Comparative phylogeography of *Phoxinus, Delminichthys, Phoxinellus*, and *Telestes* in the Dinaric karst: what factors influenced the current pattern of their distribution?

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Supplementary material

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Table S1. Mitochondrial lineages of *Phoxinus* used for the various types of analysis.

Mt (sub-)lineage/species	Distributed in Dinaric karst	Haplotype network	Diversity indices	p-distances	Divergence time dating	Ancestral biogeography
1a – *P. lumiareul*	x	x	x	x	x	x
1b – *P. lumiareul*	x	x	x	x	x	x
1c – *P. lumiareul*	x	x	x	x	x	x
1d – *P. lumiareul*		x		x	x	x
1e – *P. lumiareul*	x	x	x	x	x	x
1f – *P. lumiareul*		x		x	x	x
2	x	x	x	x	x	x
3	x	x	x	x	x	x
4	x	x	x	x	x	x
5a – *P. csikii*	x	x	x	x	x	x
6 – *P. krkae*	x	x	x	x	x	x
7 – *P. karsticus*	x	x	x	x	x	x
8	x	x	x	x	x	x

Table S2. Presented as a separate Excel file with four sheets for each genus. Details for sampling localities and source of data are given. For samples colored in black genetic data are available, while those marked in red correspond to distribution data without genetic information, collected from the literature, personal observations and personal communications.

Add Material & Methods section 2.5

1. For the phylogenetic tree reconstruction for and divergence time dating, several preliminary analyses with different datasets/outgroups were conducted. The datasets/outgroups were chosen based on 36., 54. 55. and 79.. The datasets tested: *Phoxinus* from Balkan with only one haplotype per lineage (*Phoxinus phoxinus* from Mongolia as outgroup)
2. *Phoxinus* with one haplotype per lineage with all occurring lineages in Europe
3. *Phoxinellus* only (*Phoxinus* as outgroup)
4. *Telestes* only (*Phoxinus* as outgroup)
5. *Delminichthys* only (*Phoxinus* as outgroup)
6. *Phoxinellus* and *Telestes* (with their sistergroups *Chondrostoma* and *Pseudophoxinus*)
7. *Phoxinellus*, *Delminichthys* and *Telestes* (with their sistergroups (*Chondrostom*a, *Pseudophoxinus* and *Pelasgus*), *Squalius cephalus*, *Rutilus rutilus*, *Alburnus alburnus*, *Leuciscus leucsicus*, *Vimba vimba*; outgroups: *Phoxinus*, *Cyprinus carpio* and *Carassisus auratus*)

The two datasets described in the Material & methods section of the manuscript revealed the overall highest ESS values in the trace files, as well as the highest marginal likelihood values.

Table S3. All samples with GenBank accession numbers and references included in the divergence timing analysis of *Phoxinus* mt lineages.

Species	Clade	GenBank	Reference
Alburnus alburnus		AB239593	Saitoh et al. [2]
Cyprinus carpio		NC_001606	Chang, Huang, and Lo [3]
Phoxinus lumaireul	1a	KT166665	Palandačić et al. [4]
Phoxinus lumaireul	1a	KT166757	Palandačić et al. [4]
Phoxinus lumaireul	1a	ON494605	Reier et al. [5]
Phoxinus lumaireul	1a	KT166668	Palandačić et al. [4]
Phoxinus lumaireul	1a	ON494698	Reier et al. [5]
Phoxinus lumaireul	1a	ON494839	Reier et al. [5]
Phoxinus lumaireul	1a	ON494712	Reier et al. [5]
Phoxinus lumaireul	1a	KT166604	Palandačić et al. [4]
Phoxinus lumaireul	1a	KT166722	Palandačić et al. [4]
Phoxinus lumaireul	1a	KT166647	Palandačić et al. [4]
Phoxinus lumaireul	1a	KT166734	Palandačić et al. [4]
Phoxinus lumaireul	1a	MG681503	Vučić et al. [6]
Phoxinus lumaireul	1a	KT166828	Palandačić et al. [4]
Phoxinus lumaireul	1a	KT166832	Palandačić et al. [4]
Phoxinus lumaireul	1a	ON494890	Reier et al. [5]
Phoxinus lumaireul	1b	ON494754	Reier et al. [5]
Phoxinus lumaireul	1b	ON494856	Reier et al. [5]

