

**Genetic diversity of the endemic honeybee: *Apis mellifera unicolor* (Hymenoptera: Apidae) in Madagascar**

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**Supplementary data (SD Table I).** Sites description. For each of the 76 sampled sites, code (referring to figure 1), name, administrative region of origin and number of sample (i.e colony) successfully amplified for nuclear genetic analysis are given. Asterisks indicate presence for *Varroa destructor* at the collection date.

Map codes	Sites (S)	Administrative region (R)	N	Map codes	Sites (S)	Administrative region (R)	N
1	Angahimena	D.I.A.N.A. (R1) <sup>1</sup>	3	39	Ambohidratrimo A.	Analamanga (R9)	9
2	Namakia	D.I.A.N.A. (R1) <sup>1</sup>	10	40	Ivato	Analamanga (R9)	17
3	Antanambao	D.I.A.N.A. (R1) <sup>1</sup>	4	41	Ankatso	Analamanga (R9)	2
4	Antanambao 1	D.I.A.N.A. (R1) <sup>1</sup>	5	42	Andramasina	Analamanga (R9)	10
5	Sakaramy	D.I.A.N.A. (R1) <sup>1</sup>	4	43	Tonoronala	Itasy (R10)	33
6	Antsakoabe	D.I.A.N.A. (R1) <sup>1</sup>	3	44	Tsiroanomandidy	Bongolava (R11)	5
7	Antsoha A.	D.I.A.N.A. (R1) <sup>1</sup>	9	45	Bemasoandro*	Vakinakaratra (R12)	8
8	Djamajar	D.I.A.N.A. (R1) <sup>1</sup>	6	46	Ankotsaka	Vakinakaratra (R12)	7
9	Marompamba A.	D.I.A.N.A. (R1) <sup>1</sup>	3	47	Ihazolava	Vakinakaratra (R12)	12
10	Mahavelona	D.I.A.N.A. (R1) <sup>1</sup>	6	48	Anativato 1	Vakinakaratra (R12)	7
11	Analamandrorofo	S.A.V.A. (R2) <sup>2</sup>	16	49	Anativato 2	Vakinakaratra (R12)	11
12	Analapenja	S.A.V.A. (R2) <sup>2</sup>	4	50	Mahazina	Vakinakaratra (R12)	10
13	Analapenja	S.A.V.A. (R2) <sup>2</sup>	11	51	Antsirabe	Vakinakaratra (R12)	4
14	Andranomatsioka	S.A.V.A. (R2) <sup>2</sup>	3	52	Soanirina	Amoron'i M. (R13)	11
15	Antohomarina	S.A.V.A. (R2) <sup>2</sup>	22	53	Ambositra	Amoron'i M. (R13)	10
16	Antohomarina	S.A.V.A. (R2) <sup>2</sup>	5	54	Tsararano A.	Amoron'i M. (R13)	43
17	Sarahandrano	S.A.V.A. (R2) <sup>2</sup>	5	55	Vohimasina	Amoron'i M. (R13)	22
18	Belle Rose	S.A.V.A. (R2) <sup>2</sup>	9	56	Morondava	Menabe (R14)	4
19	Beromba	Sofia (R3)	2	57	Mahabobe	Menabe (R14)	1
20	Tsararano M.1	Boeny (R4)	10	58	Tsimahavaobe	Menabe (R14)	6
21	Tsararano M.2	Boeny (R4)	15	59	Tsimahavaokely	Menabe (R14)	2
22	Tsaramandroso	Boeny (R4)	10	60	Analahiva	Menabe (R14)	3
23	Andranofasika	Boeny (R4)	5	61	Ankilozato	Menabe (R14)	1
24	Rantolava	Analanjirofo (R5)	3	62	Manitsara	Menabe (R14)	6
25	Mahambo	Analanjirofo (R5)	2	63	Androvabe	Menabe (R14)	11
26	Fenerive 1	Analanjirofo (R5)	1	64	Androvabe PM	Menabe (R14)	7
27	Fenerive 2	Analanjirofo (R5)	1	65	Manakara	Vato V. (R15)	5
28	Ambodihazomamy	Antsinanana (R6)	3	66	Toliary	Atsimo A. (R16)	30
29	Anivorano B.	Antsinanana (R7)	1	67	Bezaha	Atsimo A. (R16)	23
30	Sahavazo M.	Alaotra M. (R8)	1	68	Mahatalaky	Anosy (R17)	17
31	Masakalina	Analamanga (R9)	8	69	Mahatalaky	Anosy (R17)	8
32	Ambohitrolomahitsy	Analamanga (R9)	7	70	Ifarantsy	Anosy (R17)	10
33	Amby	Analamanga (R9)	8	71	Ifarantsy 1	Anosy (R17)	16
34	Ambotseheny	Analamanga (R9)	19	72	Ampasy N.	Anosy (R17)	10
35	Betoho	Analamanga (R9)	16	73	Ampasy N.1	Anosy (R17)	6
36	Anjepy	Analamanga (R9)	12	74	Soanierana	Anosy (R17)	21
37	Maharidaza	Analamanga (R9)	10	75	Ankaramena	Anosy (R17)	13
38	Mandraka	Analamanga (R9)	12	76	Ambovombe	Androy (R18)	15

