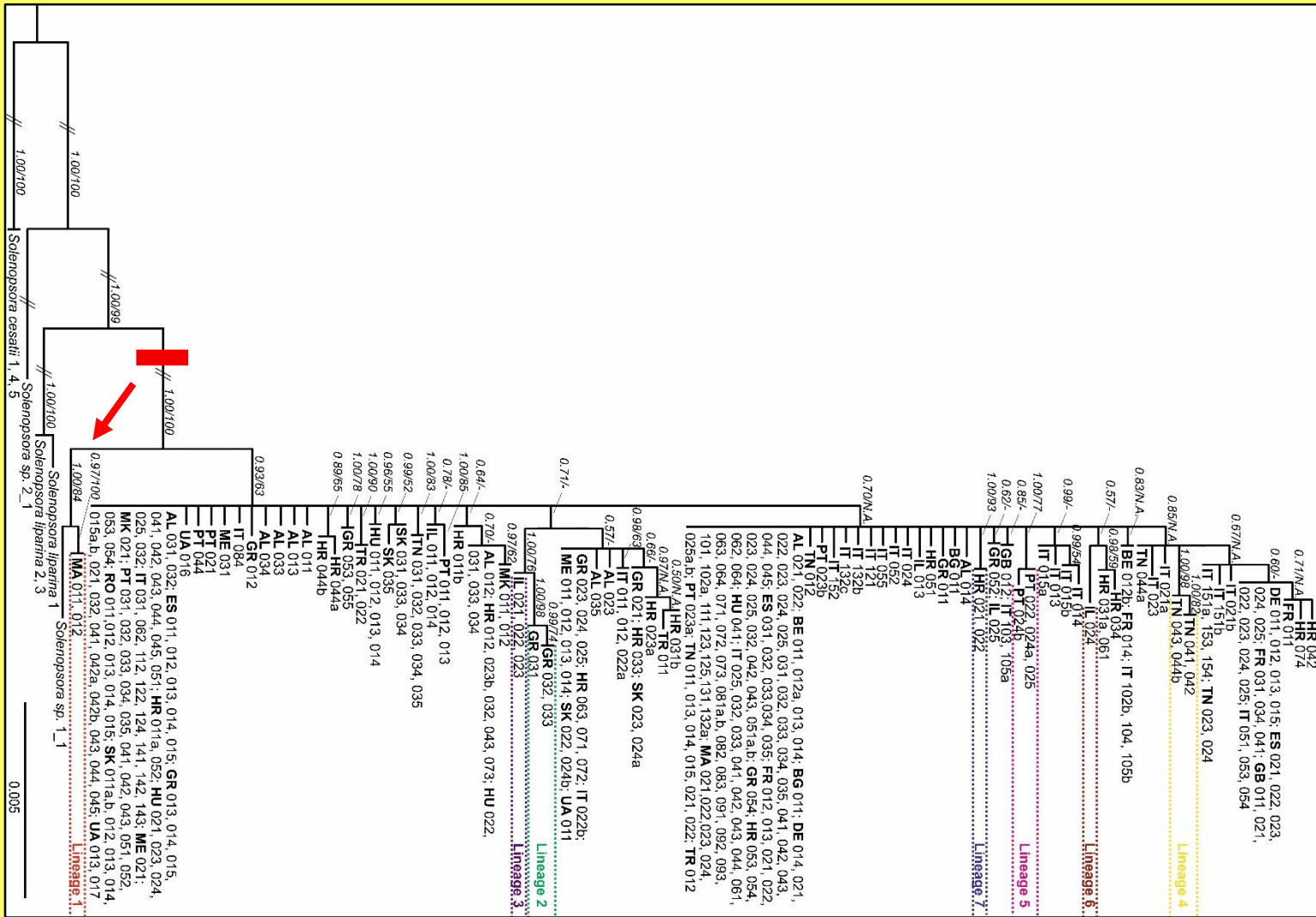
Bayesian majority-rule consensus tree inferred from the ITS nrDNA data representing genetic relationships of *Solenopsora candicans* samples and outgroup sequences. The numbers above branches refer to posterior probability values of Bayesian inference/the bootstrap support as inferred for the maximum likelihood analyses (values <50% are shown).

Phylogenetic inference and genetic structure based on β -tubulin



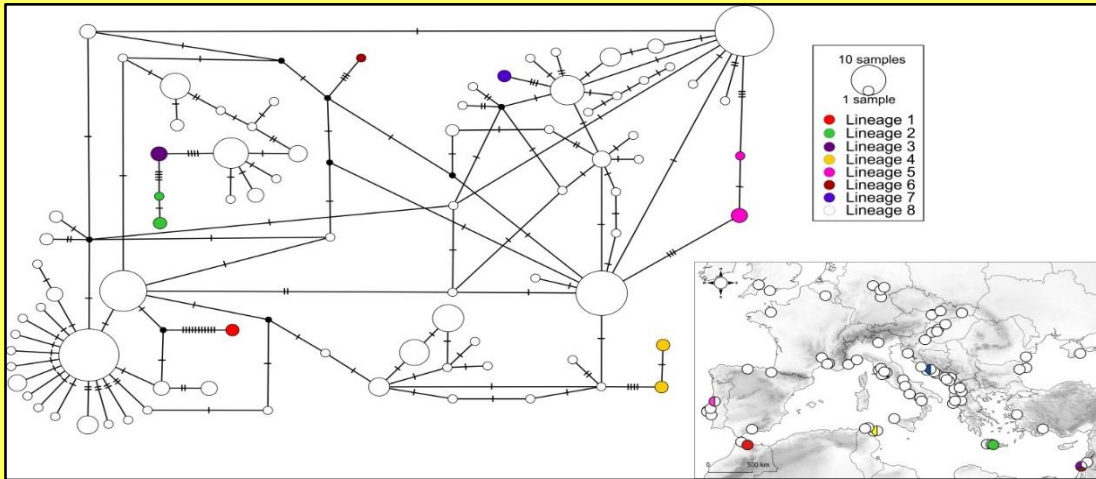
Bayesian majority-rule consensus tree inferred from the β -tubulin data representing genetic relationships of *Solenopsora candicans* samples and outgroup sequences. The numbers above branches refer to posterior probability values of Bayesian inference/the bootstrap support as inferred for the maximum likelihood analyses (values <50% are shown).