Phoxinus lumaireul	1b	KT166653	Palandačić et al. [4]
Phoxinus lumaireul	1b	KT166742	Palandačić et al. [4]
Phoxinus lumaireul	1b	KT166759	Palandačić et al. [4]
Phoxinus lumaireul	1b	KT166743	Palandačić et al. [4]
Phoxinus lumaireul	1b	KT166784	Palandačić et al. [4]
Phoxinus lumaireul	1b	KT166785	Palandačić et al. [4]
Phoxinus lumaireul	1b	KT166729	Palandačić et al. [4]
Phoxinus lumaireul	1b	KT166631	Palandačić et al. [4]
Phoxinus lumaireul	1b	MG681431	Vučić et al. [6]
Phoxinus lumaireul	1b	MG681432	Vučić et al. [6]
Phoxinus lumaireul	1b	KT166824	Palandačić et al. [4]
Phoxinus lumaireul	1b	KT166829	Palandačić et al. [4]
Phoxinus lumaireul	1c	ON494640	Reier et al. [5]
Phoxinus lumaireul	1c	ON494611	Reier et al. [5]
Phoxinus lumaireul	1c	KT166627	Palandačić et al. [4]
Phoxinus lumaireul	1c	ON495044	Reier et al. [5]
Phoxinus lumaireul	1c	ON494783	Reier et al. [5]
Phoxinus lumaireul	1c	ON494807	Reier et al. [5]
Phoxinus lumaireul	1c	KT166716	Palandačić et al. [4]
Phoxinus lumaireul	1c	ON738222	this study
Phoxinus lumaireul	1c	KT166588	Palandačić et al. [4]
Phoxinus lumaireul	1d	KT166725	Palandačić et al. [4]
Phoxinus lumaireul	1d	KT166708	Palandačić et al. [4]
Phoxinus lumaireul	1d	KT166584	Palandačić et al. [4]
Phoxinus lumaireul	1e	KT166790	Palandačić et al. [4]
Phoxinus lumaireul	1f	KT166762	Palandačić et al. [4]
Phoxinus lumaireul	1f	KT166769	Palandačić et al. [4]
Phoxinus csikii	5a	KT166638	Palandačić et al. [4]
Phoxinus csikii	5a	KT166753	Palandačić et al. [4]
Phoxinus csikii	5a	KT166553	Palandačić et al. [4]
Phoxinus sp.	2	KT166780	Palandačić et al. [4]
Phoxinus sp.	2	KT166557	Palandačić et al. [4]
Phoxinus sp.	2	KT166558	Palandačić et al. [4]
Phoxinus sp.	2	KT166813	Palandačić et al. [4]
Phoxinus sp.	2	KT166589	Palandačić et al. [4]
Phoxinus sp.	2	KT166620	Palandačić et al. [4]
Phoxinus sp.	2	KT166645	Palandačić et al. [4]
Phoxinus sp.	3	KT166691	Palandačić et al. [4]
Phoxinus sp.	3	KT166692	Palandačić et al. [4]
Phoxinus sp.	3	KT166693	Palandačić et al. [4]
Phoxinus sp.	4	KT166574	Palandačić et al. [4]
Phoxinus sp.	4	KT166575	Palandačić et al. [4]
Phoxinus csikii	5a	KT166638	Palandačić et al. [4]
Phoxinus krkae	6	KT166546	Palandačić et al. [4]
Phoxinus krkae	6	KT166548	Palandačić et al. [4]
Phoxinus krkae	6	KT166551	Palandačić et al. [4]
Phoxinus karsticus	7	KT166776	Palandačić et al. [4]

