Table S1.1 Set of microsatellites optimized for *H. molleri* combined in three multiplex reactions. For each locus we indicate the labelling dye, repeated motif, primer sequences and the volume of each primer required to prepare 100 µl of primer mix of the respective multiplex reaction. Annealing temperature was 60ºC in all cases. The total number of alleles (NA) and allele size range (in base pairs) are also shown for each marker.

Locus	Multiplex reaction	Labelling dye	Repeated motif	Primer sequences	µl primer	NA	Size range (bp)
Hmol3.3	1	6-FAM	$(AAT)_5$	5'-AATAGGACTGAAAGGAACAACGC-3'	2	4	135-146
				5'-AAGTGATCTGATCGGCTACTTTG-3'	2		
Hmol4.11	1	6-FAM	$(AGAT)_{10}$	5'-TTAAGCCTGAATGTATGGAATTGG-3'	2	2	279-290
				5'-TTTCGAGCATATTGATCCCTCCC-3'	2		
Hmol4.12	1	VIC	$(AGAT)_8$	5'-CTAAGTCATCTAGTGGTCCCTGG-3'	1	18	231-318
				5'-TTTACAAATGCGACGTTTCAACC-3'	1		
Hmol4.2	1	NED	$(ACAT)_6$	5'-GCCGAAACGTAACTCTATGTACC-3'	2	4	283-298
				5'-TGACTTGCACTGGGACTTTAAAC-3'	2		
Hmol4.1	1	PET	$(AGAT)_9$	5'-TGCAATGTATCTATTAGCCTCCAC-3'	1	11	239-290
				5'-GCCCATTTAAGCATACAGTCTAGC-3'	1		
Hmol3.7	2	6-FAM	$(ACT)_7$	5'-GAAGGAAGGGCATTAAGAGGATG-3'	1	1	140 - 149
				5'-TCCTCTGGATTAACTCAGTAGGG-3'	1		
Hmol4.22	2	6-FAM	$(AAAC)_6$	5'-GCTTCATCACCACTTAACCTGAG-3'	2	3	235-245
				5'-TGGACATGATCAGAGACCATTAC-3'	2		
Hmol3.28	2	VIC	$(AAT)_{10}$	5'-TGTACCAGAGCTTCTCCACTTAG-3'	2	4	187-200
				5'-CCTACATTGGTCAGGATTAGGTAC-3'	2		
Hmol4.29	2	NED	$(AGAT)_6$	5'-CTTTCCTTGGCTTCTTTATGCAC-3'	2	17	360-433
				5'-GTATGTGAGCTCTTTACTGCCTG-3'	2		
Hmol3.22	2	PET	$(AAT)_{10}$	5'-GACATCCATCATTCACATCCCTG-3'	2	8	293-322
				5'-TTCTGCCTTCTCTTCCCATAGAC-3'	2		
Hmol4.9	3	6-FAM	$(AGAT)_{10}$	5'-GGACAACGTTCTGCAAGTTAATC-3'	2	5	168-186
				5'-TGTCTCTTCATGTTGGTGTGATC-3'	2		
Hmol4.10	3	VIC	$(AGAT)_{10}$	5'-TATTGCCCATATCCTCCCTTCTC-3'	1	13	102-166
				5'-ATGACATCACCTCATCAGCCAG-3'	1		
Hmol4.8	3	VIC	$(AGAT)_{10}$	5'-GTTGTGCTGACCTTGAAAGTATTG-3'	2	17	387-426
				5'-CTAGGCTTGATAATGGCAGTGTG-3'	2		
Hmol4.16	3	NED	$(AGAT)_9$	5'-ATTTACTCAGGGAATGTGCATCC-3'	2	17	150-231
				5'-TCATGCTAACTGTGTTTATGTTGC-3'	2		
Hmol3.8	3	PET	$(ACT)_{12}$	5'-ATAGTCTTATGCTTGTTGGGCTG-3'	1	4	261-271
				5'-TATGGGAAACTGCACCACTCTTC-3'	1		

Table S1.2 Set of microsatellites optimized for *P. cultripes*, combined in three multiplex reactions. For each locus we indicate the labelling dye, repeated motif, primer sequences and the volume of each primer required to prepare 100 μl of primer mix of the respective multiplex reaction. Annealing temperature was 60ºC in all cases. The total number of alleles (NA) and allele size range (in base pairs) are also shown for each marker.

