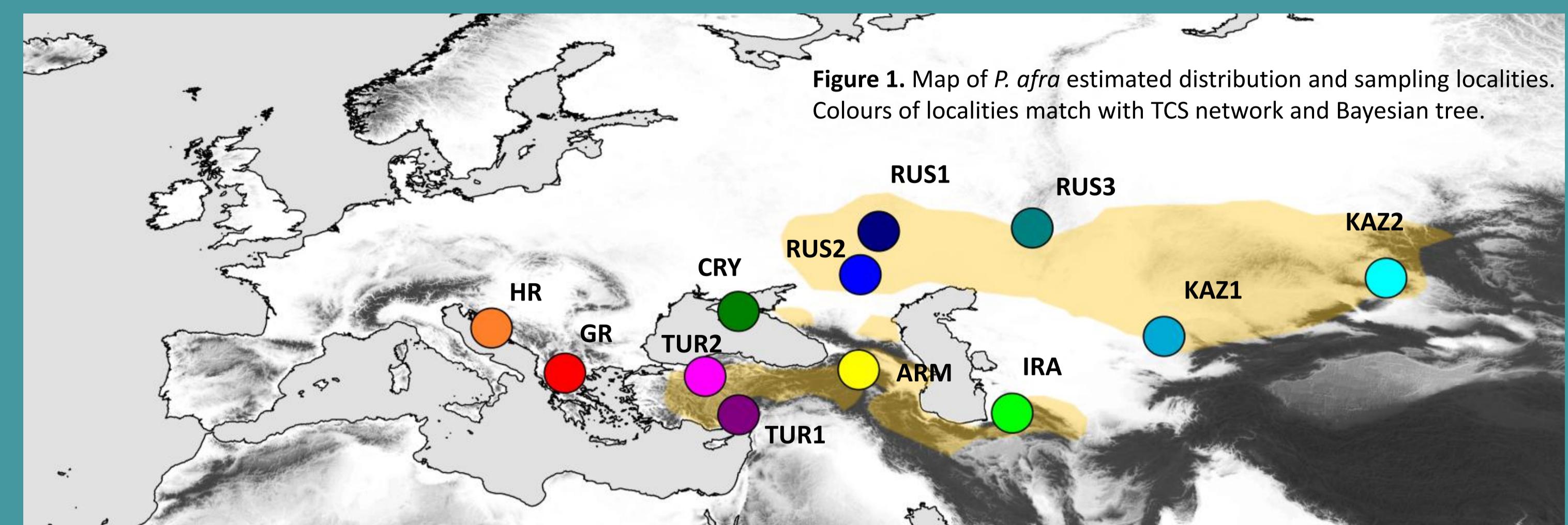
