

## Supplemental Information for:

### Multiple glacial refugia and contemporary dispersal shape the genetic structure of an endemic amphibian from the Pyrenees

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## Supplemental Methods

### *Recent dispersal (GeneClass2)*

We employed two different likelihood computation criteria for migrant detection:  $L_{\text{home}}$ , that is the likelihood of finding an individual in the population where it was sampled, and  $L_{\text{home}}/L_{\text{max}}$ , the ratio of  $L_{\text{home}}$  to the highest likelihood among all sampled populations.  $L_{\text{home}}$  is more appropriate when, as in our case, not all source populations for migrants have been sampled. However, it is less powerful than  $L_{\text{home}}/L_{\text{max}}$ . We used the Bayesian method of Rannala and Mountain (1997) coupled with the Monte-Carlo resampling method of Paetkau, Slade, Burden, and Estoup (2004). We used 10,000 simulated individuals for assignment and  $L_{\text{home}}$  computations, while 1,000 simulated individuals were employed for  $L_{\text{home}}/L_{\text{max}}$  computations, because of RAM limitations. Type I error (alpha level) was set to 0.01. A number of individuals identified by GeneClass2 as first generation migrants had similar migration probabilities for several areas and therefore could not be unambiguously assigned to a source population. This was especially evident in areas where sampling effort was low and, as a result, some of the sampled populations were apparently isolated. The individuals in question were thus classified as first generation migrants whose source locality could not be determined (Bergl & Vigilant, 2007). To visualize first generation migrants trajectories, we generated a chord diagram using the circlize R package (Gu, Gu, Eils, Schlesner, & Brors, 2014).

### *Effective population size (Colony)*

This software uses a maximum likelihood method to conduct parentage and sibship inference to estimate  $N_e$  and can accommodate null alleles and other genotyping errors. Because we could not reliably discriminate between adult and subadult individuals (and thus discriminate between putative parental and offspring genotypes), we opted to pool all sampled individuals in the offspring sample only (J. Wang, personal communication). The software was run with the null allele frequencies computed with FreeNA as allelic dropout rate and the rate of other kinds of genotyping errors was set to 0.01 at all loci (see Mokhtar-Jamai et al., 2013 for a similar approach). Mating system was assumed to be both-sex polygamy, with no sibship prior. Three medium length runs were conducted to ensure convergence of the annealing procedure, and other parameters were used as default.

### *Analysis of molecular variance (AMOVA)*

Because we had a few instances of valleys where two different genetic clusters coexisted, we repeated the first AMOVA twice: first retaining only valleys harbouring a unique genetic cluster (17 out of 22), and then including all valleys and assigning the “admixed” ones to the most representative cluster in terms of number of populations. Analyses were performed in Arlequin 3.5.2.2 (Excoffier & Lischer, 2010) with 10,000 permutations to assess statistical significance of fixation indices. Only populations with five or more genotyped individuals were considered in the analyses.

### *Approximate Bayesian Computation (DIYABC)*

Candidate scenarios were built on the basis of STRUCTURE and NJ analyses and previously published information on the population structure and putative past distribution of the species (Carranza & Amat, 2005; Valbuena-Ureña, Amat, & Carranza, 2013; Valbuena-Ureña et al.,

2018). Three types of scenarios of historical divergence were tested (Figure S1). Model selection was conducted in a hierarchical manner, in a similar fashion to Barbosa et al. (2017): each general scenario was first tested independently to examine alternative hypotheses about the timeframe of divergence among clusters (Figure S1); the configuration with the highest posterior probability for each general scenario was kept for the final analysis, where we compared the best scenarios against each other (Figure 2). We performed preliminary runs with varying priors for effective population size and divergence times ( $t$ ) to adjust them to the most appropriate values. The final parameter setting is shown in Table S3. We assumed a generation time of 2 to 3 years (Montori, 1988; Montori & Llorente, 2014), so we multiplied time estimates by two and by three to convert demographic parameters into absolute times. We generated  $10^6$  simulated datasets per scenario, assuming a 1:1 female to male sex ratio (Montori & Llorente, 2014). We used the following summary statistics (SS) for microsatellites: mean number of alleles, mean genetic diversity and mean allele size variance as one sample SS, and mean number of alleles,  $F_{st}$  and  $(d\mu)^2$  distance as two sample SS. As for *cyt-b*, the following SS were used: number of haplotypes, number of segregating sites and Tajima's  $D$  as one sample SS, and number of haplotypes and  $F_{st}$  as two sample SS. The "Pre-evaluation of scenarios and prior distributions" option was employed to ensure that our observed dataset was positioned well within the cloud of simulated datasets for all competing scenarios, through the computation of a principal component analysis (PCA) on summary statistics (Cornuet et al., 2015; Cornuet, Ravigne, & Estoup, 2010). Selection of the most supported scenario, confidence in scenario choice (type I and II errors), model checking and estimation of the posterior distribution of parameters for the most supported scenario followed Ortego, Nogueras, Gugger, and Sork (2015). Bias and precision on parameters estimation were evaluated by calculating the median of the Relative Median Absolute Deviation (RMAD) of each parameter based on 5,000 pseudo-observed datasets (Cornuet et al., 2015).

## Supporting Tables

**Table S1** Geographic information and standard genetic statistics of *Calotriton asper* sampling localities.

Population	Code	Lat.	Long.	Alt.	Habitat	N	Ar	H <sub>O</sub>	H <sub>E</sub>	F <sub>IS</sub>	N <sub>e</sub>	N mtDNA	mtDNA haps
Irati	B12	43.05	-1.06	1100	Stream	15	3.129	0.583	0.560	-0.007	-	-	-
Olhadoko	B11	42.99	-0.95	662	Stream	14	3.152	0.597	0.558	-0.032	-	-	-
Ibón Acherito	IAC	42.88	-0.71	1872	Lake	26	3.184	0.500	0.571	0.145	27	3	3(H7)
Ruisseau de Leignièrès 2	E13	42.95	-0.44	834	Stream	12	3.297	0.583	0.561	0.004	-	-	-
Ibón Espelunciecha	IES	42.79	-0.43	1951	Lake	23	2.726	0.380	0.470	0.214	28	5	5(H5)
Ruisseau de Gourzy	E11	42.95	-0.43	1137	Stream	5	3.132	0.518	0.518	0.111	-	5	5(H5)
Balsa Pertacua	BPE	42.71	-0.42	1913	Lake	-	-	-	-	-	-	1	1(H5)
Ruisseau de Leignièrès 1	E12	42.94	-0.42	1356	Stream	3	-	-	-	-	-	3	2(H5), 1(H18)
Río Barranco Mina Millor	BMM	42.78	-0.41	1800	Stream	4	-	-	-	-	-	4	4(H5)
Ibón Serrato Superior	ISA	42.76	-0.21	2459	Lake	14	1.925	0.219	0.295	0.291	-	4	4(H5)
Betharram	B7	43.10	-0.19	446	Cave	28	1.478	0.202	0.185	-0.072	11	4	4(H5)
Ruisseau Coume Rège	E15	43.08	-0.16	581	Stream	20	2.332	0.394	0.391	0.018	22	8	8(H5)
Genie Longue	B2	43.05	-0.15	669	Stream	16	2.229	0.349	0.339	0.003	24	5	5(H5)
Ruisseau de Peyrenère	E4	42.74	-0.03	1782	Stream	29	3.446	0.557	0.617	0.115	46	-	-
Río Llanos de Larri	LLA	42.70	0.09	1595	Stream	3	-	-	-	-	-	4	2(H7), 2(H8)
Gavarnie	E14	42.73	0.10	2107	Stream	30	3.549	0.584	0.626	0.084	40	10	8(H5), 2(H7)
Sarramea et Las Carraous	E2	43.04	0.11	855	Stream	5	2.290	0.259	0.353	0.367	-	-	-
Castelmouly	E1	43.04	0.13	806	Stream	1	-	-	-	-	-	-	-
Néouvielle	I	42.84	0.15	2183	Stream	15	3.202	0.587	0.574	0.013	-	-	-
Rau d'Estibère	B17	42.85	0.16	2280	Stream	8	2.764	0.419	0.437	0.106	-	-	-
Ibón Barleto Inferior	IBB	42.65	0.28	2483	Lake	4	-	-	-	-	-	5	5(H7)

