

**MALAKOLOGIJA I  
ASTAKOLOGIJA U NASTAVI  
BIOLOGIJE (208676 )  
2P+1V+0S 4 ECTS**



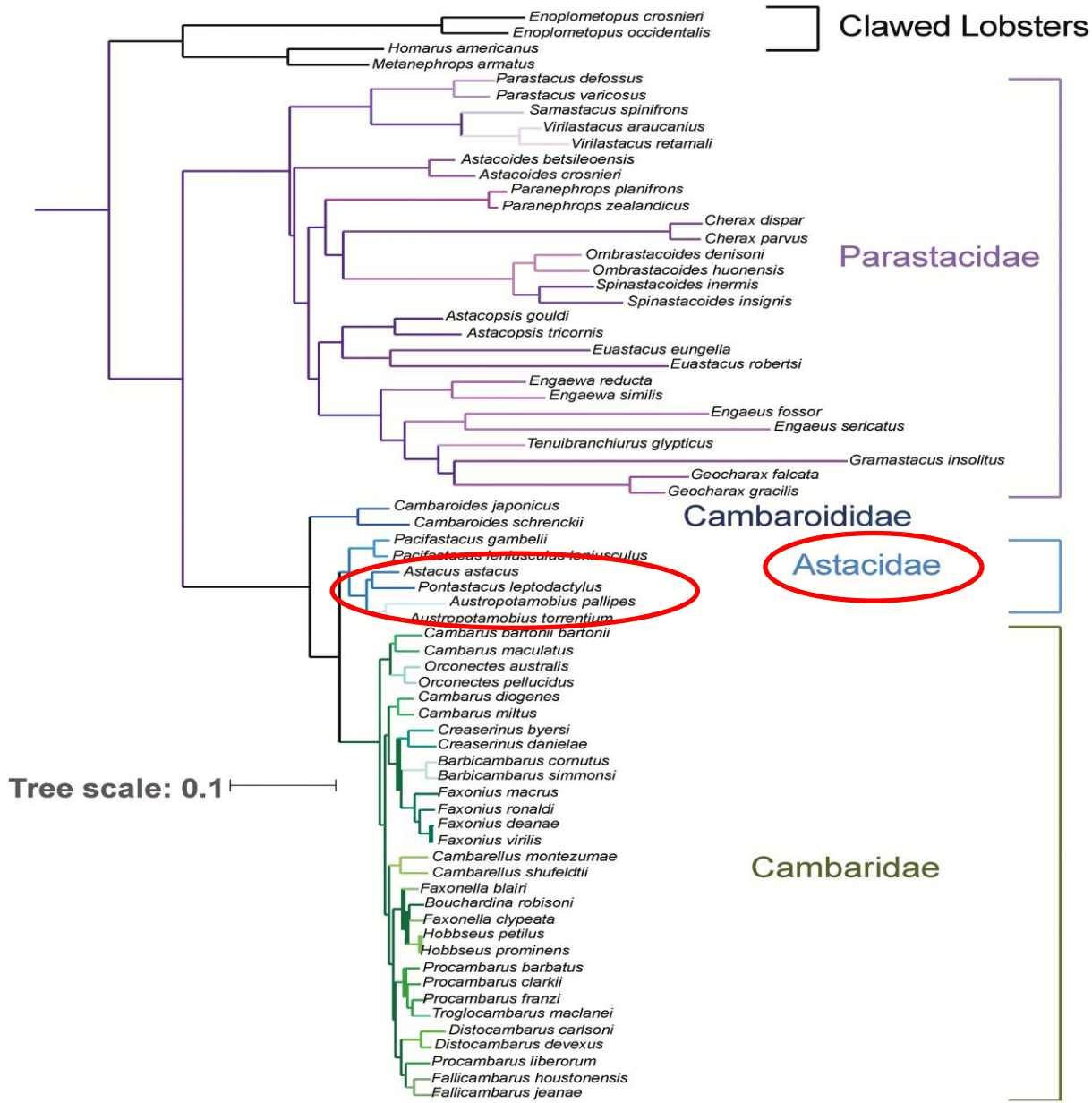


Figure 2. **Phylogenetic estimate of the freshwater crayfishes** based on a subset of data from Stern et al. (2017). Family clades of freshwater crayfish are shown in distinct colors with lobster outgroups shown in black.

**Filogenija** (grč. *phylon* i *phyle* – vrsta, rod, podrijetlo, srodstvo, *genesis*- nastajanje, postanak, rođenje) je znanstvena disciplina koje se bavi proučavanjem evolucijske (razvojne) povezanosti pojedinih skupina (taksa), koristeći se pri tome morfološkim, anatomskim, embriološkim, fiziološkim, **genetskim, molekularnim, fosilnim i geografskim homologijama**. Krajnji cilj filogenetskih istraživanja jest **rekonstrukcija rodoslovnog stabla nekog organizma (filogram)**.

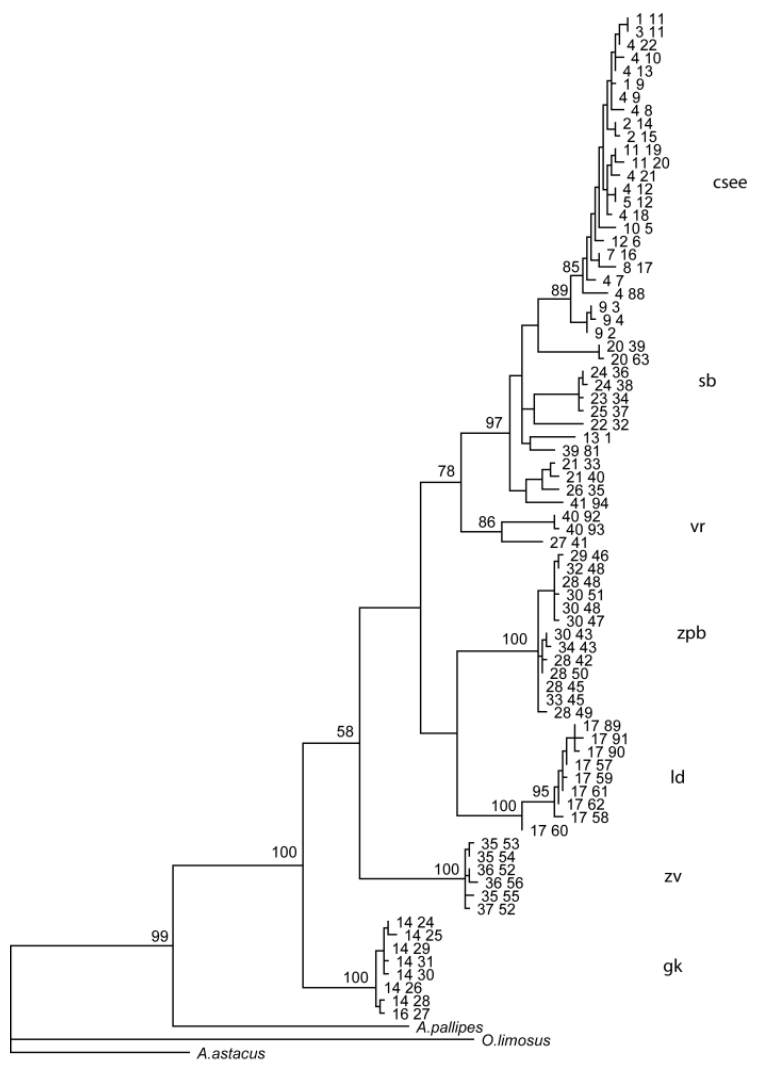
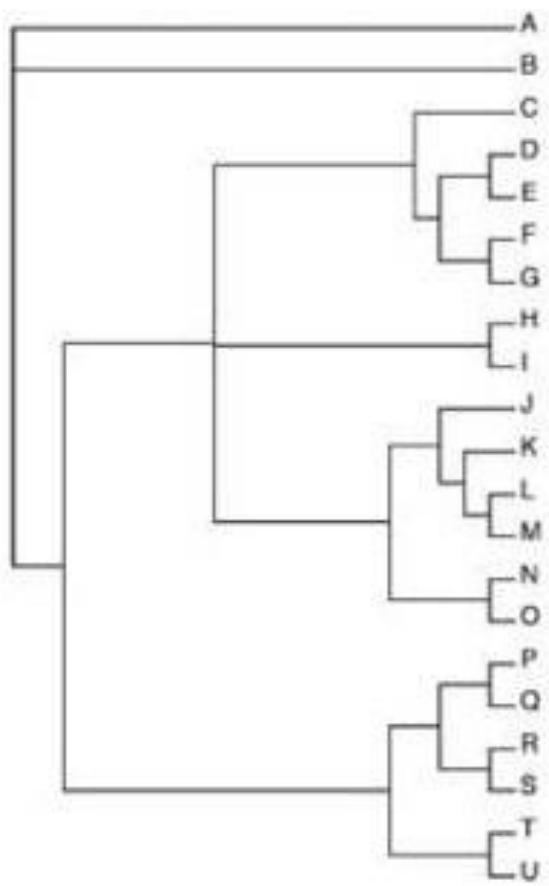
**Filogeografija** je znanstvena disciplina koja uz **pomoć filogenetskih metoda nastoji rekonstruirati povijesne promjene rasprostranjenosti vrsta na Zemlji**.

**Populacijska genetika** je grana genetike koja proučava nasljedne pojave i procese na razini reprodukcijski povezanih grupa organizama – populacija. Istražuje moguće odnose u genetičkoj strukturi populacije, faktorima održavanja i narušavanja njene ravnoteže, dinamiku i prirodu tih promjena. Ukratko, to je disciplina koja proučava genetičku raznolikost jedinki u populaciji.

**Modeli distribucije vrsta ( eng. SDM)**, ili modeli okolišnih (ili ekoloških) niša (eng. ENM), ili modeli staništa, ili predikcijski modeli distribucije u okolišu - koriste računalne algoritme kako bi predvidjeli distribuciju vrste u geografskom prostoru koristeći okolišne podatke (i distribuciju vrste)

**Kladogram** – dijagram evolucijskog odnosa potomci–predak (nema info. o evolucijskoj udaljenosti)

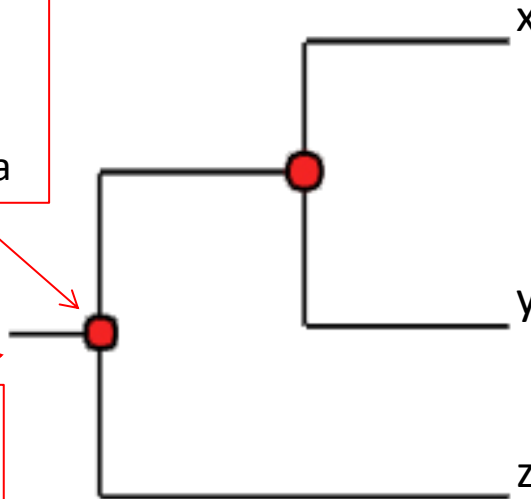
**Filogram** – daje informaciju o evolucijskim udaljenostima (na to ukazuje duljina grana)



**Čvor (node)** –  
mjesto  
neposrednog  
zajedničkog pretka

**Korijen** -  
predstavlja  
ancestralnu  
liniju

**Grana - klada**



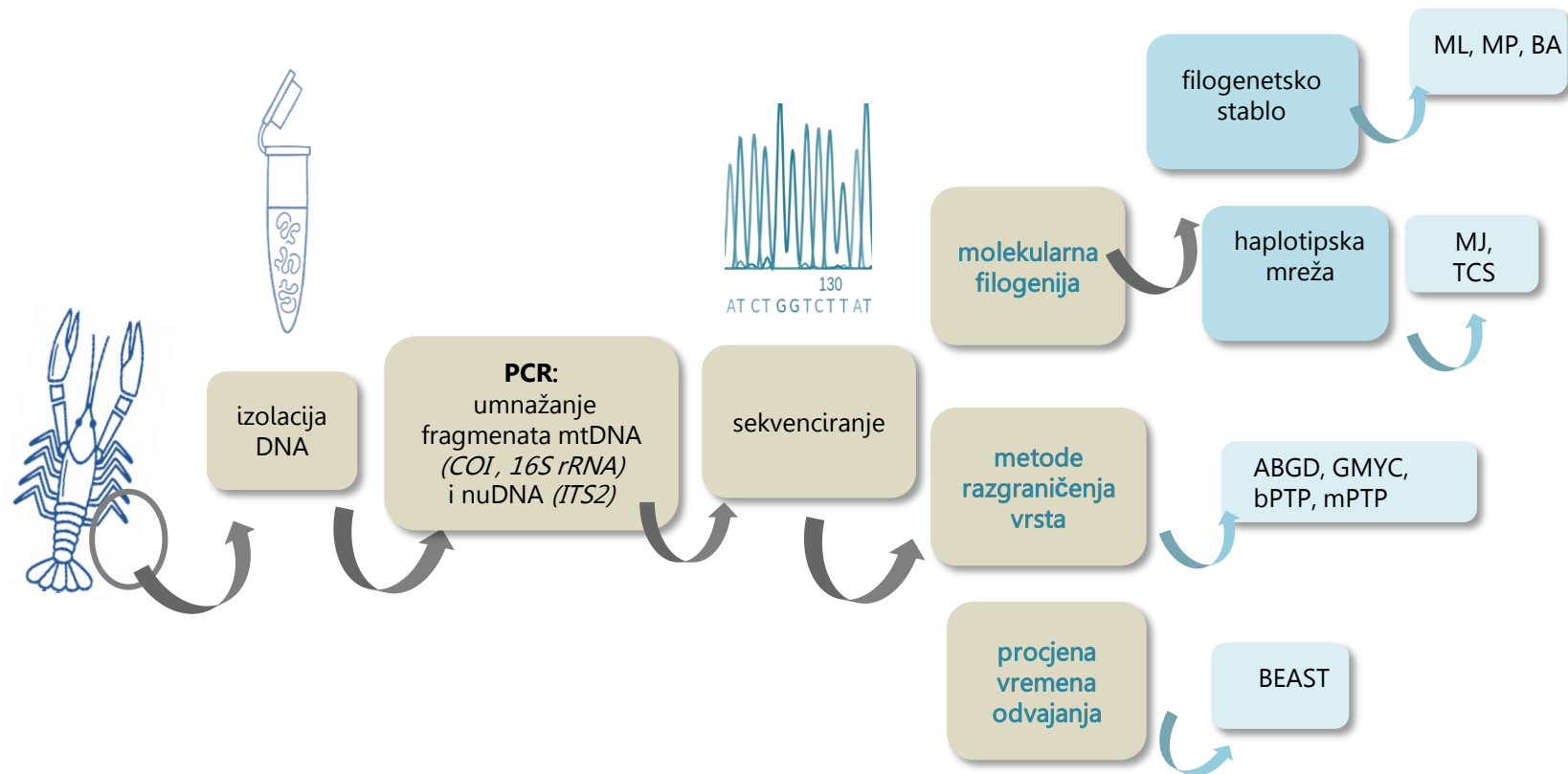
**Takson** – krajnji potomci –  
**sestrinski taksoni (x i y)** dijele  
neposrednog zajedničkog pretka

**Haplotipovi** (molekularna  
filogenija) - jedinstvena  
kombinacija genetičkih biljega  
prisutna u uzorku

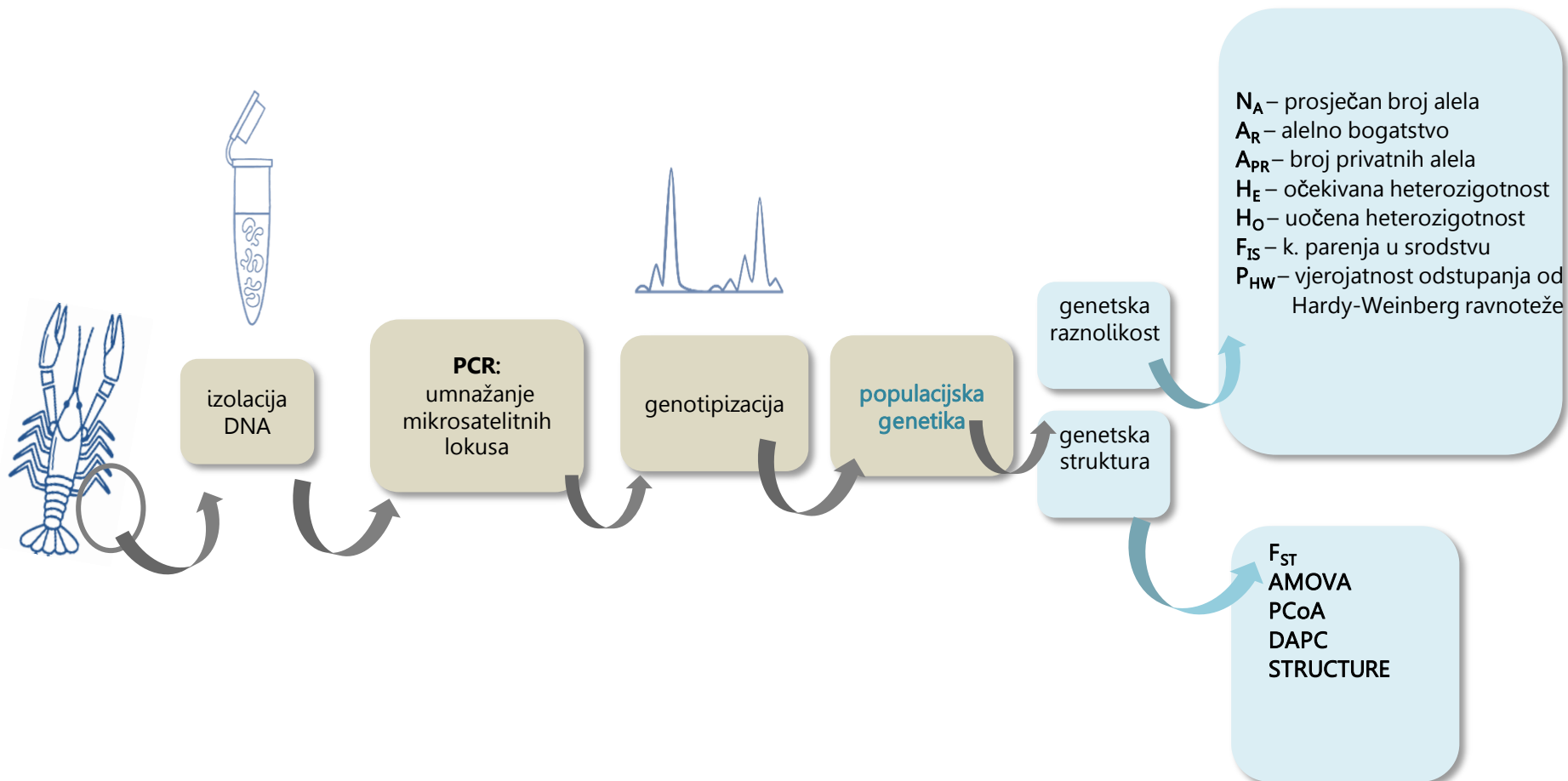
# Filogenija, filogeografija i populacijska genetika & budućnost