Locus	Multiplex reaction	Labeling dye	Repeated motif	Primer sequences	μl primer	NA	Size range (bp)
Pc3.24	1	PET	$(CCA)_6$	5'-AATCCCACCATCGTCAATGT-3'	4	4	224–239
				5'-TCCTACTACCCCAAGGCTGA-3'	4		
Pc3.3	1	VIC	$(TGG)_6$	5'-TCATTGGGAGATCTGAAGCA-3'	2	3	207–225
				5'-TAACTGGCGTCCATCATTCA-3'	2		
Pc3.7	1	PET	$(GTTT)_7$	5'-TTAGGGGAAATGCAAACCAA-3'	2	9	157–178
				5'-GAAATGCACCACCATTTTCC-3'	2		
Pc4.11	1	NED	$(GATA)_5$	5'-TCTGCTTGGCCCCTATAGTC-3'	2	6	119–139
				5'-TGCAACAAGTTTGGACCAATTA-3'	2		
Pc4.5	1	6-FAM	$(AGAA)_6$	5'-TTGCAACTGTATAGAGAGGTGATT-3'	4	9	155–187
				5'-TTTCTTTCCCTGTCCGAATG-3'	4		
Pc3.2	2	6-FAM	$(TAA)_{12}$	5'-GCTTGTTTGACCTCGTCTCTG-3'	2	10	178–205
				5'-CCTCAATGACACCTCTCATGAAC-3'	2		
Pc3.25	2	PET	$(GTT)_7$	5'-GCGTTGGTACACATTGCATC-3'	2	6	191–206
				5'-GGCAGCTGTGTAATCGACCT-3'	2		
Pc4.3	2	PET	$(GTTT)_7$	5'-TTAGGGGAAATGCAAACCAA-3'	2	9	157–178
				5'-GAAATGCACCACCATTTTCC-3'	2		
Pc4.4	2	NED	$(TGGA)_5$	5'-GGCACACCAAAACACATTGA-3'	2	6	125–145
				5'-GACTGTTTATCTATCCATCCACCC-3'	2		
Pc4.9	2	VIC	$(ATTT)_5$	5'-TGTTCACACCGTAGACAGCA-3'	2	5	133–149
				5'-CGCAATGTACAATTGACAACAG-3'	2		
Pc3.1	3	PET	$(TAT)_6$	5'-TTTGACTAGGGTCCATGCAA-3'	2	4	128–137
				5'-GGAAAGTTTTGGGTAAAGCG-3'	2		
Pc3.23	3	6-FAM	$(CCT)_5$	5'-CCCTGTAAAGGGCATCATCT-3'	4	5	178–203
				5'-TAGGGTGGGAACATCAGGAG-3'	4		
Pc3.4	3	6-FAM	$(ACA)_6$	5'-AGCATACTCCCTACTTGAACCA-3'	5	5	288–300
				5'-AGCTAATTCCCCATTCGGTT-3'	5		
Pc3.9	3	VIC	$(TAA)_6$	5'-GTGTTTCCTGCCAATTGCTT-3'	1.2	5	132–144
				5'-CGTTCACTGATGTCCCAATG-3'	1.2		
Pc4.1	3	NED	$(TAGA)_5$	5'-CAAAATGTCCAGTTGGAGTGAG-3'	3	14	151–209
				5'-GGAATTTAAGGTGGAAGAGGG-3'	3		
Pc4.7	3	6-FAM	$(GATA)_7$	5'-TTCAATCTGGGCTGAAGAGG-3'	3	11	159–207
				5'-AGGCCAAACGACTGAATTTG-3'	3		

Table S2 N_e estimates for each population, species and breeding season (left) and N_b estimates for both species in Laguna during the 2018 and 2019 breeding seasons (right), with 95% confidence intervals in parentheses (IC95%). Prior: No = no use of sibship size prior; Prior: Weak = weak sibship size prior = 1; Prob = prior probability of paternity/maternity identified among the candidate parents (genotyped adults).