<sup>1</sup>D.I.A.N.A.: Diego, Ambilobe, Nosybe, Ambanja <sup>2</sup>S.A.V.A.: Sambava, Antalaha, Vohemar, Andapa.

**Supplementary data (SD Table II).** Genetic diversity indices per geographical regions investigated in Madagascar.

<b>Geographical region</b>	<b>H<sub>O</sub></b>	<b>H<sub>S</sub></b>	<b>F<sub>IS</sub></b>	<b>F<sub>ST</sub></b>
North-East	0.365	0.403	0.094	0.019
North-West	0.371	0.432	0.140	0.013
Hauts- Plateaux (central)	0.374	0.410	0.087	0.058
Hauts- Plateaux (South)	0.374	0.398	0.061	0.003
West South	0.368	0.410	0.101	0.025
South	0.383	0.415	0.076	0.006

1 **Supplementary data (SD Table III).** Pairwise  $F_{ST}$  estimates values between pairs of 32 sites (Weir and Cockerham (1984)). Significant  $P$ -value (5%) are in  
 2 bold and highlighted in grey.

	North	North-East				North-West			High-Plateau										High-Plateau - South					West	South-West			South							
site	2	11	13	15	20	21	22	34	35	36	37	38	40	42	43	47	49	50	52	53	54	55	63	66	67	68	70	71	72	74	75				
11	0.02																																		
13	0.03	0.03																																	
15	0.01	0.01	0.01																																
20	<b>0.05</b>	<b>0.03</b>	<b>0.07</b>	<b>0.03</b>																															
21	<b>0.03</b>	0.02	<b>0.05</b>	<b>0.05</b>	<b>0.03</b>																														
22	<b>0.04</b>	0.02	<b>0.05</b>	0.03	-0.02	0.01																													
34	<b>0.04</b>	0.01	<b>0.02</b>	<b>0.02</b>	0.02	<b>0.05</b>	0.01																												
35	<b>0.03</b>	0.02	<b>0.06</b>	<b>0.03</b>	0.02	<b>0.07</b>	0.02	<b>0.02</b>																											
36	<b>0.04</b>	0.01	<b>0.06</b>	<b>0.04</b>	<b>0.05</b>	<b>0.06</b>	<b>0.06</b>	<b>0.03</b>	<b>0.04</b>																										
37	<b>0.04</b>	<b>0.05</b>	<b>0.08</b>	<b>0.05</b>	0.03	<b>0.08</b>	0.03	0.02	-0.02	<b>0.05</b>																									
38	<b>0.04</b>	0.01	<b>0.03</b>	0.02	<b>0.05</b>	0.02	0.03	0.00	<b>0.04</b>	0.01	<b>0.05</b>																								
40	<b>0.10</b>	<b>0.08</b>	<b>0.12</b>	<b>0.11</b>	<b>0.11</b>	<b>0.12</b>	<b>0.07</b>	<b>0.08</b>	<b>0.07</b>	<b>0.08</b>	<b>0.06</b>	<b>0.11</b>																							