Phylogenetic inference and genetic structure based on concatenated β -tubulin and ITS dataset



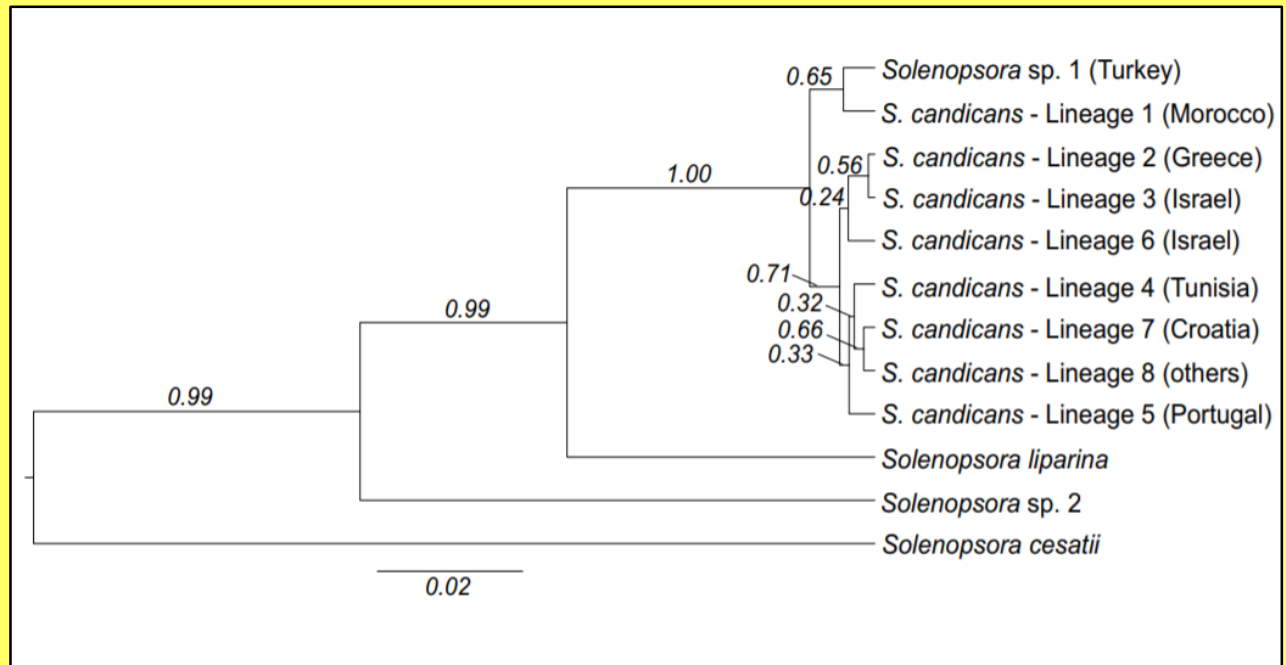
Bayesian majority-rule consensus tree inferred from the concatenated ITS nrDNA and β -tubulin data representing genetic relationships of *Solenopsis candicans* samples and outgroup sequences. The numbers above branches refer to posterior probability values of Bayesian inference/the bootstrap support as inferred for the maximum likelihood analyses (values <50% are shown).

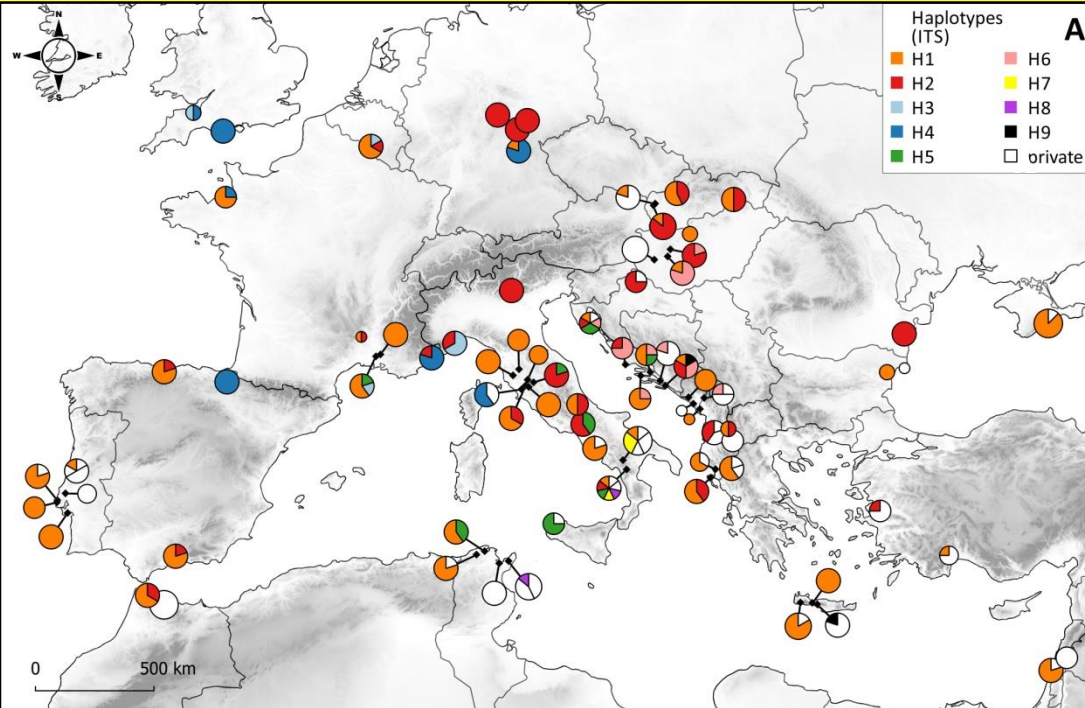
'Lineage tree' inference



Multilocus TCS network based on concatenated ITS and β -tubulin data of *Solenopsora candicans*. The colours refer to eight plausibly independently evolving lineages including *S. sp. I* (black triangle, not included in network) considered for the reconstruction of coalescence-based species tree. Haplotypes forming lineage 8 are represented by the empty circles.

