

Supplementary Information

Population expansion and individual age affect endoparasite richness and diversity in a recolonising large carnivore population

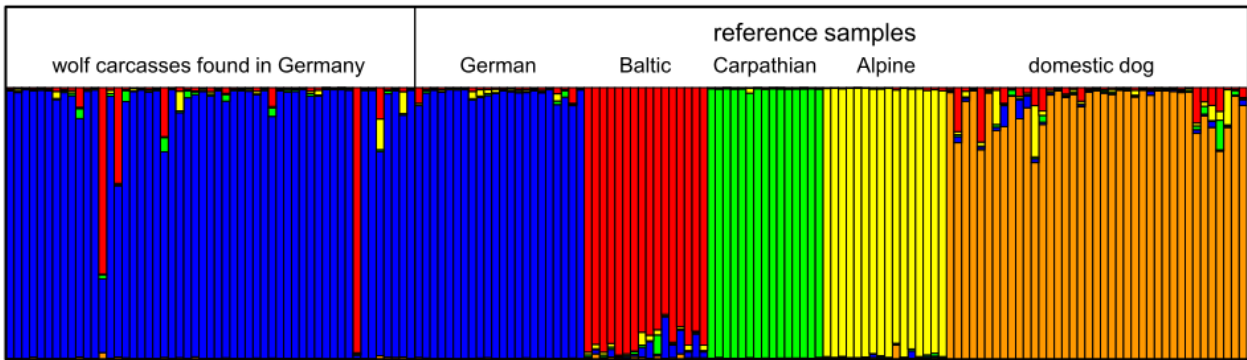
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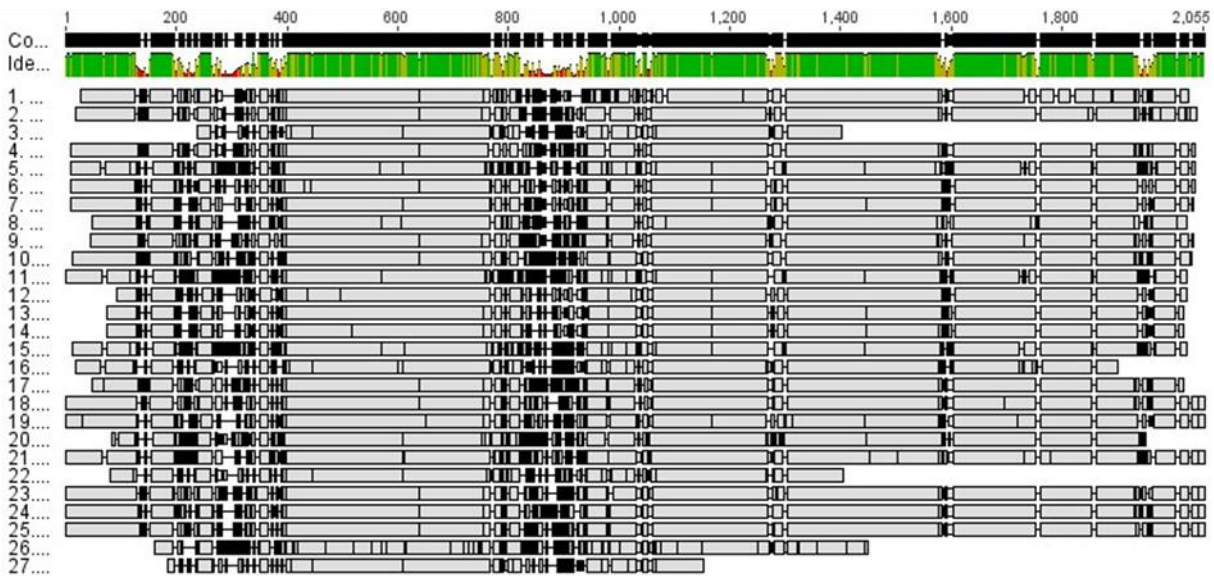
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Supplementary Figure S1: Bayesian clustering of 53 wolf carcasses found in Germany in comparison to reference wolf genotypes from Germany (blue), the Baltic region (red), the Carpathians (green), the Alpine region (yellow), and domestic dogs (orange). Each individual is represented by a vertical line divided into five coloured segments that represent the individual's estimated membership fractions in each cluster (K=5).