42	0.02	0.00	<b>0.04</b>	0.01	0.01	0.01	0.00	-0.01	0.02	0.00	0.01	-0.02	<b>0.08</b>																						
43	<b>0.06</b>	<b>0.02</b>	<b>0.05</b>	<b>0.03</b>	<b>0.05</b>	<b>0.05</b>	<b>0.05</b>	<b>0.04</b>	<b>0.04</b>	<b>0.06</b>	<b>0.06</b>	<b>0.03</b>	<b>0.13</b>	<b>0.04</b>																					
47	<b>0.03</b>	0.02	0.04	<b>0.02</b>	<b>0.05</b>	<b>0.04</b>	<b>0.05</b>	0.03	<b>0.06</b>	<b>0.03</b>	<b>0.08</b>	0.02	<b>0.12</b>	0.01	<b>0.04</b>																				
49	<b>0.04</b>	0.02	<b>0.06</b>	0.01	<b>0.07</b>	<b>0.03</b>	<b>0.04</b>	<b>0.05</b>	<b>0.07</b>	<b>0.05</b>	<b>0.10</b>	<b>0.03</b>	<b>0.10</b>	0.02	<b>0.05</b>	0.01																			
50	<b>0.07</b>	<b>0.04</b>	<b>0.09</b>	<b>0.08</b>	<b>0.07</b>	<b>0.06</b>	<b>0.09</b>	<b>0.07</b>	<b>0.08</b>	<b>0.07</b>	<b>0.11</b>	<b>0.05</b>	<b>0.17</b>	<b>0.07</b>	<b>0.07</b>	<b>0.08</b>	<b>0.12</b>																		
52	<b>0.10</b>	<b>0.05</b>	<b>0.12</b>	<b>0.13</b>	<b>0.11</b>	<b>0.13</b>	<b>0.09</b>	<b>0.05</b>	<b>0.08</b>	<b>0.07</b>	<b>0.07</b>	<b>0.08</b>	<b>0.07</b>	<b>0.03</b>	<b>0.12</b>	<b>0.13</b>	<b>0.13</b>	<b>0.16</b>																	
53	0.00	0.00	0.01	0.00	0.00	0.03	-0.01	-0.02	0.00	0.02	0.00	0.03	<b>0.08</b>	-0.02	<b>0.04</b>	0.04	<b>0.05</b>	<b>0.07</b>	<b>0.04</b>																
54	<b>0.11</b>	<b>0.05</b>	<b>0.07</b>	<b>0.09</b>	<b>0.08</b>	<b>0.12</b>	<b>0.08</b>	<b>0.03</b>	<b>0.07</b>	<b>0.08</b>	<b>0.06</b>	<b>0.07</b>	<b>0.11</b>	<b>0.03</b>	<b>0.10</b>	<b>0.13</b>	<b>0.14</b>	<b>0.14</b>	0.01	<b>0.01</b>															
55	<b>0.09</b>	<b>0.04</b>	<b>0.08</b>	<b>0.08</b>	<b>0.06</b>	<b>0.11</b>	<b>0.06</b>	<b>0.02</b>	<b>0.04</b>	<b>0.07</b>	<b>0.04</b>	<b>0.07</b>	<b>0.08</b>	<b>0.02</b>	<b>0.09</b>	<b>0.11</b>	<b>0.12</b>	<b>0.13</b>	0.00	0.00	-0.01														
63	<b>0.12</b>	<b>0.08</b>	<b>0.14</b>	<b>0.13</b>	<b>0.11</b>	<b>0.12</b>	<b>0.09</b>	<b>0.08</b>	<b>0.13</b>	<b>0.10</b>	<b>0.10</b>	<b>0.10</b>	<b>0.12</b>	<b>0.05</b>	<b>0.16</b>	<b>0.15</b>	<b>0.15</b>	<b>0.17</b>	0.02	<b>0.06</b>	<b>0.07</b>	<b>0.06</b>													
66	0.02	0.01	0.03	<b>0.03</b>	<b>0.03</b>	<b>0.02</b>	0.02	<b>0.02</b>	<b>0.04</b>	<b>0.03</b>	<b>0.06</b>	0.01	<b>0.13</b>	0.02	<b>0.03</b>	<b>0.05</b>	<b>0.05</b>	<b>0.03</b>	<b>0.13</b>	0.01	<b>0.09</b>	<b>0.09</b>	<b>0.15</b>												