Maximum clade credibility lineage tree based on ITS and β -tubulin sequences and estimated using STACEY as implemented in the software *BEAST. Values above branches represent the posterior probability values of Bayesian inference



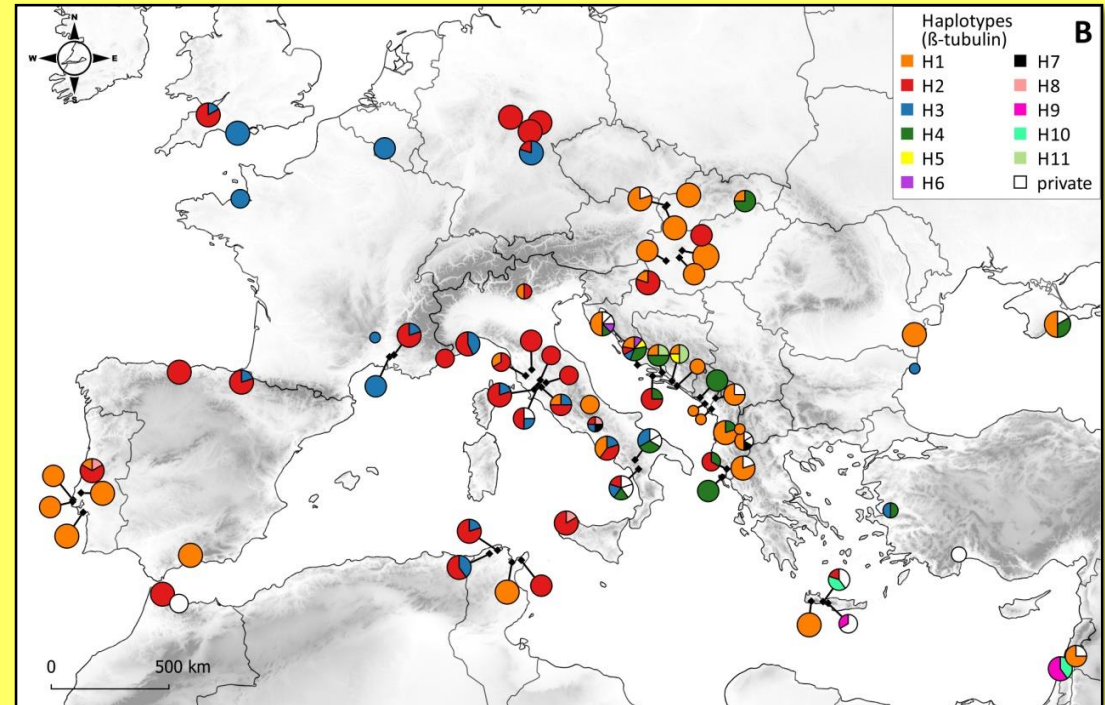
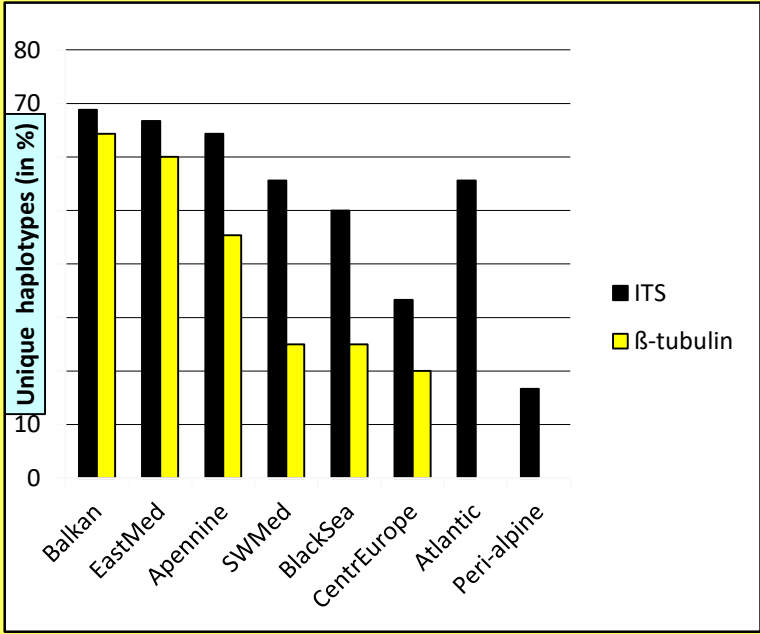