Consensus
Identity

1. gi 438889 gb L24383.1 SARRG18SZ	Sarcocystis tenella 18S ribosomal RNA (18S_rRNA) gene
2. gi 46561149 gb L76472.1 SARRGE	Sarcocystis capracanis 18S ribosomal RNA gene, partial sequence
3. gi 7593709 gb DQ146148.1	Sarcocystis canis 18S ribosomal RNA gene, partial sequence
4. gi 125662755 gb EF056010.1	Sarcocystis gruenieri isolate Sg1RtN 18S ribosomal RNA gene, complete sequence
5. gi 125662758 gb EF056013.1	Sarcocystis hardangeri isolate Sh1RtN 18S ribosomal RNA gene, complete sequence
6. gi 125662763 gb EF056018.1	Sarcocystis tarandi isolate St2RtN 18S ribosomal RNA gene, complete sequence
7. gi 167077506 gb EU282016.1	Sarcocystis silva isolate SspDAa1N 18S ribosomal RNA gene, complete sequence
8. gi 167077522 gb EU282032.1	Sarcocystis scandinavica isolate SsAa2N clone 2.6 18S ribosomal RNA gene, complete sequence
9. gi 167077523 gb EU282033.1	Sarcocystis alceslatrans isolate SaAa1C 18S ribosomal RNA gene, complete sequence
10. gi 225579514 gb FJ196261.1	Sarcocystis gracilis 18S ribosomal RNA gene, partial sequence
11. gi 225579515 gb FJ196262.1	Sarcocystis oviformis 18S ribosomal RNA gene, partial sequence
12. gi 283777696 gb GQ251016.1	Sarcocystis elongata isolate St2CeN clone 1 18S ribosomal RNA gene, partial sequence
13. gi 283777701 gb GQ251021.1	Sarcocystis truncata isolate Srf1CeN clone 1 18S ribosomal RNA gene, partial sequence
14. gi 283777730 gb GQ250986.1	Sarcocystis rangiferi isolate Srf4RtN clone 5 18S ribosomal RNA gene, partial sequence
15. gi 283777732 gb GQ250988.1	Sarcocystis ovalis isolate Ss1CeN 18S ribosomal RNA gene, partial sequence
16. gi 307073936 gb GU120092.1	Sarcocystis rileyi 18S small subunit ribosomal RNA gene, partial sequence
17. gi 345452702 gb JN226117.1	Sarcocystis capreolicanis isolate NoCc10.7 clone 1 18S ribosomal RNA gene, partial sequence
18. gi 418433444 gb JX679467.1	Sarcocystis cruzi clone 3AS.cruziArg 18S ribosomal RNA gene, complete sequence
19. gi 418433448 gb JX679471.1	Sarcocystis hominis clone 2B.HRF96A 18S ribosomal RNA gene, complete sequence
20. gi 422036446 gb JX840465.1	Sarcocystis miescheiana isolate SmJY2 18S ribosomal RNA gene, partial sequence
21. gi 425887002 gb JX855283.1	Sarcocystis hirsuta clone 6B.HRF30 18S ribosomal RNA gene, complete sequence
22. gi 568928680 gb KC877996.1	Sarcocystis muris isolate TX377 18S ribosomal RNA gene, partial sequence
23. gi 597960512 gb KF831274.1	Sarcocystis alces isolate AaC2.2 18S ribosomal RNA gene, complete sequence
24. gi 597960522 gb KF831284.1	Sarcocystis taeniata isolate AaC1.20 clone 4 18S ribosomal RNA gene, complete sequence
25. gi 597960533 gb KF831295.1	Sarcocystis hjorti isolate Aa37.8 18S ribosomal RNA gene, complete sequence
26. gi 629265895 db AB661440.1	Sarcocystis fayeri gene for 18S rRNA isolate: SF-E4-1-2
27. gi 695348385 gb J957839.1	Sarcocystis neuroña 18S small subunit ribosomal RNA gene, partial sequence



Supplementary Figure S2: The *Sarcocystis* spp. 18S rRNA consensus sequence used for the amplicon design in this study is based on the alignment of 27 different mammalian *Sarcocystis* species.