# Molekularna filogenija

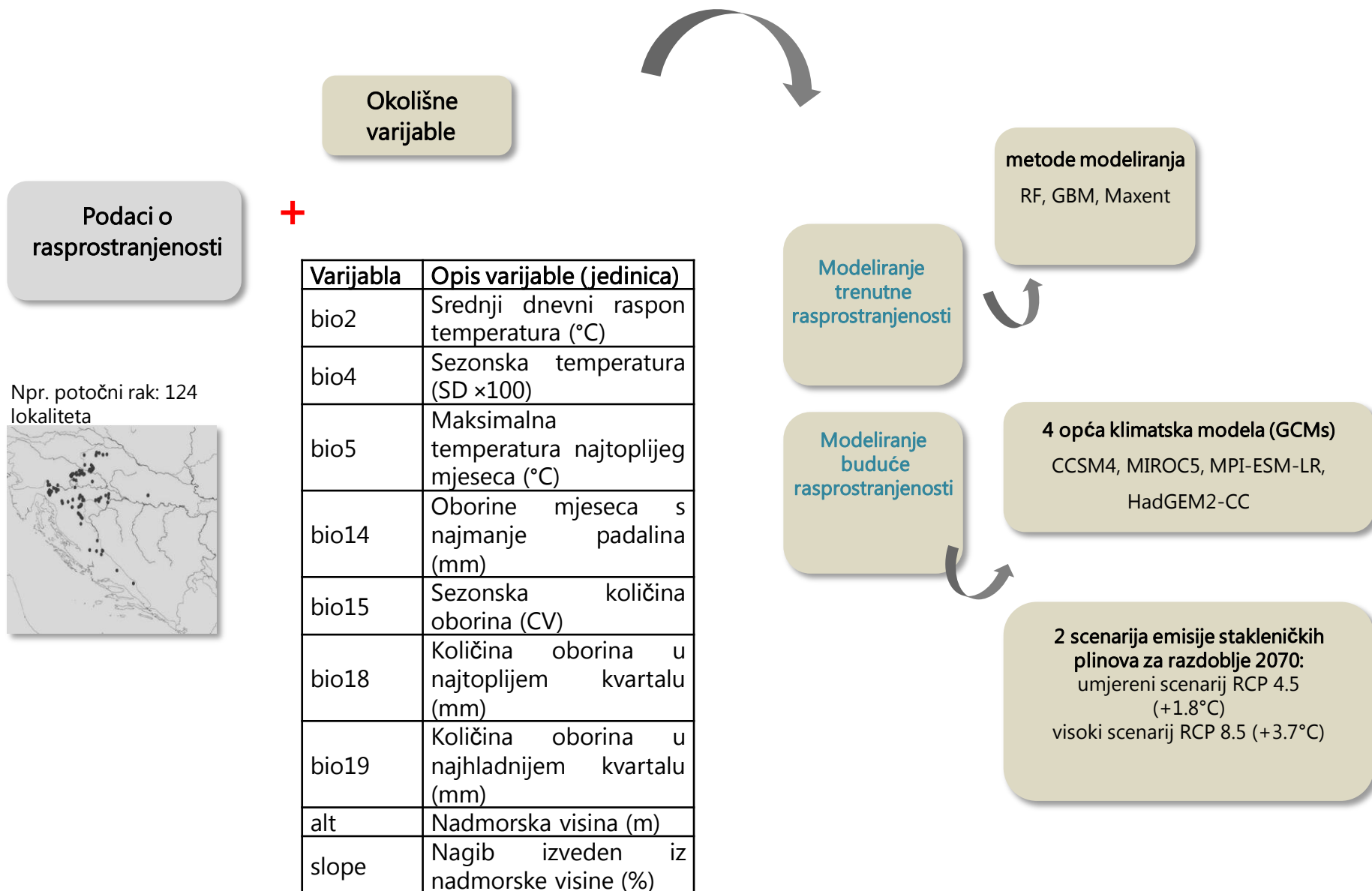


# Populacijska genetika





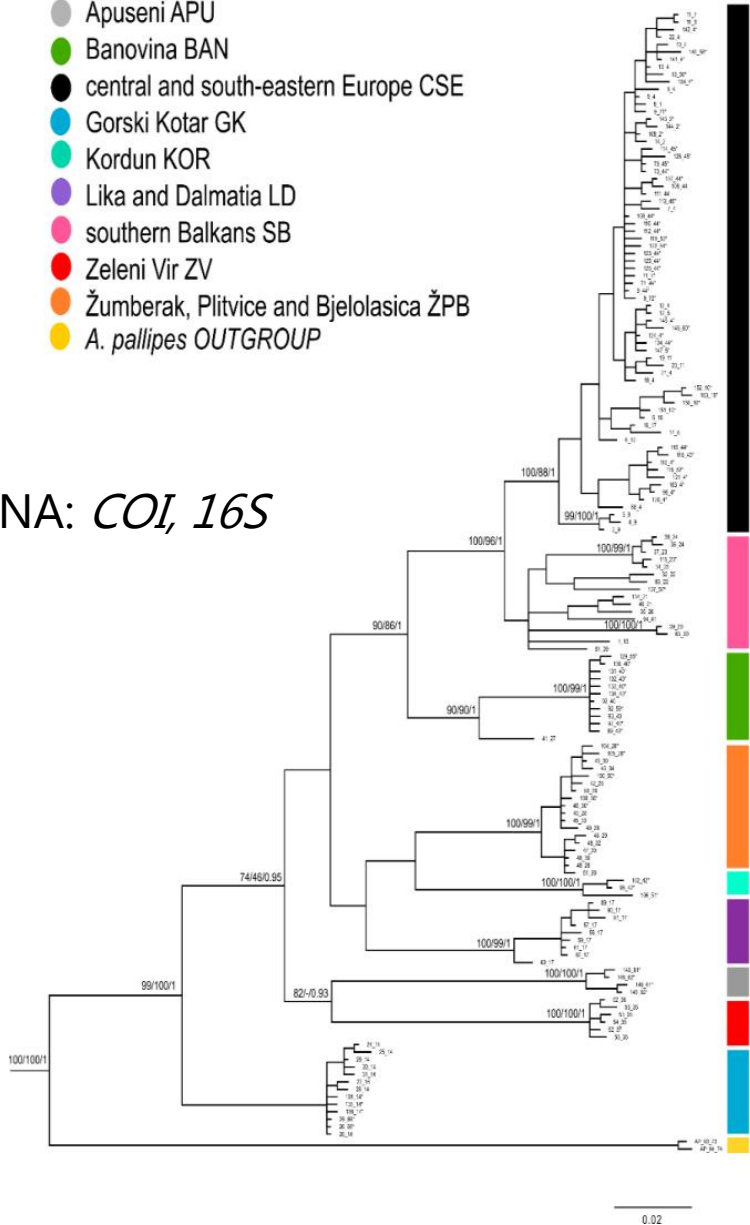
# Modeli povoljnosti staništa



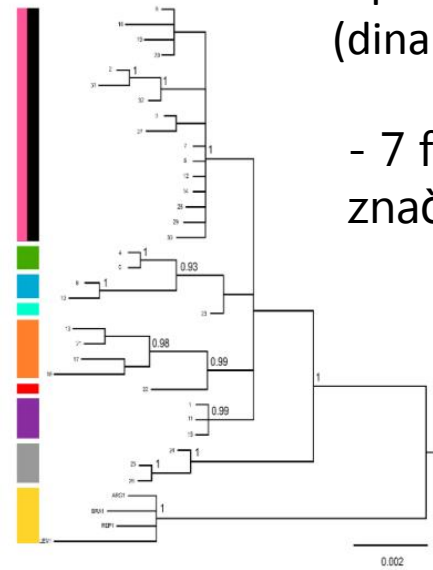
# Rekonstrukcija filogenetskih odnosa

- Apuseni APU
- Banovina BAN
- central and south-eastern Europe CSE
- Gorski Kotar GK
- Kordun KOR
- Lika and Dalmatia LD
- southern Balkans SB
- Zeleni Vir ZV
- Žumberak, Plitvice and Bjelolasica ŽPB
- *A. pallipes* OUTGROUP

mtDNA: *COI*, *16S*

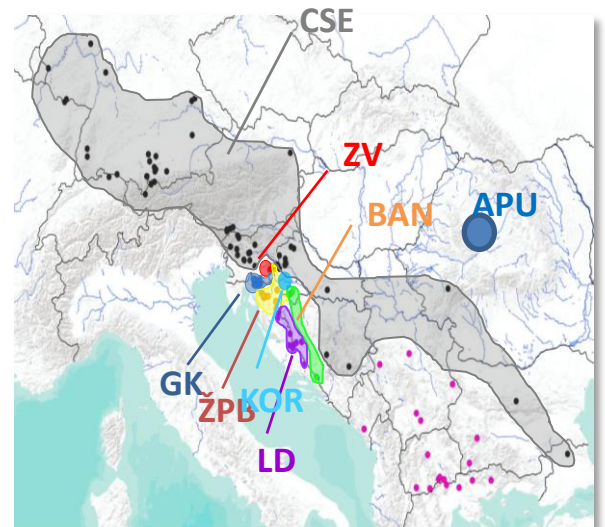


nuDNA: *ITS2*

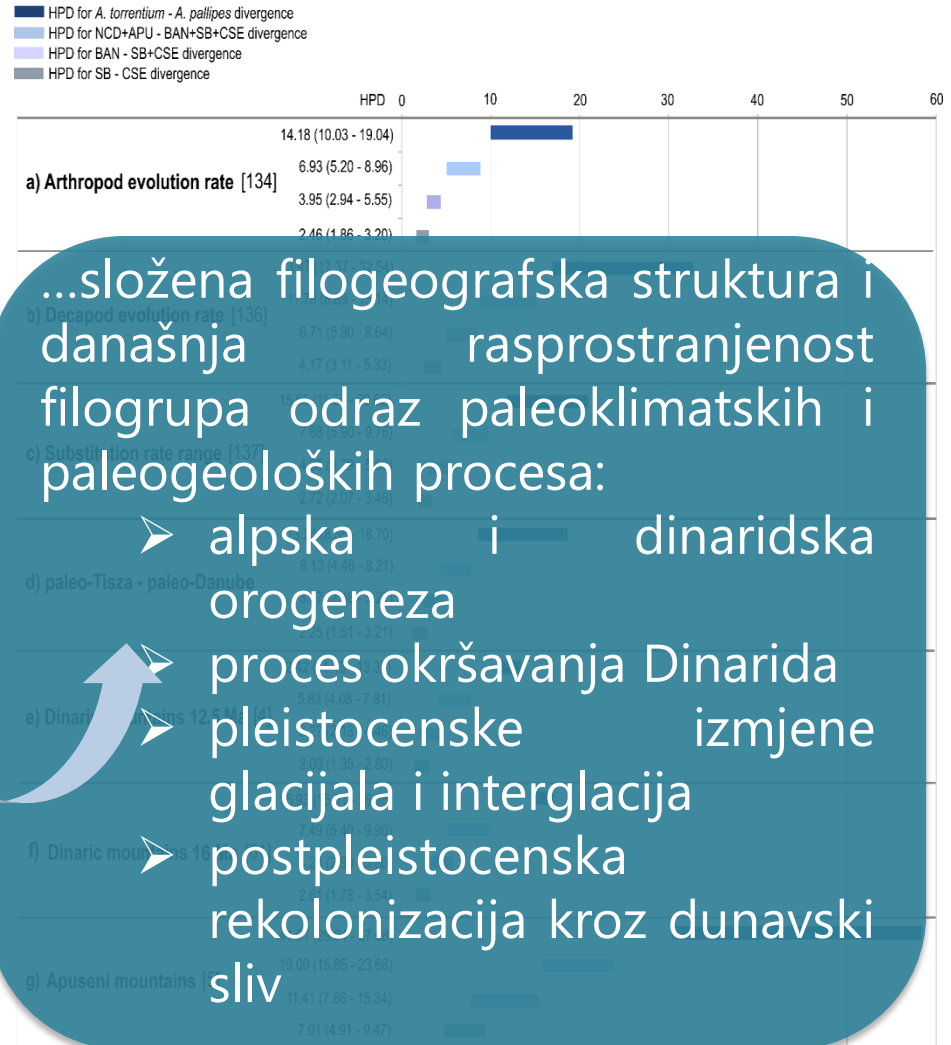
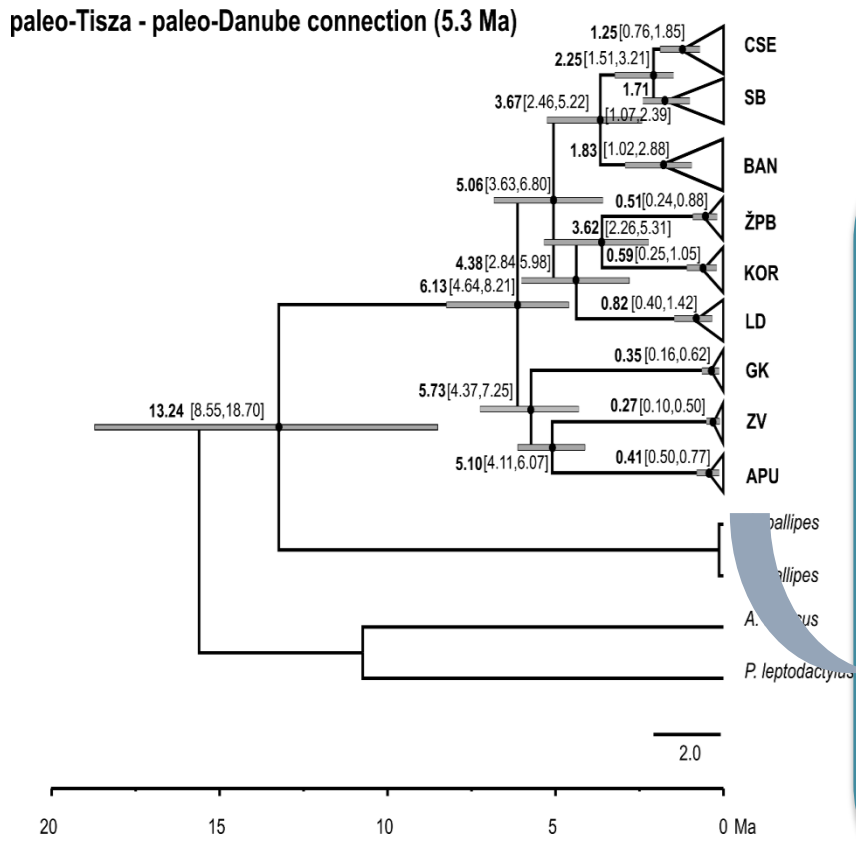


- Najveća raznolikost u Alpinskoj biogeo. regiji (dinaridski krš)

- 7 filogrupa-7 evolucijski značajnih jedinica (ESU)



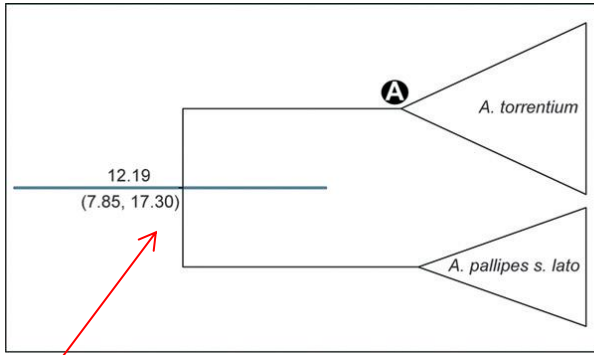
# Procjena vremena odvajanja filogrupa



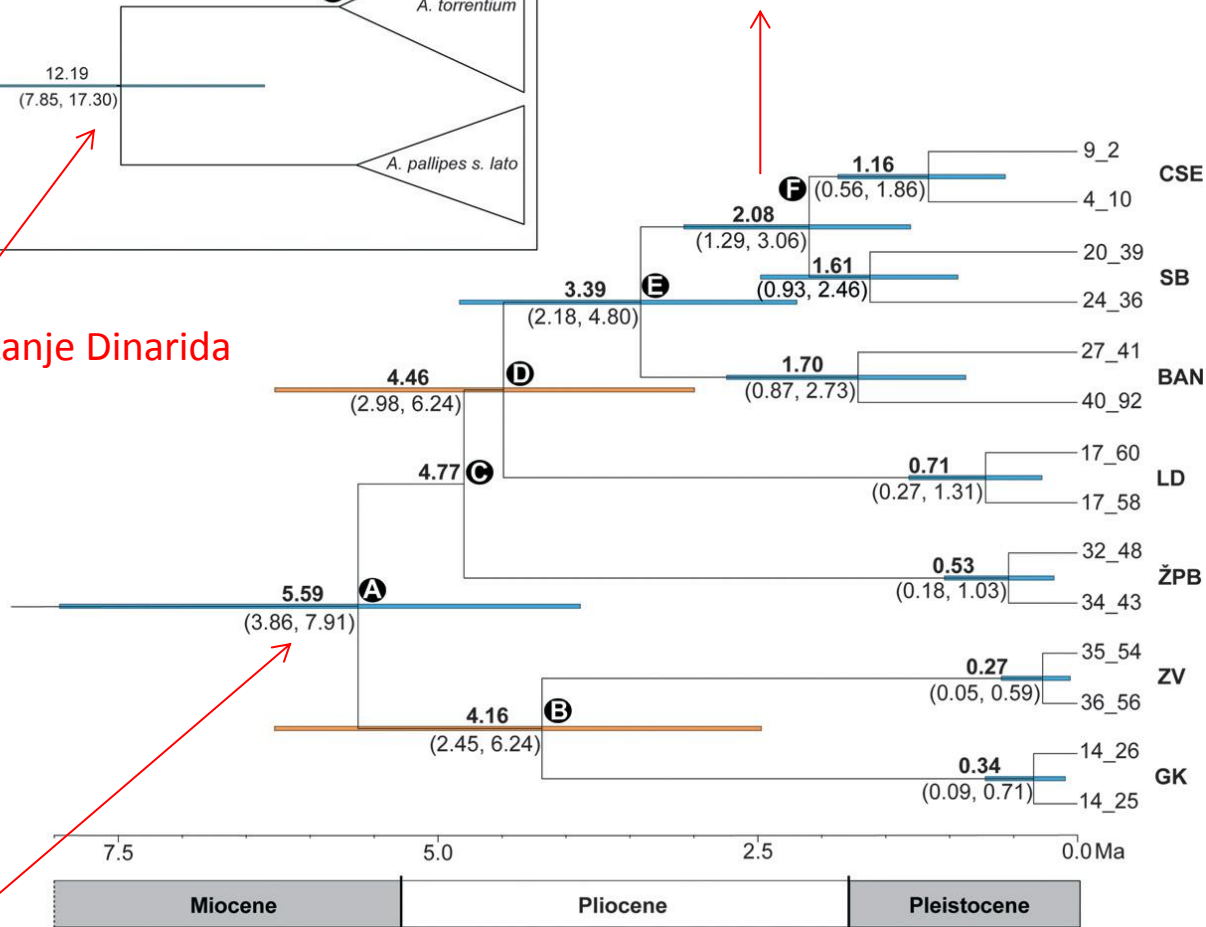
...složena filogeografska struktura i današnja rasprostranjenost filogrupa odraz paleoklimatskih i paleogeoloških procesa:

- alpska i dinaridska orogeneza
- proces okršavanja Dinarida
- pleistocenske i interglacija glacijala i interglacija
- postpleistocenska rekolonizacija kroz dunavski sliv

Formiranje Dunava – pre-pleistocensko širanje na jug (SB) i post-glacijalno širenje na sjever (CSE)



Uzdizanje Dinarida



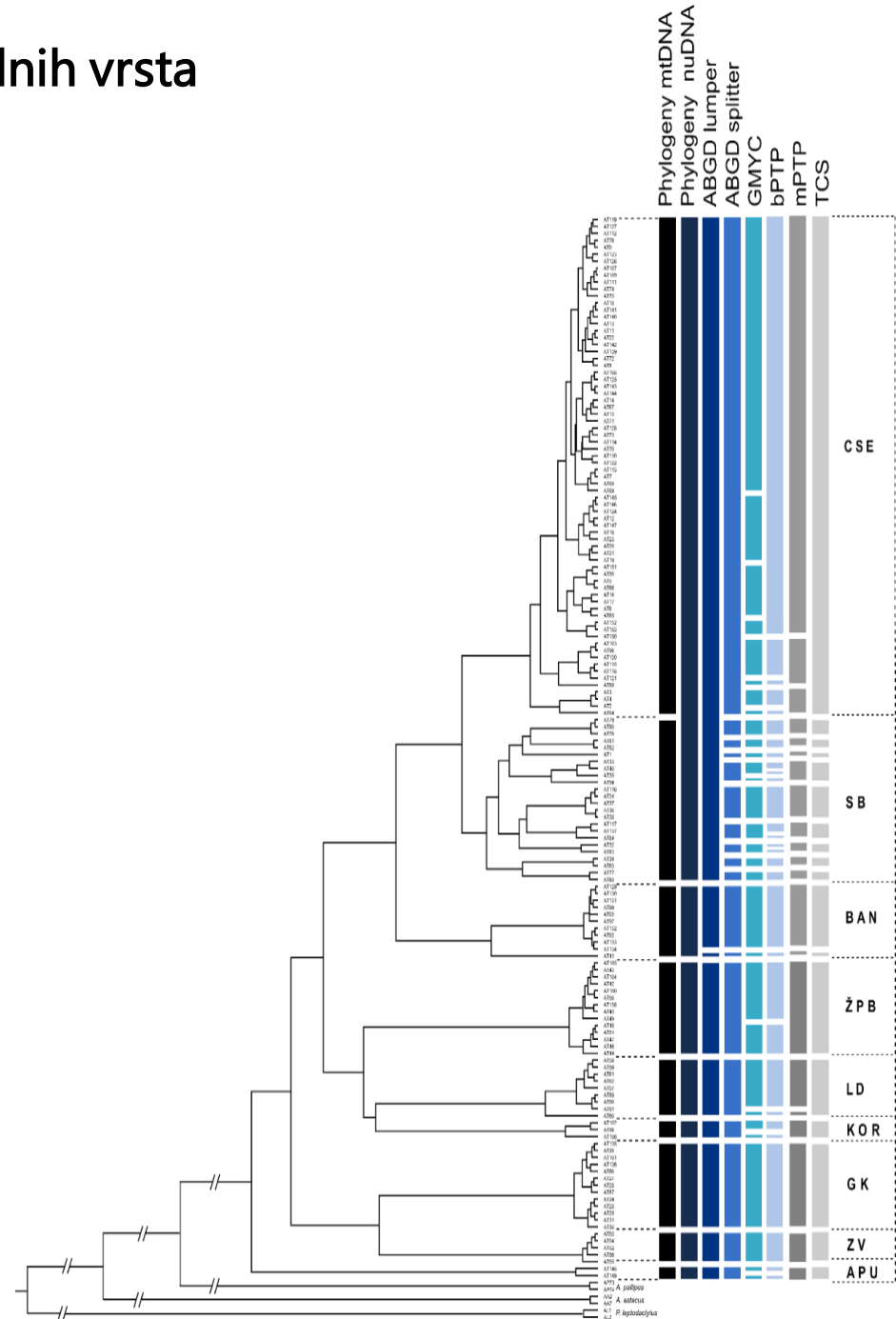
3-5 Ma intenzivna karstifikacija – fragmentirana paleohidrografija (slatkovodni otoci u kršu) – alopatrijska specijacija

NCD

Panonsko jezero (bočato) (NCD izoliran s juga Dinaridima, sa sjevera brakičnom vodom)

# Metode razgraničavanja potencijalnih vrsta

- rješavanje taksonomskog statusa filogrupa
- molekularne: ABGD, GMYC, bPTP, mPTP, TCS
  - nekongruetni rezultati
  - **nerealno velik broj potencijalnih vrsta**
- morfološke: GM, TM, meristika
  - Velika morfološka varijabilnost
  - **ne postoji determinacijska značajka koja bi pomogla razlikovanju filogrupa i omogućila opis novih vrsta**



Često se uspoređuje zabilježeni stupanj heterozigotnosti ( $H_o$ ) s očekivanim ( $H_e$ ) (prema Hardy-Weinbergovoj ravnoteži -1 gen, 2 alela A i a (učestalosti  $p + q = 1$ ); Genotipovi: AA Aa aa;  $p^2 + 2pq + q^2 = 1$ )

- ako je  **$H_o$  manji** od  **$H_e$**  – možemo pripisati srođivanju (inbreeding)/gubitak raznolikosti/veći udio homozigota – **pozitivne vrijednosti  $F_{is}$**
- ako je  **$H_o$  veća** od  **$H_e$**  – možemo pripisati miješanju dvije prethodno izol. pop./više heterozigota – **negativne vrijednosti  $F_{is}$**

Population code	N	No. of polymorphic						$F_{is}$
		loci	$N_a$	$N_{ar}$	$N_{pr}$	$H_e$	$H_o$	
KOZ	30	6	2.44	1.89	0.44	0.15	0.16	-0.04
TOL	29	2	<b>1.22</b>	<b>1.09</b>	0.11	<b>0.01</b>	<b>0.02</b>	-0.03
GLI	30	8	2.56	2.12	<b>0</b>	0.34	0.33	0.02
REK	29	8	2.22	2.09	0.11	0.36	0.34	0.08
RJE	30	8	3.00	2.44	0.22	0.44	0.42	0.06
SKO	30	8	4.00	3.01	0.33	0.50	0.48	0.06
ARG	30	7	2.00	1.91	<b>0</b>	0.29	0.24	<b>0.17</b>
BRA	39	8	<b>4.89</b>	<b>3.44</b>	0.56	<b>0.53</b>	<b>0.51</b>	0.05
PIV	26	5	1.67	1.60	<b>0</b>	0.18	0.21	<b>-0.14</b>
RAS	25	9	3.89	3.05	0.33	0.46	0.45	0.05

Reported values for intrapopulation measures of genetic variability. N – number of analysed crayfish,  $N_a$  – mean number of alleles per locus,  $N_{ar}$  – allelic richness,  $N_{pr}$  – mean number of private alleles per locus,  $H_e$  – expected heterozygosity,  $H_o$  – observed heterozygosity,  $F_{is}$  –  $F_{is}$  estimate across all loci (inbreeding coefficient). Minimum and maximum are shown in red. NA – not analysed

$F_{is}$  – koeficijent križanja u bliskom srodstvu - **pozitivan ako u populaciji postoji manje heterozigota**, a **negativan ako se radi o velikom udjelu heterozigota**

# Genetska raznolikost populacija (mikrosateliti)

Pop.	Abb.	ESU Abb.	N	P	N <sub>A</sub>	A <sub>R</sub>	A <sub>PR</sub>	uH <sub>E</sub>	H <sub>O</sub>	F <sub>IS</sub>	P <sub>HWE</sub>
Brućina	BRU	BAN	31	1.00	4.75	3.62	1 (0.40)	0.551	0.473	0.144	ns
Maja	MAJ	BAN	30	1.00	4.13	3.19	1 (1.12)	0.456	0.313	0.317*	*
					<i>BAN average</i>						
Bliznec	BLI	CSE	29	1.00	4.13	3.48	5 (2.72)	0.524	0.431	0.180*	ns
Jarak	JAS	CSE	21	1.00	3.88	2.90	3 (1.12)	0.362	0.351	0.032	ns
Okićnica	OKI	CSE	32	1.00	5.00	3.84	5 (2.88)	0.543	0.440	0.193*	*
Dolje	DOLJ	CSE	31	1.00	4.50	3.86	2 (2.08)	0.595	0.465	0.221*	*
					<i>CSE average</i>						
Delnički	DEL	GK	29	0.75	2.75	2.50	0 (0.64)	0.393	0.349	0.113	*
V. Vode	VEL	GK	31	0.88	3.13	2.49	0 (0.24)	0.344	0.363	-0.055	ns
					<i>GK average</i>						
Žrnica	ŽRN	KOR	11	0.88	3.88	3.79	3 (2.80)	0.585	0.455	0.232*	ns
Krasulja	KRA	LD	30	0.88	3.13	2.72	0 (0.64)	0.454	0.434	0.045	ns
Orašnica	ORA	LD	33	0.88	3.25	2.53	0 (0.80)	0.307	0.227	0.262*	*
Prijeboj	PRI	LD	24	1.00	3.50	3.11	0 (0.08)	0.532	0.485	0.090	*
					<i>LD average</i>						
Zeleni Vir	ZV	ZV	29	1.00	4.50	3.89	10 (8.00)	0.584	0.402	0.316*	*
Jarak	JAR	ŽPB	10	0.75	2.38	2.32	0 (0.08)	0.307	0.325	-0.061	ns
Sartuk	SAR	ŽPB	27	0.88	2.88	2.60	1 (1.52)	0.457	0.315	0.315*	*
Sopotski	SOP	ŽPB	24	0.75	2.63	2.18	2 (0.88)	0.262	0.058	0.782*	*
					<i>ŽPB average</i>						

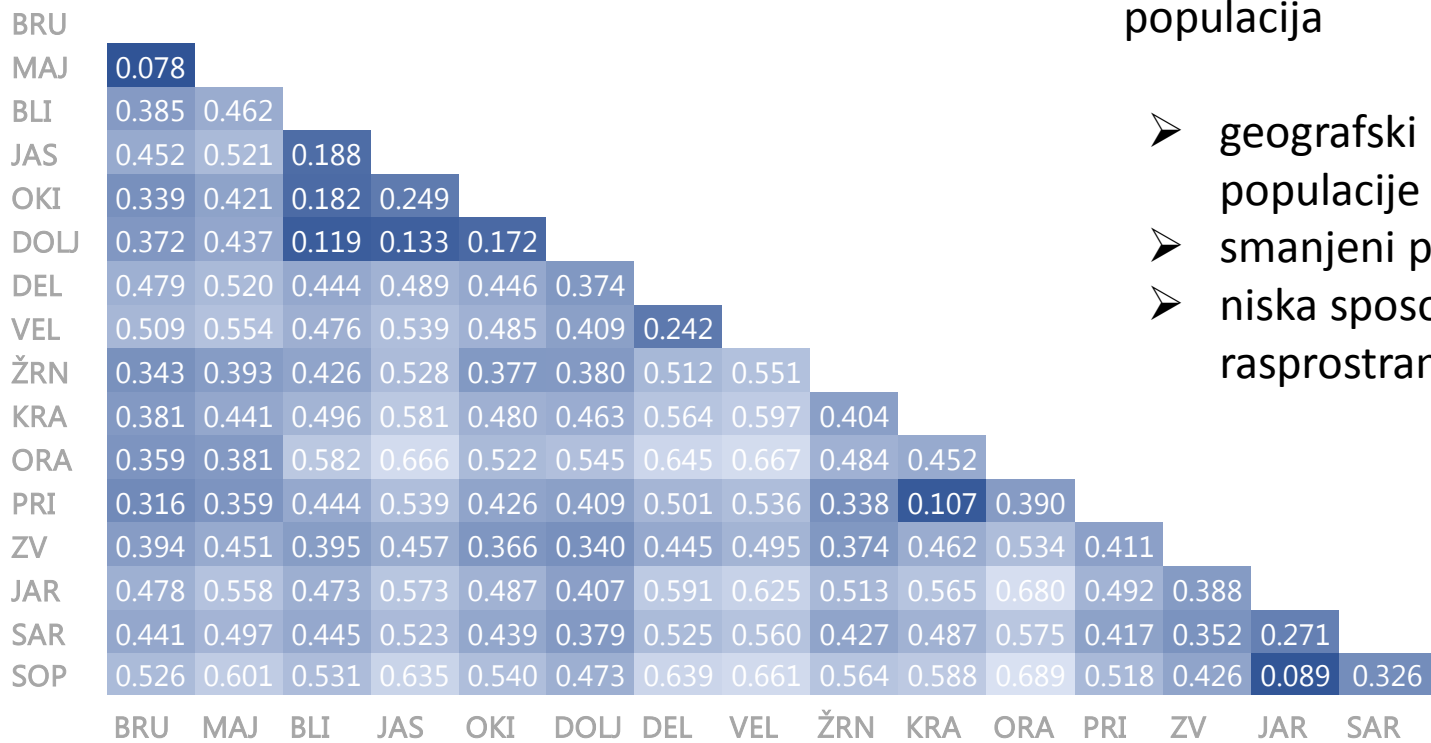
➤ umjerena genetska raznolikost

↑ visoka genetska raznolikost ↑  
ESU

↓ niska genetska raznolikost ↓  
ESU

➤ visoka vrijednost F<sub>IS</sub> ukazuje na višak homozigota u većini populacija

# Genetska diferencijacija populacija



average  $F_{ST} = 0.463$

- Vrijednosti fiksacijskih indeksa ( $F_{ST}$ ) – što su manje- povezanost kroz parenje; što su veće- manja povezanost kroz parenje

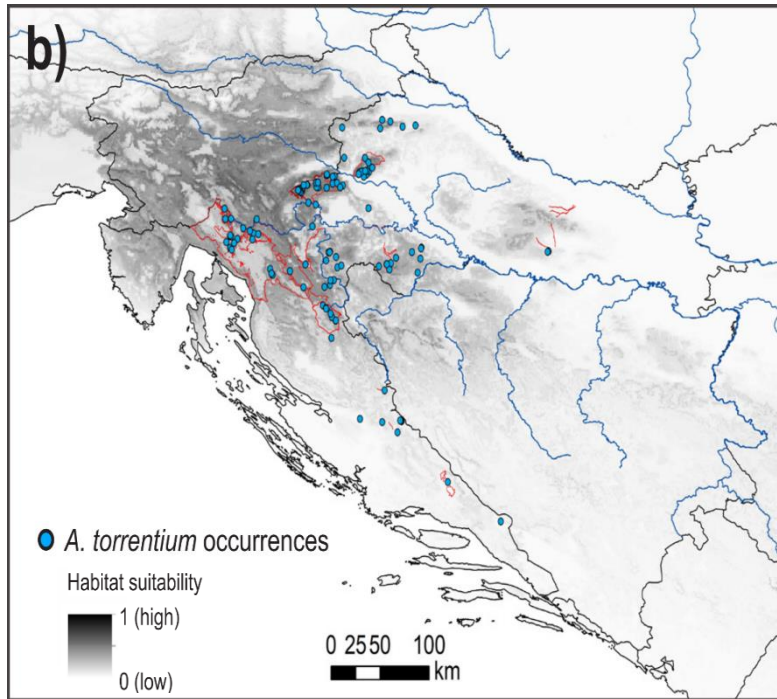
- visoka razina diferencijacije između populacija
  - geografski i genetski izolirane populacije
  - smanjeni protok gena
  - niska sposobnost rasprostranjanja

- problem u prilagođavanju klimatskim promjenama
  - limitirana mogućnost migriranja i pomicanja rasprostranjenosti prema klimatski povoljnijim staništima

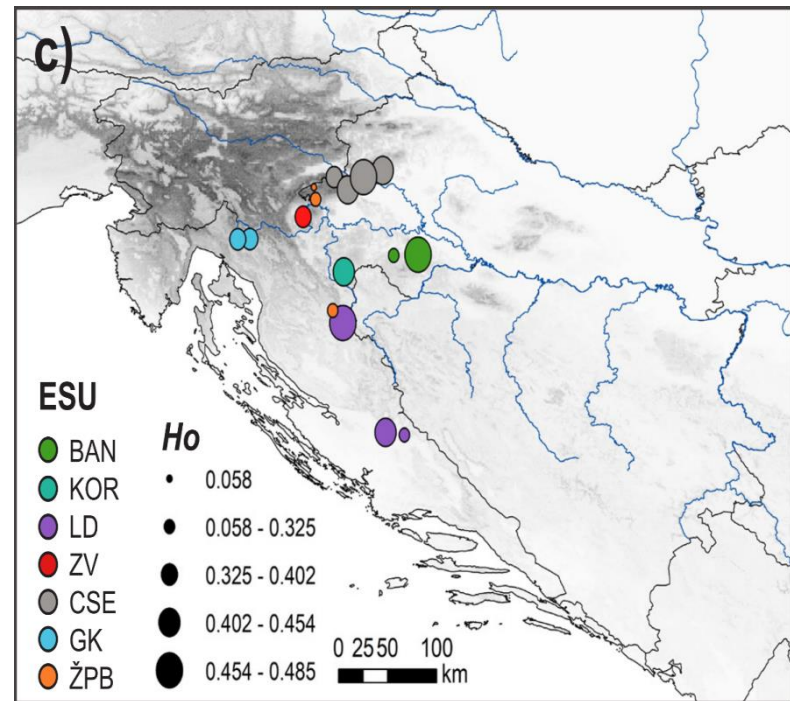


# Potencijalna trenutna (2022) i buduća (2070) rasprostranjenost

2070 (+1.8°C)

