

Supplementary Materials

The first cavefish in the Dinaric Karst? Cave colonization made possible by phenotypic plasticity in *Telestes karsticus*

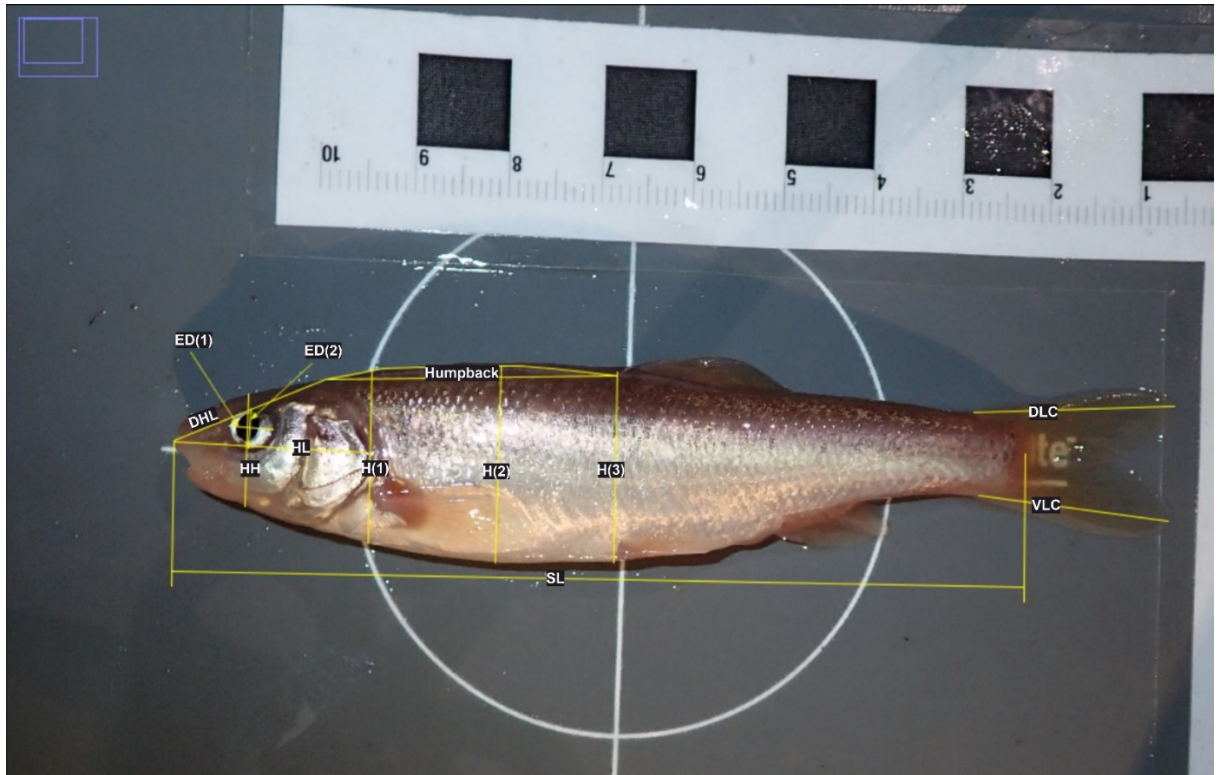
Mateo Čupić¹, Zoran Marčić², Marko Lukić^{1,3}, Romana Gračan², Helena Bilandžija^{1,3,*}