67	<b>0.04</b>	<b>0.02</b>	<b>0.05</b>	<b>0.04</b>	0.01	<b>0.05</b>	<b>0.03</b>	<b>0.01</b>	<b>0.03</b>	<b>0.03</b>	<b>0.04</b>	<b>0.03</b>	<b>0.11</b>	0.00	<b>0.06</b>	<b>0.05</b>	<b>0.07</b>	<b>0.07</b>	<b>0.08</b>	0.00	<b>0.05</b>	<b>0.05</b>	<b>0.10</b>	<b>0.02</b>											
68	0.02	0.00	0.01	<b>0.02</b>	0.02	0.01	0.01	0.00	<b>0.03</b>	0.02	<b>0.04</b>	0.00	<b>0.09</b>	-0.01	<b>0.04</b>	0.02	<b>0.04</b>	<b>0.04</b>	<b>0.05</b>	-0.01	<b>0.05</b>	<b>0.04</b>	<b>0.07</b>	0.01	0.01										
70	<b>0.03</b>	0.02	0.03	0.01	0.02	<b>0.04</b>	0.01	0.01	0.01	<b>0.04</b>	<b>0.04</b>	<b>0.03</b>	<b>0.11</b>	0.00	<b>0.04</b>	<b>0.04</b>	<b>0.07</b>	<b>0.09</b>	<b>0.10</b>	-0.02	<b>0.08</b>	<b>0.05</b>	<b>0.11</b>	0.02	<b>0.04</b>	0.00									
71	0.01	0.00	0.02	0.01	0.02	0.02	<b>0.02</b>	0.01	<b>0.03</b>	0.01	<b>0.05</b>	0.02	<b>0.10</b>	0.00	<b>0.05</b>	<b>0.02</b>	<b>0.03</b>	<b>0.05</b>	<b>0.10</b>	-0.02	<b>0.08</b>	<b>0.07</b>	<b>0.13</b>	0.01	0.01	0.00	0.01								
72	<b>0.04</b>	0.01	0.02	0.02	<b>0.04</b>	<b>0.05</b>	0.03	-0.01	0.03	0.01	0.04	0.01	<b>0.08</b>	-0.02	<b>0.06</b>	<b>0.05</b>	<b>0.07</b>	<b>0.10</b>	<b>0.04</b>	-0.02	<b>0.02</b>	0.02	<b>0.05</b>	<b>0.03</b>	<b>0.03</b>	-0.01	-0.02	0.02							
74	<b>0.03</b>	0.01	0.01	<b>0.02</b>	<b>0.03</b>	<b>0.02</b>	<b>0.03</b>	<b>0.02</b>	<b>0.04</b>	<b>0.03</b>	<b>0.07</b>	0.01	<b>0.12</b>	0.00	<b>0.03</b>	<b>0.03</b>	<b>0.05</b>	<b>0.05</b>	<b>0.11</b>	0.01	<b>0.08</b>	<b>0.08</b>	<b>0.14</b>	0.01	<b>0.03</b>	0.00	0.01	0.00	0.01						
75	<b>0.03</b>	0.01	0.03	<b>0.02</b>	0.01	<b>0.03</b>	0.03	0.02	0.02	<b>0.03</b>	<b>0.04</b>	0.01	<b>0.12</b>	0.00	<b>0.02</b>	<b>0.04</b>	<b>0.08</b>	0.03	<b>0.08</b>	0.01	<b>0.06</b>	<b>0.05</b>	<b>0.12</b>	0.01	<b>0.02</b>	0.00	0.01	0.01	0.03	-0.01					
76	<b>0.03</b>	0.01	0.02	<b>0.02</b>	0.03	-0.01	0.01	<b>0.02</b>	<b>0.03</b>	<b>0.04</b>	<b>0.05</b>	0.01	<b>0.11</b>	0.01	<b>0.02</b>	0.01	<b>0.03</b>	<b>0.06</b>	<b>0.12</b>	0.03	<b>0.11</b>	<b>0.09</b>	<b>0.12</b>	0.02	<b>0.04</b>	0.00	0.02	0.01	<b>0.03</b>	0.01	0.01				

