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Supplement of

Genetic diversity in the Alpine flatworm *Crenobia alpina*

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Table S1 *Crenobia alpina* and outgroup specimens analysed, including locality information, NCBI GenBank accession numbers of haplotypes and clade affiliation. Marked specimen were provided by *Christopher Robinson (Dübendorf, Swiss), **Larry Bellamy (Newnham on Severn, UK), ***Fahio Lepori and Christian Otto (Umeå, Sweden) ****Stefanie von Fumetti (Basel, Swiss).

Name	Country	Latitude	Longitude	Accession No.	Clade
Koralpe I	Austria	46°48'30"N	14°56'36"E	KY569139	Eastern Alps
				KY569167	
Koralpe II	Austria	46°53'45"N	15°3'53"E	KY569140	Eastern Alps
				KY569167	
Stubalpe	Austria	47°2'58"N	14°53'45"E	KY569141	Eastern Alps
				KY569167	
Radstädter Tauern I	Austria	47°14'19"N	13°34'17"E	KY569142	
				KY569168	
Radstädter Tauern II	Austria	47°15'15"N	13°31'31"E	KY569143	Eastern Alps
				KY569169	
Lammertal	Austria	47°28'45"N	13°23'55"E	KY569144	Eastern Alps
				KY569169	
Ratten	Austria	47°29'51"N	15°45'9"E	KY569150	Eastern Alps
				KY569169	
St. Aegyd	Austria	47°50'55"N	15°33'27"E	KY569151	
				KY569173	
Petit St. Bernard II	France	45°39'46"N	6°52'14"E	KY569128	Western Alps I
				KY569159	
Col d'Iseran	France	45°25'53"N	7°0'7"E	KY569128	Western Alps I
				KY569159	
Mont Cenis	France	45°12'45"N	6°57'26"E	KY569129	
				KY569160	
Col de Vars	France	44°37'26"N	6°40'59"E	KY569130	South-western Alps
				KY569161	

Col de Larche I	France	44°33'7"N	6°42'1"E	KY569131 KY569161	South-western Alps
Col de Larche II	France	44°26'43"N	6°51'31"E	KY569132 KY569161	South-western Alps
Rimella	Italy	45°54'54"N	8°10'39"E	KY569133 KY569162	Mixed Clade I
Val d'Ampola	Italy	45°50'31"N	10°37'47"E	KY569135 KY569163	Southern Alps
Monte Baldo	Italy	45°45'21"N	10°52'32"E	KY569136 KY569164	
Rio Freddo	Italy	45°57'07"N	10°48'45"E	KY569137 KY569165	Southern Alps
Passo di Costalunga	Italy	46°24'8"N	11°37'27"E	KY569137 KY569165	Southern Alps
Passo Mauria	Italy	46°31'12"N	12°1'7"E	KY569138 KY569166	Mixed Clade II
Gran St. Bernard	Italy	45°52'2"N	7°9'21"E	KY569117 KY569156	North-Western Alps
Valtournenche I	Italy	45°56'20"N	7°37'26"E	KY569125 KY569159	Western Alps I
Valtournenche II	Italy	45°55'22"N	7°37'5"E	KY569126 KY569158	Mixed Clade I
Vallpelines	Italy	45°53'57"N	7°29'2"E	KY569117 KY569156	North-western Alps
Val di Cogne	Italy	45°36'19"N	7°22'20"E	KY569127 KY569159	Western Alps I
Val di Rhemes	Italy	45°39'32"N	7° 9'25"E	KY569128 KY569159	Western Alps I
Petit St. Bernard I	Italy	45°41'39"N	6°53'59"E	KY569128 KY569156	North-western Alps
Klausenpass I	Switzerland	46°54'7"N	8°55'49"E	KY569117 KY569156	Western Alps I
Klausenpass II	Switzerland	46°52'16"N	8°51'51"E	KY569118 KY569157	Mixed Clade II