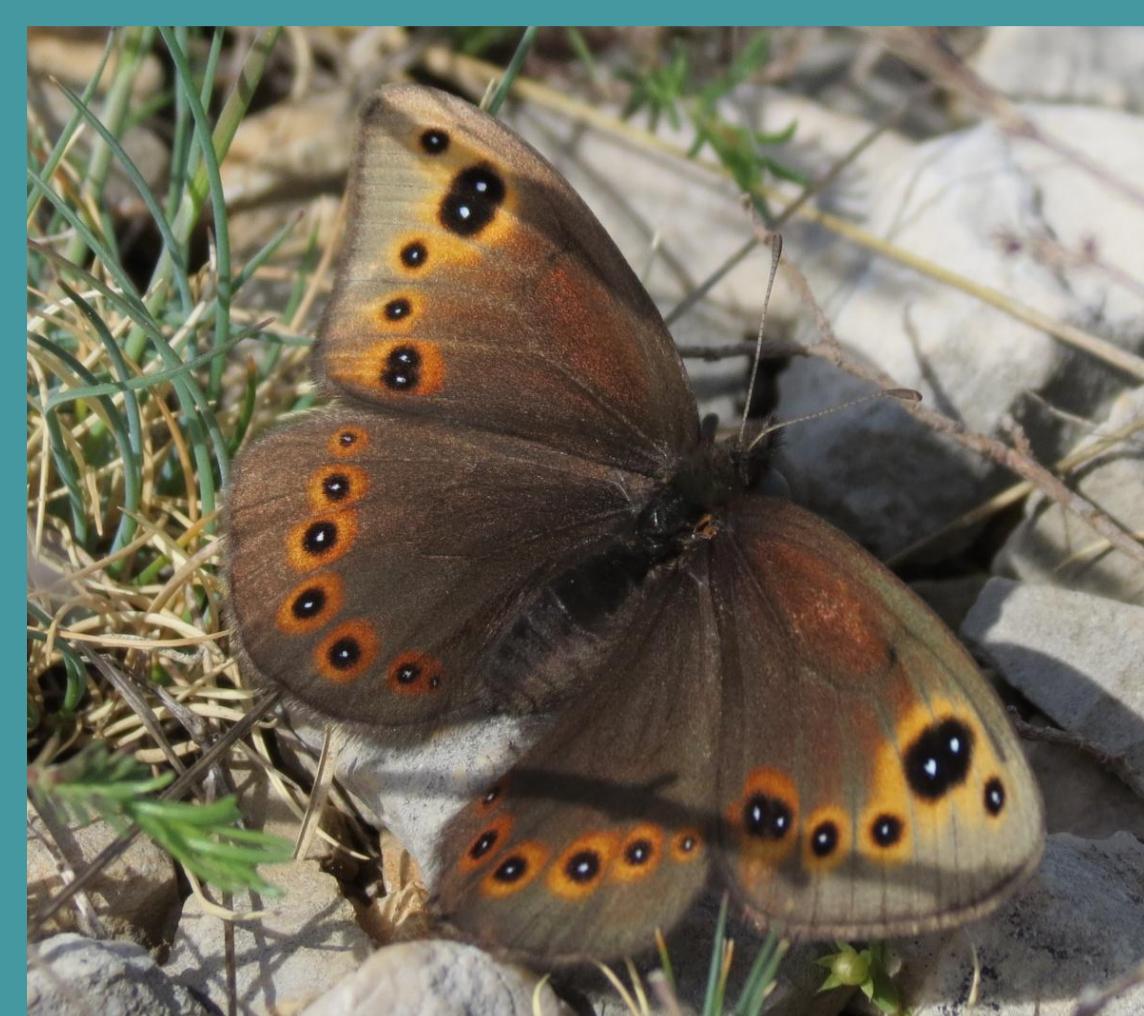