# MOLECULAR ECOLOGY

Ibón Barleto Superior	IBA	42.64	0.28	2531	Lake	16	2.571	0.393	0.467	0.194	19	5	5(H7)
Ibón Bassa de la Mora	BMO	42.54	0.33	1903	Lake	22	2.862	0.356	0.483	0.293	28	4	4(H7)
Ibón Perramó	IPE	42.64	0.50	2254	Lake	29	2.264	0.341	0.394	0.155	20	4	4(H5)
Ibón Bajo de Vallibierna	IVI	42.60	0.66	2428	Lake	25	2.379	0.386	0.422	0.106	19	4	4(H15)
Ibón Alto de Vallibierna	IVS	42.60	0.66	2474	Lake	5	2.336	0.335	0.370	0.206	-	4	2(H5), 2(H15)
Estany Cap de Llauset	CPL	42.60	0.69	2443	Lake	30	2.529	0.444	0.472	0.076	28	4	4(H5)
Arbas	A2	42.99	0.88	526	Stream	1	-	-	-	-	-	1	1(H5)
Rivert	RVT-A	42.25	0.90	932	Stream	26	2.661	0.397	0.455	0.147	23	3	2(H5), 1(H12)
Estanyet Dellui 17	IDE	42.55	0.94	2306	Lake	20	2.238	0.293	0.362	0.218	19	4	3(H5), 1(H14)
Estanyet Dellui 15	MDE	42.55	0.94	2314	Lake	39	2.651	0.355	0.448	0.221	24	5	5(H5)
Bassa Dellui	BPD	42.55	0.94	2307	Lake	2	-	-	-	-	-	2	2(H5)
Estanyet Dellui 13	NDE	42.55	0.94	2313	Lake	36	2.364	0.381	0.419	0.105	17	5	5(H5)
Estanyet Dellui 12	bfd	42.55	0.95	2314	Lake	8	2.292	0.378	0.383	0.084	-	5	5(H5)
Estany Corticelles	CTS	42.56	0.95	2278	Lake	14	2.661	0.420	0.470	0.148	-	3	3(H5)
Estany Gran Dellui	GDE	42.55	0.95	2349	Lake	-	-	-	-	-	-	1	1(H5)
Estany Redó	RQN	42.51	0.95	2411	Lake	14	2.882	0.460	0.501	0.124	-	-	-
Riu Coma de Peixerani	CPX	42.57	0.97	2297	Stream	17	2.758	0.471	0.506	0.102	32	3	3(H5)
Estany Xic de Subenuix	XSU	42.57	0.99	2272	Lake	36	2.901	0.461	0.533	0.150	33	5	4(H5), 1(H14)
Estany Subenuix	SUB	42.57	0.99	2194	Lake	5	2.718	0.459	0.413	0.007	-	3	3(H5)
Riu de Subenuix Esquerre	RSE	42.58	0.99	2142	Stream	5	2.912	0.424	0.481	0.231	-	3	3(H5)
Vall Fosca	E	42.51	0.99	1830	Stream	19	2.782	0.480	0.505	0.078	25	-	-
Riu de Subenuix	RSU	42.58	0.99	2021	Stream	21	2.839	0.504	0.513	0.046	23	3	3(H5)
Pas du Loup	B1	43.01	1.00	487	Cave	16	1.414	0.162	0.171	0.087	9	6	6(H5)
Barranc del Cap del Port de Peguera	CPP	42.54	1.03	2510	Stream	2	-	-	-	-	-	2	1(H9), 1(H13)
Estany Port d'Aulà	AUL	42.77	1.10	2128	Lake	19	3.004	0.484	0.592	0.217	21	5	5(H5)
Arcouzan	B15	42.80	1.12	1214	Stream	9	3.334	0.698	0.614	-0.077	-	-	-
Salau	G-A1	42.73	1.15	1764	Stream	39	3.415	0.586	0.621	0.068	40	-	-
Estany Buixasse Nord	BUX	42.69	1.16	2250	Lake	15	2.739	0.545	0.503	-0.047	-	3	1(H5), 2(H9)

# MOLECULAR ECOLOGY

Estany Calberante	CLB	42.69	1.17	2418	Lake	1	-	-	-	-	-	1	1(H9)
Estany Inferior de la Gallina	GIN	42.71	1.19	2268	Lake	4	-	-	-	-	-	4	4(H9)
Bassa Llavera	BSL	42.71	1.19	2193	Lake	1	-	-	-	-	-	2	2(H9)
Hoque de Fustès	E5	42.81	1.20	820	Stream	30	3.333	0.621	0.606	-0.008	35	-	-
Estanyet de Vedos	VED	42.70	1.20	2270	Lake	12	3.047	0.549	0.585	0.105	-	4	2(H5), 2(H9)
Barranc Romadriu	PAS	42.45	1.27	1684	Stream	1	-	-	-	-	-	1	1(H9)
Organyà	B-A18	42.20	1.30	758	Stream	26	2.467	0.391	0.420	0.088	26	16	15(H17), 1(H19)
Ribauí	B5	42.79	1.34	814	Stream	15	3.282	0.611	0.589	-0.003	-	5	5(H5)
Videssos ruisseau 3	V29	42.77	1.41	1718	Stream	14	2.879	0.514	0.521	0.051	-	-	-
Videssos ruisseau 4	V30	42.77	1.41	1671	Stream	16	2.594	0.498	0.477	-0.012	24	-	-
Videssos ruisseau 2	V28	42.77	1.41	1674	Stream	15	2.551	0.496	0.477	-0.005	-	-	-
Videssos ruisseau 7	V35	42.77	1.42	1667	Stream	1	-	-	-	-	-	-	-
Videssos ruisseau 6	V34	42.77	1.42	1668	Stream	4	-	-	-	-	-	-	-
Videssos ruisseau 5	V31	42.77	1.42	1677	Stream	8	2.721	0.538	0.492	-0.027	-	-	-
Courbiere	B9	42.85	1.45	1622	Stream	6	2.854	0.569	0.524	0.007	-	-	-
Comapedrosa	A4	42.58	1.45	2127	Stream	5	3.194	0.494	0.513	0.147	-	-	-
Videssos ruisseau 1	V11	42.77	1.45	1040	Stream	1	-	-	-	-	-	-	-
Riu de Mossers	A8	42.45	1.46	1322	Stream	5	3.069	0.518	0.459	-0.017	-	-	-
Riu de Turer	A6	42.57	1.47	1803	Stream	7	3.077	0.528	0.505	0.034	-	-	-
Riu enclar	A7	42.50	1.48	1435	Stream	4	-	-	-	-	-	-	-
"B17"	B3	42.77	1.49	725	Cave	7	2.753	0.543	0.469	-0.081	-	-	-
Grotte Bernard	B6-A19	43.00	1.53	564	Cave	31	1.998	0.347	0.344	0.006	18	5	5(H5)
Siech	B4	42.88	1.55	690	Cave	7	2.149	0.370	0.343	0.000	-	4	4(H5)
Labouiche	F-B8	43.00	1.57	485	Cave	39	2.593	0.497	0.478	-0.028	32	10	10(H5)
Gisclareny	A16	42.26	1.72	1200	Stream	1	-	-	-	-	-	1	1(H9)
Salana Pas	A5	42.56	1.74	1948	Stream	2	-	-	-	-	-	-	-
Bellver Cerdanya	A15	42.33	1.76	1302	Stream	1	-	-	-	-	-	1	1(H9)
Berga	A17	42.11	1.83	882	Stream	4	-	-	-	-	-	4	4(H9)

# MOLECULAR ECOLOGY

Fontestorbes 1	E6	42.87	1.89	627	Stream	14	2.692	0.465	0.460	0.026	-	5	5(H5)
Fontestorbes 2	E7	42.87	1.89	663	Stream	21	2.773	0.455	0.484	0.086	25	-	-
Rieufourcant	E16	42.88	1.94	797	Stream	24	3.415	0.507	0.602	0.179	35	-	-
Orlu	M1-H	42.66	1.97	841	Stream	58	2.760	0.428	0.474	0.106	38	5	5(H5)
Belesta	E8	42.91	1.97	506	Stream	4	-	-	-	-	-	-	-
Gombren	A14	42.26	2.07	1055	Stream	2	-	-	-	-	-	2	2(H9)
Ripoll	A13	42.20	2.16	801	Stream	2	-	-	-	-	-	2	2(H9)
Cailla	B13	42.81	2.19	725	Stream	24	2.312	0.428	0.408	-0.026	24	-	-
Ruisseau de Fabournet Puilaurens	E9	42.81	2.28	595	Stream	5	2.544	0.576	0.439	-0.210	-	-	-
Cass-Rats	E10-B14	42.88	2.32	522	Stream	28	1.851	0.303	0.286	-0.038	20	10	9(H5), 1(H7)
Vidra	A11	42.12	2.35	1046	Stream	1	-	-	-	-	-	-	-
Font de Dotz	E3-B19	42.88	2.36	484	Stream	6	2.011	0.392	0.332	-0.090	-	-	-
Camprodon	D-A12	42.28	2.36	980	Stream	24	2.186	0.305	0.323	0.077	17	10	10(H9)
Rassa de l'Areny	A3-1	42.12	2.38	906	Stream	5	2.300	0.318	0.340	0.176	-	-	-
Hostalets d'en Bas	C	42.09	2.45	626	Stream	19	2.522	0.406	0.416	0.052	25	9	9(H9)
Riu Tec	A3-2	42.09	2.45	582	Stream	3	-	-	-	-	-	-	-
Sant Esteve d'en bas	A10	42.09	2.45	511	Stream	1	-	-	-	-	-	-	-
Auriac	B16	42.93	2.49	541	Stream	6	1.651	0.294	0.253	-0.071	-	5	5(H5)
Valmanya	B10	42.53	2.54	924	Stream	10	2.048	0.363	0.320	-0.082	-	4	4(H9)
Sant Aniol Finestres	A9	42.09	2.61	367	Stream	3	-	-	-	-	-	-	-
Rassa Mosquera	A3-3	42.09	2.61	372	Stream	6	2.528	0.451	0.416	0.006	-	-	-
Total							1299					258	

Abbreviations: Lat., latitude; Long., longitude; Alt., altitude; N, sample size for microsatellites; Ar, allelic richness standardized for sample size; H<sub>O</sub>, observed heterozygosity; H<sub>E</sub>, expected heterozygosity; F<sub>IS</sub>, inbreeding coefficient; N<sub>e</sub>, effective population size; N mtDNA, sample size for mtDNA; mtDNA haps, occurrence and code (in parentheses) of mitochondrial haplotypes identified in each population.