Klausenpass III	Switzerland	46°52'24"N	8°51'16"E	KY569119 KY569156	North-western Alps
Göschener-Tal	Switzerland	46°39'15"N	8°31'1"E	KY569120 KY569158	Mixed Clade I
Furkapass	Switzerland	46°34'47"N	8°25'31"E	KY569121 KY569156	North-western Alps
Grimselfpass	Switzerland	46°33'43"N	8°20'0"E	KY569122 KY569156	North-western Alps
Lötschalpe	Switzerland	46°26'2"N	7°52'2"E	KY569123 KY569157	Mixed Clade II
Lac de Moiry	Switzerland	46°8'25"N	7°34'15"E	KY569124 KY569156	North-western Alps
Julierpass	Switzerland	46°27'51"N	9°47'40"E	KY569134 KY569157	Mixed Clade II
Graubünden*	Switzerland	46°43'45"N	10°7'49"E	KY569152 KY569174	
Corsica I	France	42°23'32"N	9°20'44"E	KY569146 KY569172	
Corsica II	France	42°21'16"N	9°21'56"E	KY569147 KY569172	
Corsica III	France	42°3'41"N	9°11'29"E	KY569148 KY569172	
Corsica IV	France	42°3'14"N	9°1'33"E	KY569149 KY569172	
St. Anthony`s Well**	Britain	51°50'11"N	2°27'51"W	KY569153 KY569174	
Abisko I***	Sweden	68°21'N	18°49'E	KY569145 KY569170	
Abisko II***	Sweden	68°21'N	18°49'E	KY569145 KY569156	
Abisko III***	Sweden	68°21'N	18°49'E	KY569145 KY569156	
Ohrid (<i>C. a. montenegrina</i>)	Macedonia	41°6'38"N	20°49'8"E	KY569154 KY569171	

Arisdorf****	Switzerland	47°31'19"N	7°46'8"E	KY569155
(<i>P. felina</i> , outgroup)				KY569175

Table S2: Haplotype assignation of samples listed in Table S1.

Specimen	COI tree	18S tree	Combined tree
Koralpe I (A)	Koralpe I (A)	H XI	Koralpe I (A)
Koralpe II (A)	Koralpe II (A)	H XI	Koralpe II (A)
Stubalpe (A)	Stubalpe (A)	H XI	Stubalpe (A)
Radstädter Tauern I (A)	Radstädter Tauern I (A)	Radstädter Tauern I (A)	Radstädter Tauern I (A)
Radstädter Tauern II (A)	Radstädter Tauern II (A)	H XII	Radstädter Tauern II (A)
Lammertal (A)	Lammertal (A)	H XII	Lammertal (A)
Ratten (A)	Ratten (A)	H XII	Ratten (A)
St. Aegydt (A)	St. Aegydt (A)	St.Aegydt (A)	St. Aegydt (A)
Petit St. Bernard II (F)	H II	H VIII	H II
Col d'Iseran (F)	H II	H VIII	H II
Mons Cenis (F)	Mons Cenis (F)	Mons Cenis (F)	Mons Cenis (F)
Col de Vars (F)	Col de Vars (F)	H IX	Col de Vars (F)
Col de Larche I (F)	Col de Larche I (F)	H IX	Col de Larche I (F)
Col de Larche II (F)	Col de Larche II (F)	H IX	Col de Larche II (F)
Rimella (I)	Rimella (I)	Rimella (I)	Rimella (I)
Val d'Ampola (I)	Val d'Ampola (I)	Val d'Ampola (I)	Val d'Ampola (I)
Monte Baldo (I)	Monte Baldo (I)	Monte Baldo (I)	Monte Baldo (I)
Rio Freddo (I)	H III	H X	H II
Passo di Costalunga (I)	H III	H X	H II
Passo Mauria (I)	Passo Mauria (I)	Passo Mauria (I)	Passo Mauria (I)
Gran St. Bernard (I)	H I	H V	H III
Valtournenche I (I)	Valtournenche I (I)	H VIII	Valtournenche I (I)
Valtournenche II (I)	Valtournenche II (I)	H VII	Valtournenche II (I)
Vallpelines (I)	H I	H V	H III
Val di Cogne (I)	Val di Cogne (I)	H VIII	Val di Cogne (I)
Val di Rhemes (I)	H II	H VIII	Val di Rhemes (I)
Petit St. Bernard I (I)	H I	H V	H III
Klausenpass I (CH)	H I	H V	Klausenpass I (CH)
Klausenpass II (CH)	Klausenpass II (CH)	H VI	Klausenpass II (CH)
Klausenpass III (CH)	Klausenpass III (CH)	H V	Klausenpass III (CH)
Göschener Tal (CH)	Göschener Tal (CH)	H VII	Göschener Tal (CH)
Furkapass (CH)	Furkapass (CH)	H V	Furkapass (CH)
Grimselfpass (CH)	Grimselfpass (CH)	H V	Grimselfpass (CH)
Lötschalpe (CH)	Lötschalpe (CH)	H VI	Lötschalpe (CH)
Lac de Moiry (CH)	Lac de Moiry (CH)	H V	Lac de Moiry (CH)
Julierpass (CH)	Julierpass (CH)	H VI	Julierpass (CH)
Graubünden (CH)	Graubünden (CH)	H XIV	Graubünden (CH)
Corsica I (F)	Corsica I (F)	H XIII	Corsica I (F)
Corsica II (F)	Corsica II (F)	H XIII	Corsica II (F)
Corsica III (F)	Corsica III (F)	H XIII	Corsica III (F)
Corsica IV (F)	Corsica IV (F)	H XIII	Corsica IV (F)
St. Anthony's Well (GB)	St. Anthony's Well (GB)	HXIV	St. Anthony's Well (GB)
Abisko I (S)	H IV	Abisko I (S)	Abisko I (S)
Abisko II (S)	H IV	H V	Abisko II (S)
Abisko III (S)	H IV	H V	Abisko III (S)
Ohrid (MK)	Ohrid (MK)	Ohrid (MK)	Ohrid (MK)
Arisdorf (CH)	Arisdorf (CH)	Arisdorf (CH)	Arisdorf (CH)