Phoxinus karsticus	7	KT166778	Palandačić et al. [4]
Phoxinus karsticus	7	MG681299	Vučić et al. [6]
Phoxinus karsticus	7	MG681489	Vučić et al. [6]
Phoxinus karsticus	7	MG681490	Vučić et al. [6]
Phoxinus karsticus	7	KT166806	Palandačić et al. [4]
Phoxinus karsticus	7	KT166820	Palandačić et al. [4]
Phoxinus karsticus	7	KT166649	Palandačić et al. [4]
Phoxinus sp.	7	KT166815	Palandačić et al. [4]
Phoxinus sp.	7	KT166817	Palandačić et al. [4]
Phoxinus sp.	8	KT166678	Palandačić et al. [4]
Phoxinus sp.	8	KT166679	Palandačić et al. [4]
Phoxinus sp.	8	KT166683	Palandačić et al. [4]
Rutilus rutilus		AP010775	Saitoh et al. [2]
Squalius cephalus		NC031540	Iwasaki et al. [7]

Table S4. All samples with GenBank accession numbers and references included in the divergence timing analysis of *Delminichthys, Phoxinellus* and *Telestes* and sister/outgroups.

Species	**GenBank**	**Reference**
Barbus barbus	NC_008654	Saitoh et al. [8]
Carassius auratus	KJ874430	Cheng, Liang, and Sun [9]
Chondrostoma nasus	MG806657	Schönhuth et al. [10]
Chondrostoma phoxinus	MG806658	Schönhuth et al. [10]
Cyprinus carpio	NC_001606	Chang, Huang, and Lo (1994)
Delminichthys adspersus	JQ315974	Palandačić et al. [11]
Delminichthys ghetaldii	MG806663	Schönhuth et al. [10]
Delminichthys jadovensis	ON738200	this study
Delminichthys krbavensis	ON738195	this study
Pachychilon macedonicum	MG806671	Schönhuth et al. [10]
Pelasgus minutus	MZ230788	Viñuela Rodríguez et al. [12]
Pelecus cultratus	AY838938	Freyhof et al. [13]
Phoxinellus alepidotus	ON738208	this study
Phoxinellus dalmaticus	ON738213	this study
Phoxinellus pseudoalepidotus	ON738206	this study
Pseudophoxinus anatolicus	AY494754	Hrbek et al. [14]
Rutilus rutilus	AP010775	Saitoh et al. (2011)
Rutilus rutilus	MG806695	Schönhuth et al. [10]
Squalius cephalus	MG806701	Schönhuth et al. [10]
Squalius cephalus	NC031540	Iwasaki et al. [7]
Telestes beoticus	MG372658	Buj et al. [15]
Telestes croaticus	AY509828	Ketmaier et al. [16]
Telestes dabar	MG372497	Buj et al. [15]
Telestes fontinalis	MG372533	Buj et al. [15]
Telestes karsticus	JN188371	Marčić et al. [17]
Telestes metohiensis	MK482048	Benovics et al. [18]

Telestes miloradi	MG372657	Buj et al. [15]
Telestes muticellus	MG372593	Buj et al. [15]
Telestes montenegrinus	AY509849	Ketmaier et al. [16]
Telestes pleurobipunctatus	MG372650	Buj et al. [15]
Telestes polylepis	HM560222	Perea et al. [19]
Telestes souffia	AY509861.1	Ketmaier et al. [16]
Telestes souffia	MG372582	Buj et al. [15]
Telestes turskyi	MG372555	Buj et al. [15]
Telestes ukliva	MG372543	Buj et al. [15]
Vimba melanops	MG806725	Schönhuth et al. [10]

Table S5. Genetic diversity parameters of the mitochondrial cytochrome b marker of the four fish genera. Abbreviations: *h*, number of haplotypes; *Hd*, haplotype diversity; *π*, nucleotide diversity; *S*, number of polymorphic sites; *k*, mean number of pairwise differences; N, number of sequences.