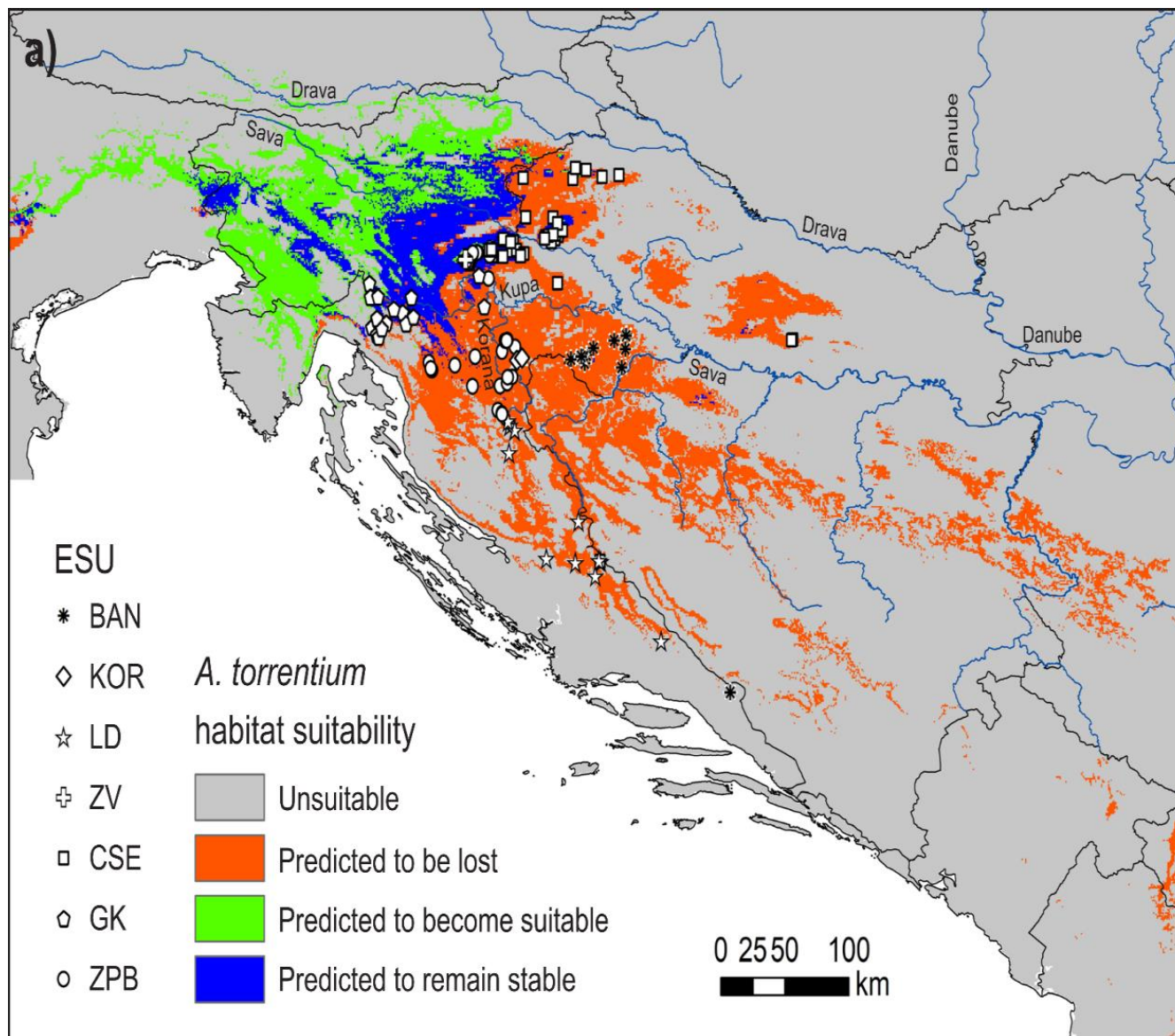


2070 (+3.7°C)



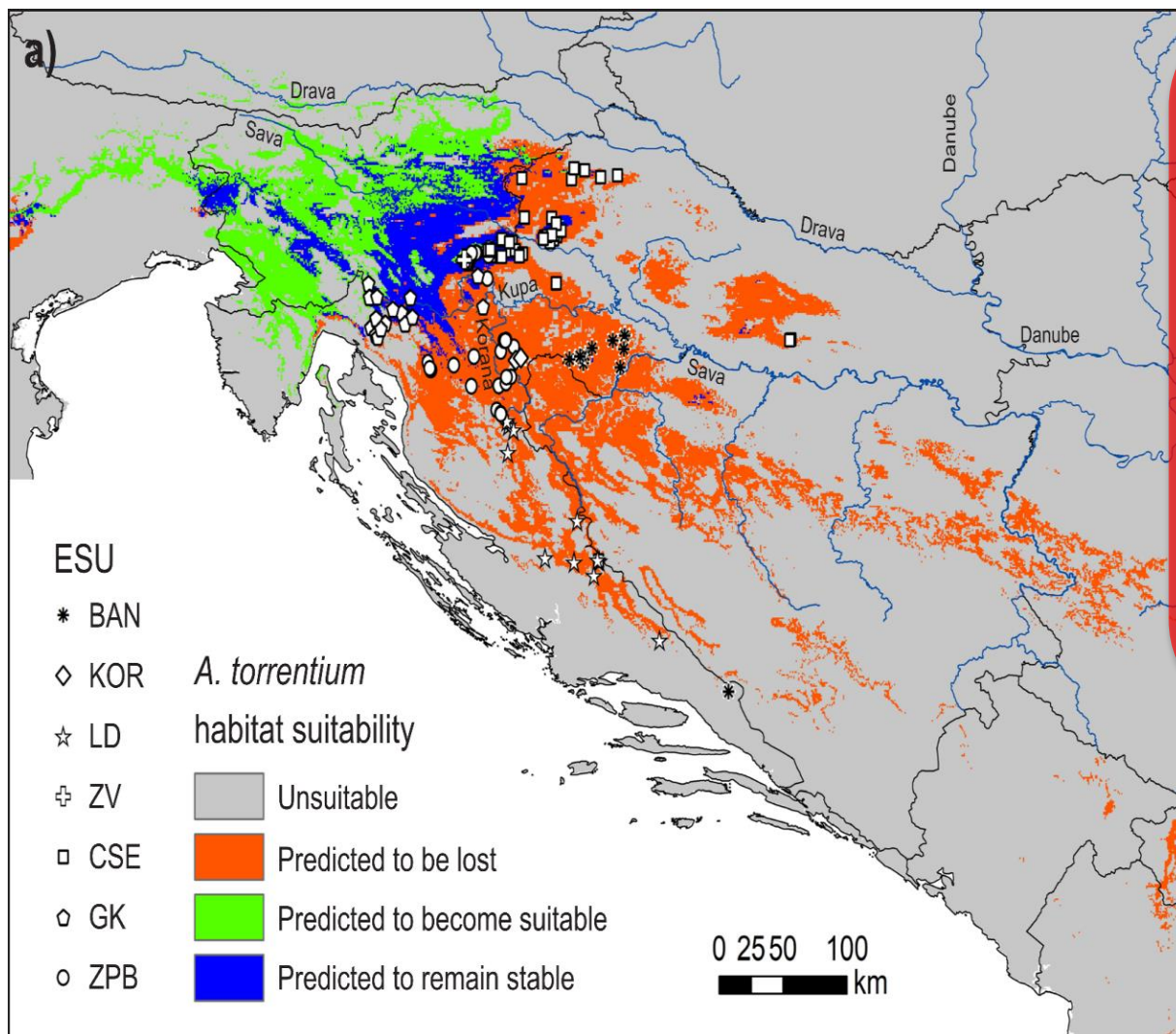
- značajno smanjenje povoljnih staništa zbog klimatskih promjena
- pomicanje potencijalne buduće rasprostranjenosti prema SZ

# Preklapanje podataka o potencijalnoj trenutnoj i budućoj rasprostranjenosti



...gubitak  
80% trenutno  
povoljnih staništa  
za potočnog raka  
do 2070  
(+3.7°C)

# Preklapanje potencijalne buduće rasprostranjenosti i genetske raznolikosti



...**44%** populacija s visokom ili jedinstvenom genetskom raznolikosti smještene u područjima koja su predviđena kao nepovoljna u budućnosti, stoga potencijalno izgubljena

... uključujući **3** evolucijski značajne jedinice

## Potencijalni klimatski refugiji

- potencijalni gubitak velikog dijela genetske raznolikosti potočnog raka zbog klimatskih promjena
  - populacije s najvećom i jedinstvenom genetskom raznolikosti smještene u područjima koja su predviđena kao nepovoljna u budućnosti
- predloženi klimatski refugiji za potočnog raka uz sjeverozapadnu granicu i prema staništima na višim nadmorskim visinama
  - nedostupni zbog prirodnih granica širenja
  - programi repopulacije i reintrodukcije
  - prekogranična suradnja u konzervaciji

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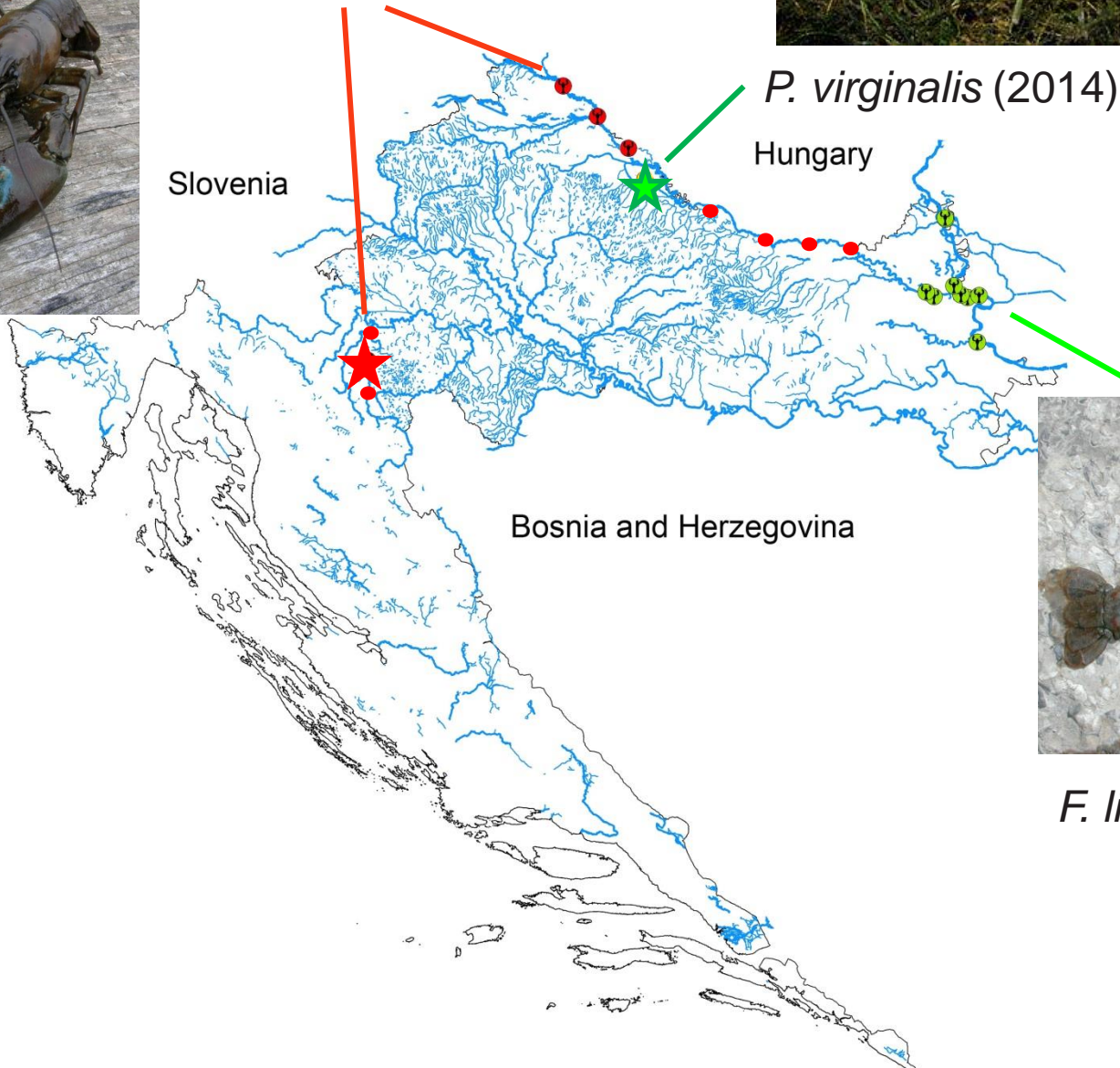
# Hrvatska - 3 strane invazivne vrste



*P. leniusculus* (2008; 2012)



*P. virginalis* (2014)



Slovenia

Hungary

Bosnia and Herzegovina



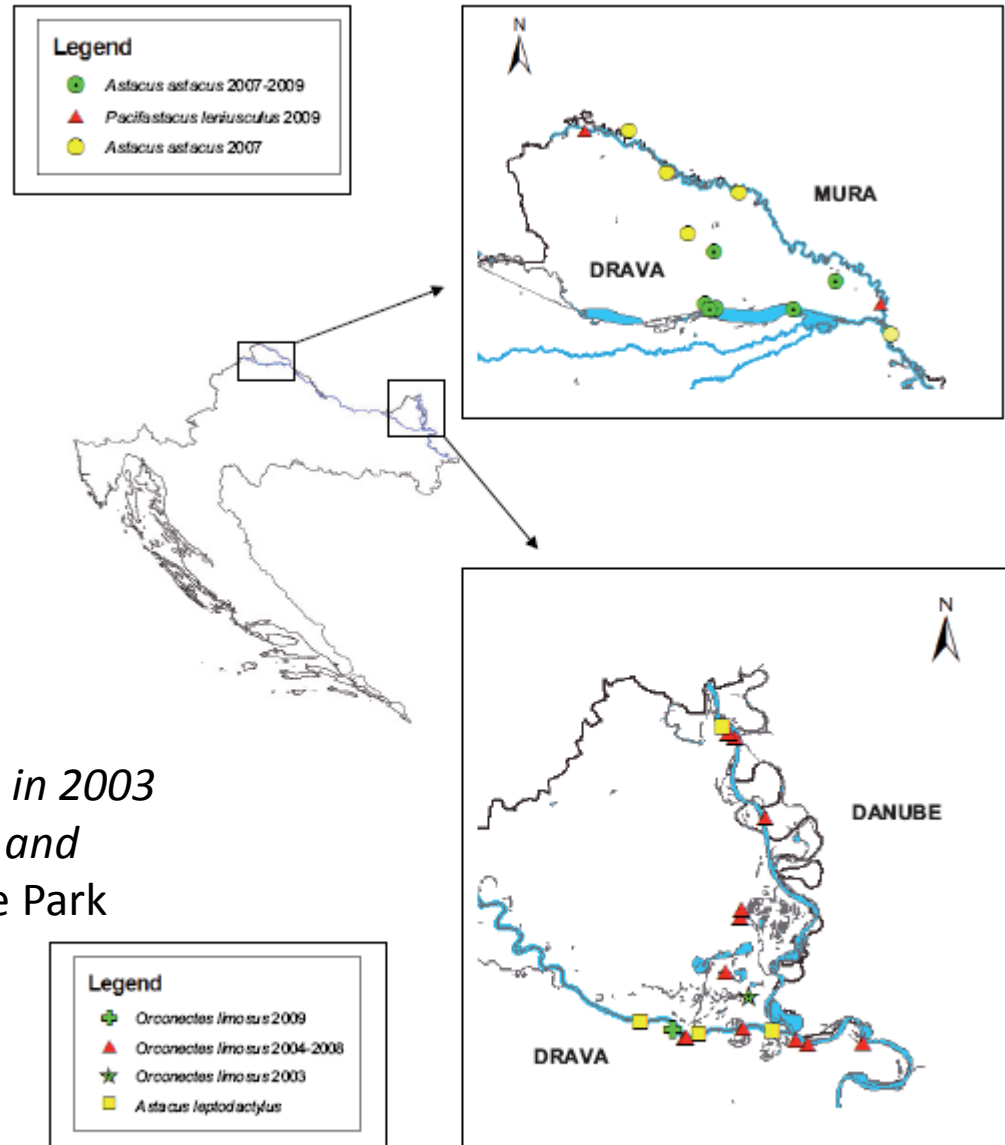
*F. limosus* (2003)

The first record of the signal crayfish in Croatia dates from 2008 (Maguire *et al.*, 2008). Until now, signal crayfish records in Croatia have been found in the Mura River and the the Drava River.

**18-24.4 km/god.  
(najbrže u Europi)**

*O. limosus* was first recorded in Croatia in 2003 (Maguire and Klobucar, 2003; Maguire and Gottstein-Matocec, 2004) in the Nature Park Kopacki rit, where it spread from the Hungarian section of the Danube River

**> 2.5 km/god.**

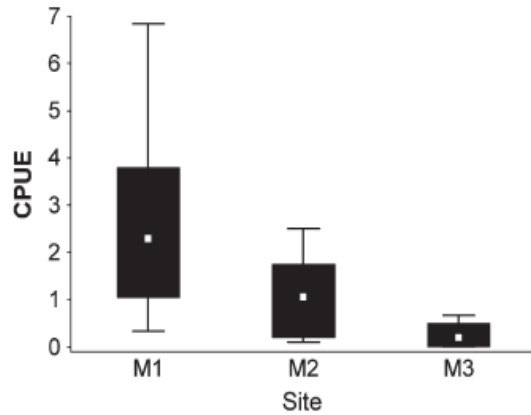


**Figure 1**

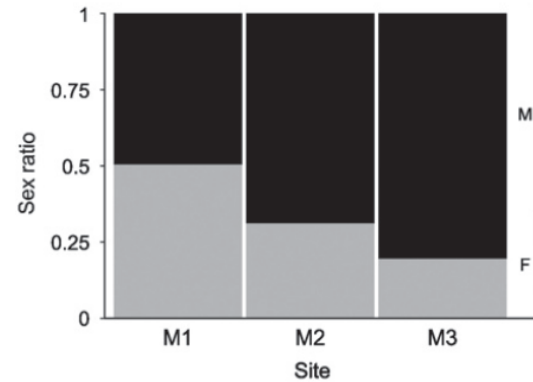
Distribution of the invasive signal crayfish and the native noble crayfish in the Drava and the Mura rivers, and distribution of the invasive spiny-cheek crayfish and the native narrow-clawed crayfish in the Drava and the Danube rivers.



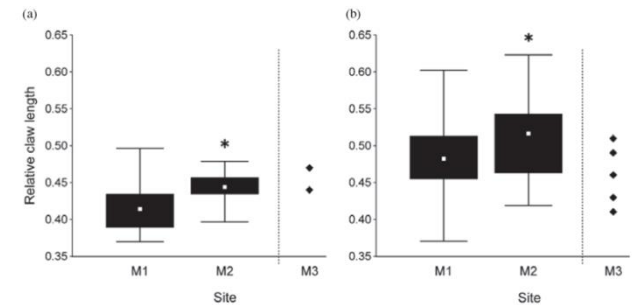
**Fig. 1.** The position of examined sites in the lower section of the Mura River, Croatia. M1 represents the most upstream examined site (source population), M3 invasion front, while M2 represents the intermediate point between these two sites. G1 and G2 represent two gauging stations from which continuous measurements of water temperature and discharge were obtained.



**Fig. 2.** Differences in catch per unit effort (CPUE; equal to the number of caught crayfish per trap per number of trapping nights) between examined sites during same trapping periods (August, October and November 2009). The boxes show medians and quartiles, whiskers show minimum and maximum.



**Fig. 3.** Sex ratio (M = males; F = females) at three examined sites during the same trapping periods (August, October and November 2009). The bar on the right shows the sex ratio for a composite sample of all three sites.