Figure S3: Bayesian phylogeny of *C. alpina* inferred from the sequenced COI gene fragment (450 bp). For sampling locations, see Table S1; and for haplotype assignment, see Table 2. Values on nodes represent posterior probabilities (> 0.8).

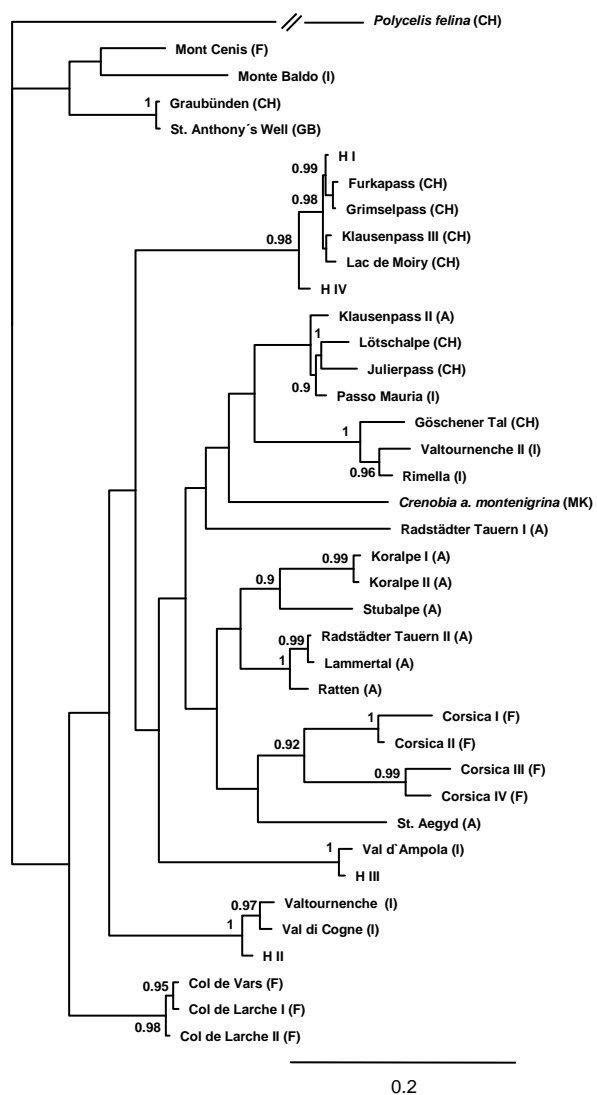
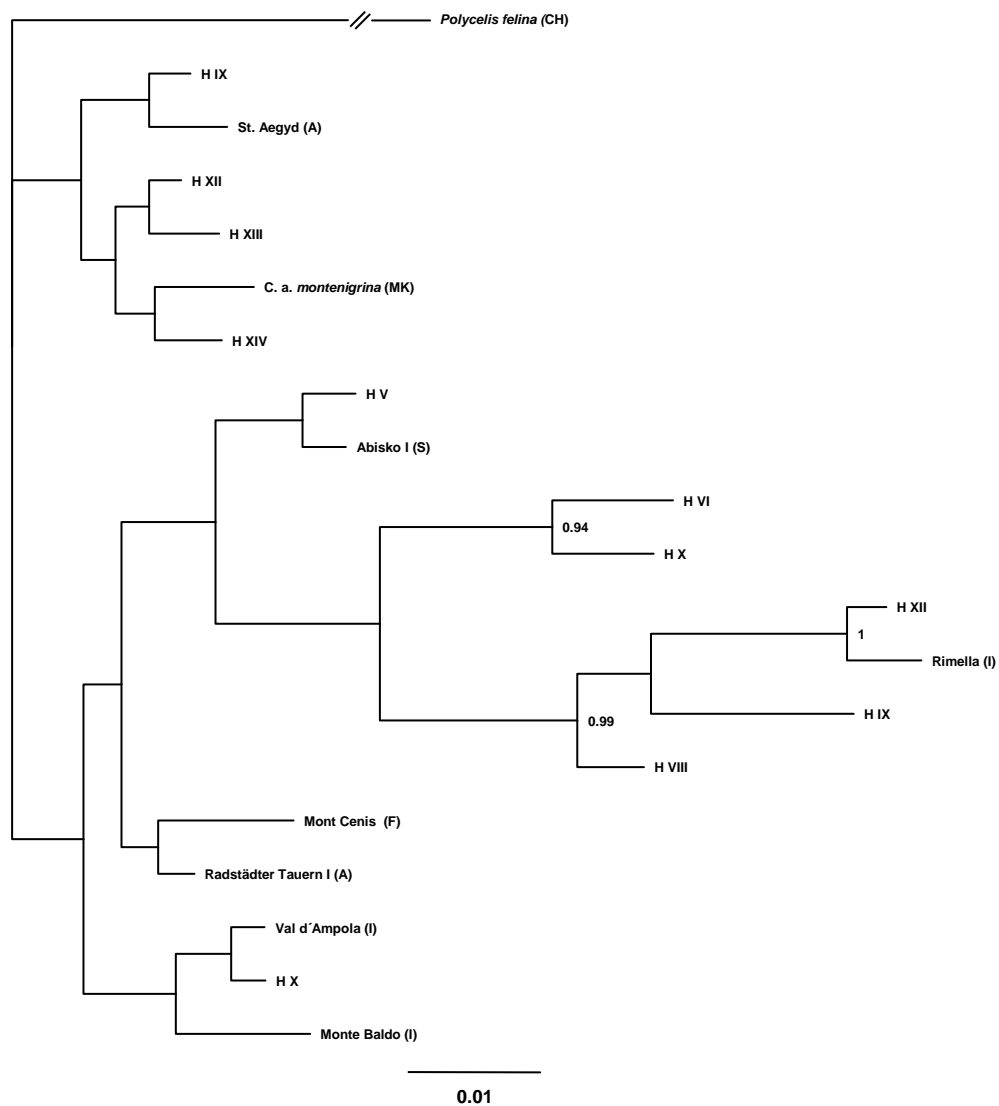