ID	***h***	***Hd***	**π**	***S***	***k***	**N**
Phoxinus						
all	297					1,008
1	225	0.95	0.0183	243	19.95	886
1a	88	0.81	0.0072	149	7.81	393
1b	84	0.96	0.0085	124	9.23	267
1c	40	0.83	0.0077	81	8.43	186
1e	9	0.94	0.0026	13	2.82	12
2	22	0.91	0.0111	80	12.10	40
3	4	1.00	0.0029	6	3.17	4
4	2	0.67	0.0012	2	1.33	3
5a	13	0.94	0.0074	31	8.10	21
6	3	0.42	0.0006	3	0.67	9
7	19	0.92	0.0032	26	3.53	41
8	3	0.60	0.0006	2	0.67	6
Delminichthys						
all	84					359
adspersus	74	0.74	0.0012	78	1.23	340
ghetaldii	3	0.70	0.0022	5	2.2	5
jadovensis	2	0.33	0.0003	1	0.33	6

krbavensis	5	0.86	0.0032	9	3.18	8
Phoxinellus						
all	16					17
alepidotus	4	1.00	0.0024	5	2.67	4
dalmaticus	6	0.95	0.0021	6	2.29	7
pseudoaleptidotus	6	1.00	0.0035	10	3.93	6
Telestes						
all	96					197
croaticus	20	0.97	0.0147	75	15.30	25
dabar	9	0.74	0.0023	11	2.40	55
fontinalis	8	0.97	0.0042	16	4.39	9
karsticus	9	0.95	0.0036	17	3.72	13
miloradi	1					1
methionensis	11	0.78	0.0072	85	7.49	26
montenegrinus	15	0.86	0.0047	38	4.94	42
polylepis	4	0.80	0.0017	4	1.80	6
turskyi	6	0.95	0.0036	11	3.71	7
ukliva	12	1.00	0.0057	26	5.97	12

Table S6. Pairwise uncorrected p-distances between species/lineages of each genera. Species names of *Delminichthys*, *Phoxinellus* and *Telestes* are abbreviated in the horizontal line.

Phoxinus	1a	1b	1c	1e	5	2	3	4	6	7
1a										
1b	0.02									
1c	0.02	0.02								
1e	0.03	0.03	0.03							
5	0.06	0.06	0.06	0.06						
2	0.04	0.05	0.04	0.05	0.05					
3	0.05	0.06	0.05	0.06	0.05	0.04				
4	0.05	0.06	0.05	0.06	0.05	0.04	0.05			
6	0.07	0.07	0.07	0.07	0.08	0.06	0.08	0.07		
7	0.08	0.09	0.08	0.08	0.09	0.07	0.08	0.08	0.08	
8	0.08	0.09	0.09	0.09	0.09	0.07	0.08	0.09	0.09	0.08
Delminichthys	*ads*	*ghe*	*jad*							

adspersus									
ghetaldii	0.02								
jadovensis	0.04	0.04							
krbavensis	0.04	0.04	0.02						
Phoxinellus	*ale*	*pse*	*dal*						
alepidotus									
pseudoalepidotus	0.02								
dalmaticus	0.04	0.05							
Telestes	*cro*	*dab*	*fon*	*kar*	*met*	*mil*	*mon*	*pol*	*tur*
croaticus									
dabar	0.11								
fontinalis	0.03	0.11							
karsticus	0.10	0.10	0.09						
metohiensis	0.11	0.01	0.11	0.09					
miloradi	0.11	0.03	0.11	0.10	0.03				
montenegrinus	0.09	0.10	0.10	0.07	0.10	0.10			
polylepis	0.09	0.09	0.08	0.04	0.09	0.09	0.07		
turskyi	0.10	0.10	0.10	0.07	0.10	0.10	0.08	0.07	
ukliva	0.10	0.08	0.09	0.07	0.08	0.08	0.07	0.06	0.05