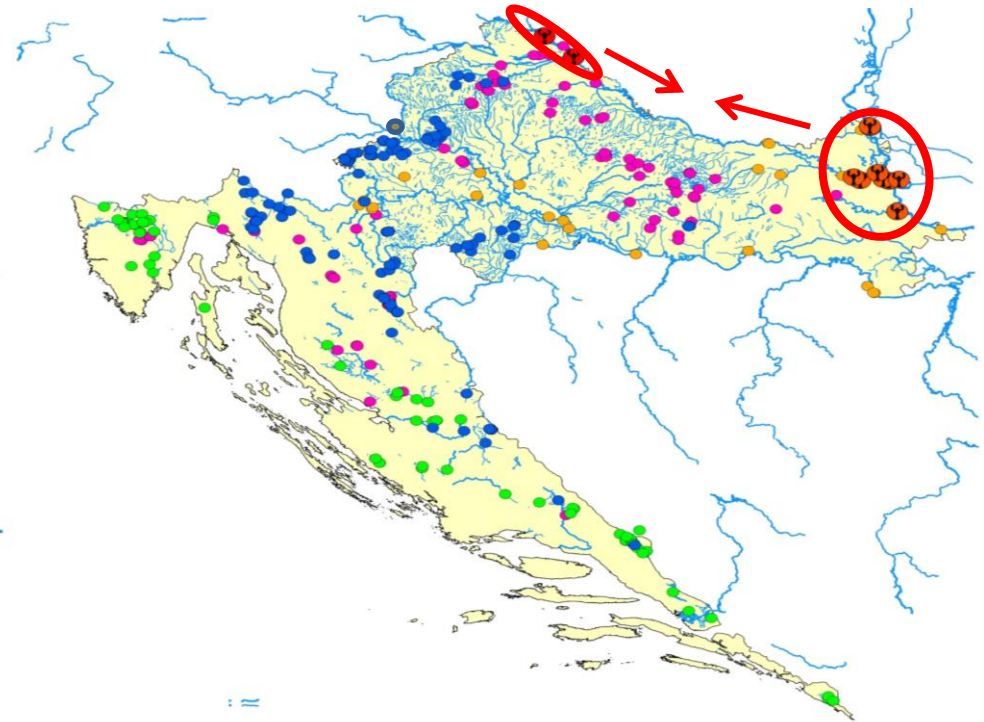
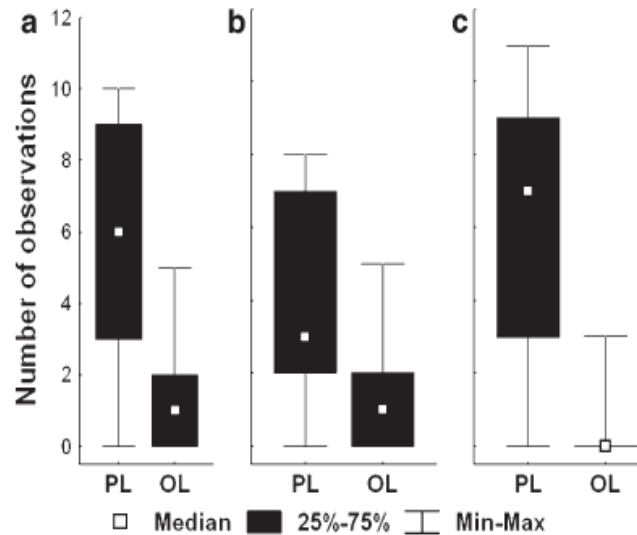


**Fig. 4.** Relative claw length (CLL) at two segments of signal crayfish invasive range for size class 1 (a) and size class 2 (b) males. Data for the third site (M3) were added for reference only and were not included in the quantitative analyses. The boxes show medians and quartiles, whiskers show minimum and maximum. \*Indicates significantly higher relative CLL.

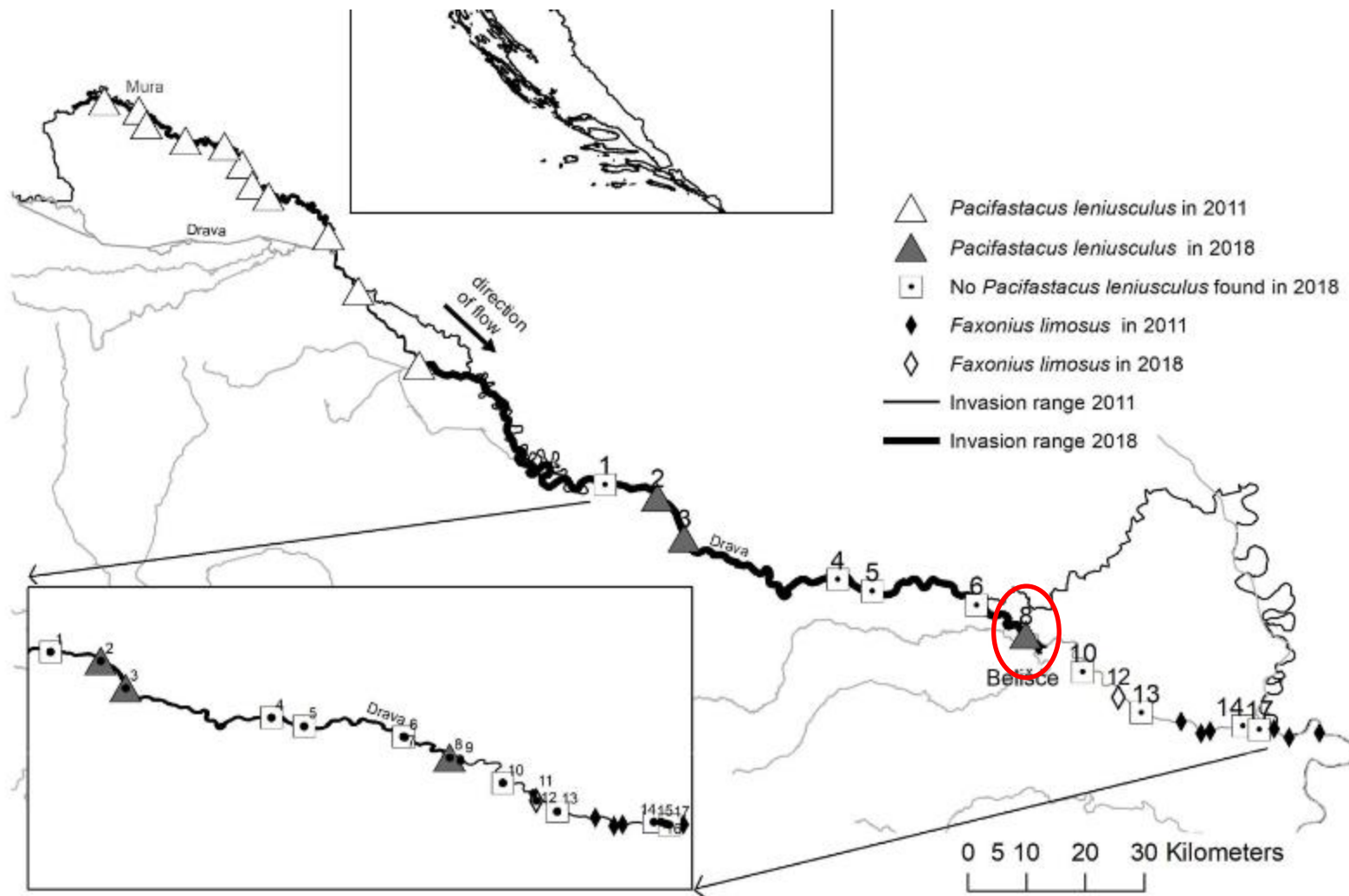


# Competitive interactions between co-occurring invaders: identifying asymmetries between two invasive crayfish species

Sandra Hudina · Nika Galić · Ivo Roessink ·  
Karlo Hock



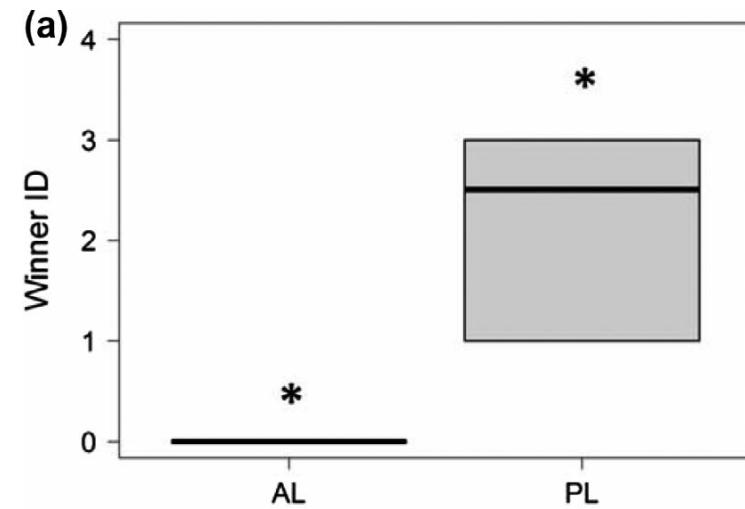
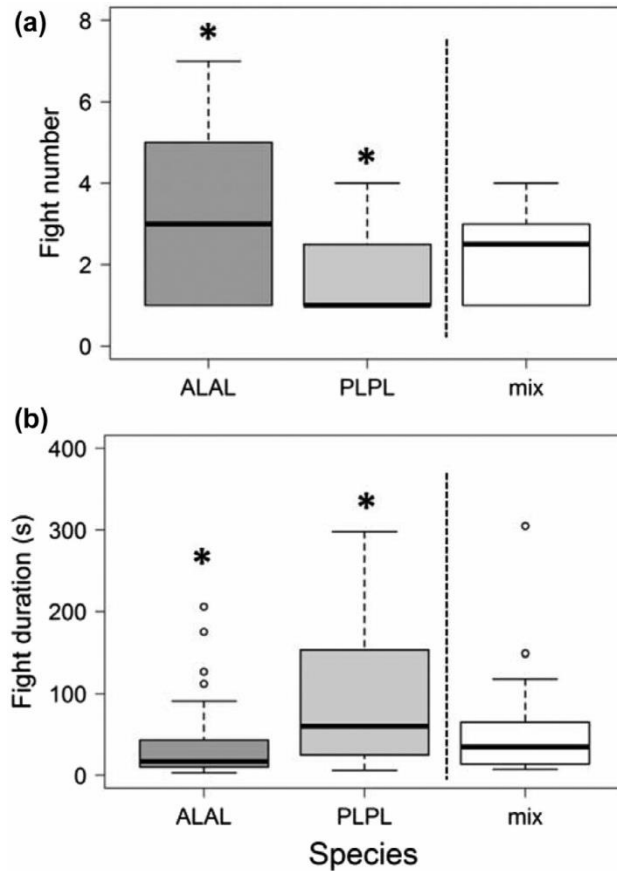
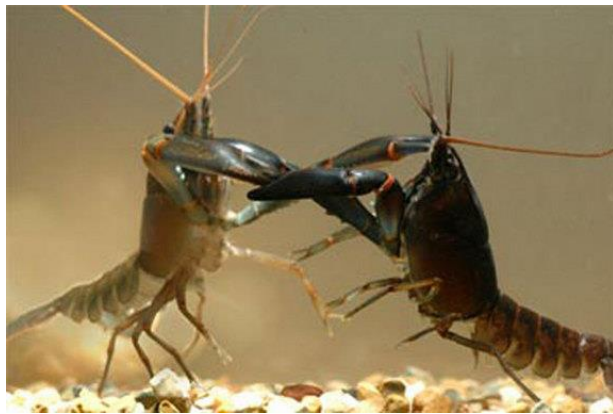
**Fig. 1** Counts and identity of **a** initiator of agonistic interaction, **b** initiator of physical contact and **c** interaction winner, in 25 heterospecific dyads of *P. leniusculus* (PL) and *O. limosus* (OL). PL initiated significantly more interactions and physical contacts than OL and was identified as interaction winner significantly more frequently



**Update on the signal crayfish, *Pacifastacus leniusculus* (Dana, 1852) range expansion in Croatia: a 10-year report**

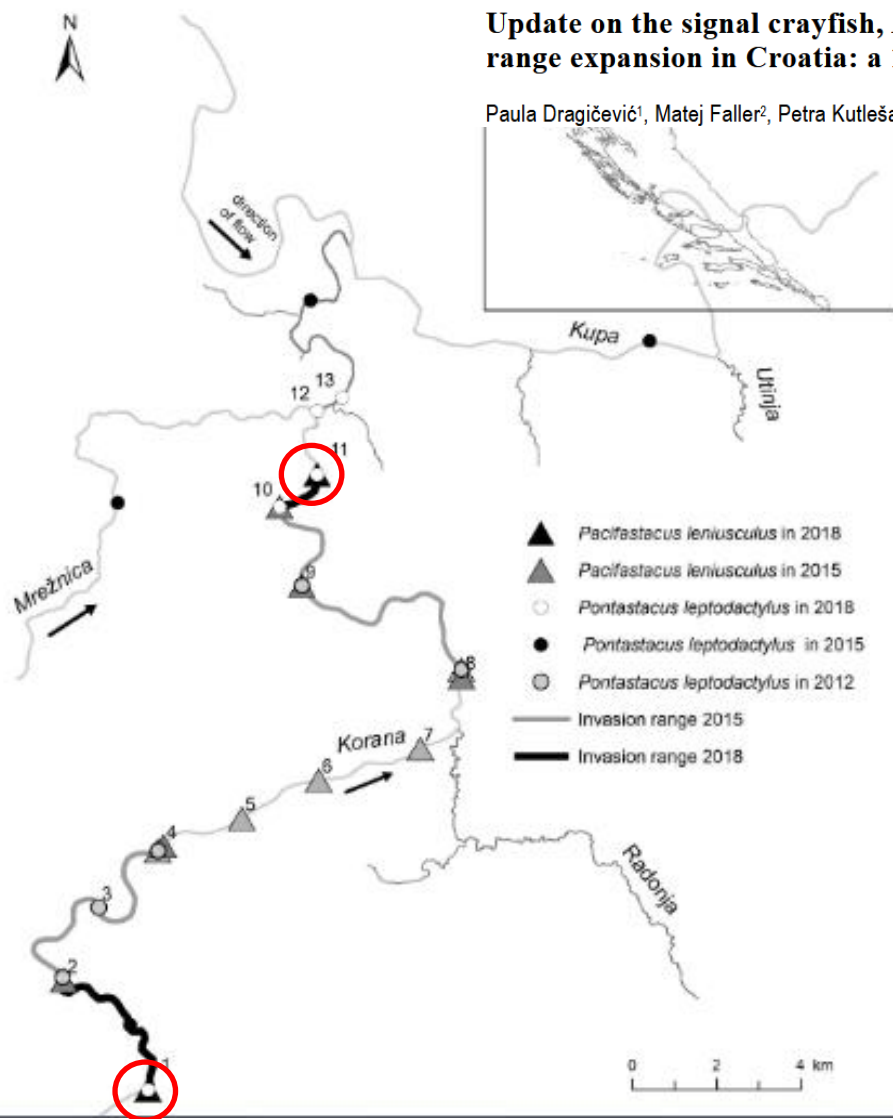
## Species-specific differences in dynamics of agonistic interactions may contribute to the competitive advantage of the invasive signal crayfish (*Pacifastacus leniusculus*) over the native narrow-clawed crayfish (*Astacus leptodactylus*)

Sandra Hudina<sup>a</sup>, Karlo Hock<sup>b</sup>, Andreja Radović<sup>c</sup>, Goran Klobučar<sup>a</sup>, Jelena Petković<sup>a</sup>, Mišel Jelić<sup>a</sup> and Ivana Maguire<sup>a</sup>



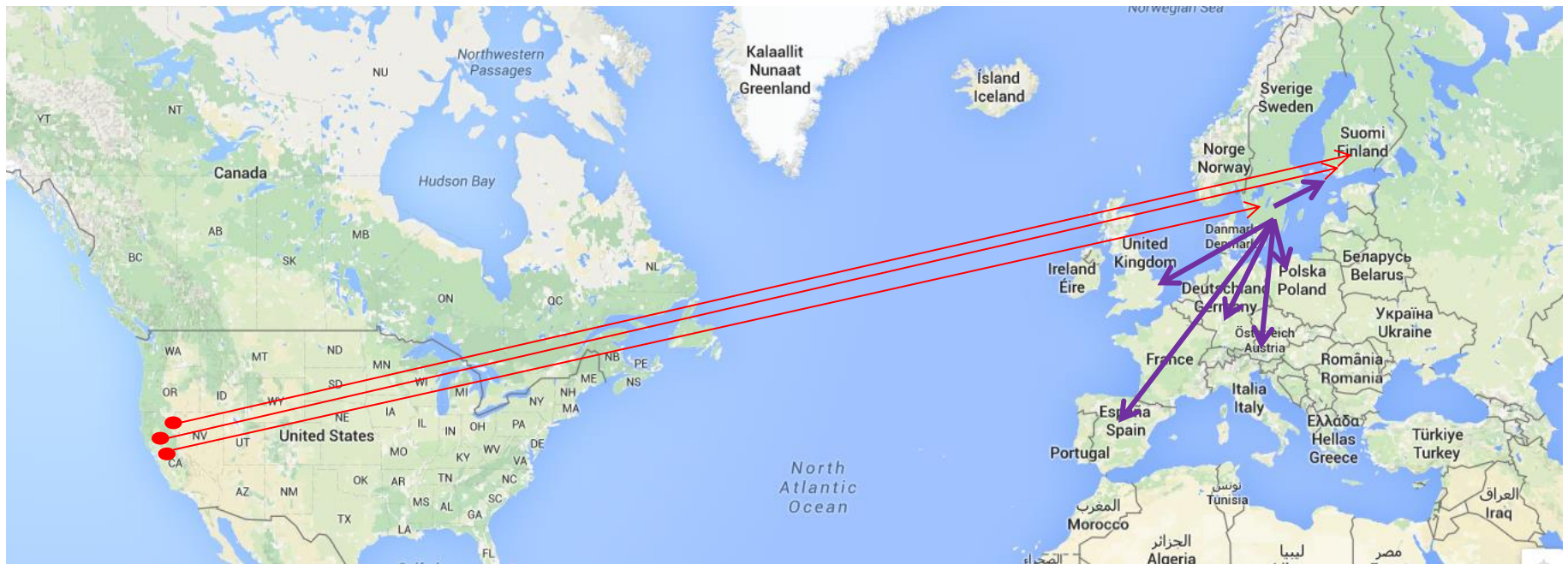
## Research Article

## Update on the signal crayfish, *Pacifastacus leniusculus* (Dana, 1852) range expansion in Croatia: a 10-year report

 Paula Dragičević<sup>1</sup>, Matej Faller<sup>2</sup>, Petra Kutleša<sup>3</sup> and Sandra Hudina<sup>1\*</sup>