**Table S2** Posterior parameters (median and 95% confidence intervals) and RMedAD (Relative Median Absolute Deviation) estimated in the pilot runs with DIYABC for the best supported scenario (scenario 3) when considering all 1,299 individuals screened for microsatellites (simple sequence repeats – SSRs). See Figures 2 and S1 for more information on the tested scenarios.

Parameter	Microsatellites			RMedAD
	Median	$Q_{2.5}$	$Q_{97.5}$	
$N_1$	8 170	5 190	9 800	0.126
$N_2$	4 640	2 740	6 920	0.158
$N_3$	5 750	2 780	9 150	0.146
$N_4$	8 950	6 610	9 920	0.130
$N_5$	5 290	2 470	9 040	0.165
$N_{135}$	3 400	361	9 310	0.410
$N_{241}$	2 210	154	11 600	0.560
$t_1$	2 570	907	6 020	0.278
$t_2$	4 850	1 660	9 410	0.238
$t_3$	8 160	3 150	14 400	0.229
Mean $\mu_{(SSRs)}$	$2.80 \times 10^{-4}$	$1.56 \times 10^{-4}$	$5.63 \times 10^{-4}$	0.394
Mean $P_{(SSRs)}$	0.275	0.159	0.300	0.209

Abbreviations: N, effective population size for each analysed deme (1 – cluster 1; 2 – cluster 2; 3 – cluster 3; 4 – cluster 4, 5 – cluster 5; 135 – central clusters; 241 – three oldest glacial refugia: eastern, western and central); t, time of events in generations ( $t_1$  – time to the most recent split;  $t_2$  – time to the intermediate split;  $t_3$  – time to the most ancient split); mean  $\mu$ , mean mutation rate; mean  $P$ , mean coefficient  $P$ ;  $Q_{2.5}$ , quantile 2.5%;  $Q_{97.5}$ , quantile 97.5%.



**Table S3** Parameters used in DIYABC analysis and respective priors for the best supported scenario (scenario 3). See Figures 2 and S1 for more information on tested scenarios.

Parameter	Conditions	Distribution [min-max]
$N_1$		Uniform [10 - 15 000]
$N_2$		Uniform [10 - 15 000]
$N_3$		Uniform [10 - 15 000]
$N_4$		Uniform [10 - 15 000]
$N_5$		Uniform [10 - 15 000]
$N_{135}$		Uniform [10 - 15 000]
$N_{241}$		Uniform [10 - 20 000]
$t_1$		Uniform [10 - 10 000]
$t_2$	$t_2 > t_1$	Uniform [10 - 10 000]
$t_3$	$t_3 > t_2$	Uniform [10 - 20 000]
Mean $\mu_{(SSRs)}$		Uniform [ $10^{-4}$ - $10^{-3}$ ]
Individual locus $\mu_{(SSRs)}$		Gamma [ $10^{-5}$ - $10^{-2}$ ]
Mean $P_{(SSRs)}$		Uniform [ $10^{-1}$ - $3 \times 10^{-1}$ ]
Individual locus $P_{(SSRs)}$		Gamma [ $10^{-2}$ - $9 \times 10^{-1}$ ]
SNI <sub>(SSRs)</sub>		Log-u [0]
Mean $\mu_{(cyt-b)}$	K2P	Uniform [ $10^{-10}$ - $10^{-6}$ ]
Individual locus $\mu_{(cyt-b)}$	K2P	Gamma [ $10^{-10}$ - $10^{-6}$ ]
Mean $kI_{(cyt-b)}$	K2P	Uniform [0.05 - 20]
Individual locus $kI_{(cyt-b)}$	K2P	Gamma [0.05 - 20]

Abbreviations: Parameters: N, effective population size for each analysed deme (1 – cluster 1; 2 – cluster 2; 3 – cluster 3; 4 – cluster 4, 5 – cluster 5; 135 – central clusters; 241 – three oldest glacial refugia: eastern, western and central); t, time of events in generations ( $t_1$  – time to the most recent split;  $t_2$  – time to the intermediate split;  $t_3$  – time to the most ancient split); Microsatellite (SSRs) and mitochondrial (cyt-b) parameters: mean  $\mu$ , mean mutation rate; individual locus  $\mu$ , individual locus mutation rate; mean  $P$ , mean coefficient  $P$ ; individual locus  $P$ , individual locus coefficient  $P$ ; SNI, Single Nucleotide Insertion rate; mean  $kI$ , mean coefficient  $kI$ ; individual locus  $kI$ , individual locus coefficient  $kI$ . Conditions: sequence data were simulated under a Kimura two-parameter (K2P) mutation model. Distribution: parameter distributions were left as default, with the exception of maximum N values, which were set to 15,000 or 20,000, maximum t value for the most ancient split, which was set to 20,000, SNI rate that was set to zero, and minimum and maximum mutation rate for the cyt-b gene that were set to  $10^{-10}$  and  $10^{-6}$ , respectively.

**Table S4** Assignment of individuals to populations of origin and admixture proportions to other sampling localities in *Calotriton asper* as estimated with GeneClass2. Numbers in parentheses indicate minor assignment proportions (only assignment probabilities  $\geq 0.1$  were considered). For population codes see Table S1.

# MOLECULAR ECOLOGY

					Cluster 1																
		Highest AProb	Lowest AProb	Mean AProb	G-A1	B6-A19	B5	F-B8	B13	E10-B14	E5	E6	E7	E16	M1-H	V28	V29	V30	AUL	VED	
Cluster 1	G-A1	0.94	0.01	0.38	39	-	(10)	-	-	-	(17)	-	-	(1)	-	-	-	-	-	-	(1)
	B6-A19	0.99	0	0.45	(5)	30	-	-	-	-	-	-	-	(2)	-	-	-	-	-	-	-
	B5	0.87	0.01	0.38	4(5)	-	11(3)	-	-	-	(9)	-	-	-	-	-	(1)	-	(2)	(1)	-
	F-B8	0.99	0.05	0.50	-	-	(2)	38	-	-	-	-	-	-	-	-	-	-	-	-	(2)
	B13	0.93	0.01	0.45	(4)	-	(1)	-	16(4)	-	(1)	(5)	(4)	4(18)	3(10)	-	-	-	-	-	-
	E10-B14	0.92	0	0.51	(6)	-	-	-	(11)	11(15)	-	(5)	(15)	14(12)	1(19)	-	(1)	-	-	-	-
	E5	0.97	0.01	0.40	6(9)	-	1(4)	-	-	-	22(7)	-	-	-	-	-	-	-	-	(1)	-
	E6	0.94	0	0.40	(2)	-	-	-	-	-	-	3(9)	6(6)	4(8)	(4)	-	-	-	-	-	-
	E7	0.95	0	0.45	(1)	-	-	-	-	-	(1)	2(12)	10(7)	8(12)	(4)	-	-	-	-	-	-
	E16	0.97	0	0.41	(2)	-	(2)	-	-	-	-	-	(5)	24	(3)	-	-	-	-	-	-
	M1-H	0.99	0.01	0.55	(10)	-	(4)	-	-	-	(2)	(4)	(3)	1(21)	57	-	-	-	-	-	-
	V28	0.95	0.01	0.48	(8)	-	(2)	-	-	-	(1)	-	-	-	-	3(9)	5(8)	6(8)	-	-	-
	V29	0.90	0.02	0.37	3(3)	-	(6)	-	-	-	(4)	-	-	(1)	-	(3)	10(3)	(5)	-	-	-
	V30	0.93	0.01	0.46	1(9)	-	(1)	-	-	-	(4)	-	-	-	-	8(7)	5(10)	2(12)	-	-	-
AUL	0.97	0.06	0.42	(1)	-	-	-	-	-	(1)	-	-	-	-	-	-	-	-	18	-	
VED	0.78	0.04	0.38	(1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12	
Cluster 2	D-A12	0.98	0	0.46	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	B-A18	0.94	0	0.49	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	B10	0.76	0.01	0.38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	C	0.92	0	0.51	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Cluster 3	B1	1	0	0.61	-	-	-	-	-	-	-	-	-	-	-	-	-	-	(1)	-	
	IBA	0.93	0.04	0.43	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	BMO	0.98	0.01	0.45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	IPE	1	0.05	0.61	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	IVI	0.96	0.10	0.43	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	CPL	1	0.01	0.55	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Cluster 4	B2	0.91	0.02	0.48	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	B7	1	0	0.70	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	B11	0.98	0.01	0.32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	B12	0.78	0	0.34	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	E4	0.98	0	0.38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	E13	0.76	0.02	0.27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	E14	0.93	0.01	0.41	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	E15	1	0.01	0.40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	I	0.83	0.03	0.41	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	ISA	0.99	0.01	0.48	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Cluster 5	XSU	0.99	0.01	0.54	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	(3)	
	MDE	1	0	0.57	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	(1)	
	NDE	1	0.02	0.57	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	IDE	1	0	0.56	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	BUX	0.99	0.04	0.61	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	CPX	1	0.01	0.50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	(1)	(1)	
	CTS	0.85	0.01	0.43	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	RQN	0.99	0	0.46	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	RSU	1	0.07	0.63	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	RVT-A	0.99	0	0.54	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
E	0.93	0	0.39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		