Genetic diversity in studied regions

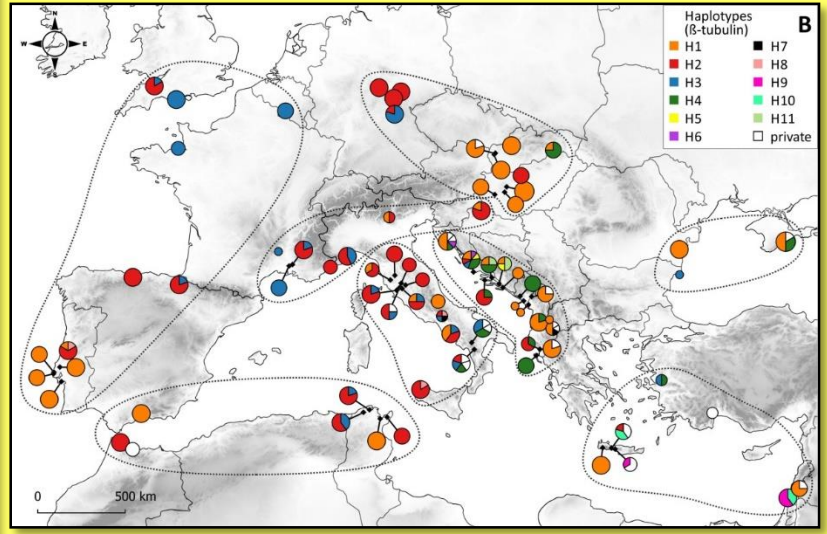
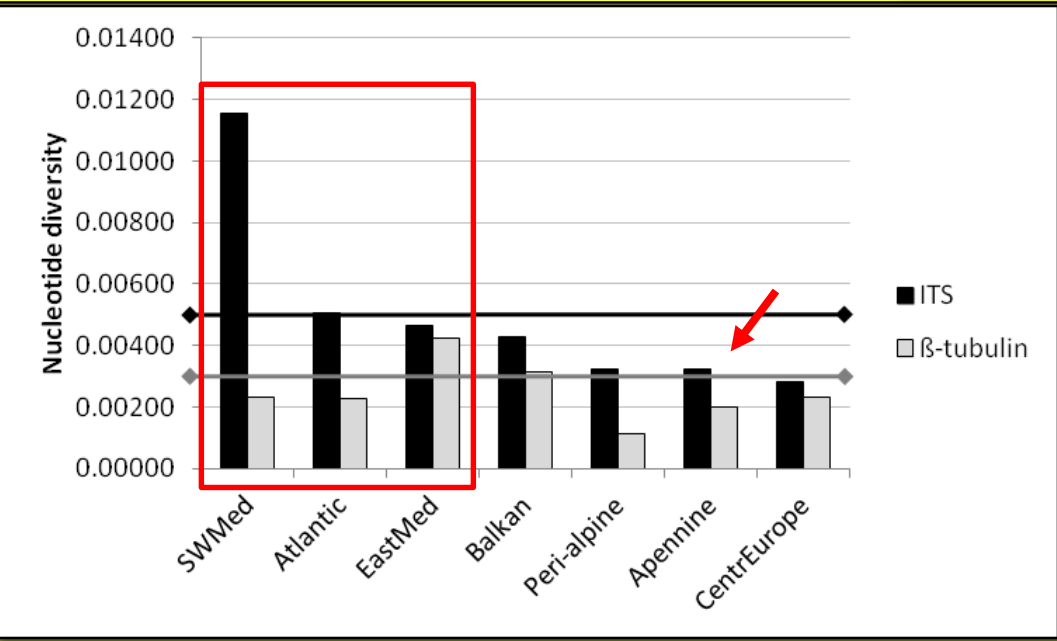
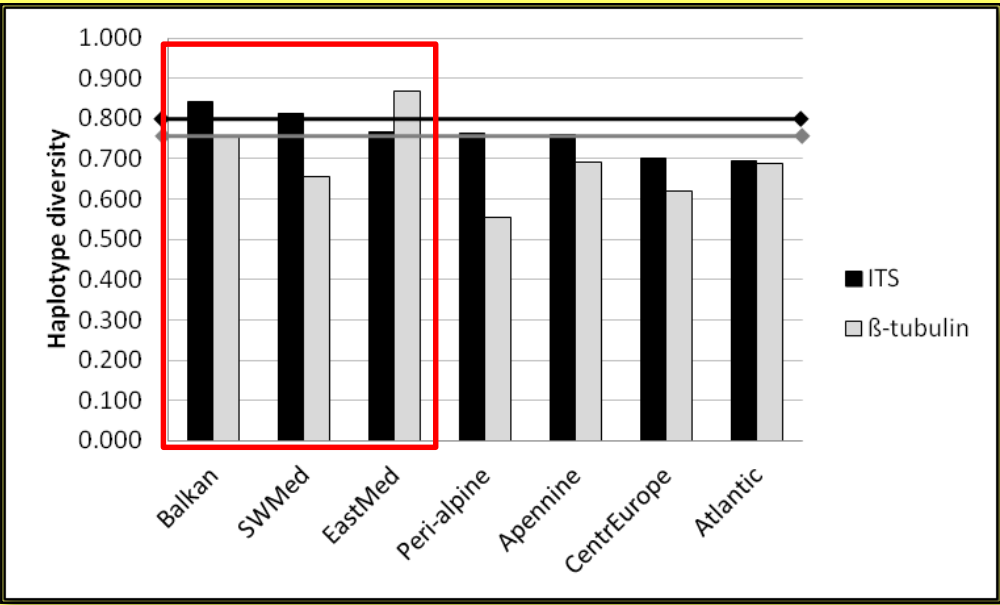
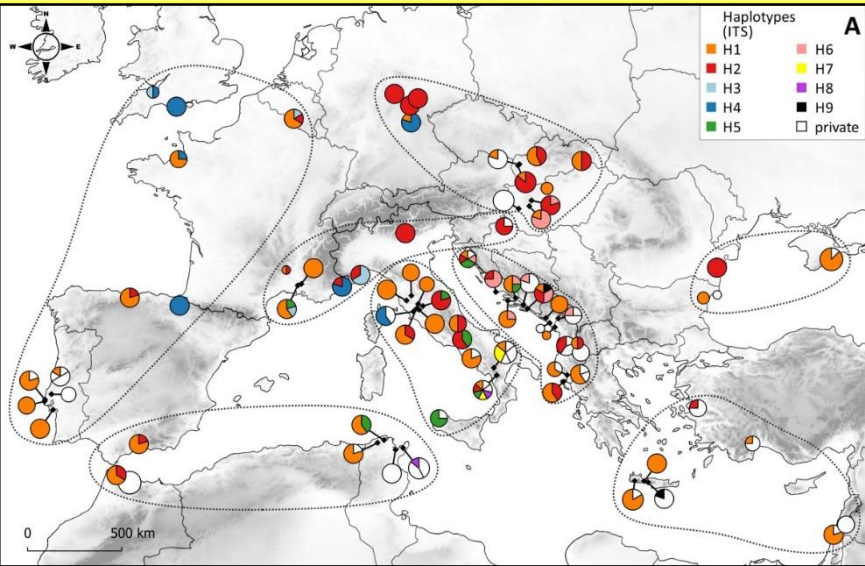
Distribution of ITS ribotypes