Lake Natoma (Kalifornija) - Švedska

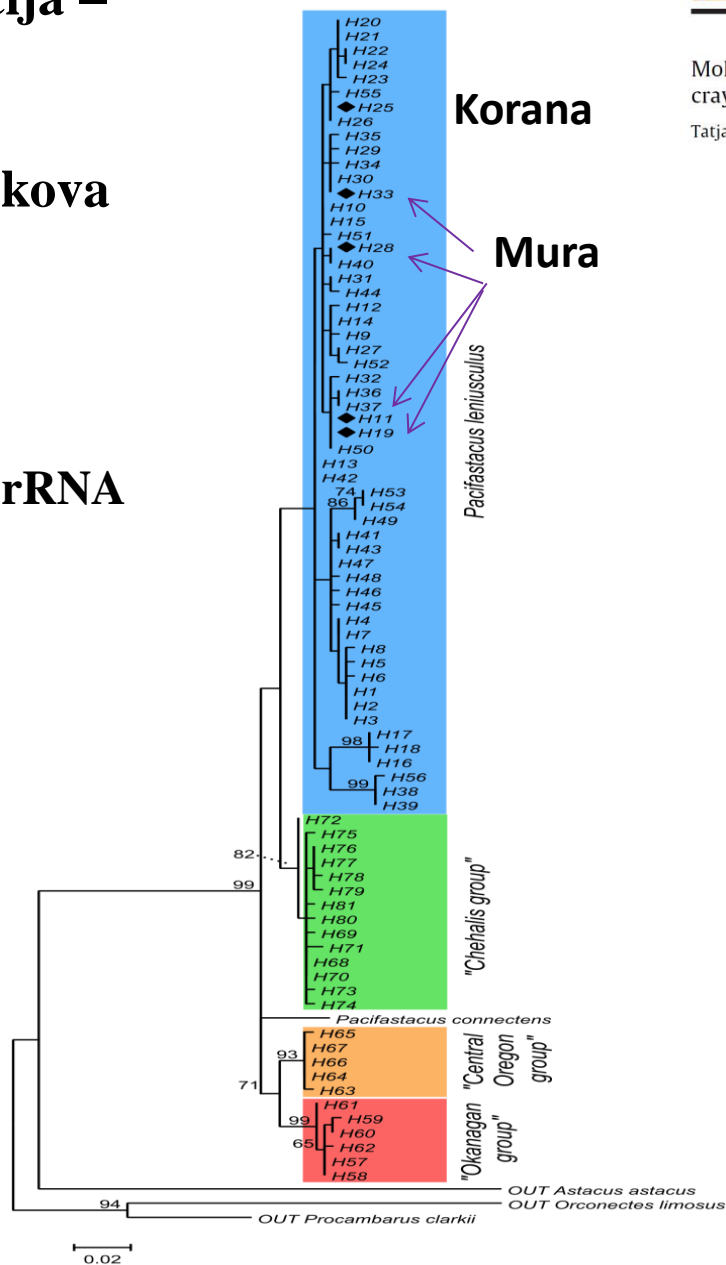
Lake Tahoe, Lake Hennessey (Kalifornija) - Finska



Austrija – unos iz Kalifornije (Lake Tahoe + Švedske) – pritoke Drave i Mure – Slovenija - Hrvatska

# Filogenetska rekonstrukcija – pozicija hrvatskih signalnih rakova

MtDNA – 16S rRNA



Molecular and morphometric characterisation of the invasive signal crayfish populations in Croatia

Tatjana Mijošek<sup>a</sup>, Mišel Jelić<sup>b</sup>, Vedrana Mijošek<sup>c</sup>, Ivana Maguire<sup>b,\*</sup>



## Srednje vrijednosti genetskih parametara

$N_a$  – alelna bogatstvo,  $H_E$  – očekivana heterozigotnost,  $H_O$  – uočena heterozigotnost,  $F_{IS}$  – koeficijent križanja u bliskom srodstvu,  $F_{ST}$  – fiksacijski indeks; GB – Great Britain, PT – Portugal, FI – Finland, SW – Sweden

Locality	$N_a$	$H_E$	$H_O$	$F_{IS}$	$F_{ST}$
Korana	4.13	0.526	0.496	0.003	0.026
Mura	6.00	0.577	0.530	0.117	
GB	4.36	0.530	0.622	-0.154	0.127
PT	3.83	0.572	0.611	-0.056	
FI	4.16	0.529	0.432	0.240	
SW	3.63	0.500	0.507	0.108	

- Broj Alela ( $N_a$ ) najveći u Muri
- Uočena heterozigotnost ( $H_o$ ) nešto manja u Hrvatskoj – Mura veća od Korane (duže etablirane populacije, opetovane introdukcije – veća raznolikost),  
-Niže vrijednosti  $H_o$  indicira srođivanje – gubitak raznolikosti i porast homozigota
- Fiksacijski indeks ( $F_{st}$ ) – što je manji – veći protok gena (povezanost kroz parenje) – Kod nas manji nego u EU – ali populacije geo odvojene – indicira nedavnu povezanost – antropogeni utjecaj – možebit **uneseni u Koranu iz Mure**

## Phylogeographic insights into the invasion history and secondary spread of the signal crayfish in Japan

Nisikawa Usio<sup>1</sup>, Noriko Azuma<sup>2</sup>, Eric R. Larson<sup>3,4</sup>, Cathryn L. Abbott<sup>5</sup>, Julian D. Olden<sup>4</sup>, Hiromi Akanuma<sup>6</sup>, Kenzi Takamura<sup>7</sup> & Noriko Takamura<sup>7</sup>



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Limnologia

journal homepage: [www.elsevier.com/locate/limno](http://www.elsevier.com/locate/limno)



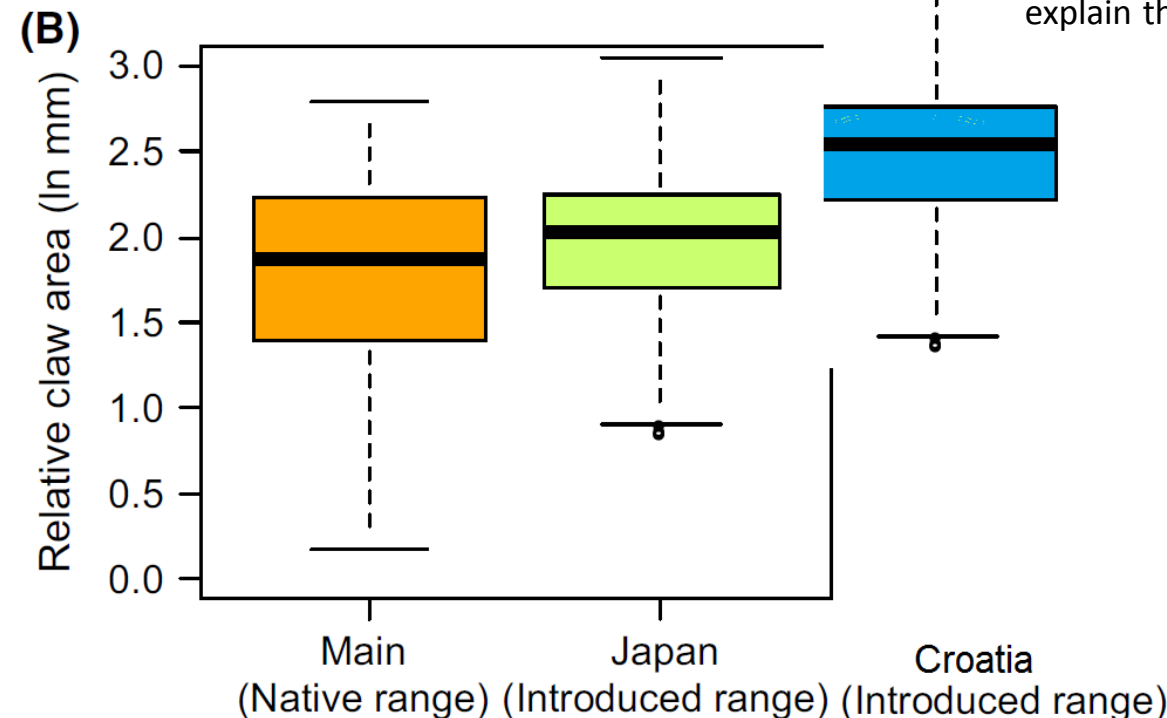
Veličina klijesta – agresivno ponašanje  
– bolje invazivni uspjeh

Molecular and morphometric characterisation of the invasive signal crayfish populations in Croatia



Tatjana Mijošek<sup>a</sup>, Mišel Jelić<sup>b</sup>, Vedrana Mijošek<sup>c</sup>, Ivana Maguire<sup>b,\*</sup>

- claws are larger compared to signal crayfish from the native range, what is one of the features that gives them competitive advantage over other species, and could explain their invasive success

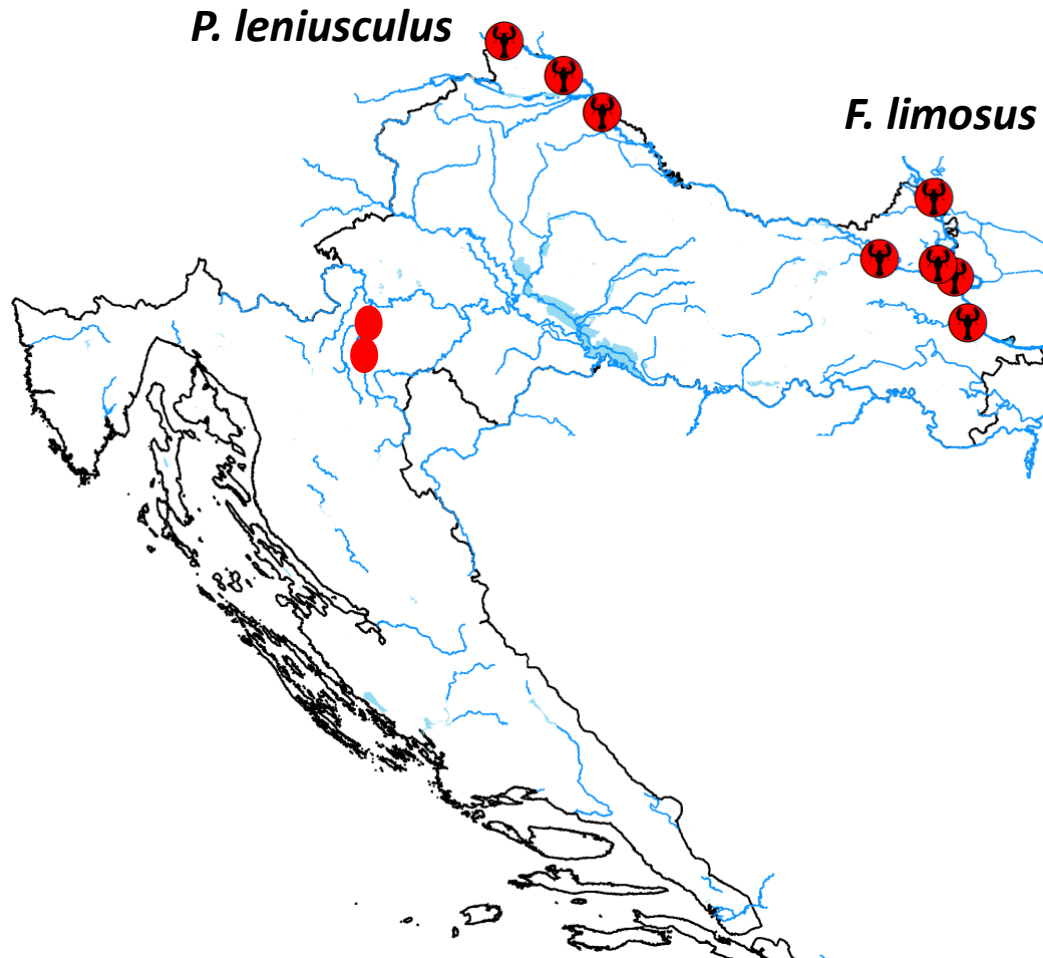
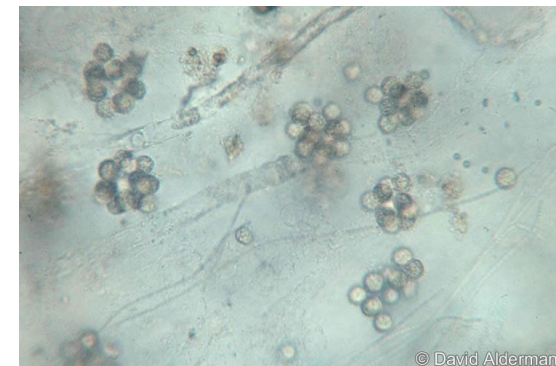


Kolbe et al. (2004) showed that substantial increases in genetic diversity of invasive populations can occur and can be explained by **multiple introductions**, especially when **source populations are genetically divergent**. Both interspecific hybridization and **intraspecific admixture** seem to be possible stimuli of invasion success in those cases.

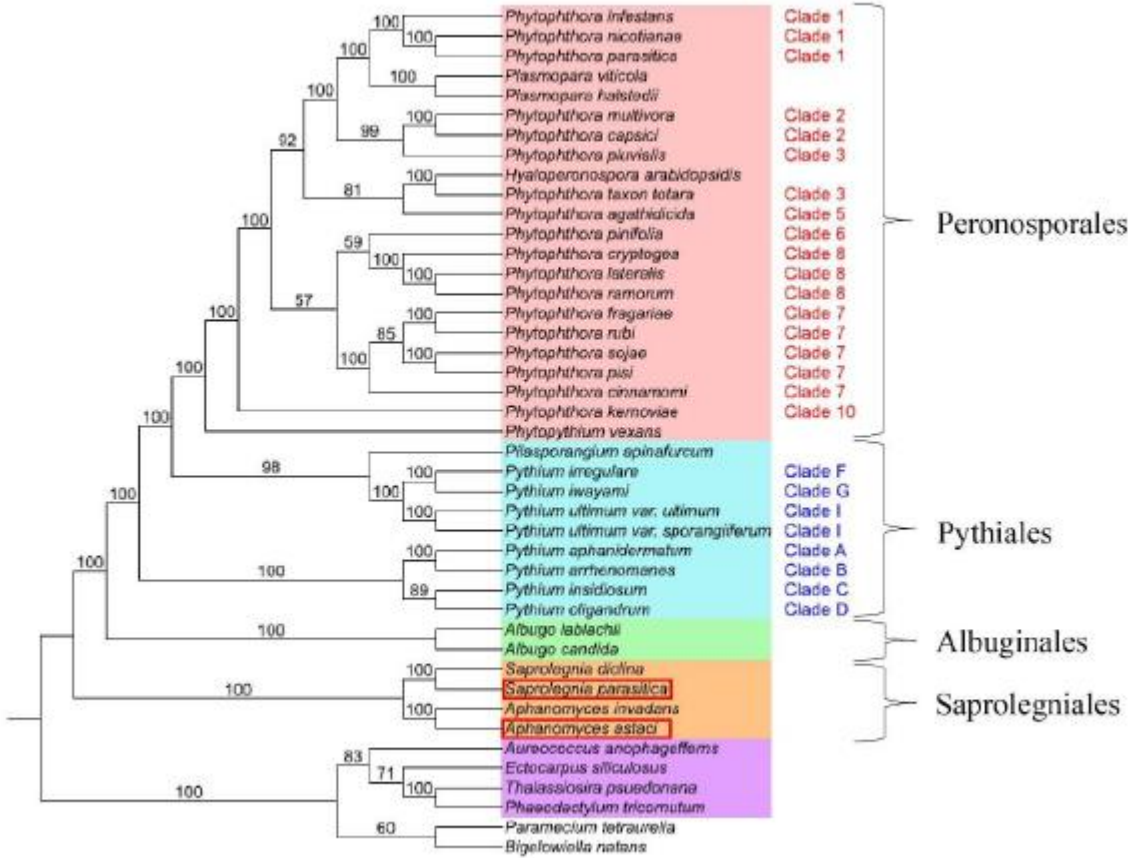


# Strane vrste prenosiooci patogena *Aphanomyces astaci* RAČJA KUGA

- Letalna po native vrste
- *Aphanomyces astaci* – jedna od 100 najinvazivnijih vrsta na svijetu