Figure S4: Bayesian phylogeny of *C. alpina* inferred from the sequenced 18S rRNA fragment (531 bp). For sampling locations, see Appendix S1; and for haplotype assignment, see Table S2. Values on nodes represent posterior probabilities (> 0.8).



Additional methods S5

Secondary structures

Ribosomal RNA (rRNA) 18S sequences were aligned using their secondary structure with RNAsalsa 0.8.1, as outlined in Stocsits *et al.* (2009). As the initial input, we used constraint files based on the secondary structures of *Calicophoron calicophorum* (accession number gi289869) downloaded from www.rna.ccbb.utexas.edu (EF648647) and *Apis mellifera* 28S rRNA downloaded from the European Protein Database. The sequences were aligned with our dataset using the MUSCLE algorithm in MEGA 5.05 (Tamura *et al.*, 20011). Starting with this initial alignment and the constraint file, RNAsalsa implements a workflow for both RNA secondary structure prediction and enhanced structural alignment that results in a final multiple sequence alignment together with a consensus structure. To exclude ambiguously aligned sites, we used Aliscore v1.0 (downloaded at www.zfmk.de/web/Forschung/Abteilungen/AG_Wgele/Software/Aliscore/index.en.html), treating gaps as ambiguities (option -N), using a window size of 6 (option -w 6) and comparing all sequences pairwise (option -r 100000). Other parameters were kept at default settings.

Phylogenetic analyses

We chose substitution models following the approach of Minin *et al.* (2003), as implemented in the Perl script DT-ModSel. In contrast to the likelihood ratio tests implemented in MrModeltest 2.2 (Nylander, 2004), this approach of performance-based model selection uses the Bayesian information criterion and considers the relative branch-length error as a performance measure in a consistent framework with a penalty for overfitting based on decision theory (Minin *et al.*, 2003). DT-ModSel selected the GTR I+ Γ model for the 18S rRNA loop region. For the rRNA stem regions, the doublet model proposed by Schöniger &

von Haeseler (1999) was assigned in the Bayesian analysis. For this procedure, unambiguous stem pairs were derived on the basis of the consensus structure from RNAsalsa and specified in the MrBayes input file.

MrBayes was run for 10^7 generations, sampling trees every 1,000th generation and using a random tree as a starting point. Inspection of the standard deviation of split frequencies after the final run indicated convergence of Markov chains (at least < 0.05). Two parallel Markov chain Monte Carlo simulations with four chains (one cold and three heated) were run. The first 10% of the samples were discarded as burn-in. Based on the sampled trees, consensus trees were produced using the sumt command in MrBayes.

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