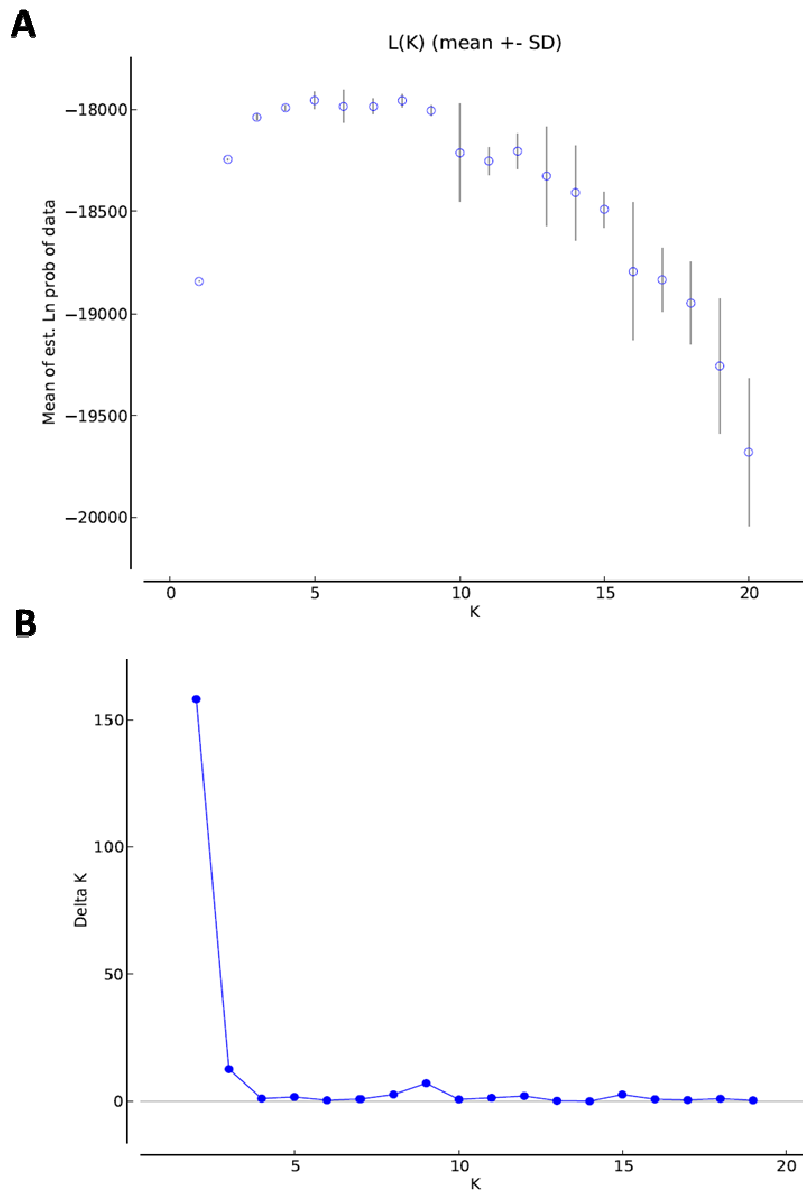
**Supplementary data (SD Table IV).** Hierarchical analysis of molecular variance (AMOVA) determining the proportion of genetic variation partitioned among the 6 geographical regions containing at least two sites with more than 10 individuals, among sites within regions and within regions.