# MOLECULAR ECOLOGY

		Highest AProb	Lowest AProb	Mean AProb	Cluster 2				Cluster 3					
					D-A12	B-A18	B10	C	B1	IBA	BMO	IPE	IVI	CPL
Cluster 1	G-A1	0.94	0.01	0.38	-	-	-	-	-	-	-	-	-	-
	B6-A19	0.99	0	0.45	-	-	-	-	-	-	-	-	-	-
	B5	0.87	0.01	0.38	-	-	-	-	-	-	-	-	-	-
	F-B8	0.99	0.05	0.50	-	-	-	-	-	-	-	-	-	-
	B13	0.93	0.01	0.45	-	-	-	-	-	-	-	-	-	-
	E10-B14	0.92	0	0.51	-	-	-	-	-	-	-	-	-	-
	E5	0.97	0.01	0.40	-	-	-	-	-	-	-	-	-	-
	E6	0.94	0	0.40	-	-	-	-	-	-	-	-	-	-
	E7	0.95	0	0.45	-	-	-	-	-	-	-	-	-	-
	E16	0.97	0	0.41	-	-	-	-	-	-	-	-	-	-
	M1-H	0.99	0.01	0.55	-	-	-	-	-	-	-	-	-	-
	V28	0.95	0.01	0.48	-	-	-	-	-	-	-	-	-	-
	V29	0.90	0.02	0.37	-	-	-	-	-	-	-	-	-	-
	V30	0.93	0.01	0.46	-	-	-	-	-	-	-	-	-	-
AUL	0.97	0.06	0.42	-	-	-	-	-	-	-	-	-	-	
VED	0.78	0.04	0.38	-	-	-	-	-	-	-	-	-	-	
Cluster 2	D-A12	0.98	0	0.46	15(8)	-	1(6)	7(14)	-	-	-	-	-	-
	B-A18	0.94	0	0.49	-	25	-	-	-	-	-	-	-	-
	B10	0.76	0.01	0.38	(7)	-	3(6)	7(3)	-	-	-	-	-	-
	C	0.92	0	0.51	(3)	-	(3)	19	-	-	-	-	-	-
Cluster 3	B1	1	0	0.61	-	-	-	-	15	-	-	-	-	-
	IBA	0.93	0.04	0.43	-	-	-	-	-	16	(1)	-	-	-
	BMO	0.98	0.01	0.45	-	-	-	-	-	(1)	22	-	-	-
	IPE	1	0.05	0.61	-	-	-	-	-	-	-	29	-	-
	IVI	0.96	0.10	0.43	-	-	-	-	-	-	-	-	25	-
	CPL	1	0.01	0.55	-	-	-	-	-	-	-	-	-	30
Cluster 4	B2	0.91	0.02	0.48	-	-	-	-	-	-	-	-	-	-
	B7	1	0	0.70	-	-	-	-	-	-	-	-	-	-
	B11	0.98	0.01	0.32	-	-	-	-	-	-	-	-	-	-
	B12	0.78	0	0.34	-	-	-	-	-	-	-	-	-	-
	E4	0.98	0	0.38	-	-	-	-	-	-	-	-	-	-
	E13	0.76	0.02	0.27	-	-	-	-	-	-	-	-	-	-
	E14	0.93	0.01	0.41	-	-	-	-	-	-	-	-	-	-
	E15	1	0.01	0.40	-	-	-	-	-	-	-	-	-	-
	I	0.83	0.03	0.41	-	-	-	-	-	-	-	-	-	-
	ISA	0.99	0.01	0.48	-	-	-	-	-	-	-	-	-	-
Cluster 5	IES	0.99	0.01	0.60	-	-	-	-	-	-	-	-	-	-
	IAC	0.98	0.01	0.54	-	-	-	-	-	-	-	-	-	-
	XSU	0.99	0.01	0.54	-	-	-	-	-	-	-	-	-	-
	MDE	1	0	0.57	-	-	-	-	-	-	-	-	-	-
	NDE	1	0.02	0.57	-	-	-	-	-	-	-	-	-	-
	IDE	1	0	0.56	-	-	-	-	-	-	-	-	-	-
	BUX	0.99	0.04	0.61	-	-	-	-	-	-	-	-	-	-
	CPX	1	0.01	0.50	-	-	-	-	-	-	-	-	-	-
	CTS	0.85	0.01	0.43	-	-	-	-	-	-	-	-	-	-
	RQN	0.99	0	0.46	-	-	-	-	-	-	-	-	-	-
Cluster 5	RSU	1	0.07	0.63	-	-	-	-	-	-	-	-	-	-
	RVT-A	0.99	0	0.54	-	-	-	-	-	-	-	-	-	-
	E	0.93	0	0.39	-	-	-	-	-	-	-	-	-	-