		H. molleri	***P. cultripes***
Population	**Prior**	**N_e (IC95%)**	**N_e (IC95%)**
Bustarviejo 2020	No	17 (9-34)	27 (16-47)
		17 (10-34)	27 (16-48)
	Weak	13 (7-30)	12 (6-26)
		13 (7-30)	12 (6-28)
Roblellano 2019	No	20 (11-39)	26 (15-46)
		20 (11-42)	24 (14-46)
	Weak	14 (7-30)	18 (10-34)
		14 (7-31)	21 (12-39)
Laguna 2013	No	57 (39-87)	45 (30-71)
		56 (40-85)	52 (35-77)
	Weak	54 (37-84)	37 (24-59)
		48 (32-74)	31 (20-52)
Laguna 2018 + 2019	No	59 (42-84)	61 (44-89)
		67 (47-98)	60 (41-88)
	Weak	57 (40-86)	36 (23-59)
		57 (39-83)	44 (30-70)
Gravera 2018 + 2019	No	41 (25-69)	21 (11-44)
		41 (26-71)	21 (12-43)
	Weak	32 (19-56)	11 (6-26)
		32 (19-56)	11 (5-28)

		H. molleri	***P. cultripes***
Prior	**Prob**	**N_b (IC95%)**	**N_b (IC95%)**
No prior	0.2	57 (39-87)	35 (22-58)
	0.2	63 (43-94)	35 (22-56)
	0.5	55 (37-83)	35 (23-57)
	0.5	59 (41-88)	35 (23-56)
	0.8	69 (46-111)	33 (21-54)
	0.8	58 (39-88)	33 (21-53)
Weak prior	0.2	65 (44-100)	33 (21-55)
	0.2	65 (43-98)	35 (23-58)
	0.5	68 (45-105)	34 (22-56)
	0.5	72 (50-109)	34 (22-56)
	0.8	72 (48-113)	34 (21-56)
	0.8	63 (43-95)	34 (21-57)

Table S3 Mean and variance (in parentheses) of residence and migration rates per generation in the four sampled populations of *H. molleri* and *P. cultripes*. Displayed values represent the proportion of individuals of each population source (rows) whose ancestry has been assigned to each of the sampling localities (columns). Cells were grey-shaded according to estimated values.

		Laguna	**Gravera**	**Bustarviejo**	**Roblellano**
H. molleri	**Laguna**	0.7415 (0.0004)	0.2459 (0.0004)	0.0041 (0.0000)	0.0085 (0.0000)
	Gravera	0.1420 (0.0022)	0.8029 (0.0017)	0.0267 (0.0004)	0.0284 (0.0005)
	Bustarviejo	0.0698 (0.0008)	0.0515 (0.0007)	0.8605 (0.0012)	0.0181 (0.0002)
	Roblellano	0.0551 (0.0011)	0.0796 (0.0015)	0.0177 (0.0003)	0.8476 (0.0020)
P. cultripes	**Laguna**	0.9817 (0.0112)	0.0016 (0.0016)	0.0095 (0.0000)	0.0071 (0.0001)
	Gravera	0.2097 (0.0014)	0.6800 (0.0017)	0.0899 (0.0011)	0.0204 (0.0004)
	Bustarviejo	0.0743 (0.0032)	0.0084 (0.0001)	0.8794 (0.0049)	0.0380 (0.0023)
	Roblellano	0.2103 (0.0139)	0.0062 (0.0000)	0.0129 (0.0018)	0.7707 (0.0141)