Steppe species under the glacial cycles: Phylogeography of *Proterebia afra*

Alena Bartoňová^{1,2}, Zdeněk Faltýnek Fric² & Martin Konvička^{1,2}

¹ Faculty of Science, University of South Bohemia in České Budějovice, Czech Republic

² Institute of Entomology, Biology Centre CAS, Czech Republic



Intro

- **Steppes:** the most endangered biome of the world (causes: agriculture, land use change)
- Ice Ages: dry climate, enhanced continentality, the most extensive biome: cold mammoth steppe
- Interglacial period: humidity, retreat
- ***Proterebia afra* (Fabricius, 1787)** (Nymphalidae: Satyrinae): steppe species with disjunct distribution: refugial for Europe
- Two isolated populations within EU borders: Dalmatia (Croatia; HR) and Askion Mts. (NW Greece; GR)

Material & Methods

- 120 samples from 12 localities
- DNA extraction, amplification of 4 markers: mtDNA: cytochrome c oxidase subunit I and II (COI, COII), nDNA: arginin kinase, wingless
- Phylogeography: BEAST 1.8
- Population Genetics: TCS (PopArt), DNAsP, Arlequin

Results

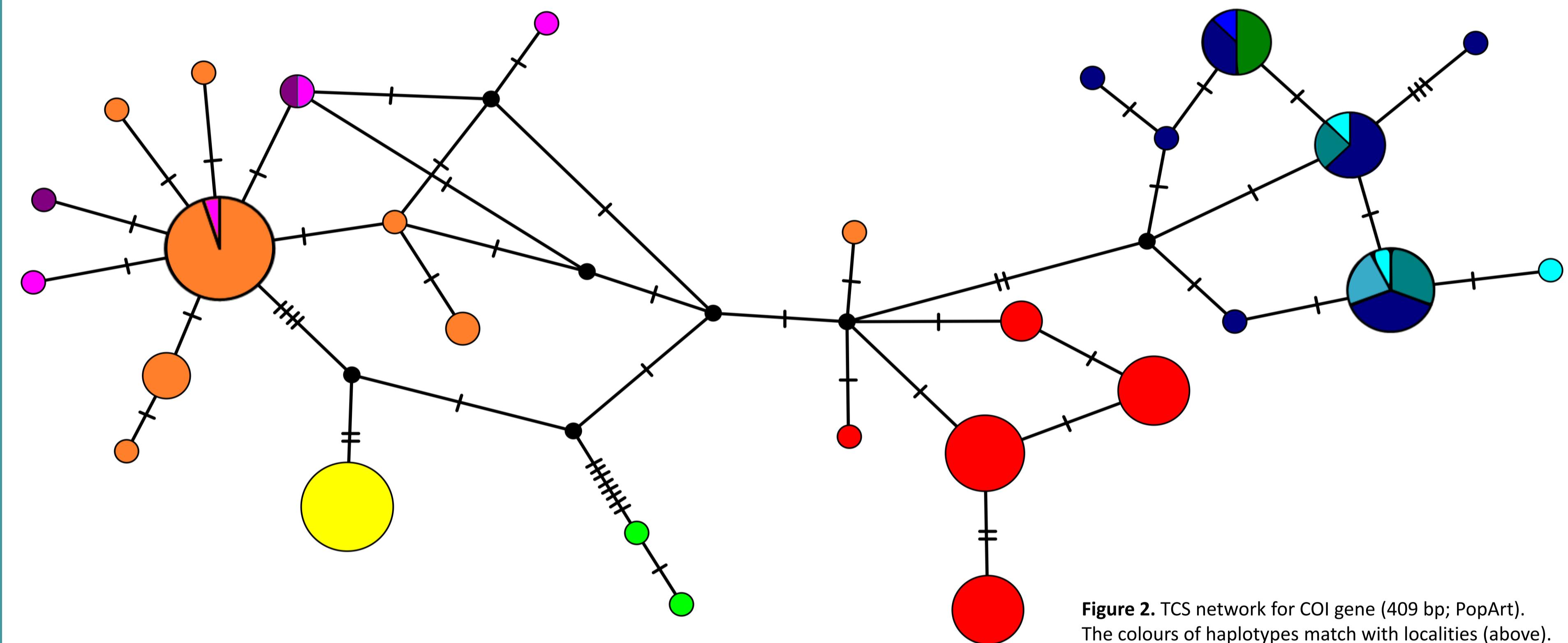


Figure 2. TCS network for COI gene (409 bp; PopArt). The colours of haplotypes match with localities (above).