# MOLECULAR ECOLOGY

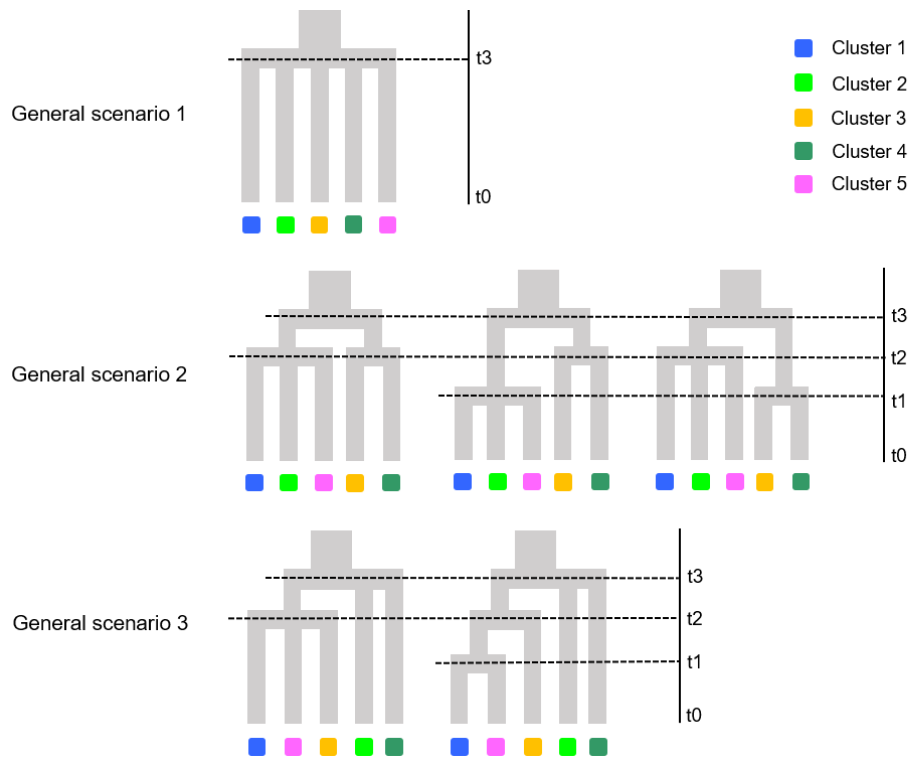
		Highest AProb	Lowest AProb	Mean AProb	Cluster 4											
					B2	B7	B11	B12	E4	E13	E14	E15	I	ISA	IES	IAC
Cluster 1	G-A1	0.94	0.01	0.38	-	-	-	-	-	-	-	-	-	-	-	-
	B6-A19	0.99	0	0.45	-	-	-	-	-	-	-	-	-	-	-	-
	B5	0.87	0.01	0.38	-	-	-	-	-	-	-	-	-	-	-	-
	F-B8	0.99	0.05	0.50	-	-	-	-	-	-	-	-	-	-	-	-
	B13	0.93	0.01	0.45	-	-	-	-	-	-	-	-	-	-	-	-
	E10-B14	0.92	0	0.51	-	-	-	-	-	-	-	-	-	-	-	-
	E5	0.97	0.01	0.40	-	-	-	-	-	-	-	-	-	-	-	-
	E6	0.94	0	0.40	-	-	-	-	-	-	-	-	-	-	-	-
	E7	0.95	0	0.45	-	-	-	-	-	-	-	-	-	-	-	-
	E16	0.97	0	0.41	-	-	-	-	-	-	-	-	-	-	-	-
	M1-H	0.99	0.01	0.55	-	-	-	-	-	-	-	-	-	-	-	-
	V28	0.95	0.01	0.48	-	-	-	-	-	-	-	-	-	-	-	-
	V29	0.90	0.02	0.37	-	-	-	-	-	-	-	-	-	-	-	-
	V30	0.93	0.01	0.46	-	-	-	-	-	-	-	-	-	-	-	-
AUL	0.97	0.06	0.42	-	-	-	-	-	-	-	-	-	-	-	-	
VED	0.78	0.04	0.38	-	-	-	-	-	-	-	-	-	-	-	-	
Cluster 2	D-A12	0.98	0	0.46	-	-	-	-	-	-	-	-	-	-	-	-
	B-A18	0.94	0	0.49	-	-	-	-	-	-	-	-	-	-	-	-
	B10	0.76	0.01	0.38	-	-	-	-	-	-	-	-	-	-	-	-
	C	0.92	0	0.51	-	-	-	-	-	-	-	-	-	-	-	-
Cluster 3	B1	1	0	0.61	-	-	-	-	-	-	-	-	-	-	-	-
	IBA	0.93	0.04	0.43	-	-	-	-	-	-	-	-	-	-	-	-
	BMO	0.98	0.01	0.45	-	-	-	-	-	-	-	-	-	-	-	-
	IPE	1	0.05	0.61	-	-	-	-	-	-	-	-	-	-	-	-
	IVI	0.96	0.10	0.43	-	-	-	-	-	-	-	-	-	-	-	-
CPL	1	0.01	0.55	-	-	-	-	-	-	-	-	-	-	-	-	
Cluster 4	B2	0.91	0.02	0.48	16	-	-	-	-	-	-	-	-	-	(1)	-
	B7	1	0	0.70	2(4)	26(1)	-	-	-	-	-	-	-	-	(1)	-
	B11	0.98	0.01	0.32	-	-	11	1(4)	-	-	-	-	-	-	-	-
	B12	0.78	0	0.34	-	-	1(8)	13(1)	-	-	-	-	-	-	-	-
	E4	0.98	0	0.38	-	-	-	-	29	-	(2)	-	-	-	-	-
	E13	0.76	0.02	0.27	-	-	-	-	-	12	-	-	-	-	-	-
	E14	0.93	0.01	0.41	-	-	-	-	(1)	-	30	-	-	-	-	-
	E15	1	0.01	0.40	(1)	-	-	-	-	(3)	-	18	-	-	-	-
	I	0.83	0.03	0.41	-	-	-	-	-	-	-	-	14	-	-	-
	ISA	0.99	0.01	0.48	-	-	-	-	-	-	-	-	-	14	-	-
	IES	0.99	0.01	0.60	-	-	-	-	-	1	-	-	-	-	22	-
IAC	0.98	0.01	0.54	-	-	-	-	-	-	-	-	-	-	-	26	
Cluster 5	XSU	0.99	0.01	0.54	-	-	-	-	-	-	-	-	-	-	-	-
	MDE	1	0	0.57	-	-	-	-	-	-	-	-	-	-	-	-
	NDE	1	0.02	0.57	-	-	-	-	-	-	-	-	-	-	-	-
	IDE	1	0	0.56	-	-	-	-	-	-	-	-	-	-	-	-
	BUX	0.99	0.04	0.61	-	-	-	-	-	-	-	-	-	-	-	-
	CPX	1	0.01	0.50	-	-	-	-	-	-	-	-	-	-	-	-
	CTS	0.85	0.01	0.43	-	-	-	-	-	-	-	-	-	-	-	-
	RQN	0.99	0	0.46	-	-	-	-	-	-	-	-	-	-	-	-
	RSU	1	0.07	0.63	-	-	-	-	-	-	-	-	-	-	-	-
	RVT-A	0.99	0	0.54	-	-	-	-	-	-	-	-	-	-	-	-
E	0.93	0	0.39	-	-	-	-	-	-	-	-	-	-	-	-	