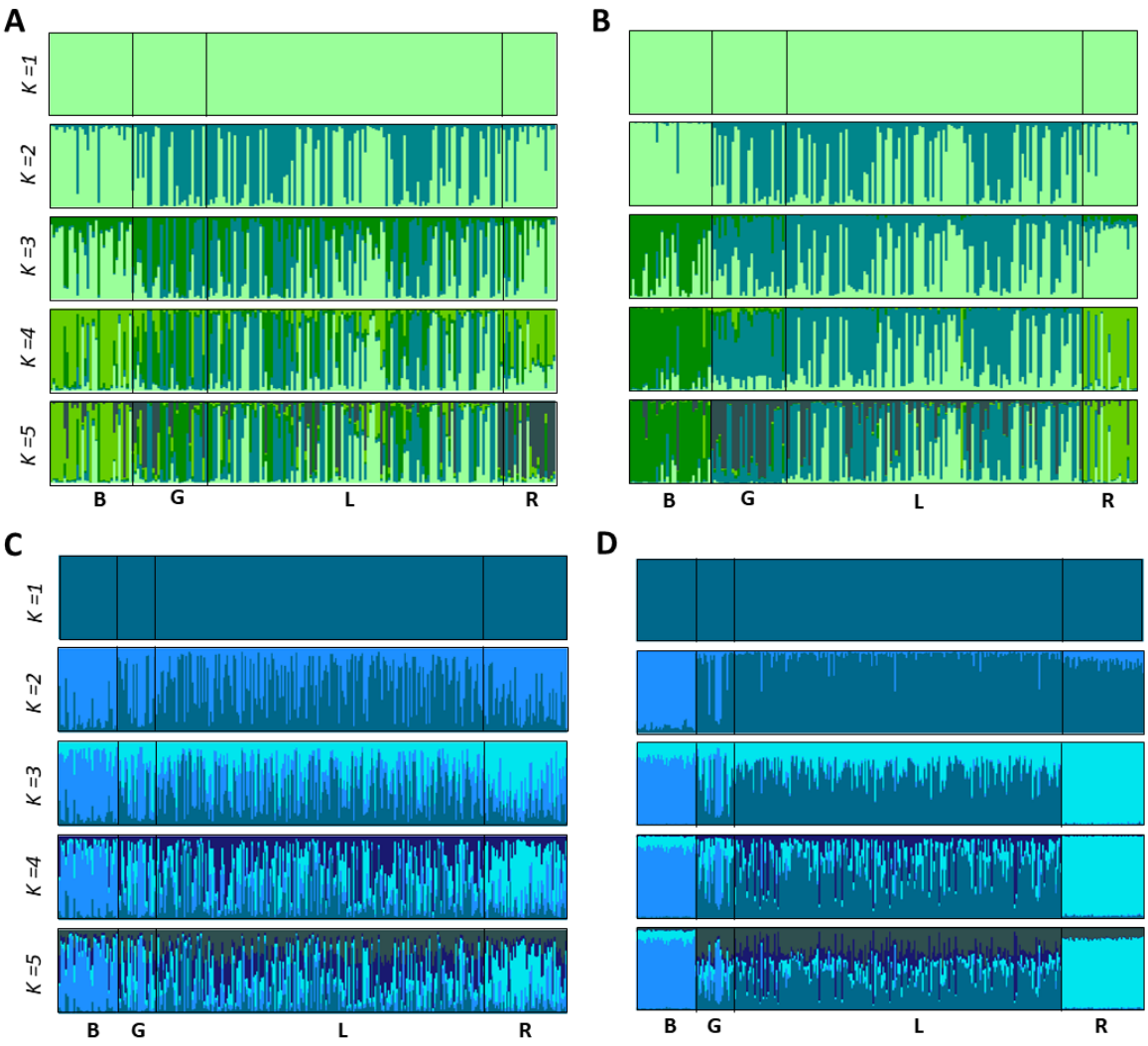

Figure S1 Assignment probabilities of each individual of *H. molleri* (green, top) and *P. cultripes* (blue, bottom) (vertical bars) to each genetic cluster (K = 1 - 5), not using (A and C) or using (B and C) the locprior option in STRUCTURE. Abbreviations: L=Laguna, G=Gravera, R=Roblellano and B=Bustarviejo.