# Što je *A. astaci* i kud spada?



**Crayfish plague**

**Scientific classification**

Kingdom: **Chromista**

Phylum: **Oomycota**

Order: **Saprolegniales**

Family: **Leptolegniaceae**

Genus: ***Aphanomyces***

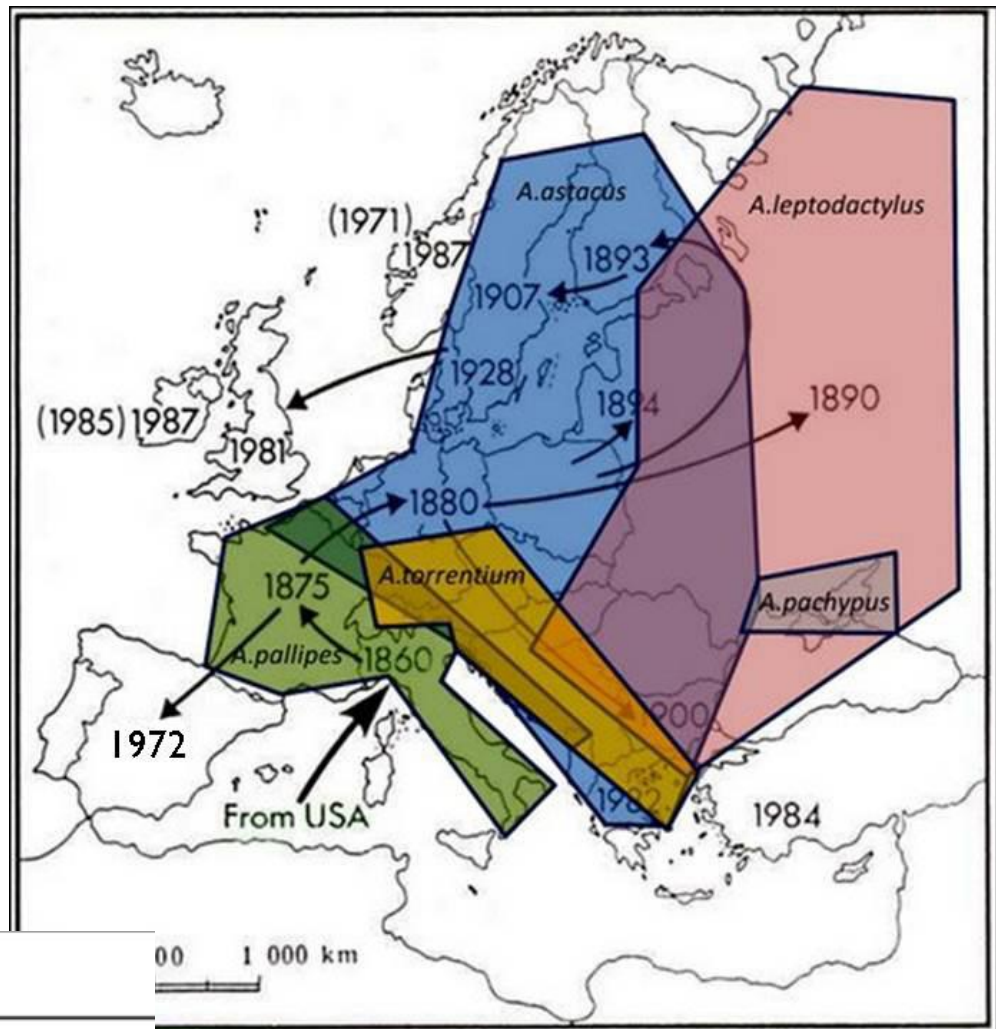
Species: ***A. astaci***

**Binomial name**

***Aphanomyces astaci***

Schikora, 1906 <sup>[1]</sup>

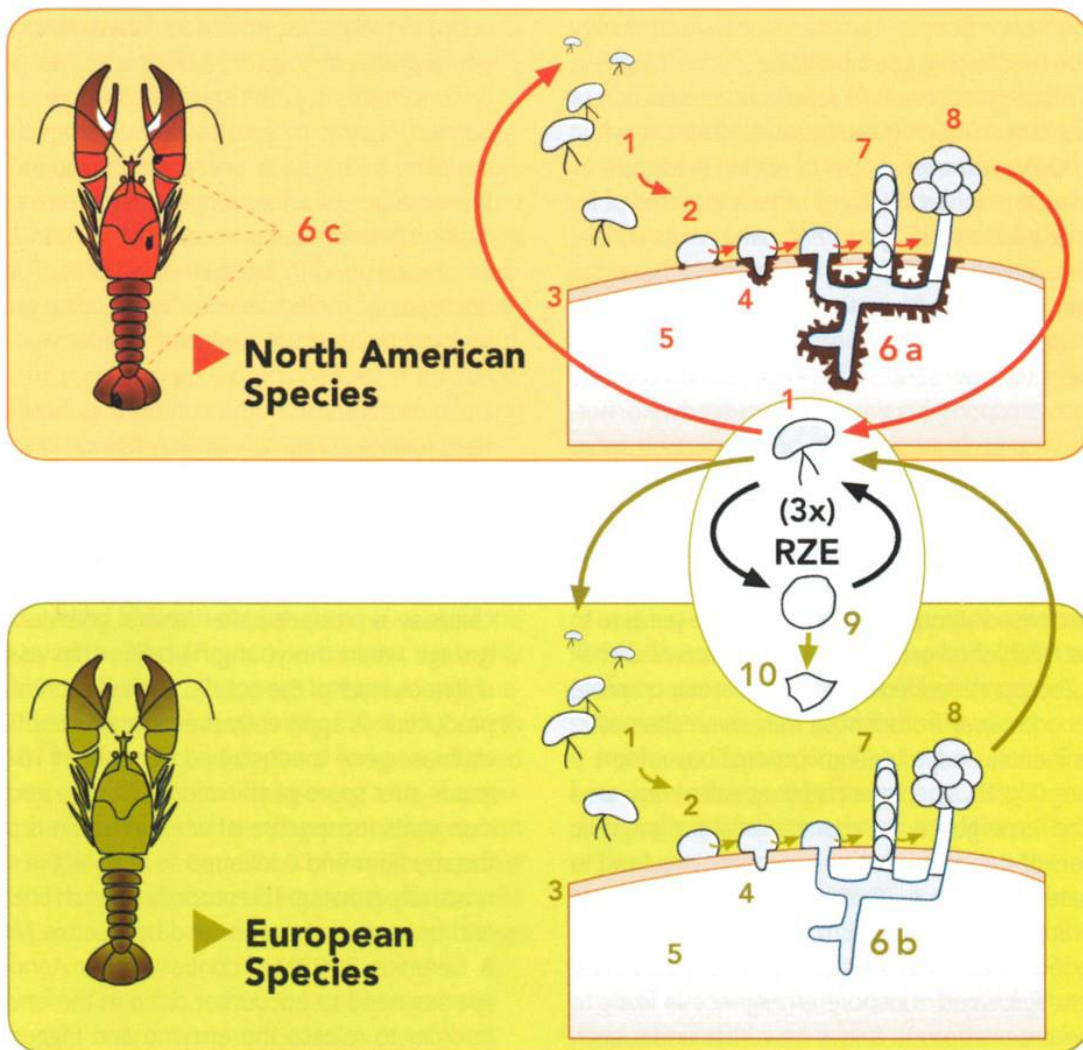
McCarthy and Fitzpatrick, 2017 (Pavić, 2023)



Genotype	Original host
----------	---------------

A	As	unknown
B	PsI	<i>P. leniusculus</i> (Lake Tahoe)
C	PsII	<i>P. leniusculus</i> (Lake Pitt)
D	Pc	<i>P. clarkii</i>
E	Or	<i>O. limosus</i>

- Zašto nativni oboljevaju i ugibaju?

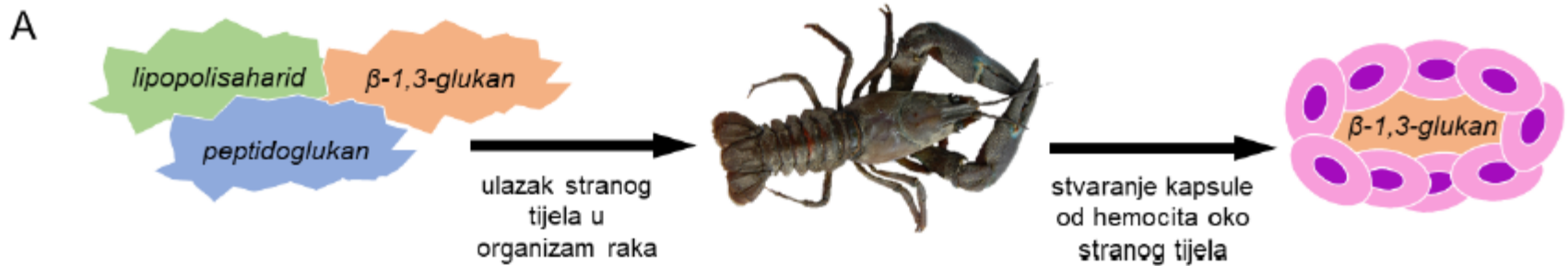


Američke vrste imaju stalno aktivirani proPO sistem (melanizacija patogena)

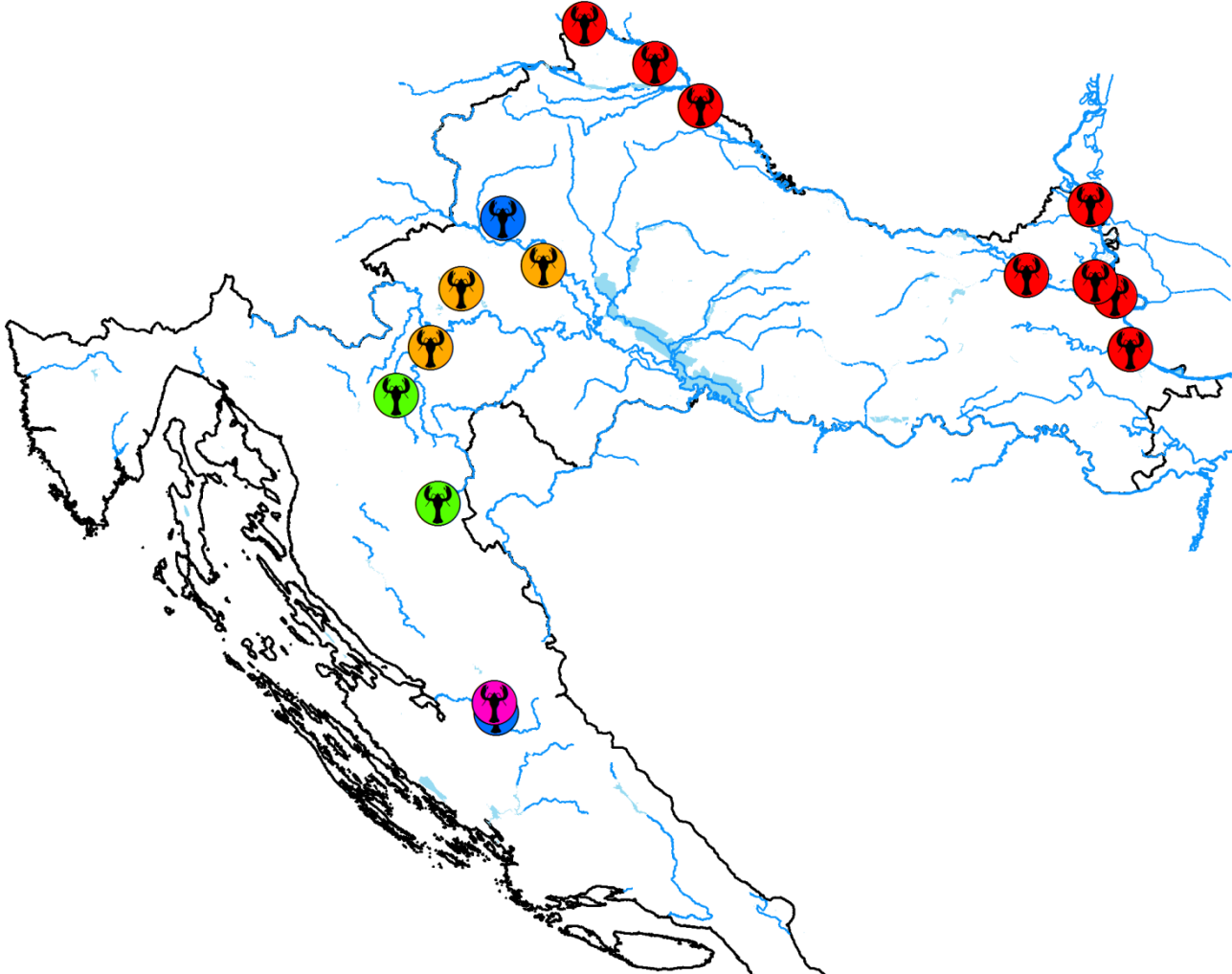
*Life cycle of A. astaci*

- 1) secondary zoospore (the infective unit),
- 2) encysting zoospore,
- 3) crayfish epicuticle,
- 4) germinating cyst,
- 5) cuticle penetration,
- 6a) melanized hyphae (chronic infection in the North-American crayfish),
- 6b) unmelanized hyphae (acute infection in the native European crayfish species or in the immune stressed North-American crayfish),
- 6c) melanized spots in the crayfish cuticle as a macroscopic sign of an infection of *A. astaci*,
- 7) sporangium of *A. astaci*,
- 8) clusters of primary cysts,
- 9) secondary cyst - form a new zoospore
- 10) Non-viable cyst

Diéguez-Urbeondo et al. (2006)

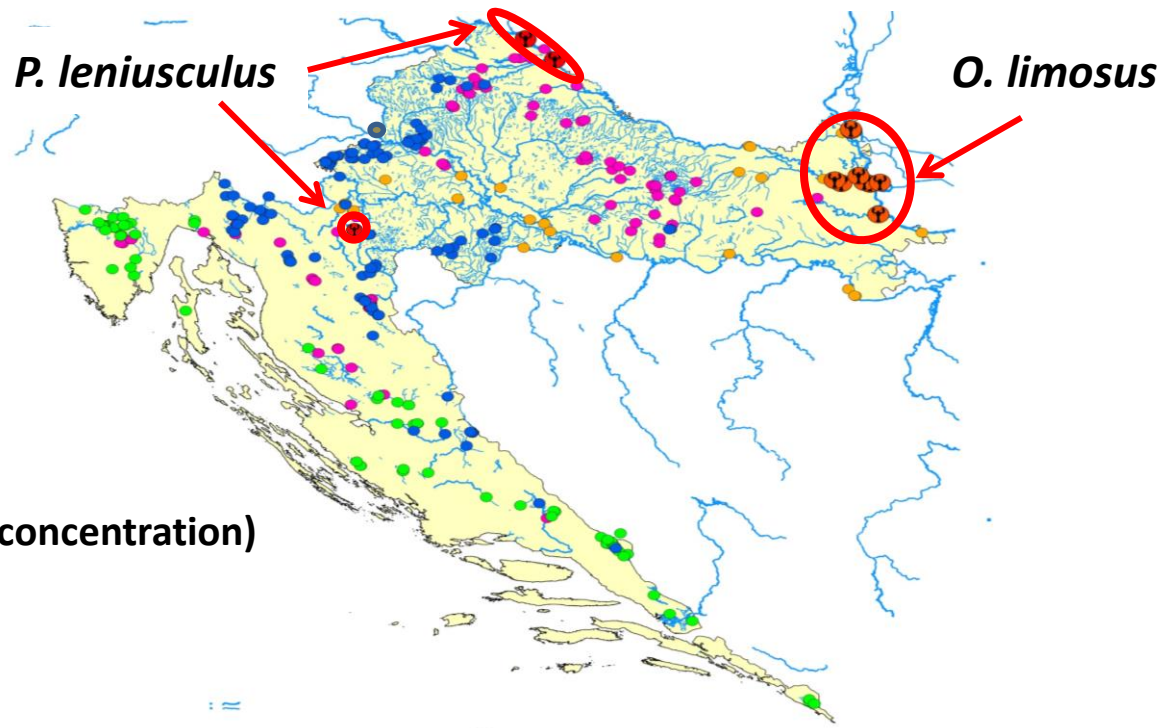


# Pojavnost *Aphanomyces astaci* u Hrvatskoj



vrsta	<i>A. pallipes</i>	<i>A. torrentium</i>	<i>A. astacus</i>	<i>P. leptodactylus</i>	<i>F. limosus</i>	<i>P. leniusculus</i>	<i>P. virginalis</i>
zaraza	+	+	+	+	+	+	-

Prisutnost patogena *Aphanomyces astaci* u invazivnih vrsta

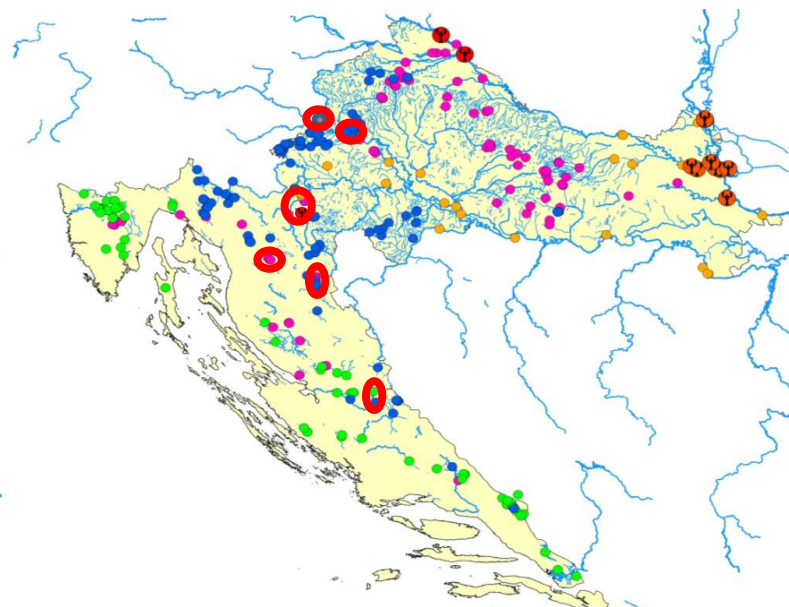


Infection level (pathogen concentration)

- A0 – no infection
- A1-A2 – low infection
- > A3 – strong infection

vrsta	<i>O. limosus</i>	<i>P. leniusculus</i>
Br.infic./br.testiranih	14 / 24	7 / 26
% zaraženih	58%	27%
Nivo zaraze	A0(10), A1(1), A2(2), A3(11)	A0(19), A2(4), A3(1), A4(1), A5(1)
Soj /genotip/haplogrupa	?	B (Ps)

# Prisutnost patogena *Aphanomyces astaci* u nativnim vrstama



## Zaključak

- Patogen prisutan i u nativnih
- Nivo zaraze invazivnih niži (samo 1 jedinka A5 - Ps genotip)
- Nivo zaraze nativnih (ako pozitivan) viši – moguća genotipizacija – ako u kontaktu s invazivnim Ps genotip, ako ne As genotip (stari europski nepoznatog podrijetla)

vrsta	<i>A. pallipes</i>	<i>A. torrentium</i>	<i>A. astacus</i>	<i>A. leptodactylus</i>
Br zaraženih/Br tesiranih	1 / 1	2 / 5	7 / 9	12 / 52
% zaraženosti	100%	40%	77%	23%
Soj/genotip/hapologrupa	A3(1)	A0(3), A3(1), A6(1)	A0(2), A1(1), A2(1), A3(3), A4(1), A6(1)	A0(40), A1(1), A3(4), A4(7)
Nivo zaraze	?	A (As)*	A (As)	B (Ps)



# Metode detekcije

## Invazivna metoda



Foto: Ljubej L.

Žrtvovanje rakova – izrezivanje komadića kutikule



## Neinvazivna metoda



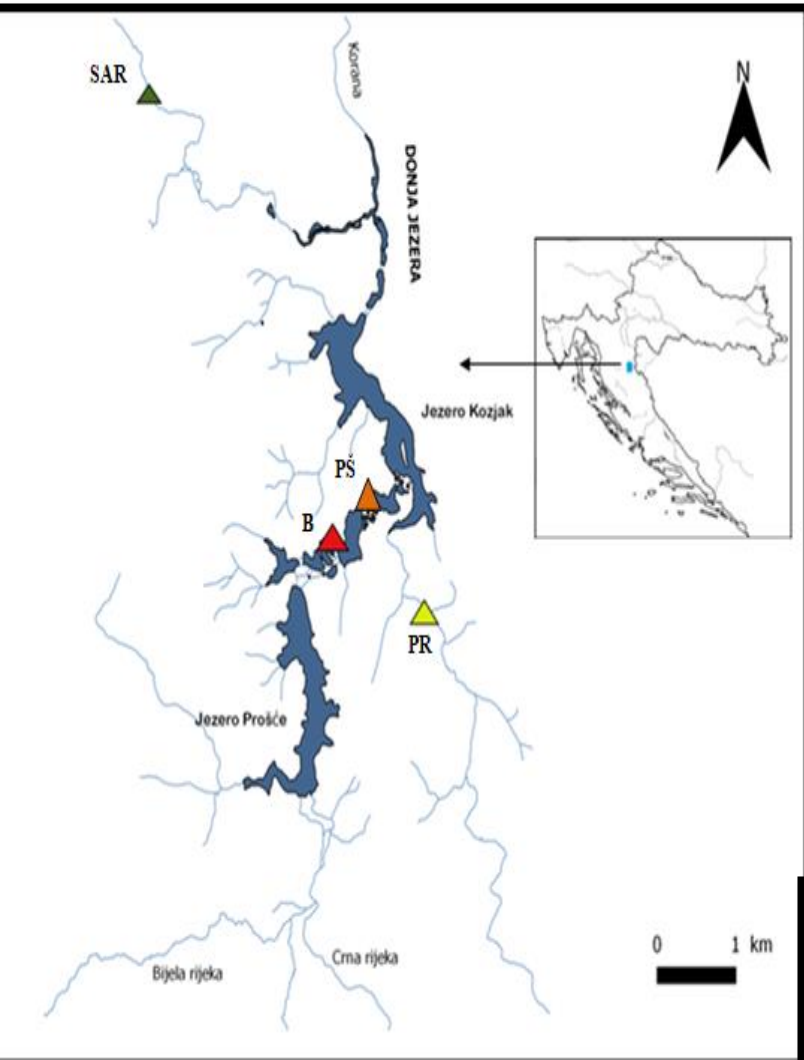
Foto: Hudina S.