<b>Source of variation</b>	<b>Degrees of freedom</b>	<b>Sum of square</b>	<b>Variance component</b>	<b>Percentage of variation</b>	<b>Fixation indices</b>
Between geographical regions	5	86.575	0.081	2.33	$F_{CT}$ 0.023***
Among sites within regions	24	151.004	0.116	3.34	$F_{SC}$ 0.034***
Within sites	944	2776.455	3.261	94.33	$F_{ST}$ 0.057***
	973	3014.033	3.457	100	

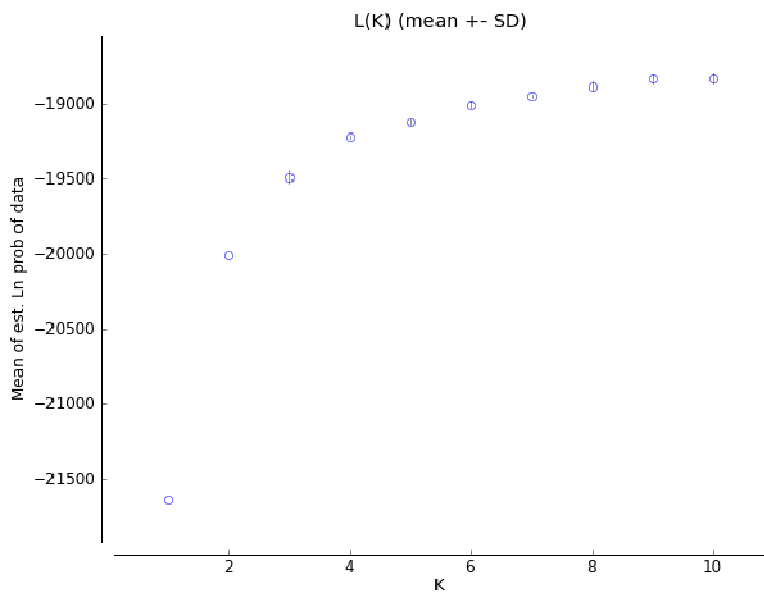
**Supplementary data (SD Table V).** Description of the 18 mitochondrial haplotypes obtained through sequencing of the COI-COII region from *A. m. unicolor* from Madagascar. For each haplotype are indicated: code, accession number, number of samples per haplotype and distribution among geographical region S(), n= number of samples per haplotype.

Names	Accession number	N	North S1-10	North-East S11-18	North West S19-23	East S24-29; S65	Hauts Plateaux S30-55	South-west S66-67	South S68-76
A1_Mad1	KF976992	14				S65 (2)	S31 (5) S32 (3) S35 (1) S33 (2)		
A1_Mad2	KF976993	17					S41 (1) S32 (2) S35 (4) S65 (5) S39 (3) S41 (3)		
A1_Mad3	KF976994	121	S2 (1) S3 (3) S4 (3) S5 (1) S7 (1) S8 (3) S9 (2) S10 (1)	S14 (2) S15 (5) S16 (2)	S19 (3) S20/21(10) S22 (4) S23 (2)	S24 (6) S25 (3) S28 (4) S29 (1) S65 (3)	S30 (1) S31 (3) S32 (1) S33 (1) S34 (1) S35 (1) S36 (1) S37 (3) S38 (1) S40 (8) S41 (2) S42 (3) S43 (4) S44 (2) S45 (3) S48 (4) S49 (3) S50 (2) S53 (1)	S66 (3) S67 (4)	S68 (3) S73 (2) S74 (2) S75 (2)
A1_Mad4	KF976995	1			S19 (1)				
A1_Mad5	KF976996	2		S18 (1) S14 (1)					
A1_Mad6	KF976997	1	S9 (1)						
A1_Mad7	KF976998	1		S18 (1)					
A1_Mad8	KF976999	1		S11 (1)					
A1_Mad9	KF977000	2		S11 (1)					S74 (1)
A1_Mad10	KF977001	3	S7 (2)		S22 (1)				
A1_Mad11	KF977002	1					S41 (1)		
A1_Mad12	KF977003	1						S66 (1)	
A1_Mad13	KF977004	2			S22 (1)	S29 (1)			
A1_Mad14	KF977005	1					S44 (1)		
A1_Mad15	KF977006	2	S4 (1)	S11 (1)					
A1_Mad16	KF977007	1							S73 (1)
A4_Mad1	KF977008	1						S66 (1)	
A4_Mad2	KF977009	1			S23 (1)				
	<b>Total</b>	173							