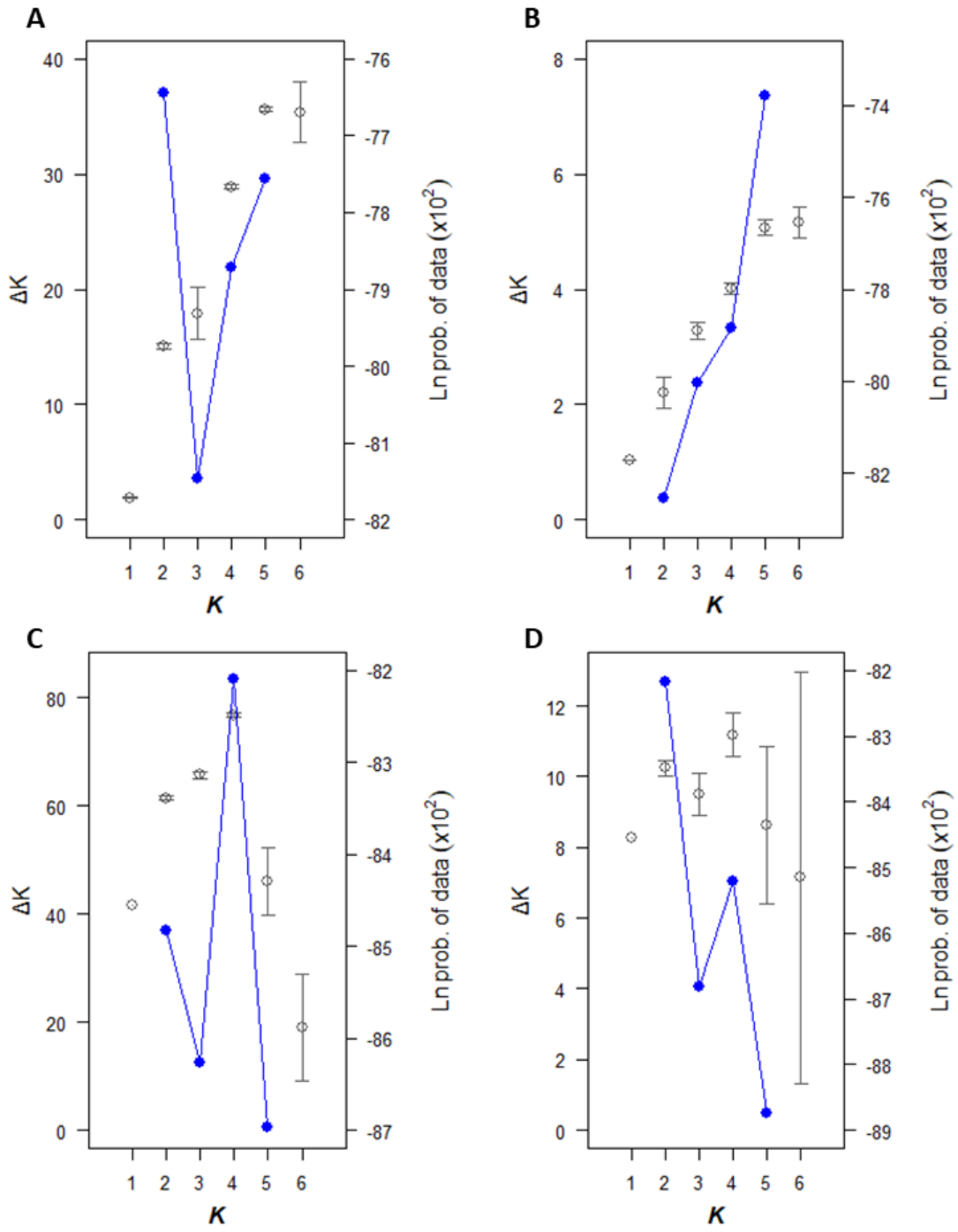

Figure S2 ΔK and likelihood (L(K)) scores of Structure runs for *Hyla molleri* (top) and *Pelobates cultripes* (bottom). A, C: locprior = OFF; B, D: locprior = ON.