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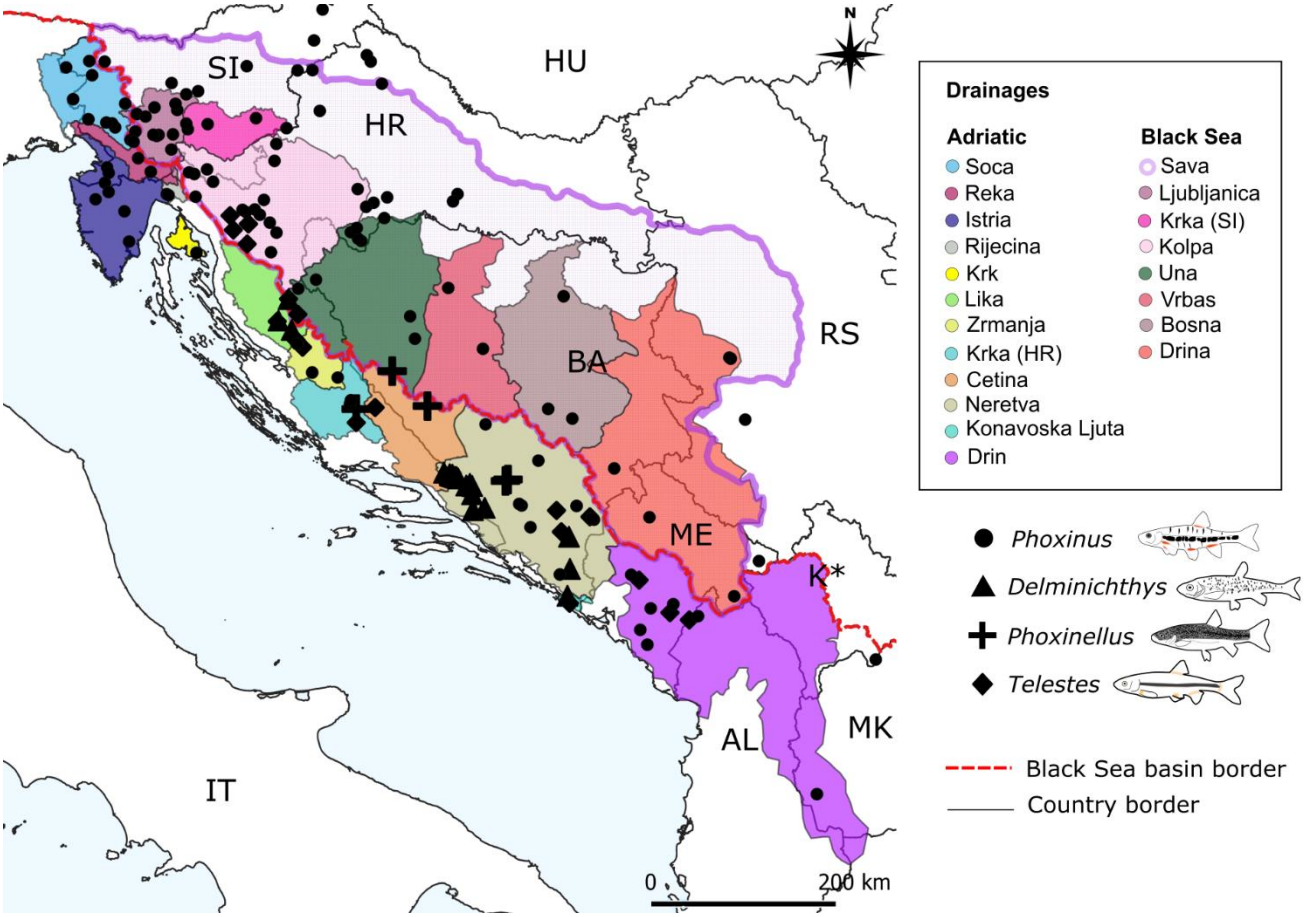

Figure S1. Major river drainages in the Dinaric karst with the distribution of the four fish genera *Phoxinus*, *Delminichthys*, *Phoxinellus* and *Telestes*). Each Symbols correspond to species (see legend).