Supplementary Table S1 Genetic identification of wolf helminths was performed for a subset of parasites. Helminth species were determined based on *cox1* and 18S rRNA sequences which were searched in the NCBI nucleotide database. If there was no distinct best hit species, the search was either repeated using an alternative marker gene or morphological indicators were used to determine the species. *Trichinella* spp. were identified by the National Reference Laboratory for *Trichinella* and are not included in this table.

This table is provided as separate online csv file

Supplementary Table S2 Target-specific primers for the amplification of the variable regions of the *Sarcocystis* spp. 18S rRNA gene. *designed for this study

#	primer name	sequence (5'-3')	source	primer position	~product size [bp]
1	G18S4	GCTTGTCTCAAAGATTAAGCCN	1	forward	377
	Euk360_CR	TCTCAKGCKCCYTCTCCG	2	reverse	
2	G18S4	GCTTGTCTCAAAGATTAAGCCN	1	forward	437
	SSU22R	GCCTGCTGCCTTCCTTGG	1	reverse	
3	proti15	TGCCAGTAGTCATATGCTTGTYT	*	forward	378
	proti440_R	CAGGCYCSCTCTCCGGA	*	reverse	
4	proti15	TGCCAGTAGTCATATGCTTGTYT	*	forward	421
	proti482_R	KTTSCGCGCCTGCTGCC	*	reverse	
5	proti15	TGCCAGTAGTCATATGCTTGTYT	*	forward	572
	proti643_R	GAGCTGGAATTACCGCGG	*	reverse	
6	proti89	CKGCGVATGGCTCATTAMAWC	*	forward	485
	proti628_R	GGSTGCTGGCACCAGAC	*	reverse	
7	proti89	CKGCGVATGGCTCATTAMAWC	*	forward	501
	proti643_R	GAGCTGGAATTACCGCGG	*	reverse	
8	F-556	CAGCAGCCGCGGTAATTCC	3	forward	383
	Nem_0425_4R	ARACATTCTTGGCAAATGCYTTC	*	reverse	
9	18S rRNA#1	TGGTGCCAGCAGCCGC	4	forward	577
	Mach2Gen	TCCGTCAATTTCTTTAAGTTTCAG	*	reverse	
10	18S rRNA#1	TGGTGCCAGCAGCCGC	4	forward	389
	Nem_0425_4R	ARACATTCTTGGCAAATGCYTTC	*	reverse	
11	18S rRNA#1	TGGTGCCAGCAGCCGC	4	forward	391
	Nem_0425_2	GAARACATTCTTGGCAAATGCY	*	reverse	
12	18S r DNA549-566	GAGGGCAAGTCTGGTGCC	5	forward	637
	R-1200	CCCGTGTTGAGTCAAATTAAGC	3	reverse	
13	proti628	GTCTGGTGCCAGCASC	*	forward	604
	18S rDNA 1141-1161	GGTGCCCTTCCGTCAATTC	5	reverse	
14	18S r RNA549-566	GAGGGCAAGTCTGGTGCC	5	forward	588
	Mach2Gen	TCCGTCAATTTCTTTAAGTTTCAG	*	reverse	
15	18S r DNA573-589	GCCGCGGTAATTCAGCT	5	forward	378
	Nem_0425_4R	ARACATTCTTGGCAAATGCYTTC	*	reverse	

Supplementary Table S3 Comparison of helminth species richness (HSR) and helminth prevalence (%) between wolves from the CEL wolf population (this study) and the Baltic wolf population. Red p-values indicate significant differences.