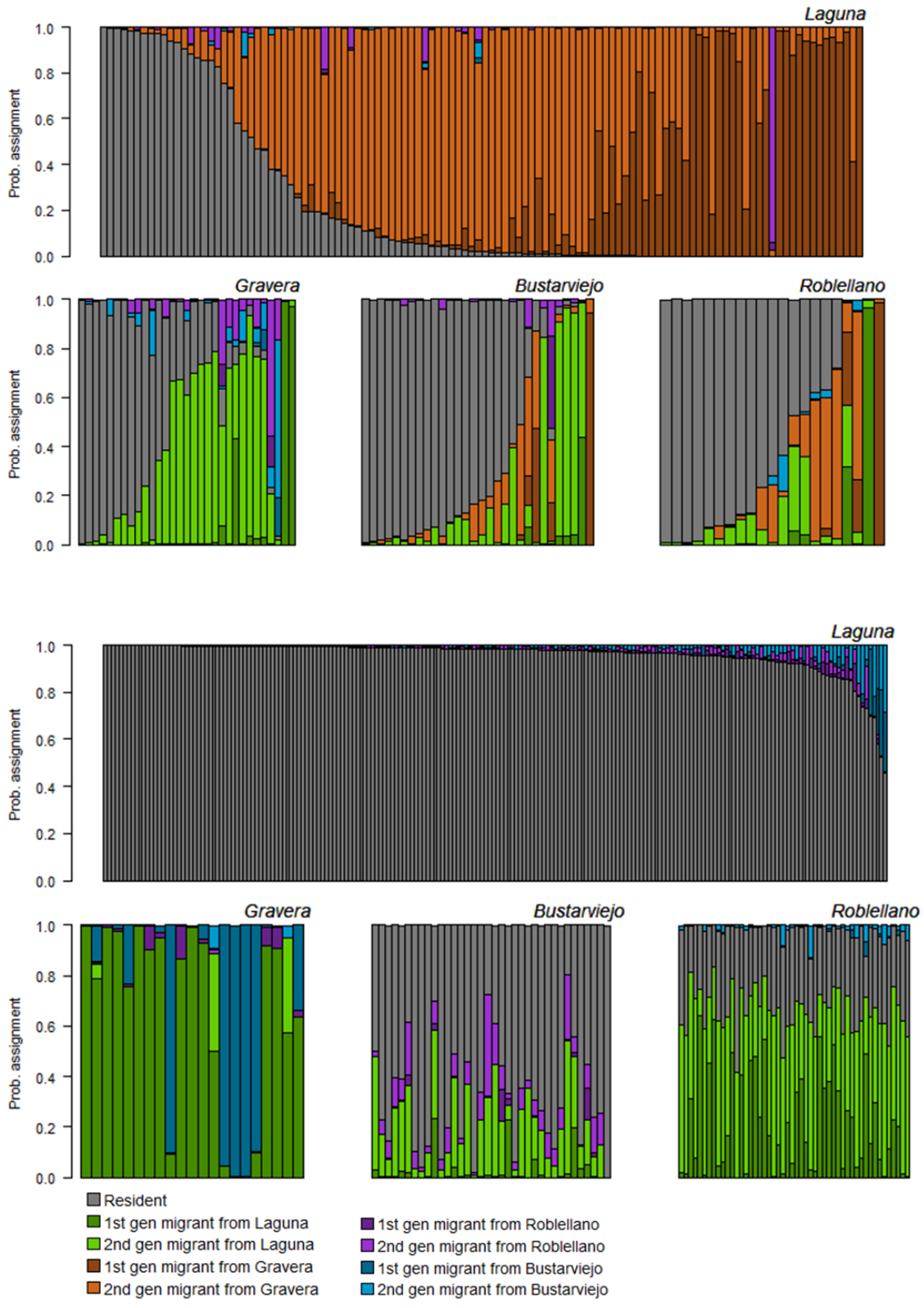

Figure S3 Probability of classification of each individual of *H. molleri* (top) and *P. cultripes* (bottom) genotyped in the four study populations to different genetic ancestry categories (resident, first or second generation migrant from other populations).