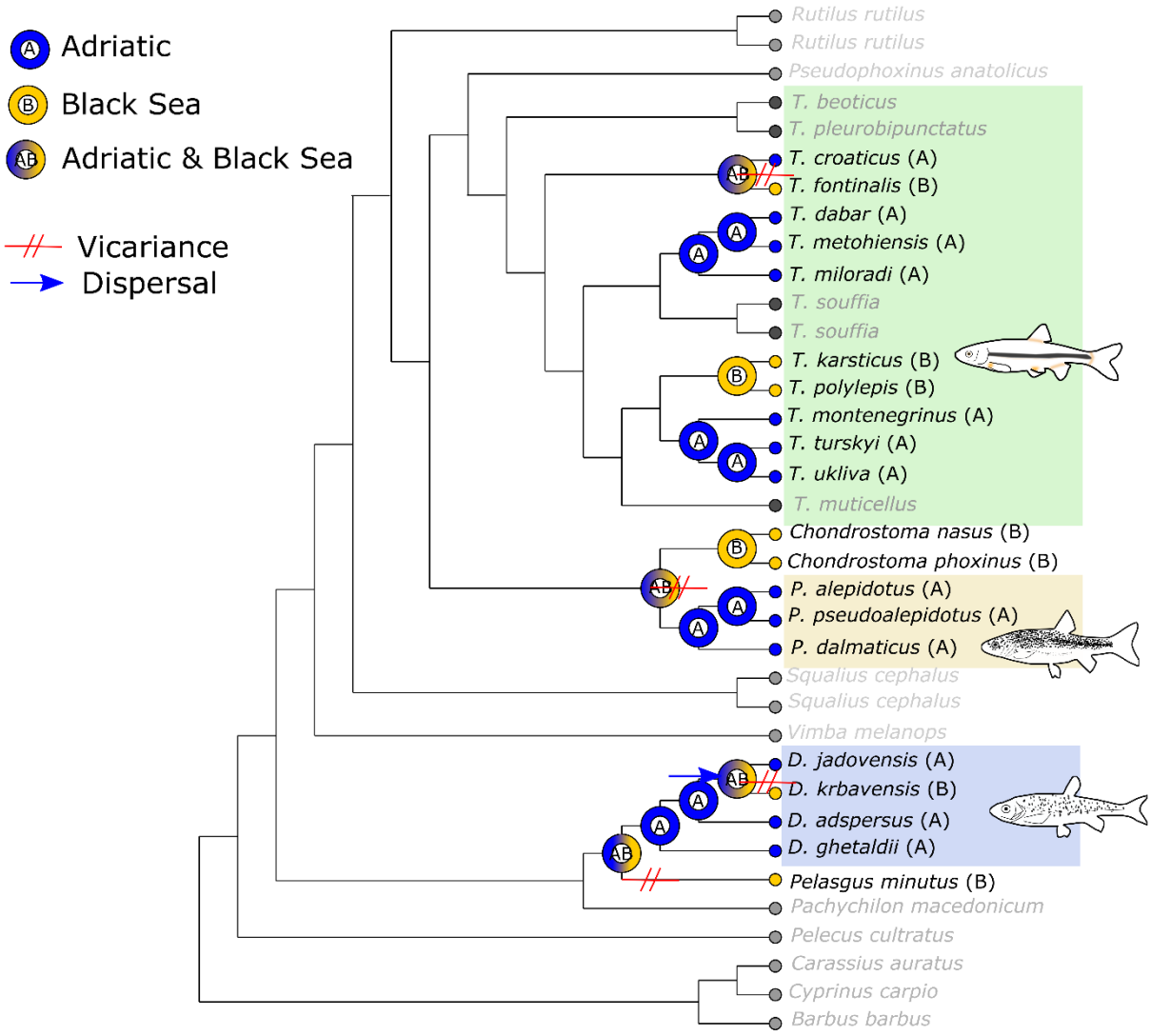

Figure S2. Reconstruction of ancestral areas (RASP) analyses of *Delminichthys*, *Phoxinellus* and *Telestes* (together with sister genera and outgroups, for details see Table S4) using two biogeographic areas: Adriatic and Black Sea basin (see legend) and based on cytochrome b fragment. The analysis was performed with S-DIVA [20] and implemented in RASP [21] based on the phylogenetic trees calculated in the divergence timing analysis (BEAST [1] see Materials and Methods for details). Pie chart at node represent the most likely ancestral distributions and letters denote which regions (Adriatic, Black Sea, or both) are coded by each color. Letters following tip labels indicate the recent distribution of each tip. Only ancestral areas with a probability >0.5 are shown. Vicariance and/or dispersal events are given next to the pie chart.