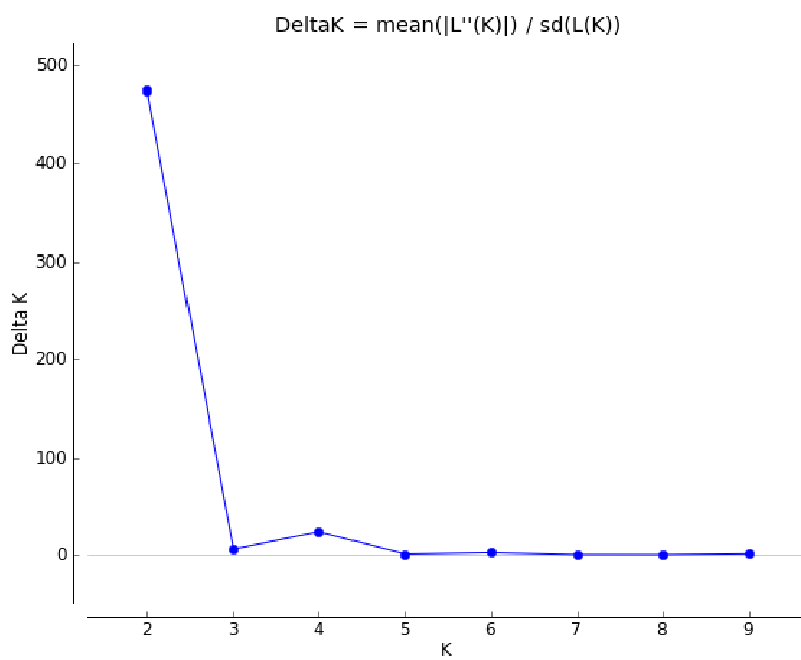
**Figure supplementary data (SD Fig. 1): A & A'.** absolute values of the likelihood distribution (mean±SD) calculated according to the formula:  $|L''(K)|=|L'(K+1)-L'(K)|$  (Evanno et al. 2005), implemented in Structure Harvester on the web, for the Malagasy dataset (A) and full dataset including Madagascar and South African individuals (A') **B.** delta K calculated as  $(\Delta k) = m|L''(K)|/s[L(K)]$  according to Evanno et al. (2005), for the Malagasy dataset (B) and the Malagasy and full dataset including Madagascar and South African individuals (B') The modal value of this distribution is the K=2 in both cases.