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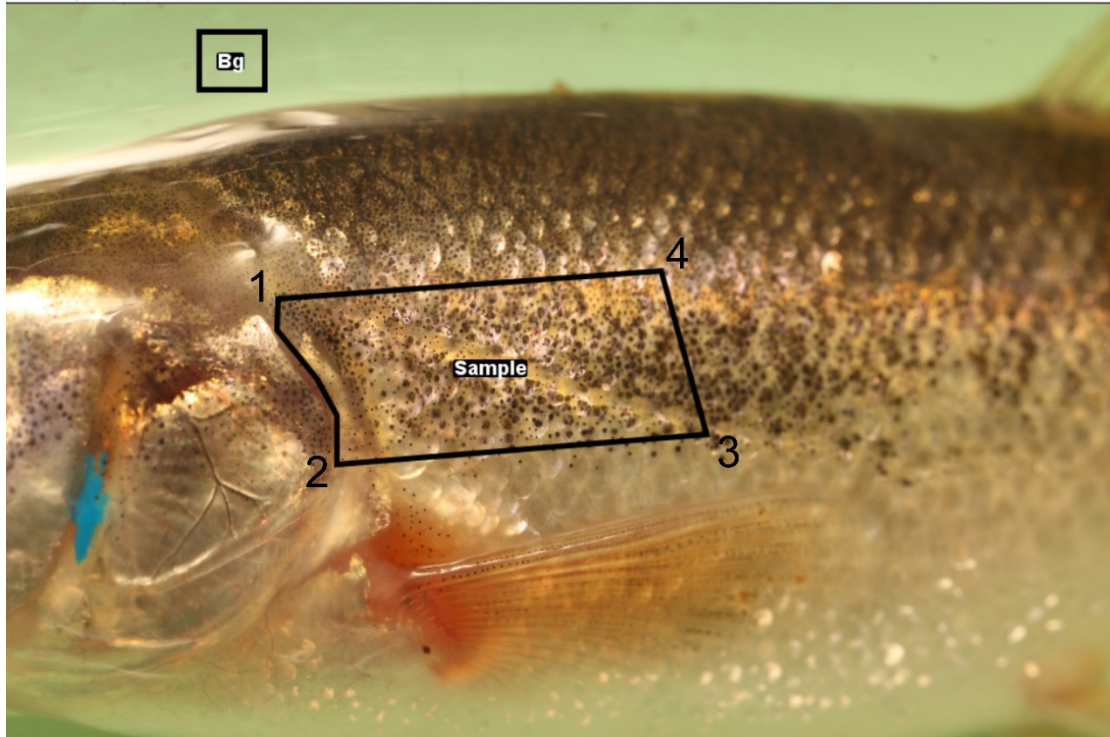
²Department of Biology, Faculty of Science, Zagreb 10000, Croatia

³Croatian Biospeleological Society, Zagreb 10000, Croatia

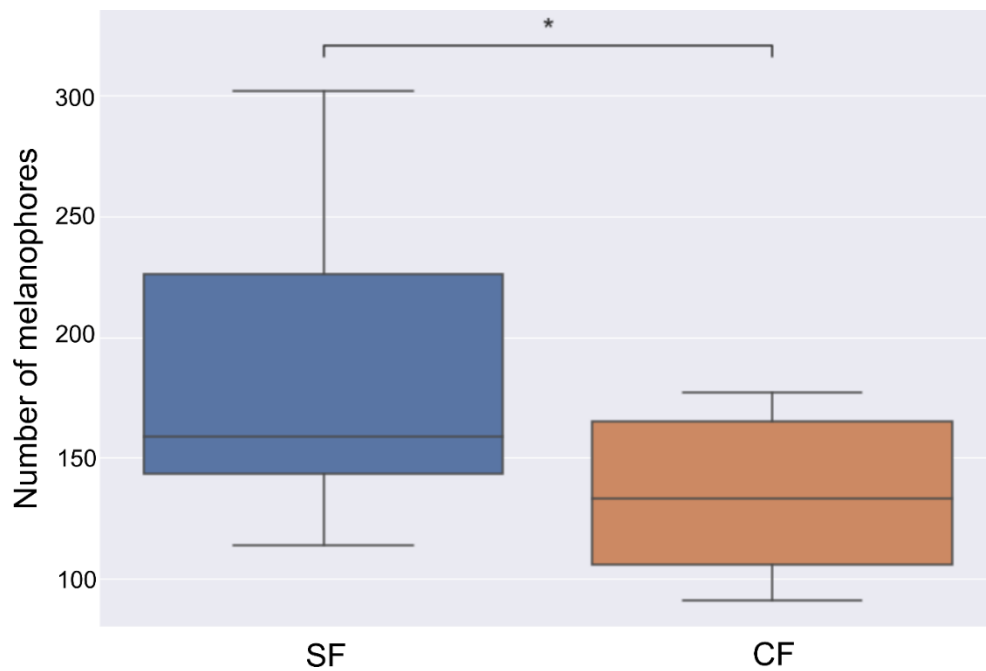
*Corresponding author, E-mail: hbilandz@irb.hr