White symbols represent haplotypes unique for single population

Distribution of β -tubulin haplotypes



Genetic diversity in studied regions



Genetic differentiations

ITS	Apennine	Atlantic	Balkan	CentrEurope	EastMed	PeriAlpine	SWMed
Apennine	-	< 0.001	< 0.001	< 0.001	< 0.001	< 0.01	< 0.001
Atlantic	0.033*	-	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Balkan	0.066*	0.100*	-	< 0.001	< 0.001	< 0.001	< 0.001
CentrEurope	0.046*	0.099*	0.051*	-	< 0.001	< 0.001	< 0.001
EastMed	0.081*	0.097*	0.032*	0.099*	-	< 0.001	< 0.001
PeriAlpine	0.007	0.026	0.071*	0.031	0.097*	-	< 0.001
SWMed	0.130*	0.125*	0.146*	0.143*	0.116*	0.104*	-
Bt tubulin	Apennine	Atlantic	Balkan	CentrEurope	EastMed	PeriAlpine	SWMed
Apennine	-	< 0.01	< 0.001	< 0.001	< 0.001	0.698±0.010	0.126±0.006
Atlantic	0.045*	-	< 0.001	< 0.05	< 0.001	< 0.05	< 0.05
Balkan	0.277*	0.179*	-	< 0.001	< 0.001	< 0.001	< 0.001
CentrEurope	0.183*	0.061*	0.071*	-	< 0.001	< 0.001	< 0.05
EastMed	0.295*	0.200*	0.073*	0.109*	-	< 0.001	< 0.001
PeriAlpine	0.029	0.131*	0.386*	0.324*	0.393*	-	< 0.01
SWMed	0.068*	-0.002	0.151*	0.023	0.160*	0.199*	-

Tab. Genetic differentiation of *S. candicans* between investigated geographical regions. Above diagonal: probabilities for rejection of the null hypothesis of no pairwise differentiation between regions calculated by an exact test of differentiation. Below diagonal: pairwise fixation index (F_{ST}) and its significance ($P < 0.05$ marked by *).

Differentiation among regions:

<i>Low</i>
<i>Middle</i>
<i>High</i>
<i>Very high</i>

F_{ST} :
 0.0 - identical allelic composition
 1.0 - absence of any shared alleles

Fixation index reflects probability that two alleles in subpopulation are of the identical origin

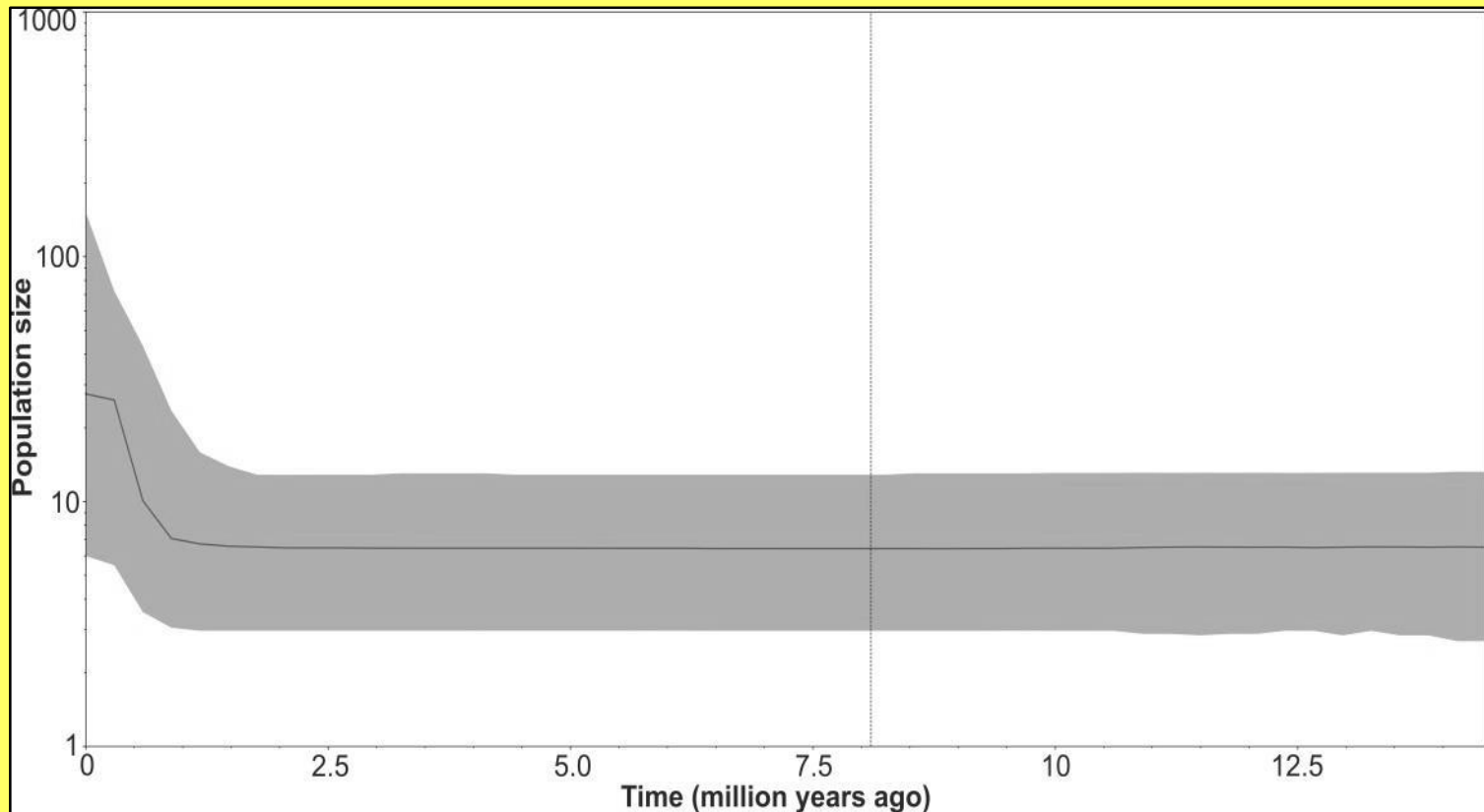
Selective neutrality hypothesis

- Significantly negative values of the Fu's F_S in Apennine and Balkan peninsulas are evidence for an excess number of allele as would be expected from a recent population expansion or genetic hitchhiking