Uzimanje brisa kutikule živog raka



- izolacija DNA
- PCR – utvrđivanje prisutnosti patogena
- qPCR – informacije o intezitetu infekcije (A0-A7)
- genotipizacija

# Metoda testirana na populacijama *A. astacus* i *A. torrentium* u NP Plitvička jezera



## PCR- test

- proveden za sve uzorke
- patogen utvrđen kod 9/115 jedinki
- samo na vrsti *A. astacus*
- usporedba metoda – 7/9

Oznaka	Vrsta	Lokacija	PCR
B5	<i>A. astacus</i>	Burgeti	-(B), +(K)
B9	<i>A. astacus</i>	Burgeti	+(B)
B10	<i>A. astacus</i>	Burgeti	-(B), +(K)
B21	<i>A. astacus</i>	Burgeti	+(B)
B24	<i>A. astacus</i>	Burgeti	+(B), +(K)
B37	<i>A. astacus</i>	Burgeti	+(B)
B60	<i>A. astacus</i>	Burgeti	+(B)
B67	<i>A. astacus</i>	Burgeti	+(B)
PŠ1	<i>A. astacus</i>	Slap Prštavac	+(B)

# qPCR

- tri para uzorka – određen intezitet infekcije

Oznaka	Vrsta	Lokacija	PCR	qPCR
B5	<i>A. astacus</i>	Burgeti	-(B), +(K)	A0(B), A3(K)
B9	<i>A. astacus</i>	Burgeti	+ (B)	N.T.
B10	<i>A. astacus</i>	Burgeti	-(B), +(K)	A1(B), A3(K)
B21	<i>A. astacus</i>	Burgeti	+(B)	N.T.
B24	<i>A. astacus</i>	Burgeti	+ (B), + (K)	A4(B), A5(K)
B37	<i>A. astacus</i>	Burgeti	+(B)	N.T.
B60	<i>A. astacus</i>	Burgeti	+(B)	N.T.
B67	<i>A. astacus</i>	Burgeti	+(B)	N.T.
PŠ1	<i>A. astacus</i>	Slap Prštavac	+(B)	N.T.

# Genotipizacija

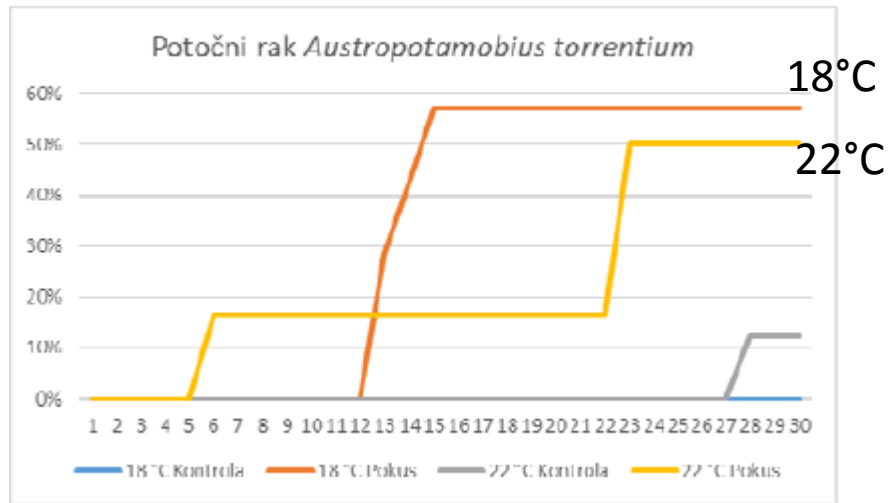
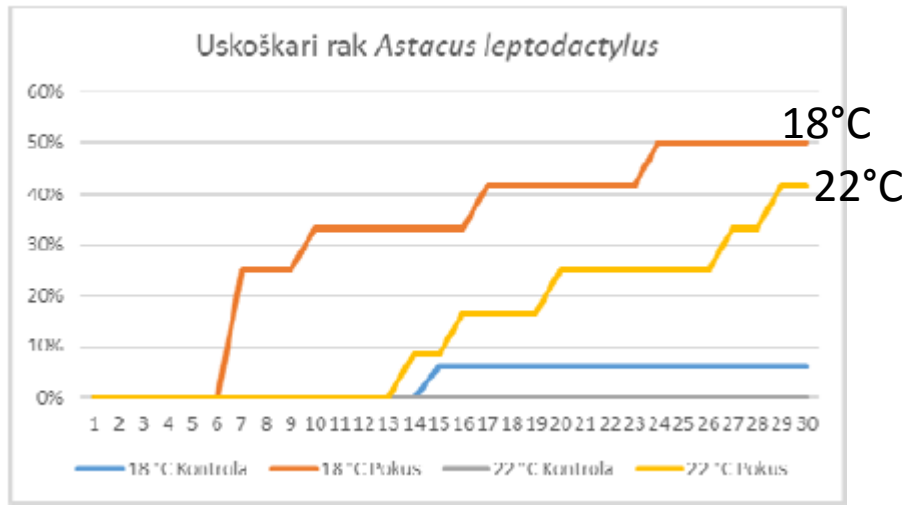
- analizom dužine mikrosatelitnih biljega – određen genotip
- A4 ili više
- soj A

- niska razina virulencije soja A
- adaptacija imunog sustava
- dugi boravak soja A

Oznaka	Vrsta	Lokacija	PCR	qPCR	Soj (genotip)
B5	<i>A. astacus</i>	Burgeti	-(B), +(K)	A0(B), A3(K)	N.T.
B9	<i>A. astacus</i>	Burgeti	+(B)	N.T.	N.T.
B10	<i>A. astacus</i>	Burgeti	-(B), +(K)	A1(B), A3(K)	N.T.
B21	<i>A. astacus</i>	Burgeti	+(B)	N.T.	N.T.
B24	<i>A. astacus</i>	Burgeti	+(B), +(K)	A4(B), A5(K)	A(B), A(K)
B37	<i>A. astacus</i>	Burgeti	+(B)	N.T.	N.T.
B60	<i>A. astacus</i>	Burgeti	+(B)	N.T.	N.T.
B67	<i>A. astacus</i>	Burgeti	+(B)	N.T.	N.T.
PŠ1	<i>A. astacus</i>	Slap Prštavac	+(B)	N.T.	N.T.

**Populacije zavičajnih rakova kronično zaražene, ali vijabilne.**

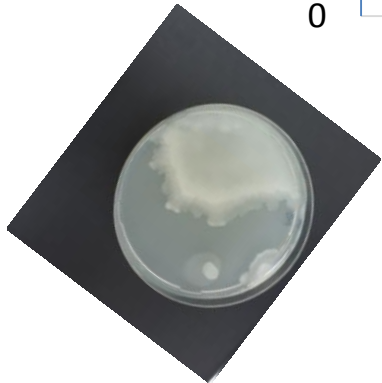
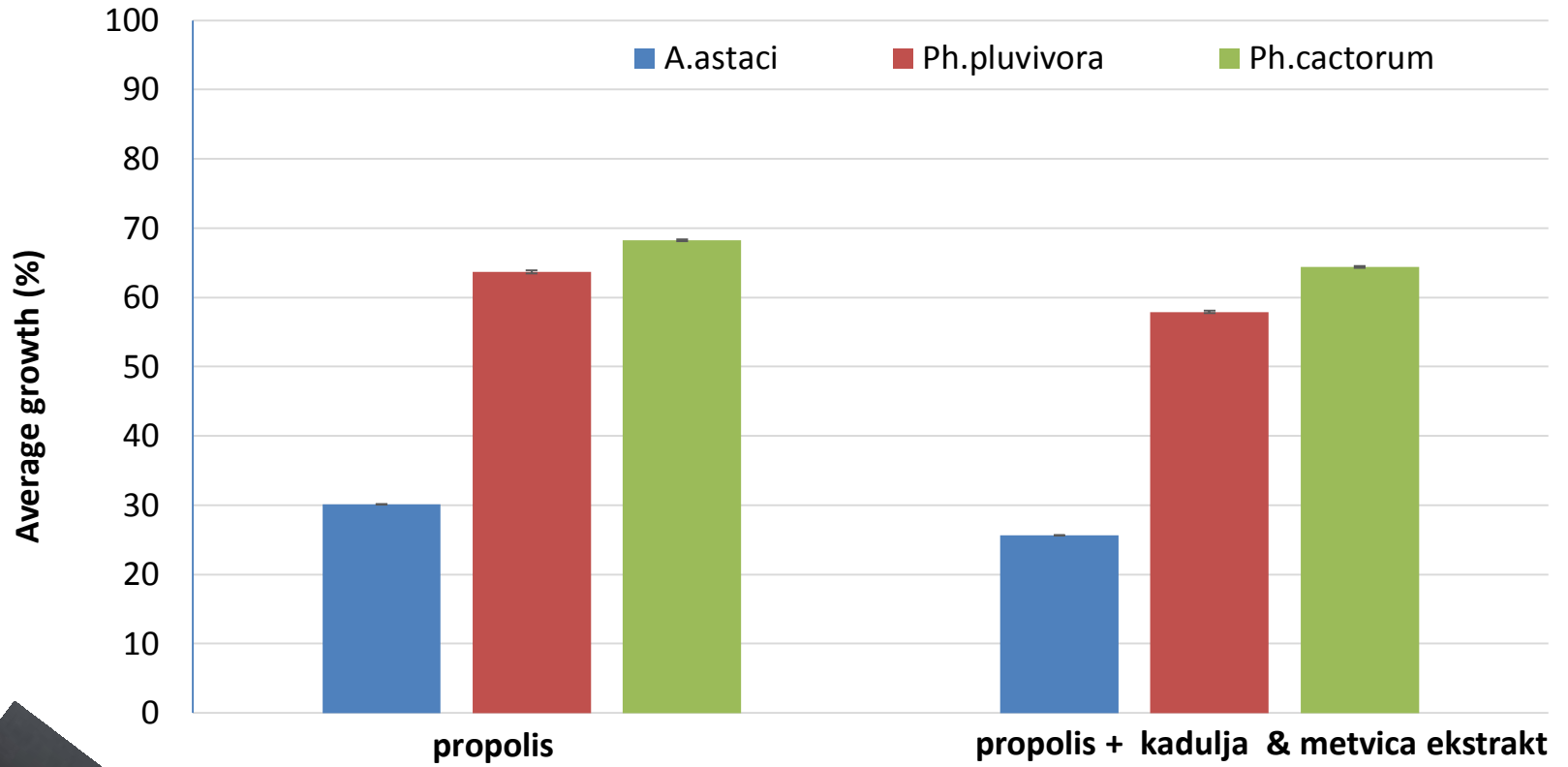
# Klimatske promjene i račja kuga



- *A. astaci* (B ili PsII soj) osjetljiv na porast temperature iznad 22°C
- treba testirati ostale sojeve

# Interactions of freshwater pathogenic oomycetes and their environment

- Mogućnot prevencije kuge



### 3. invazivna vrsta - *Procambarus virginalis*

Winter Issue,  
December 2014  
Volume 36, Issue 4

# Crayfish NEWS

ISSN: 1023-8174 (print), 2150-9235 (online)

The Official Newsletter of the International Association of Astacology

- Akvaristika
- Poplave

## Short Articles

### The First Record of the Marbled Crayfish (*Procambarus fallax* (Hagen, 1870) f. *virginalis*) in Croatia

Here we report the first record of the non-indigenous parthenogenetic species, the marble crayfish in the wild from Croatia. It is the third non-indigenous crayfish (NICS) recorded in Croatia – with four indigenous crayfish, Croatian astacofauna now contains 7 crayfish species. The marble crayfish were discovered in the Šoderica gravel pit lake, located at far northwest of Croatia, close to the Drava River and Hungarian border (Figure 1).

Previous studies recorded presence of the native *Astacus astacus* in the Šoderica, and invasive *Pacifastacus leniusculus* in the nearby Drava River (Hudina et al, 2009; Maguire et al, 2011). In the autumn 2013, during scuba-diving in the Šoderica gravel pit lake numerous crayfish were observed under submerged boat. Some juvenile specimens were taken to the school laboratory under presumption that they are juvenile specimens of *A. astacus*. However, after their close examination they were identified as the marble crayfish, *Procambarus fallax* (Hagen, 1870) f. *virginalis* (Figure 2) (Chucholl et al, 2012; Martin et al, 2010; Souty-Grosset et al, 2006). We assume that the widespread and lively on-line marble crayfish pet trade, and its release to the gravel pit lake, was the introduction pathway of this species to Croatia.

The nearest recorded marble crayfish locations in the wild

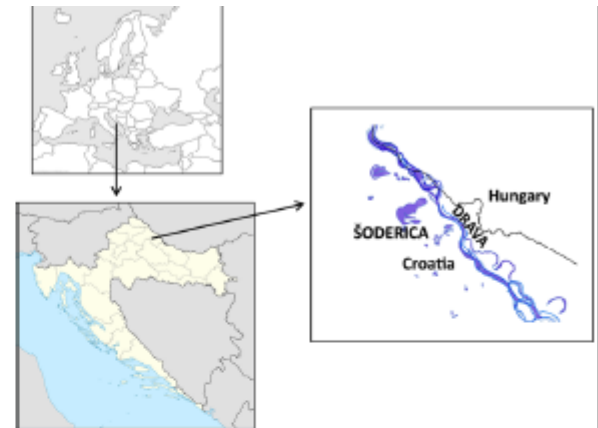
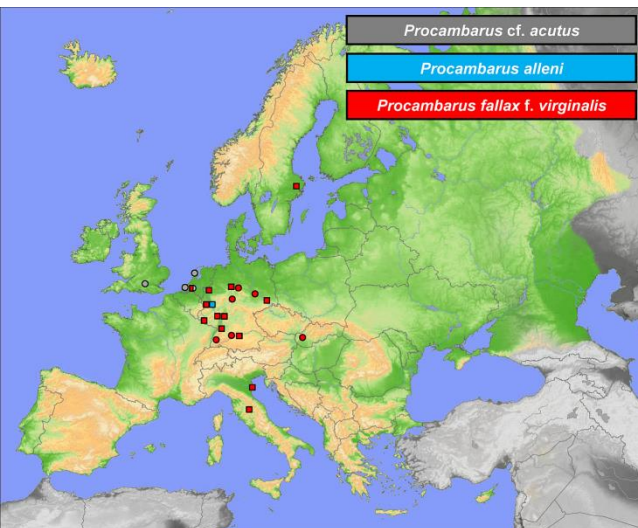


Figure 1. Position of the Šoderica gravel pit lake (coordinates 46.175; 17.025) where the marble crayfish were caught in autumn 2013.





## *Procambarus fallax*



## *Procambarus (fallax f.) virginalis*

- akvaristika sredina 1990ih Njemačka
- jedina vrsta za koju se zna da je partenogenetska



Elizabeth Pennisi

(26 August 2015) *Science* | DOI: 10.1126/science.aad1673

- researchers show that the *Procambarus fallax* males can't fertilize marbled crayfish eggs, a hallmark of a species split, and that the clones contain enough genetic differences to justify designating them a separate species

- researchers are now analyzing these so-called epigenetic differences in more detail and are proposing this new species be called *Procambarus virginalis*—the virgin form of the genus *Procambarus*

## The marbled crayfish (Decapoda: Cambaridae) represents an independent new species

December 2017 · Zootaxa 4363(4):544

DOI: 10.11646/zootaxa.4363.4.6

FRANK LYKO

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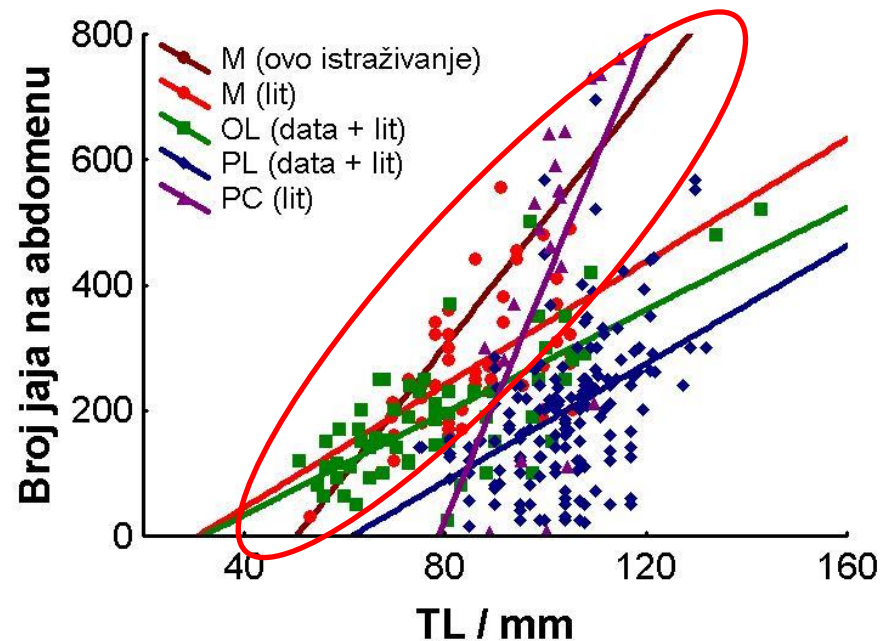
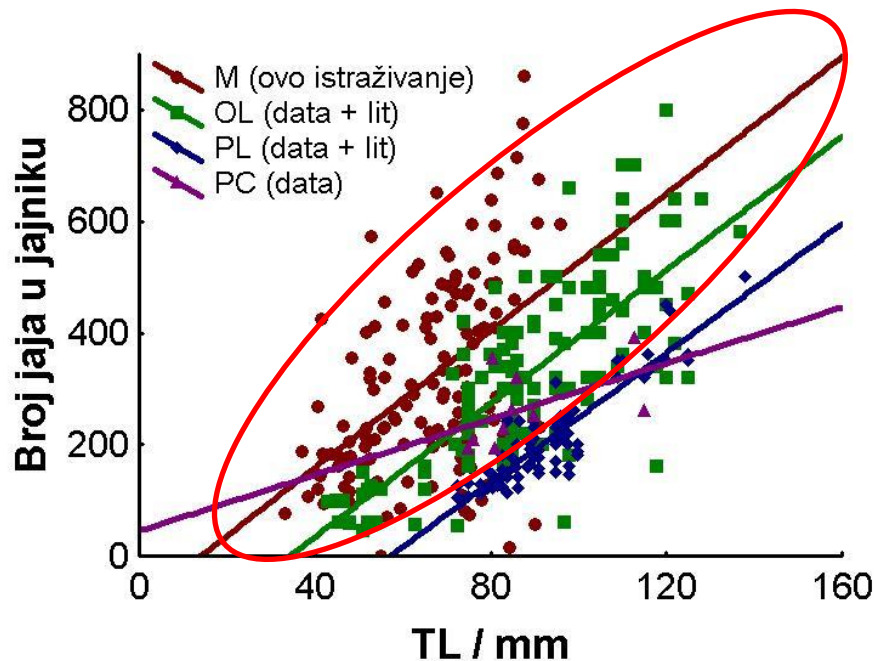
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NEWS · 06 FEBRUARY 2018 · CORRECTION 07 FEBRUARY 2018

## Geneticists unravel secrets of super-invasive crayfish

<https://www.nature.com/articles/d41586-018-01624-y>

## ■ Usporedba fekunditeta raznih invazivaca u Europi



**M** – mramorni rak, **OL** – bodljobradi rak, **PL** – signalni rak, **PC** – *P. clarkii*



*...will live happily ever after*



## Zaključak

- Hrvatske populacije čuvaju značajnu genetsku raznolikost slatkovodnih rakova – rezervoar na europskom nivou
- Najveća raznolikost potočnog raka – Alpska biogeografska regija
- Najveća raznolikost riječnog raka – Kontinentalna biogeo. regija
- Najveća raznolikost bjelonogog raka – Mediteranska biogeo. regija
- **Filogenija + filogeografija + populacijska genetika + SDM – neophodni za izradu konzervacijskih planova za ove ugrožene/osjetljive native vrste**