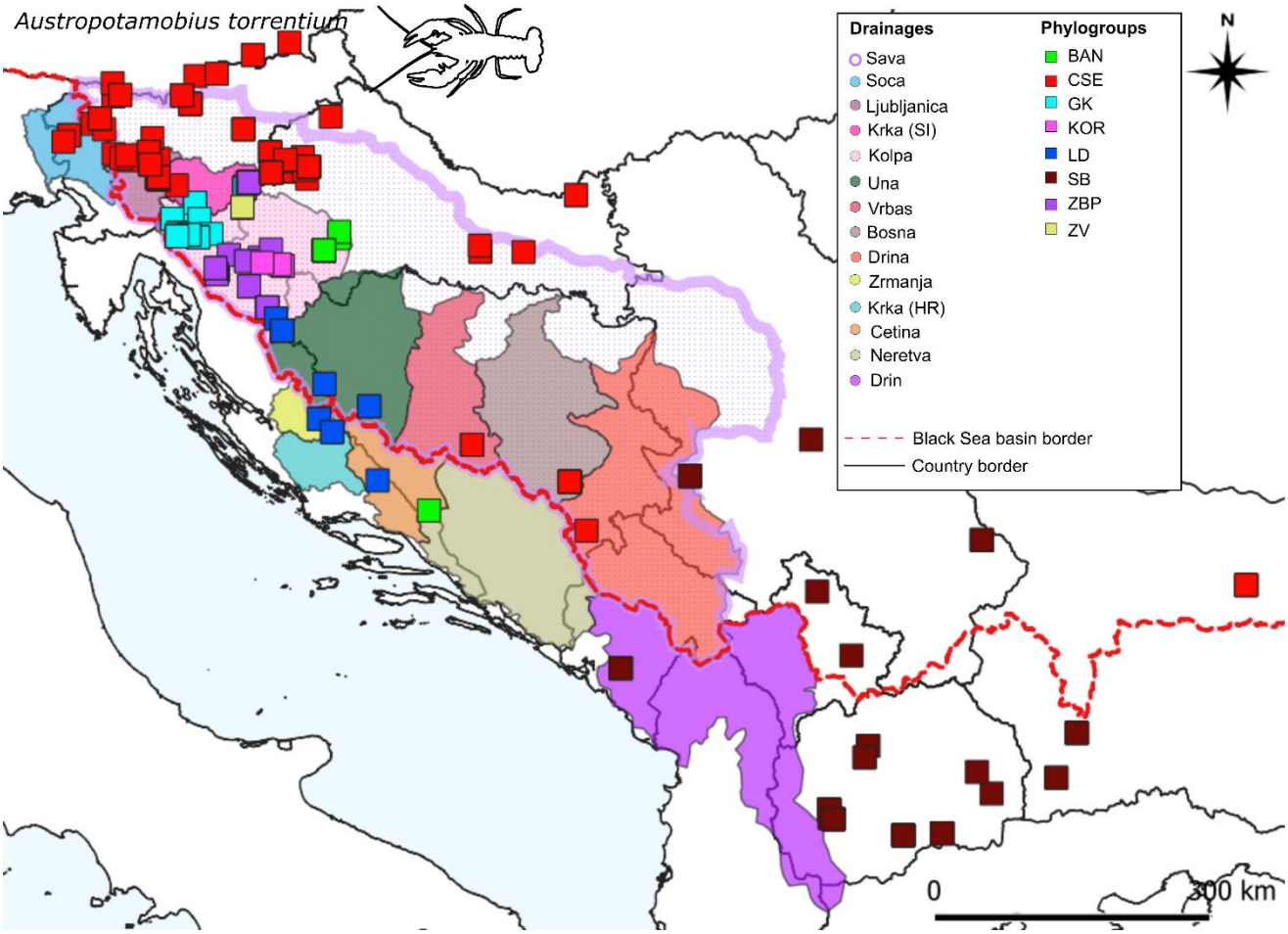

Figure S3. Distribution map of the mitochondrial lineages of stone crayfish *Astopotamobius torrentium* summarized according to [22, 23, 24]; based on cytochrome oxidase I. The lineages are coded in Klobucar et al. [22] and were thereafter used with these names throughout the literature. CSE – central and south-eastern Europa, SB – southern Balkans, GK – Gorski Kotar, BAN - Banovina, ZV – Zeleni vir, KOR - Kolpa, LD – Lika and Dalmacija, ZPB – Zumberak, Plitvice and Bjelolasica. The red dashed line represents the Adriatic-Black Sea basin divide.

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