	Regions	Tajima's D	P	Fu's F_S	P
ITS	Apennine	-1.320	0.078	-6.534	0.003
	Atlantic	-0.365	0.413	-0.261	0.500
	Balkan	-1.091	0.133	-6.700	0.006
	Central Europe	-0.418	0.394	0.296	0.595
	East Mediterranean	-0.439	0.371	-1.377	0.259
	Peri-alpine	0.243	0.648	-0.144	0.498
	SW Mediterranean	0.585	0.776	2.735	0.862
	Black Sea coast	-	-	-	-
	Overall	-2.106	0.001	-26.494	0.000
β-tubulin	Apennine	0.939	0.953	-3.689	0.047
	Atlantic	0.066	0.230	2.944	0.907
	Balkan	0.938	0.983	-3.401	0.089
	Central Europe	0.406	0.457	2.015	0.840
	East Mediterranean	0.517	0.416	-1.368	0.269
	Peri-alpine	0.283	0.298	1.226	0.750
	SW Mediterranean	0.216	0.147	2.258	0.877
	Black Sea coast	-	-	-	-
	Overall	-0.920	0.180	-13.294	0.003

Population demographics

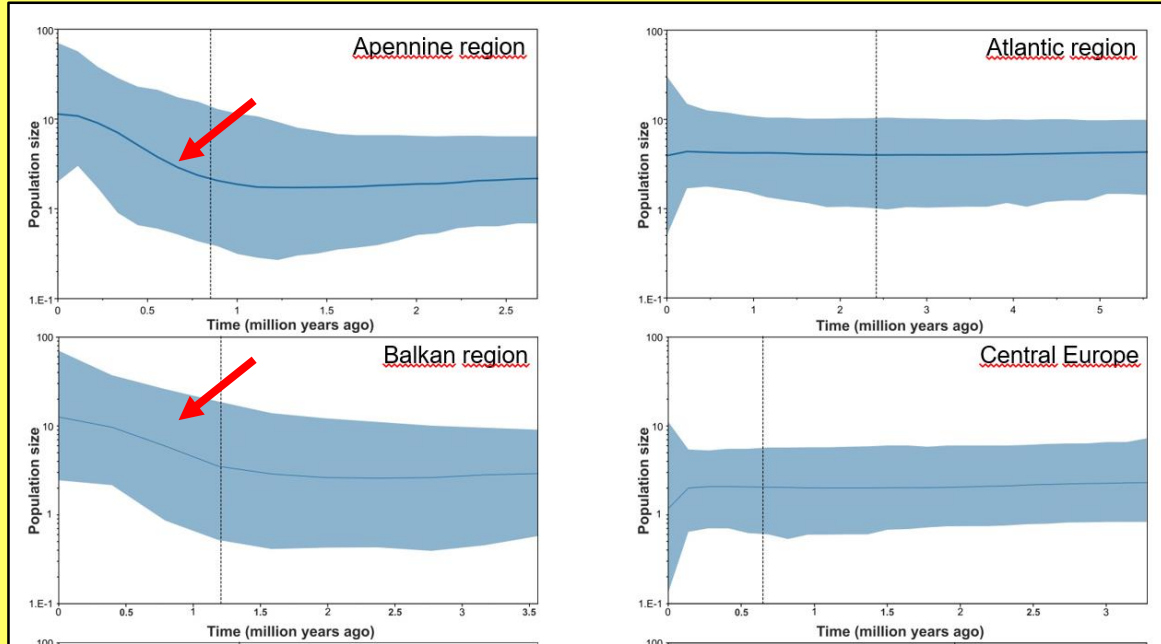
Bayesian skyline plots



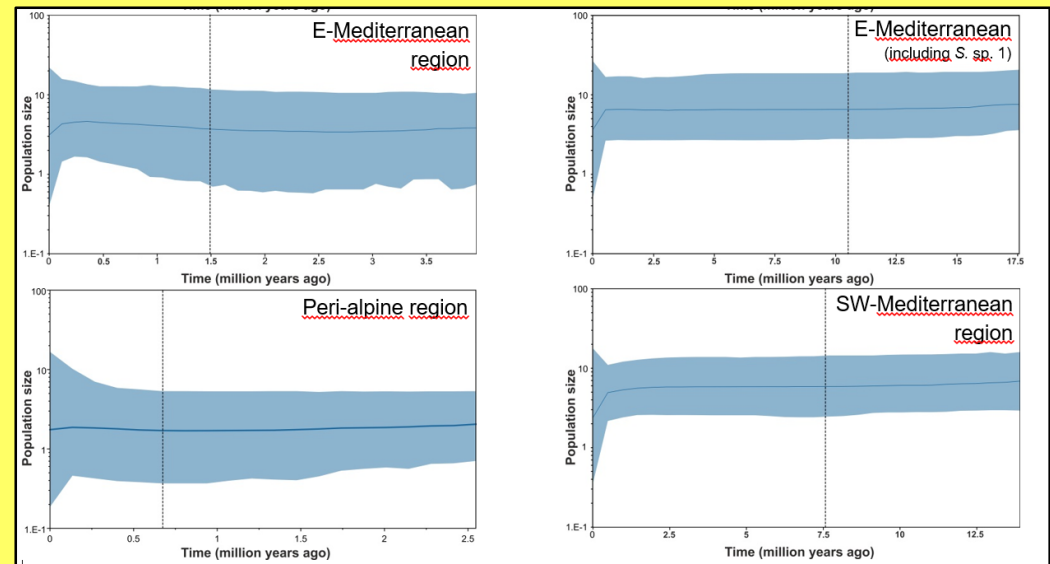
Bayesian skyline plot showing population size changes of *Solenopsis candicans* over time inferred with ITS data. The x axis display times in millions of years. Since generation times for *S. candicans* are not exactly known the axis y represent only relative changes instead of population sizes. The grey solid horizontal line represents the median value of 95% highest posterior density (HPD) and shaded areas represent the upper and lower 95% credible intervals