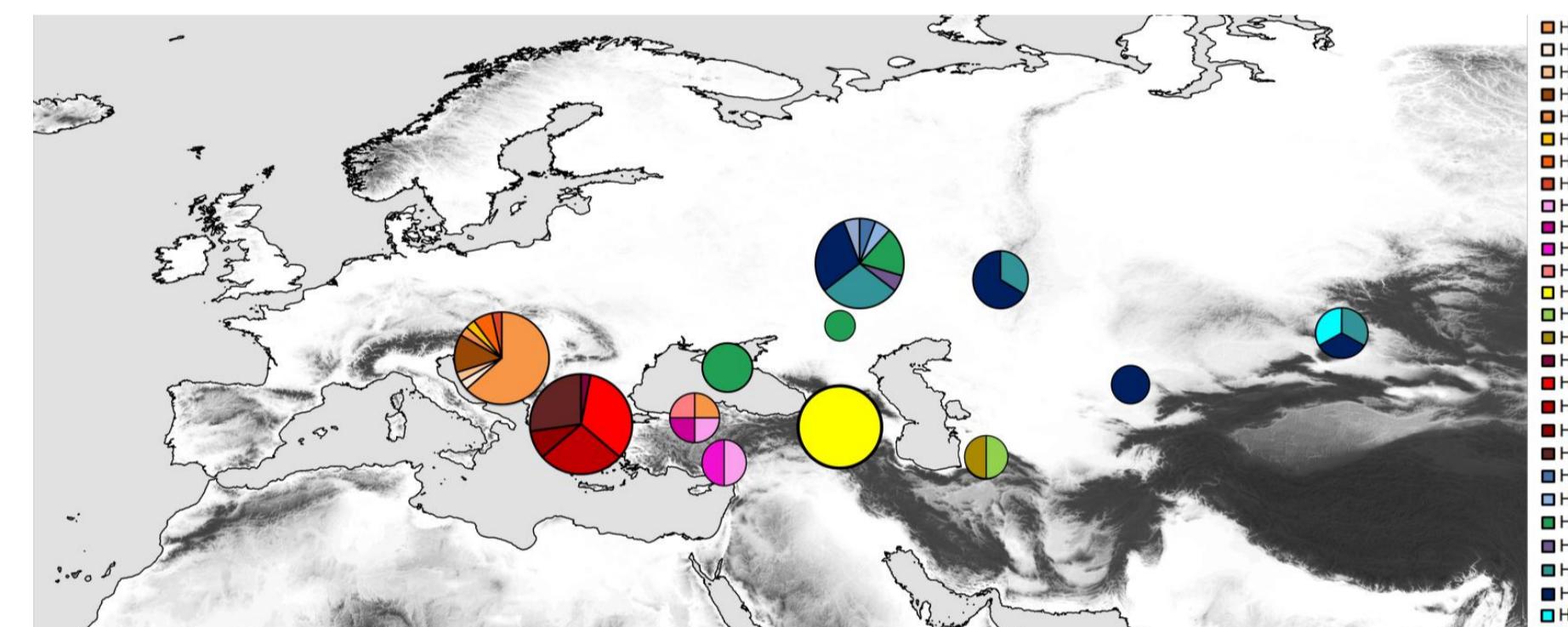


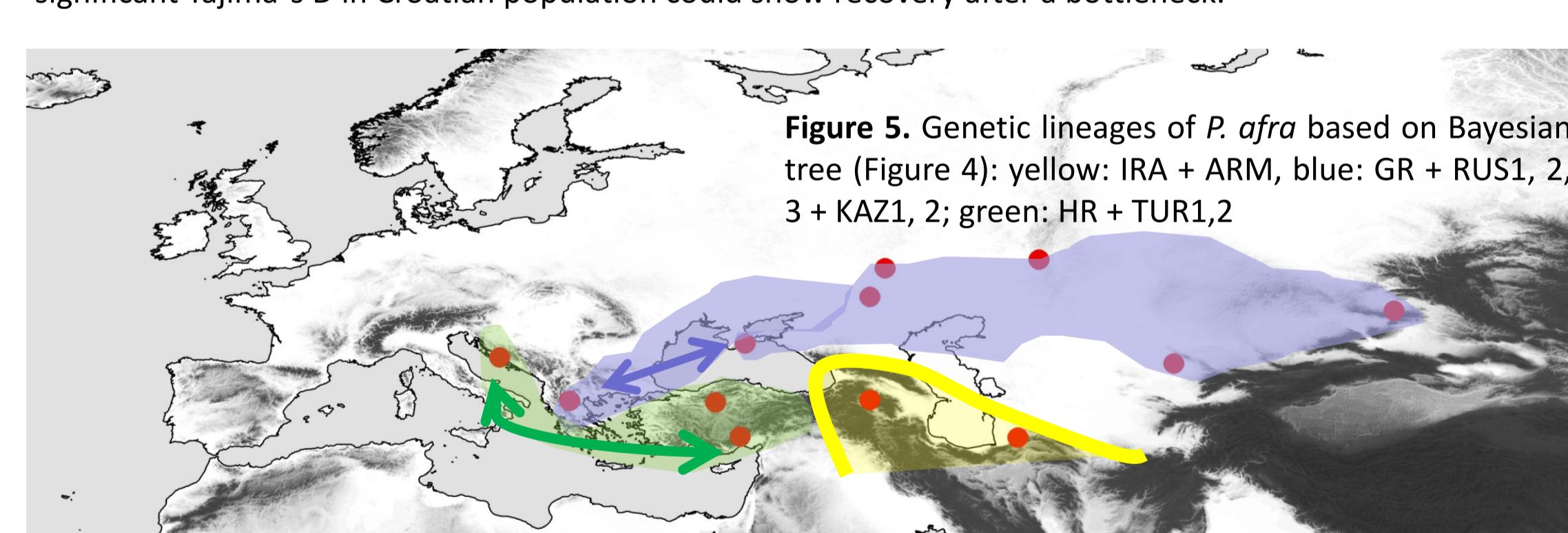
Figure 3. Haplotype diversity in each region (PopArt). The size of a pie chart corresponds to the sampling size.