# MOLECULAR ECOLOGY

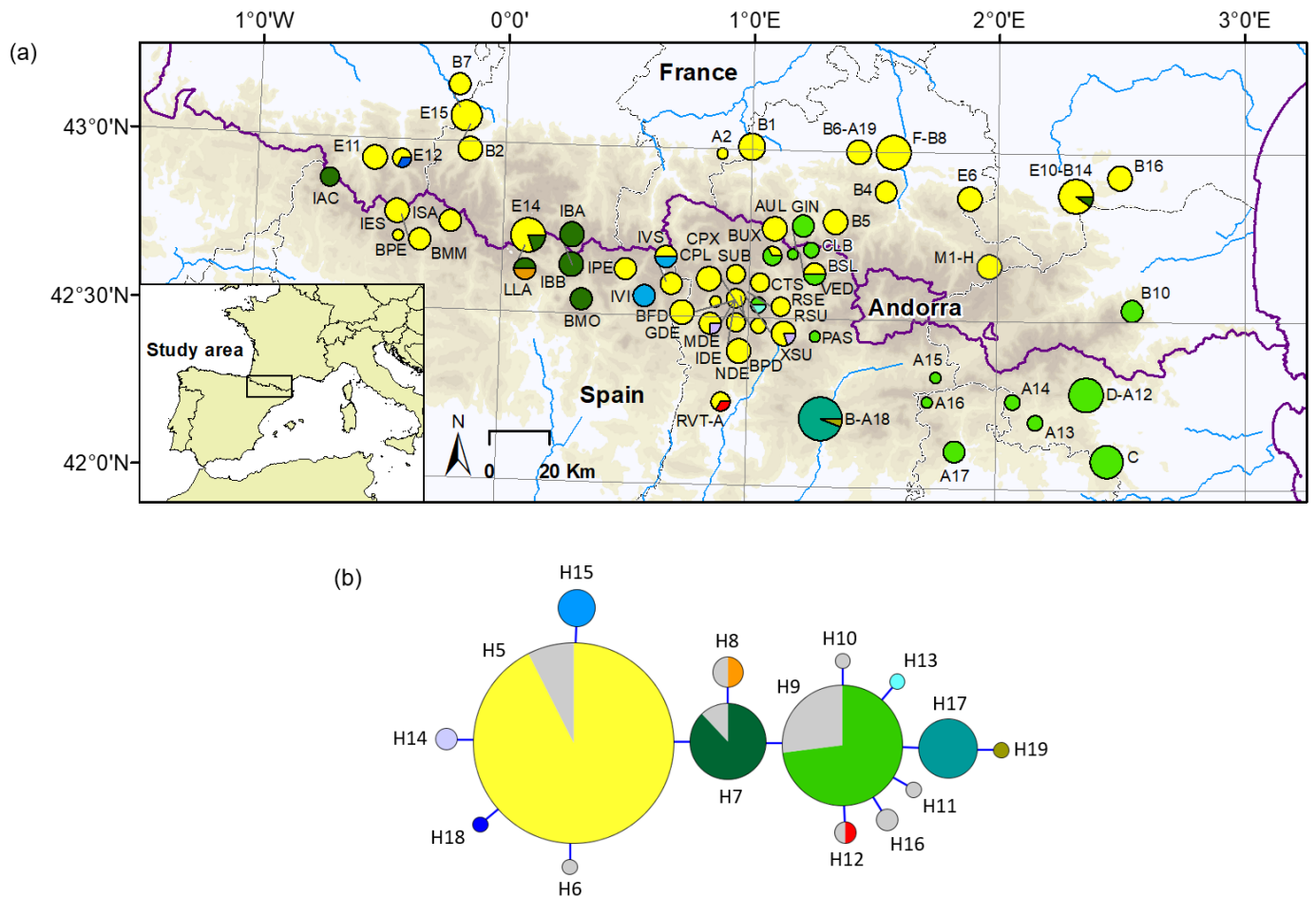
		Highest AProb	Lowest AProb	Mean AProb	Cluster 5										
					XSU	MDE	NDE	IDE	BUX	CPX	CTS	RQN	RSU	RVT-A	E
Cluster 1	G-A1	0.94	0.01	0.38	-	-	-	-	-	-	-	-	-	-	-
	B6-A19	0.99	0	0.45	-	-	-	-	-	-	-	-	-	-	-
	B5	0.87	0.01	0.38	-	-	-	-	-	-	-	-	-	-	-
	F-B8	0.99	0.05	0.50	-	-	-	-	-	-	-	-	-	-	-
	B13	0.93	0.01	0.45	-	-	-	-	-	-	-	-	-	-	-
	E10-B14	0.92	0	0.51	-	-	-	-	-	-	-	-	-	-	-
	E5	0.97	0.01	0.40	-	-	-	-	-	-	-	-	-	-	-
	E6	0.94	0	0.40	-	-	-	-	-	-	-	-	-	-	-
	E7	0.95	0	0.45	-	-	-	-	-	-	-	-	-	-	-
	E16	0.97	0	0.41	-	-	-	-	-	-	-	-	-	-	-
	M1-H	0.99	0.01	0.55	-	-	-	-	-	-	-	-	-	-	-
	V28	0.95	0.01	0.48	-	-	-	-	-	-	-	-	-	-	-
	V29	0.90	0.02	0.37	-	-	-	-	-	-	-	-	-	-	-
	V30	0.93	0.01	0.46	-	-	-	-	-	-	-	-	-	-	-
AUL	0.97	0.06	0.42	-	-	-	-	-	-	-	-	-	-	-	
VED	0.78	0.04	0.38	-	-	-	-	-	-	-	-	-	-	-	
Cluster 2	D-A12	0.98	0	0.46	-	-	-	-	-	-	-	-	-	-	-
	B-A18	0.94	0	0.49	-	-	-	-	-	-	-	-	-	-	-
	B10	0.76	0.01	0.38	-	-	-	-	-	-	-	-	-	-	-
	C	0.92	0	0.51	-	-	-	-	-	-	-	-	-	-	-
Cluster 3	B1	1	0	0.61	-	-	-	-	-	-	-	-	-	-	-
	IBA	0.93	0.04	0.43	-	-	-	-	-	-	-	-	-	-	-
	BMO	0.98	0.01	0.45	-	-	-	-	-	-	-	-	-	-	-
	IPE	1	0.05	0.61	-	-	-	-	-	-	-	-	-	-	-
	IVI	0.96	0.10	0.43	-	-	-	-	-	-	-	-	-	-	-
CPL	1	0.01	0.55	-	-	-	-	-	-	-	-	-	-	-	
Cluster 4	B2	0.91	0.02	0.48	-	-	-	-	-	-	-	-	-	-	-
	B7	1	0	0.70	-	-	-	-	-	-	-	-	-	-	-
	B11	0.98	0.01	0.32	-	-	-	-	-	-	-	-	-	-	-
	B12	0.78	0	0.34	-	-	-	-	-	-	-	-	-	-	-
	E4	0.98	0	0.38	-	-	-	-	-	-	-	-	-	-	-
	E13	0.76	0.02	0.27	-	-	-	-	-	-	-	-	-	-	-
	E14	0.93	0.01	0.41	-	-	-	-	-	-	-	-	-	-	-
	E15	1	0.01	0.40	-	-	-	-	-	-	-	-	-	-	-
	I	0.83	0.03	0.41	-	-	-	-	-	-	-	-	-	-	-
ISA	0.99	0.01	0.48	-	-	-	-	-	-	-	-	-	-	-	
IES	0.99	0.01	0.60	-	-	-	-	-	-	-	-	-	-	-	
IAC	0.98	0.01	0.54	-	-	-	-	-	-	-	-	-	-	-	
Cluster 5	XSU	0.99	0.01	0.54	31(3)	-	-	-	-	(1)	-	-	4(19)	-	-
	MDE	1	0	0.57	(1)	30(7)	1(14)	1(16)	-	(3)	3(21)	-	3(9)	-	-
	NDE	1	0.02	0.57	(3)	18(15)	14(20)	3(12)	-	(3)	(11)	-	(6)	-	-
	IDE	1	0	0.56	(2)	11(7)	(11)	6(12)	-	-	1(13)	-	(7)	-	-
	BUX	0.99	0.04	0.61	-	-	-	-	15	-	-	-	-	-	-
	CPX	1	0.01	0.50	(2)	-	-	-	-	16	(1)	-	(4)	-	-
	CTS	0.85	0.01	0.43	1(1)	(9)	(2)	(3)	-	(3)	8(3)	-	3(6)	-	-
	RQN	0.99	0	0.46	-	-	-	-	-	-	-	14	-	-	(1)
	RSU	1	0.07	0.63	3(6)	-	-	-	(1)	(2)	(1)	-	18	-	-
	RVT-A	0.99	0	0.54	-	-	-	-	-	-	-	-	-	26	-
	E	0.93	0	0.39	-	-	-	-	-	-	-	(2)	-	-	19

Abbreviations: AProb, probability of assignment to the population of collection.

## Supporting Figures



**Figure S1** Phylogeographic models of *Calotriton asper* evolution tested with DIYABC during phase 1. The best scenario for each general topology was selected for phase 2, where we compared the best trees for each general scenario against each other. See Table S3 for more information on parameters for effective population size and time of events ( $t$ ), as well as prior distributions.

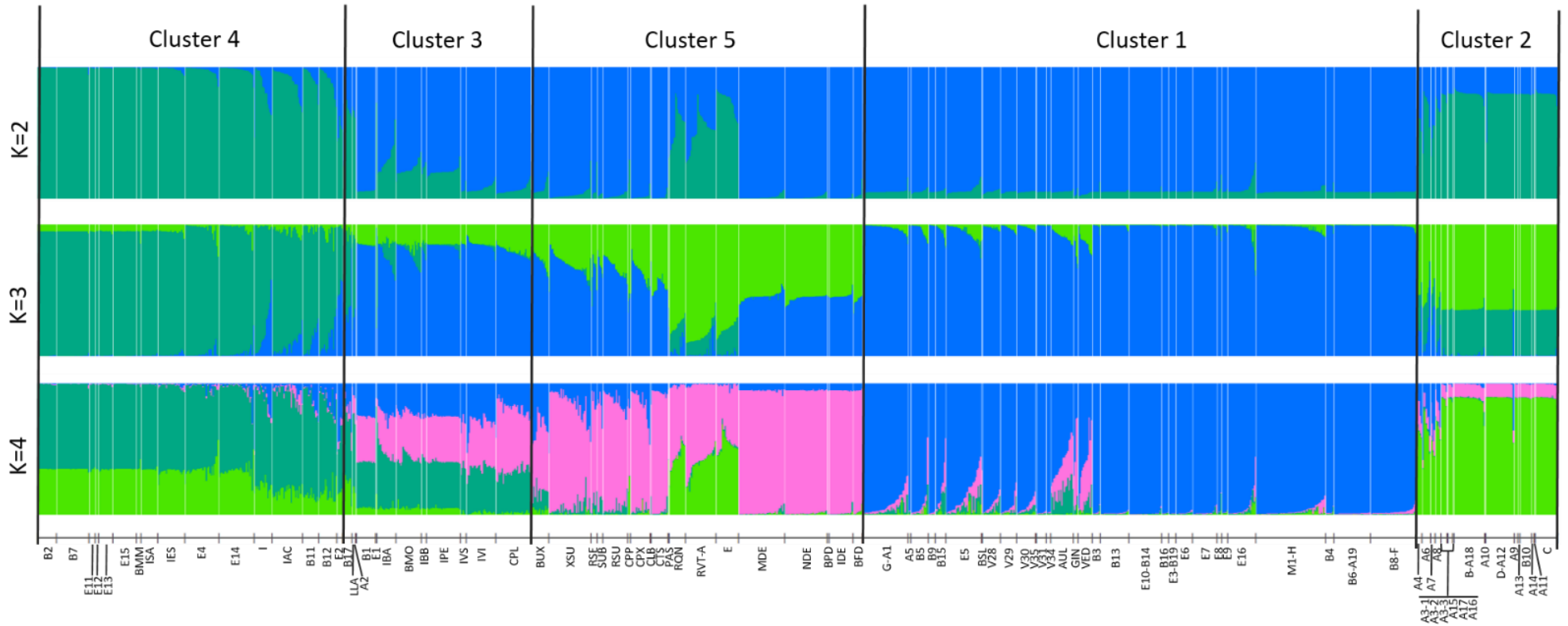


**Figure S2** Results of the network analysis of the cytochrome *b* (*cyt-b*) mtDNA across *Calotriton asper* distribution range. Panel (a) shows the geographic distribution of *cyt-b* haplotypes identified from 258 individuals. The size of circles is proportional to the number of analysed sequences. For population codes, as well as number of analysed sequences in each population, see Table S1. Panel (b) shows the haplotype network inferred from *cyt-b* sequences. Each circle represents a unique haplotype and the circle area is proportional to the number of sequences of a given haplotype. Connecting lines correspond to one mutational step. Colours and codes of haplotypes follow Valbuena-Ureña et al. (2013). Sequences depicted in grey were retrieved from GenBank.