Population demographics

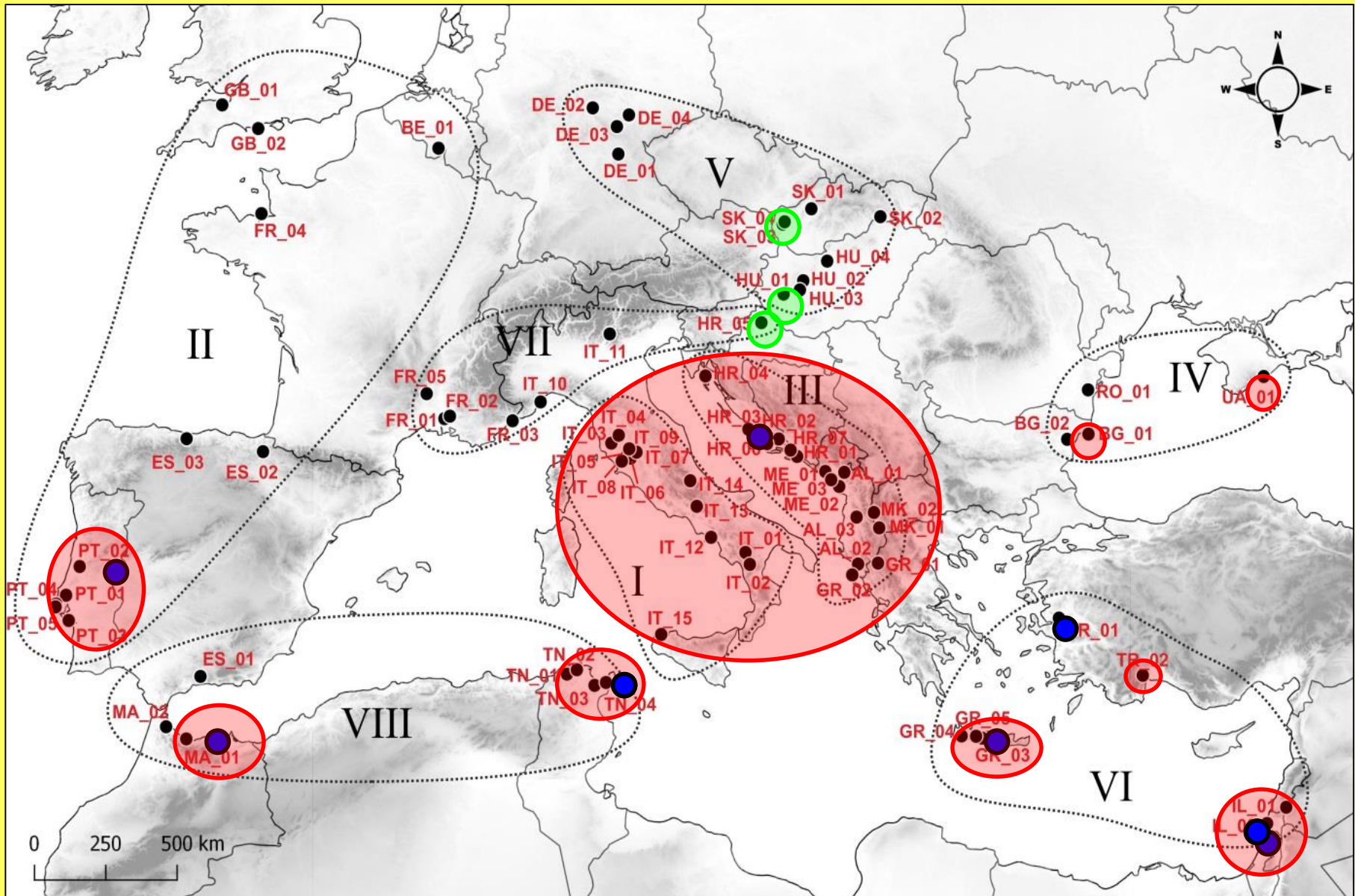
Bayesian skyline plots



Bayesian skyline plots showing population size dynamics of *Solenopsis candicans* in studied geographical regions over time inferred with ITS data. The x axis display times in millions of years. The axis y represent relative changes instead of population sizes. The dark blue solid line represents the median value of 95% highest posterior density (HPD) and shaded areas represent the upper and lower 95% credible intervals. A

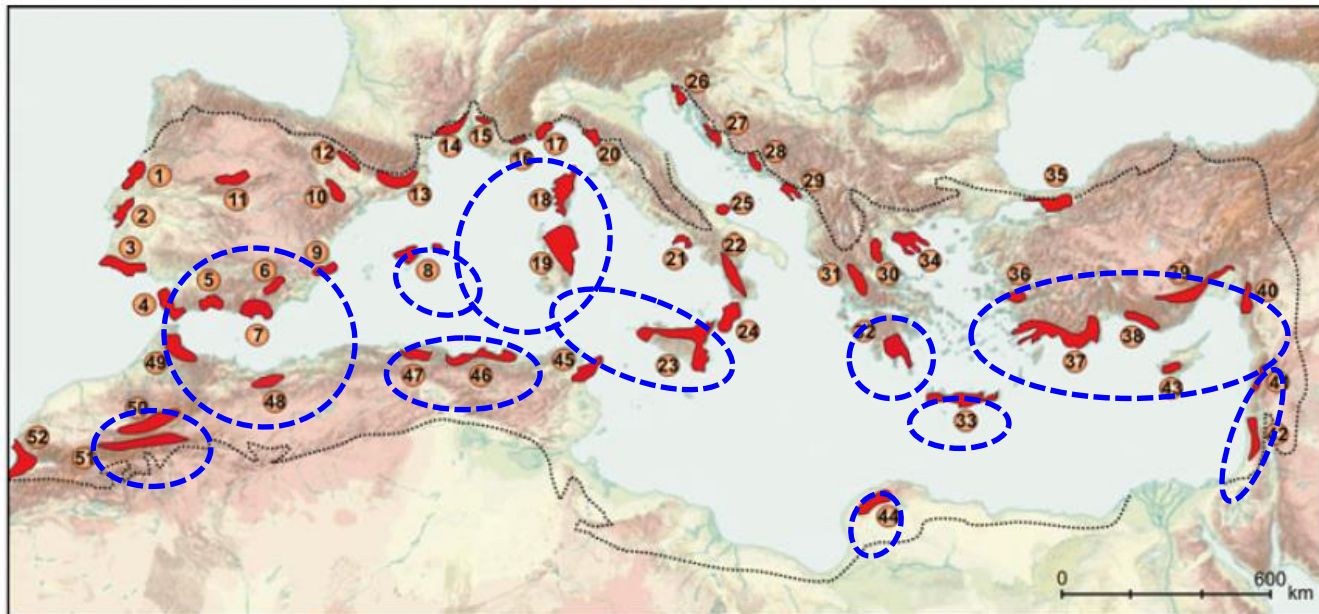


Phylogeographic history of *S. candicans*



The distribution of the most divergent lineages, mostly of a pre-Pleistocene origin, was restricted to the eastern or western extremities of the Mediterranean exhibiting **Kiermack disjunction**