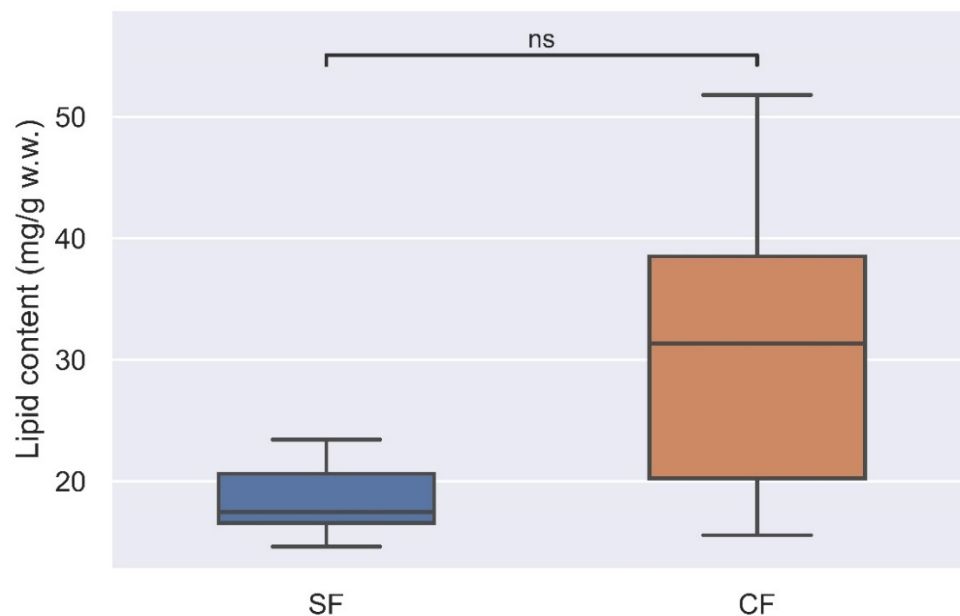
Supplementary Figure S1A. Example of *Telestes karsticus* for morphological analyses. A total of 12 variables were taken along with weight: SL – standard length, ED(1) – Anterior-posterior axis of the eye, ED(2) – Dorsoventral axis of the eye, H(1) – body height at operculum end, H(2) – maximum body height, H(3) – body height at ventral fin base, DLC – dorsal lobe of caudal fin, VLC – ventral lobe of caudal fin, HL – head length, DHL – dorsal head length, HH – head height.



Supplementary Figure S1B. Example of *Telestes karsticus* for pigmentation analyses. Bg – background for normalization. Four points were used to make a polygon: From the most dorsal point of operculum (1) to the most posterior point of the operculum (2) connecting posteriorly to the lateral line point (3), and proceeding dorsally to point (4). Blue mark on the operculum is the injected dye from Visible Implant Elastomer Tags.



Supplementary Figure S1C. The number of manually counted melanophores in *Telestes karsticus* surface fish (SF) and cave fish (CF), (N: surface fish=10, cave fish=10). Melanophores were counted in the area between points 1, 2, and 3 used for the grey scale value measurements (see Supplementary Figure 1b). Independent sample *t*-test: $p=0,046$.



Supplementary Figure S2. Mean concentration of lipids from whole body of *Telestes karsticus* surface fish (SF) and cavefish (CF), (N: SF=5, CF=5). Independent sample *t*-test: ns – not significant.

Supplementary Table S1. Summary statistics of wild *Telestes karsticus* for morphological analysis, body condition index, pigmentation level and lipid content. M – mean, SD – standard deviation, SF – surface fish, CF – cavefish, SL – standard length. The remaining variables are shown as the ratio of the morphometric measurement to SL (in mm): ED(1) – Anterior-posterior axis of the eye, ED(2) – Dorsoventral axis of the eye, H(1) – body height at operculum end, H(2) – maximum body height, H(3) – body height at ventral fin base, DLC – dorsal lobe of caudal fin, VLC – ventral lobe of caudal fin, HL – head length, DHL – dorsal head length, HH – head height