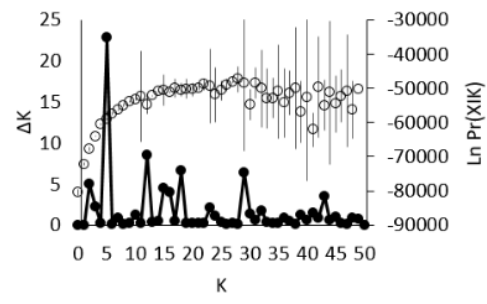


# MOLECULAR ECOLOGY

(a)

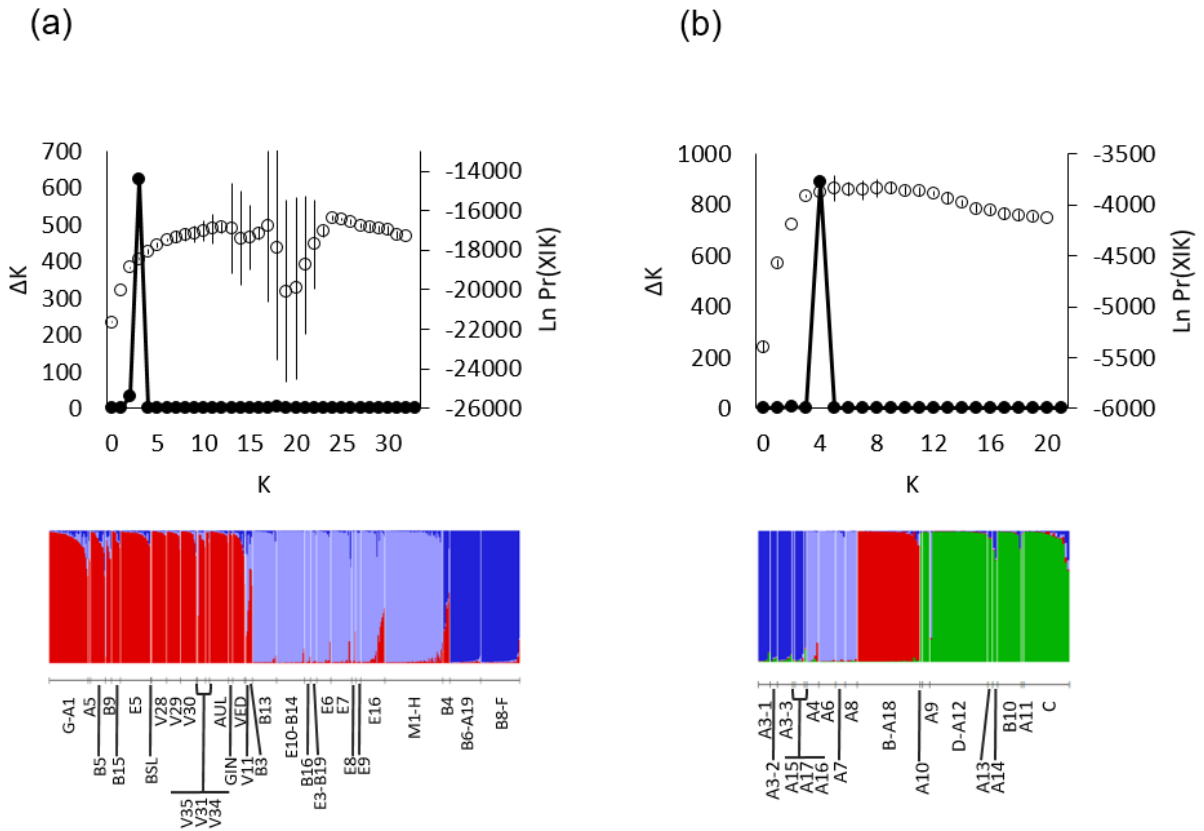


(b)

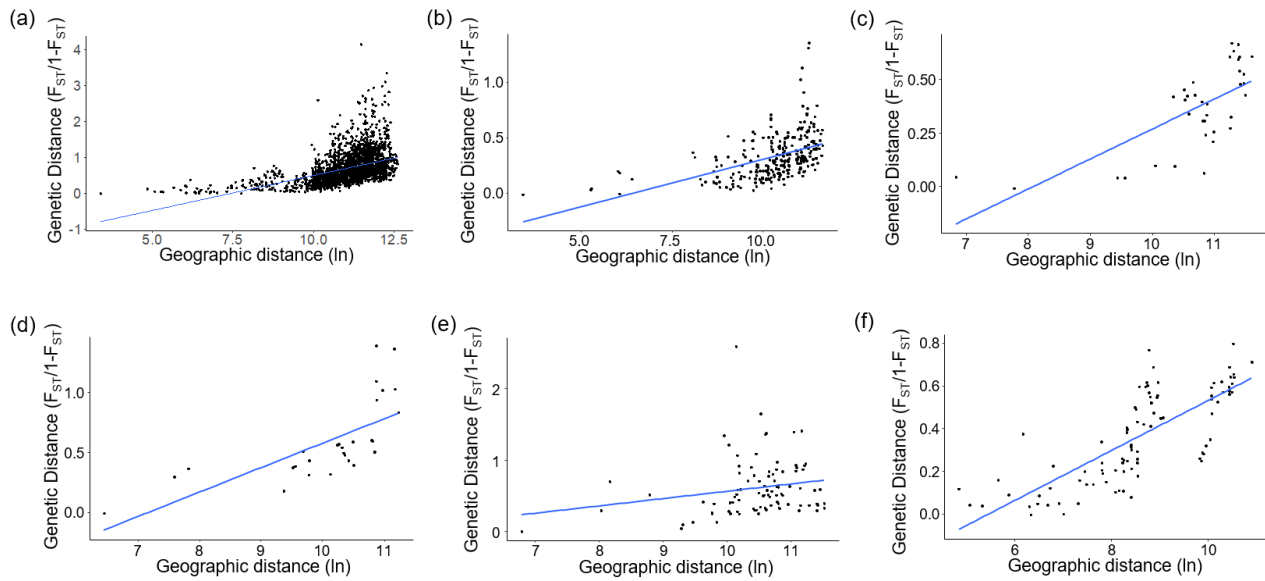


# MOLECULAR ECOLOGY

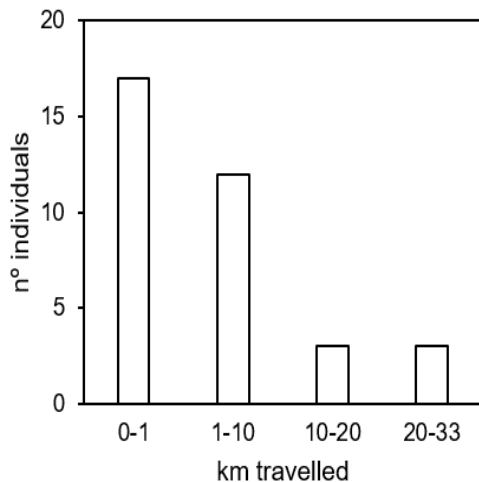
**Figure S3** Resulting plots from STRUCTURE analysis for the complete dataset. Panel (a) shows barplots of membership assignment for  $K = 2-4$  genetic clusters: each individual is represented by a vertical bar corresponding to the sum of assignment probabilities to the  $K$  cluster. White lines separate populations and black lines separate clusters. Further information on population codes and sampling sites are given in Table S1. In panel (b), the right axis (open dots with error bars) displays mean ( $\pm$  SD) log probability of the data [ $\ln \Pr(X|K)$ ] over 20 runs, for each value of  $K$ ; the left axis (black dots) shows  $\Delta K$  values as a function of  $K$ , calculated according to Evanno, Regnaut, and Goudet (2005).