	GR	HR	CRY	RUS1	RUS3	TUR	ARM
GR	0						
HR	0.77897*	0					
CRY	0.77106*	0.88265*	0				
RUS1	0.71002*	0.83279*	0.22668*	0			
RUS3	0.75227*	0.87184*	0.80488*	0.03508	0		
TUR	0.72466*	0.22517*	0.83203*	0.76993*	0.82449*	0	
ARM	0.86462*	0.88611*	1*	0.90514*	0.98533*	0.90885*	0

Table 1. FST values (Arlequin). Almost all the populations are highly differentiated from each other ($p < 0.05$, **), except for RUS1 and RUS3.

Population	N of sequences	N of haplotypes	N of segregating sites	Haplotype diversity	Nucleotide diversity	Tajima's D
GR	33	5	5	0.754	0.00399	0.74321
HR	30	9	12	0.669	0.00325	-1.84402*
CRY	4	1	0	0	0	/
RUS1	17	8	9	0.875	0.00598	-0.55805
RUS2	1	1				
RUS3	6	3	2	0.733	0.0022	-0.05002
TUR	6	5	5	0.993	0.00542	-0.65543
ARM	15	1	0	0	0	/
IRA	2	2				
KAZ1	3	1				
KAZ2	3	3				

Table 2. Population genetics measurements for *P. afra* based on COI fragment (409 bp; DNAsP). Populations with less than 4 individuals were not included. Note that DNAsP produces more haplotypes than PopArt. Armenian and Crimean population are impoverished, which suggest recent bottleneck or founder effect. In other populations, the haplotype diversity is high, interestingly, also in Greek population. Negative significant Tajima's D in Croatian population could show recovery after a bottleneck.



Box 1. Habitats of *P. afra* in Askion Mts. (up) and Dalmatia (down). In Askion Mts., the species forms abundances up to 100 indiv./50 m², on grassy plains with a lot of dry biomass. In Dalmatia, the maximal abundances are lower (max 30 indiv./50 m²), and the preferred biotopes are plains with a lot of dry biomass and rock outcrops.

Phylogeography of a latitudinally-distributed species complicated:

- Repeated expansions and retreats, leaving isolated populations
- Greece connected to Russia, Croatia to Turkey
- First separation: Caucasus Mts., Central Asian deserts
- Croatia: population expansion
- High haplotype diversity of isolated populations (HR, GR)
- Mountain population in Armenia: impoverishment
- Both older and more recent separations (less than 0.5 Mya)
- “Northern lineage” (RUS, CRY, KAZ) not separable by standard gene markers



Acknowledgement

We thank to The Explorers Club Exploration Fund (USA), FAN(B) (Germany), Faculty of Science of University of South Bohemia in České Budějovice (04-168/2013/P), and Croatian Ministry of Environmental and Nature Protection.

Many thanks also to Martina Sasic, Stan Korb, Milan Hluchý, Jana Šlancarová, Jana Marešová, Pavel Sucháček, Radek Kučmerčík, and Vojtěch Kolář. CALL FOR SAMPLES of the 2016 season!