	Group	M	SD	df	t	95% CI																																																																																																																																																		
Weight (g)	SF	1.460	0.910	59.64	-7.37	[-14.15, -8.11]																																																																																																																																																		
	CF	12.590	11.670				SL (mm)	SF	43.720	8.460	69.02	-10.135	[-44.81, 30.07]	CF	81.160	27.470	ED(1)	SF	0.065	0.005	89.3	9.45	[0.006, 0.015]	CF	0.053	0.009	ED(2)	SF	0.057	0.005	105.2	8.02	[0.006, .001]	CF	0.049	0.007	H(1)	SF	0.217	0.005	94.501	4.48	[0.003, 0.0008]	CF	0.211	0.009	H(2)	SF	0.231	0.009	102.575	-6.446	[-0.016, -0.009]	CF	0.243	0.013	H(3)	SF	0.216	0.009	103.581	-8.09	[-0.02, -0.012]	CF	0.232	0.013	DLC	SF	0.240	0.013	95.41	-1.6	[-0.012, 0.00128]	CF	0.246	0.022	VLC	SF	0.232	0.014	107.295	1.497	[-0.002, 0.011]	CF	0.227	0.020	HL	SF	0.249	0.009	90.694	10.944	[-0.022, .031]	CF	0.223	0.017	DHL	SF	0.221	0.009	123	7.944	[0.009, .015]	CF	0.195	0.011	HH	SF	0.134	0.007	125	14.579	[-0.023, .03]	CF	0.122	0.010	Humpback	SF	0.144	0.043	65	-11.49	[-0.295, -0.21]	CF	0.396	0.161	Fulton body index	SF	1.560	0.180	102	-6.55	[0.0435, 0.068]	CF	1.830	0.260	Pigmentation	SF	1.980	0.610	103	2.12	[0.02, 0.55]	CF	1.690	0.770	Lipid content (mg/g w.w.)	SF	70.540	11.660	20	2.29
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Supplementary Table S2. Summary statistics of laboratory kept *Telestes karsticus* for morphological analysis, body condition index, pigmentation level and lipid content. M – mean, SD – standard deviation, SF – surface fish, CF – cavefish, SL – standard length. The remaining variables are shown as the ratio of the morphometric measurement to SL (in mm): ED(1) – Anterior-posterior axis of the eye, ED(2) – Dorsoventral axis of the eye, H(1) – body height at operculum end, H(2) – maximum body height, H(3) – body height at ventral fin base, DLC – dorsal lobe of caudal fin, VLC – ventral lobe of caudal fin, HL – head length, DHL – dorsal head length, HH – head height

	Group	M	SD	df	F	95% CI (Total)
SL (mm)	SFLD	88.537	6.346	3,26	4.29	[91.17, 99.64]
	SFDD	90.420	3.455			
	CFLD	98.348	8.331			
	CFDD	103.630	14.858			
ED(1)	SFLD	0.057	0.005	3,26	0.78	[0.054, 0.057]
	SFDD	0.058	0.003			
	CFLD	0.055	0.004			
	CFDD	0.056	0.002			
ED(2)	SFLD	0.052	0.004	3,26	0.46	[0.051, 0.053]
	SFDD	0.054	0.003			
	CFLD	0.052	0.003			
	CFDD	0.052	0.003			
H(1)	SFLD	0.201	0.007	3,26	1.69	[0.201, 0.206]
	SFDD	0.207	0.006			
	CFLD	0.200	0.009			
	CFDD	0.205	0.005			
H(2)	SFLD	0.210	0.006	3,26	5.84	[0.213, 0.22]
	SFDD	0.224	0.009			
	CFLD	0.214	0.009			
	CFDD	0.220	0.006			
H(3)	SFLD	0.196	0.005	3,26	4.58	[0.198, 0.205]
	SFDD	0.211	0.013			
	CFLD	0.198	0.006			
	CFDD	0.203	0.006			
DLC	SFLD	0.235	0.014	3,26	1.77	[0.221, 0.231]
	SFDD	0.227	0.013			
	CFLD	0.224	0.016			
	CFDD	0.220	0.012			
VLC	SFLD	0.223	0.014	3,26	2.33	[0.209, 0.220]
	SFDD	0.218	0.012			
	CFLD	0.205	0.018			
HL	SFLD	0.211	0.013	3,26	0.17	[0.226, 0.234]
	SFDD	0.231	0.013			
	SFDD	0.228	0.012			