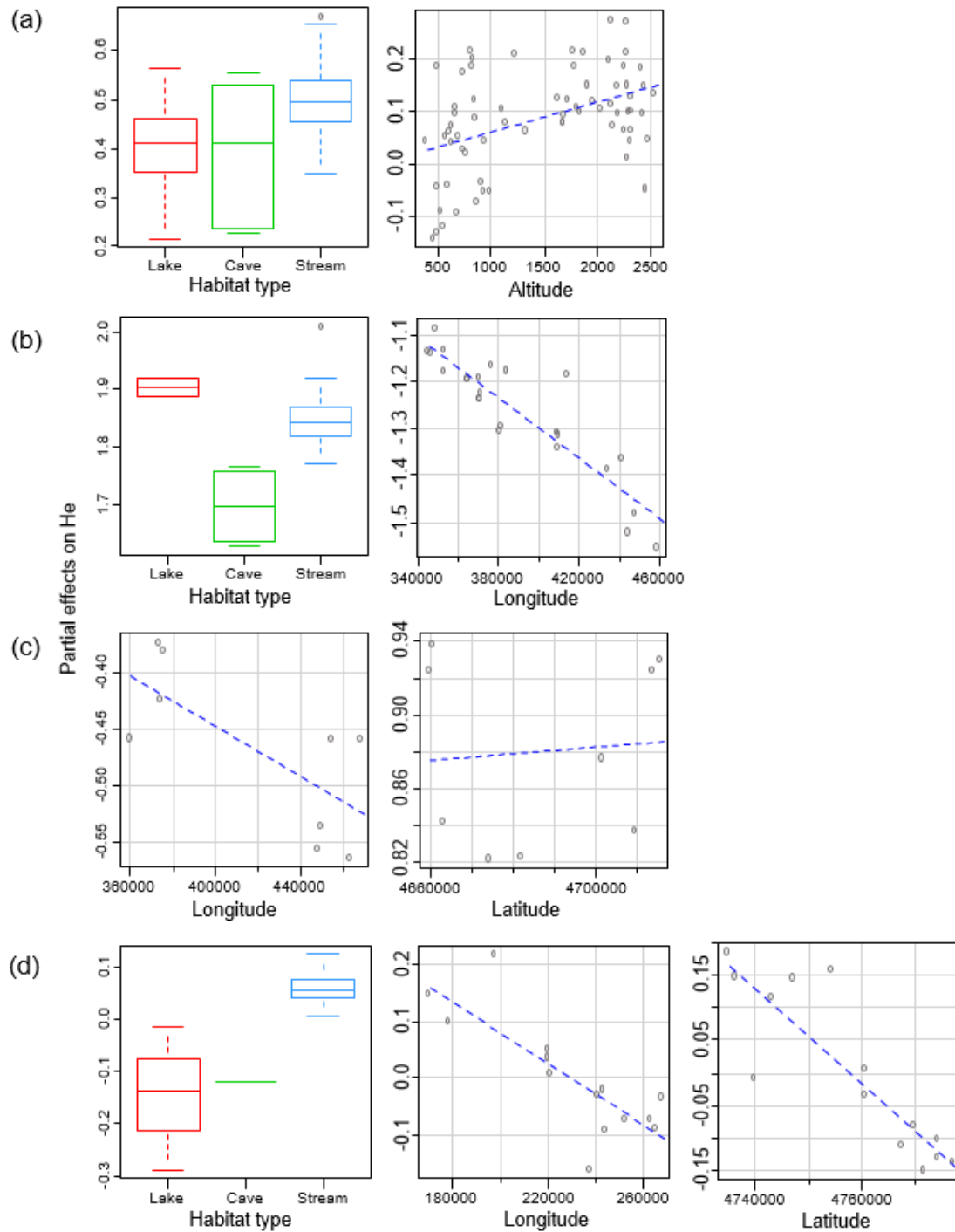
**Figure S4** Resulting plots from STRUCTURE analysis for cluster 1 (eastern French populations; a) and cluster 2 (eastern Spanish populations; b). In the upper plots, the right axis (open dots with error bars) displays mean ( $\pm$  SD) log probability of the data [ $\ln \text{Pr}(X|K)$ ] over 20 runs, for each value of  $K$ ; the left axis (black dots) shows  $\Delta K$  values as a function of  $K$ , calculated according to Evanno et al. (2005). Lower plots represent barplots of membership assignment for  $K = 3$  (cluster 1; a) and  $K = 4$  (cluster 2; b): each individual is represented by a vertical bar corresponding to the sum of assignment probabilities to the  $K$  cluster. White lines separate populations. Further information on population codes and sampling sites are given in Table S1.



**Figure S5** IBD (isolation by distance) analysis over all *Calotriton asper* population pairs (a) and for each genetic cluster as inferred by STRUCTURE (b, cluster 1; c, cluster 2; d, cluster 3; e, cluster 4; f, cluster 5).

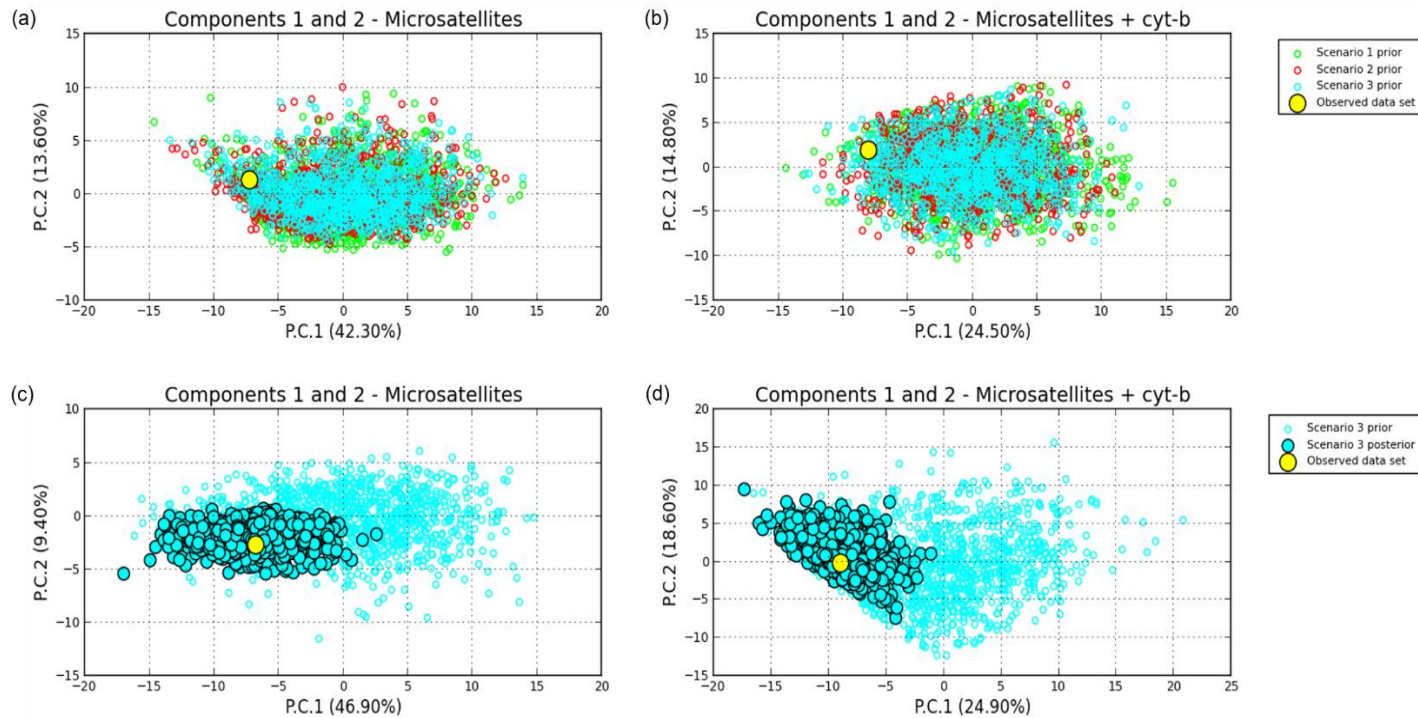


**Figure S6** Histogram showing the Euclidean distance covered by *Calotriton asper* first generation migrants as inferred by GeneClass2. Only individuals whose source locality could be determined are shown.



**Figure S7** Partial effects of environmental (habitat type) and geographic (latitude, longitude, altitude) variables on expected heterozygosity ( $H_E$ ). (a), all populations; (b), cluster 1; (c), cluster 2; (d), cluster 4. Only variables that had a significant effect on  $H_E$  as determined by linear models selection are drawn. Latitude and longitude are in UTM coordinates and altitude is expressed in meters.

# MOLECULAR ECOLOGY



**Figure S8** Resulting plots from DIYABC analysis. Panels (a) and (b) show the pre-evaluation step of scenarios tested during phase 2, conducted through a PCA on summary statistics of simulated and observed datasets. Panels (c) and (d) show the model checking step for the most probable scenario (scenario 3), through performance of a PCA on the simulated datasets generated from posterior parameter distributions and the observed dataset. Simulations were performed only including microsatellites (panels a and c) and considering both mtDNA and microsatellite markers (panels b and d). The observed dataset (yellow dot) falls within the cloud of simulated points, indicating a good fit between observed and simulated datasets under the chosen priors. See Figure S1 for details on the tested scenarios.

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