Mediterranean refugia



- | | | | | |
|--------------------------|--------------------|----------------------|-------------------------|----------------------------|
| 1 Beira litoral | 11 Sistema central | 21 Campania | 31 C. Greece (Pindos) | 42 Israel/Palestine |
| 2 Estramadura | 12 S. Pyrenees | 22 S. Apennines | 32 Peloponnese | 43 Cyprus |
| 3 Algarve | 13 S.E. Pyrenees | 23 Sicilia | 33 Crete | 44 Cyrenaic (Lybia) |
| 4 Cadiz/Algeciras region | 14 S. Cévennes | 24 S. Calabria | 34 Chalkidiki peninsula | 45 J. Zaghouan/Cap Bon |
| 5 Serrania de Ronda | 15 Mont Ventoux | 25 Gargano | 35 Izmit region | 46 Petite Kabylie/de Collo |
| 6 Sierra Cazorla/Segura | 16 E. Provence | 26 N. Istria | 36 Boz/Aydin dag | 47 Grande Kabylie |
| 7 Sierra Nevada/Gata | 17 Maritime Alps | 27 Velebit Mountains | 37 S.W. Anatolia | 48 Tlemcen Mountains |
| 8 Balearic Islands | 18 Corsica | 28 S. Bosnia/Biokovo | 38 C. Taurus | 49 Rif Mountains |
| 9 Valencia region | 19 Sardinia | 29 Montenegro | 39 E. Taurus | 50 Middle Atlas |
| 10 Ebro Valley | 20 Alpi Apuani | 30 Olympe/Katalympos | 40 Amanus | 51 High Atlas |
| | | | 41 Lebanon range | 52 Souss/W. Anti Atlas |

Fine-scale geographical distribution of the 52 putative refugia within the Mediterranean obtained from the analysis of the phylogeographical patterns of 82 plant species (41 trees and 41 herbs).

Médail and Diadema 2009, Journal of Biogeography

Extra-Mediterranean refugia for *S. candicans*

Unhospitable for *S. candicans* during Pleistocene glacial cycles



Mammoth steppe



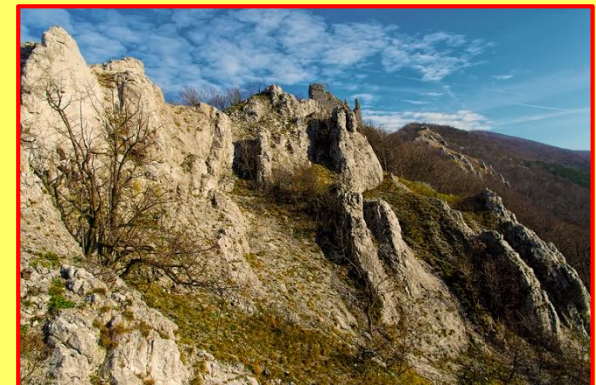
Stipa steppe

BUT!

Assumed microrefugia for *S. candicans* during Pleistocene glacial cycles



small patches of deciduous and evergreen trees

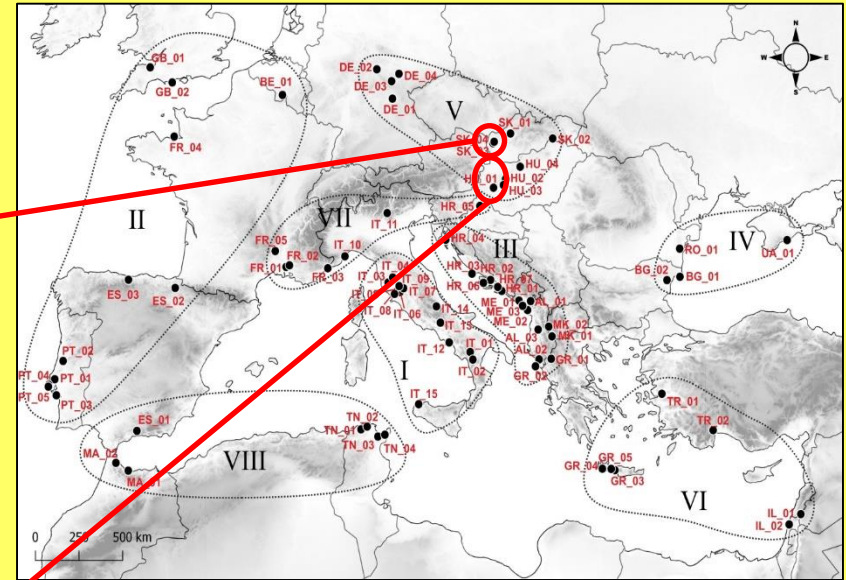


Lower altitudinal calcareous outcrops with SW exposition

Extra-Mediterranean refugia for *S. candicans*



Malé Karpaty Mts



Balaton lake



Concluding remarks

- **Tertiary (Oligocene)** origin for *S. candicans*, with formation of intraspecies diversity initiated in the **Late Miocene**
- The distribution of the most divergent lineages, mostly of a pre-Pleistocene origin, was restricted to the eastern or western extremities of the Mediterranean exhibiting **Kiermack disjunction**.
- The population genetic diversity analyses indicated **multiple diversity centres and refugia** for *S. candicans* across the entire Mediterranean Basin.
- South Mediterranean regions harboured both the **Tertiary and Quaternary** born diversity - '**cumulative refugia**' paradigm.
- the Apennine and Balkan Peninsulas hosted mostly younger Pleistocene haplotypes and lineages.
- The **recent population expansion** of *S. candicans* might have occurred in the middle Pleistocene with a population burst in the **Apennine and Balkan** peninsulas.
- The presence of unique haplotypes in **Central Europe** indicates the existence of **extra-Mediterranean microrefugia**.

Thanks for your attention

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