	CFLD	0.230	0.010			
	CFDD	0.232	0.010			
	SFLD	0.201	0.013			
DHL	SFDD	0.200	0.011	3,26	0.592	[0.195, 0.202]
	CFLD	0.194	0.008			
	CFDD	0.199	0.009			
	SFLD	0.123	0.008			
HH	SFDD	0.120	0.005	3,26	1.95	[0.117, 0.121]
	CFLD	0.118	0.003			
	CFDD	0.117	0.004			
	SFLD	0.346	0.043			
Humpback	SFDD	0.343	0.054	3,26	4.42	[0.36, 0.432]
	CFLD	0.416	0.040			
	CFDD	0.469	0.131			
	SFLD	1.759	0.524			
Pigmentation	SFDD	1.142	0.187	3,26	3.21	[91.17, 99.64]
	CFLD	1.286	0.221			
	CFDD	1.393	0.465			
	SFLD	63.851	19.316			
Lipid content (mg/g w.w.)	SFDD	116.559	26.101	3,24	5.23	[64.21, 88.39]
	CFLD	75.430	19.330			
	CFDD	65.588	32.954			

Supplementary Table S3. Summary statistics of Illumina reads for *de novo* transcriptome assembly of *Telestes karsticus*: number of raw reads, quality trimmed reads, overall mapping rate, contigs and BUSCO statistics.

	Wild <i>T. karsticus</i>	Laboratory <i>T. karsticus</i>
Illumina reads	78406034 - 96788520	41748480 - 49792758
Cleaned reads	78403010 - 96786460	41747612 - 49790960
Mapping rate	68.32% - 77.51%	67.47% - 80.16%
# contigs	223184	160100
Largest contig	20785	30976
N50	1594	2199
GC (%)	43.64	44.07
Complete BUSCOs	84.40%	87.80%
Complete and single-copy BUSCOs	48.80%	57.60%
Complete and duplicated BUSCOs	35.60%	30.20%
Fragmented BUSCOs	2.60%	2.60%
Missing BUSCOs	13.00%	9.60%

Supplementary Table S4 and S5 are listed as separate Excel files due to their large size.

Supplementary Table S6. Significantly enriched GO terms related to Molecular Function according to Fisher method for wild caught cavefish (CF) and surface fish (SF), and experimental surface fish (SF in DD/LD conditions) and cavefish (CFDD/CFLD)

	GO.ID	Term	Annotate d	Significan t	Expecte d	classicFisher
Wild CFvSF						
1	GO:0003677	DNA binding	582	84	50.05	2.10E-08
2	GO:0003777	microtubule motor activity	46	16	3.96	9.70E-07
3	GO:0008017	microtubule binding	61	16	5.25	3.90E-05
4	GO:0009982	pseudouridine synthase activity	14	7	1.2	6.80E-05
5	GO:0005524	ATP binding	1220	141	104.91	7.60E-05
		ATP-dependent chromatin remodeler				
6	GO:0140658	activity	24	8	2.06	0.00061
7	GO:0008408	3'-5' exonuclease activity	9	5	0.77	0.00285
		extracellular ligand-gated ion channel				
8	GO:0005230	activity	5	3	0.43	0.00555
9	GO:0003887	DNA-directed DNA polymerase activity	5	3	0.43	0.00555
10	GO:0004930	G protein-coupled receptor activity	42	10	3.61	0.00584
SFDDvSFLD						
1	GO:0003677	DNA binding	510	141	103.64	3.10E-07
2	GO:0008017	microtubule binding	39	21	7.93	3.60E-06
3	GO:0016887	ATP hydrolysis activity	49	24	9.96	6.50E-06
4	GO:0008168	methyltransferase activity	94	40	19.1	1.30E-05
5	GO:0003723	RNA binding	284	92	57.71	2.20E-05
6	GO:0005524	ATP binding	932	234	189.39	7.20E-05