	this study	Shimalov <i>et al.</i> ⁶			Bagrađe <i>et al.</i> ⁷			Szczęsna-Staśkiewicz ⁸			Moks <i>et al.</i> ⁹		
	Germany	Belarus	p-value	t	Latvia	p-value	t	Poland	p-value	t	Estonia	p-value	t
n wolves	53	52			34			18			26		
HSR	13	24			17			17			13		
mean HSR _{individual}	2.57±0.26	NA			4.6±0.35	0.002	-3.422	4.6±0.54	2.024e⁻⁵	-4.5796	NA		
max. HSR _{individual}	8	NA			8			9			NA		
<i>Taenia</i> spp richness	2	5			7			5			5		
	%	%	p-value	X²	%	p-value	X²	%	p-value	X²	%	p-value	X²
<i>C. plica</i>	25	14	0.232	1.429	41	0.161	1.961	NA	NA	NA	0	0.015	5.954
<i>C. vulpis</i>	25	8	0.038	4.312	9	0.118	2.438	17	0.716	0.132	0	0.015	5.954
<i>C. aerophila</i>	15	0	0.011	6.487	36	0.054	3.701	17	1.000	3137e ⁻³⁰	NA	NA	NA
<i>U. stenocephala</i>	11	15	0.745	0.106	41	0.003	8.810	72	2.196e⁻⁶	22.415	77	2.46e⁻⁸	31.093
<i>T. canis</i>	11	21	0.270	1.126	6	0.634	0.227	11	1.000	5.480e ⁻³⁰	8	0.916	0.011
<i>T. leonina</i>	4	14	0.154	2.029	0	0.680	0.170	6	1.000	5.304e ⁻³⁰	8	0.841	0.040
<i>Trichinella</i> spp.	4	19	0.052	3.785	70	5.103e⁻⁶	20.798	33	0.371	0.800	50	3.887e⁻⁶	21.320
<i>T. krabbei</i>	77	8	2.298e⁻¹²	49.212	9	1.756e⁻⁹	36.227	NA	NA	NA	15	6.171e⁻⁷	24.858
<i>T. hydatigena</i>	15	27	0.212	1.561	41	0.013	6.141	56	0.002	9.584	12	0.934	0.007
<i>M. litteratus</i>	9	0	0.070	3.281	0	0.170	1.884	NA	NA	NA	0	0.260	1.269
<i>E. multilocularis</i>	2	0	1.000	1.262e ⁻²⁹	6	0.693	0.156	0	1.000	4.819e ⁻²⁹	0	1.000	1.478e ⁻²⁹
<i>A. alata</i>	53	17	0.001	12.998	85	0.004	8.279	33	0.247	1.340	89	0.004	8.818
total	89	80	0.392	0.733	100	0.110	2.559	100	0.317	1.003	100	0.183	1.777

Supplementary Table S4 Comparison of cysticercoses prevalence (%) between ungulates from Germany and Eastern Europe. Red p-values indicate significant differences.

host species	this study	Murai <i>et al.</i> ¹⁰			Kuzmina <i>et al.</i> ¹¹			Onac <i>et al.</i> ¹²			
	Germany	Hungary			Ukraine			Romania			
n roe deer (<i>Capreolus capreolus</i>)	177	345			92			NA			
n red deer (<i>Cervus elaphus</i>)	102	70			NA			21			
n wild boar (<i>Sus scrofa</i>)	126	44			NA			267			
n fallow deer (<i>Dama dama</i>)	35	35			NA			NA			
cestode species	%	%	p-value	X ²	%	p-value	X ²	%	p-value	X ²	
roe deer	<i>T. krabbei</i>	0	33	8.64e⁻¹⁴	55.654	0	0.177	1.825	NA	NA	NA
	<i>T. hydatigena</i>	2	18	2.89e⁻⁰⁷	26.325	2	1	1.64e ⁻²⁹	NA	NA	NA
red deer	<i>T. krabbei</i>	4	19	0.004	8.4254	NA	NA	NA	NA	NA	NA
	<i>T. hydatigena</i>	5	36	4.98e⁻⁰⁷	25.271	NA	NA	NA	NA	NA	NA
	<i>E. granulosus</i>	0	0	NA	NA	NA	NA	NA	12	0.0282	4.818
wild boar	<i>T. hydatigena</i>	4	43	6.42e⁻¹⁰	38.189	NA	NA	NA	NA	NA	NA
	<i>E. granulosus</i>	0	36	9.63e⁻¹²	46.403	NA	NA	NA	10	8.55e⁻⁰⁵	15.432
fallow deer	<i>T. krabbei</i>	0	6	0.4731	0.51471	NA	NA	NA	NA	NA	NA
	<i>T. hydatigena</i>	0	20	0.017	5.7143	NA	NA	NA	NA	NA	NA

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