**A'**

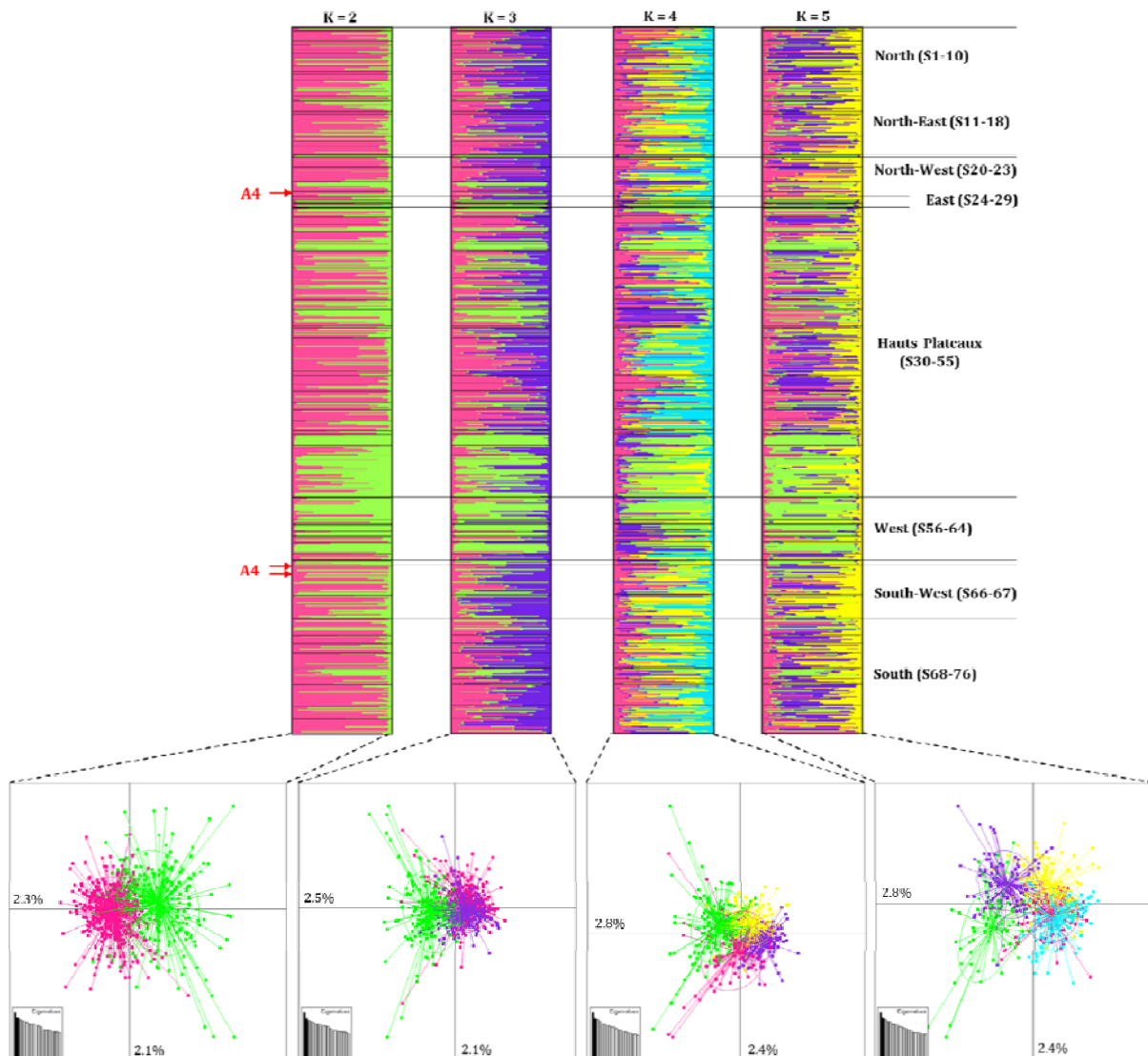


**B'**





**Figure Supplementary data (SD Fig. 2).** Intra-Madagascar population structure and Euclidean distances among genetic clusters based on 17 microsatellites *loci*. Top: STRUCTURE barplots (K = 2, 3, 4 and 5) of the 710 individuals order by sampling sites (S1-76). Bottom: PCAs of Euclidean distances among individual genotypes assigned to the different clusters (K = 2, 3, 4 and 5). Inertia percentage of each axis is indicated (using 131 variables).



**Figure Supplementary data (SD Fig. 3).** Minimum-spanning network depicting relationships between the mtDNA A1 haplotypes (519 bp) detected in Madagascar. Plain lines represent one substitution/deletion event. Dashed lines represent the deletion of 4 adjacent bases, that could result in one mutational event.