7	GO:0030983	mismatched DNA binding ATP-dependent chromatin remodeler activity	8	7	1.63	9.30E-05
8	GO:0140658		23	13	4.67	0.00014
9	GO:0009982	pseudouridine synthase activity	9	7	1.83	0.00034
10	GO:0003777	microtubule motor activity	36	16	7.32	0.00089

CFDDvCFLD

1	GO:0009982	pseudouridine synthase activity	9	4	0.68	3.00E-03
2	GO:0001733	galactosylceramide sulfotransferase activity hydrolase activity, acting on acid anhydrides, in phosphorus-containing	2	2	0.15	5.70E-03
3	GO:0016818	anhydrides	177	20	13.37	7.00E-03
4	GO:0008168	methyltransferase activity	94	16	7.1	1.02E-02
5	GO:0004376	glycolipid mannosyltransferase activity	3	2	0.23	1.62E-02
6	GO:0008017	microtubule binding	39	7	2.94	2.49E-02
7	GO:0003677	DNA binding	510	49	38.51	2.70E-02
8	GO:0003887	DNA-directed DNA polymerase activity	4	2	0.3	0.0308
9	GO:0008173	RNA methyltransferase activity	10	3	0.76	0.0344
10	GO:0003916	DNA topoisomerase activity	37	7	2.79	0.0551

Supplementary Table S7. Significantly enriched GO terms related to Biological Process according to Fisher method for wild caught cavefish (CF) and surface fish (SF), and experimental surface fish (SF in DD/LD conditions) and cavefish (CFDD/CFLD)

	GO.ID	Term	Annotate d	Significan t	Expecte d	classicFisher
Wild CFvSF						
1	GO:0001522	pseudouridine synthesis	14	7	1.25	8.50E-05
2	GO:0032508	DNA duplex unwinding	11	6	0.98	1.50E-04
3	GO:0007018	microtubule-based movement	37	10	3.31	1.11E-03
4	GO:0006260	DNA replication	26	9	2.32	1.75E-03
5	GO:0030488	tRNA methylation	4	3	0.36	2.65E-03
6	GO:0006364	rRNA processing	22	7	1.97	0.00636
7	GO:0007059	chromosome segregation	10	5	0.89	0.00787
8	GO:0000725	recombinational repair	2	2	0.18	0.00797
9	GO:0007076	mitotic chromosome condensation	2	2	0.18	0.00797
10	GO:0006471	protein ADP-ribosylation	7	4	0.63	0.01146
SFDDvSFLD						
1	GO:0006281	DNA repair	49	26	10.03	1.20E-08
2	GO:0006364	rRNA processing	16	12	3.27	5.30E-05
3	GO:0032508	DNA duplex unwinding	8	7	1.64	9.70E-05
4	GO:0001522	pseudouridine synthesis	9	7	1.84	3.60E-04
5	GO:0006260	DNA replication	20	11	4.09	4.74E-03
6	GO:0006357	regulation of transcription by RNA polymerase II transcription initiation at RNA	37	15	7.57	5.75E-03
7	GO:0006367	polymerase II promoter	5	4	1.02	7.29E-03

8	GO:0006400	tRNA modification	13	10	2.66	0.00836
9	GO:0030488	tRNA methylation	3	3	0.61	0.00854
		nucleobase-containing compound				
10	GO:0006139	metabolic process	780	229	159.63	0.01137
CFDDvCFD						
1	GO:0001522	pseudouridine synthesis	9	4	0.74	4.10E-03
2	GO:0009247	glycolipid biosynthetic process	7	4	0.58	6.70E-03
3	GO:0006396	RNA processing	95	15	7.85	1.56E-02
4	GO:0006281	DNA repair	49	10	4.05	1.90E-02
5	GO:0006306	DNA methylation	3	2	0.25	1.93E-02
6	GO:0030488	tRNA methylation	3	2	0.25	1.93E-02
7	GO:0051382	kinetochore assembly	3	2	0.25	1.93E-02
8	GO:0006351	DNA-templated transcription	387	37	32	0.0296
9	GO:0006506	GPI anchor biosynthetic process	5	2	0.41	0.0576
		nucleobase-containing compound				
10	GO:0006139	metabolic process	780	95	64.49	0.0634