# The mitochondrial DNA diversity of captive ruffed lemurs (*Varecia* spp.): implications for conservation

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SUPPLEMENTARY MATERIAL 1

**Methods**

*Extended molecular biology techniques*

All tissue, fresh blood and hair samples were stored at 4 °C until analysis. Blood in Whatman FTA cards was stored at room temperature. DNA from dried blood samples from Whatman FTA cards was obtained by removing two punches of approximately 3 mm in diameter and placing them in 0.2 mL tubes. The tubes were placed in a thermal cycler for 15 minutes at 80 °C. The punches were removed and allowed to cool down for 10 minutes, 1 mL of molecular grade water was added and vortexed for 5 seconds. All the water in the tubes were removed and this step was repeated one more time. We added 100 µL of water to each sample and placed them in a thermal cycler for 20 minutes at 96 °C. After 20 minutes, the DNA was re-suspended and the punches were removed from the solution. The tubes containing DNA were then stored at -20 °C.

To amplify the D-loop region, PCR was done in 50 µL final volume, consisting of 5 µL 10X Maxima Hot Start Taq buffer, 5 µL dNTP Mix (2 mM each), 1 µL 10 µM forward and reverse primers [Heavy strand dLp5 (5'-CCATCGWGATGTCTTATTTAAGRGGAA-3') (Baker et al., 1993) and Light strand dLp1.5 (5'-GCACCCAAAGCTGARRTTCTA-3') (Wyner et al., 1999)], 1.2 µL 25 mM $MgCl_2$, 0.3 µL 5U/µL Maxima Hot Start Taq DNA Polymerase (ThermoFisher Scientific, Waltham, USA), 1 µL DNA sample, and 27.5 µL molecular grade water. PCR conditions were 95 °C for 5 min followed by 95 °C for 1 min, 47 °C for 1 min and 72 °C for 1 min 30 s (35 cycles), and a final elongation step at 72 °C for 10 min. PCR products were visualised under UV light after electrophoresis in 1% agarose gels containing SYBR Safe DNA gel stain (Invitrogen). Successful amplifications were purified using the GeneJET PCR Purification Kit (ThermoFisher Scientific, Waltham, USA) and sent for Sanger sequencing to DBS Genomics (Durham University).

| SUPPLEMENTARY TABLE 1 Samples used for the analysis of mitochondrial DNA (mtDNA) diversity of wild and captive ruffed lemurs (*Varecia* spp.). | | | | | | |
|---|---|---|---|---|---|---|
| Sample ID | Museum register number | Studbook number | Source | Origin | Haplotype[1] | Group |
| L1_Mas | | ISB1271 | This study | ZooParc de Beauval, France | **Hap_1** | *V. rubra* (C) |
| L2_Rubra | | ISB1272 | This study | ZooParc de Beauval, France | **Hap_1** | *V. rubra* (C) |
| L3_Diego | | ISB1130 | This study | ZooParc de Beauval, France | **Hap_1** | *V. rubra* (C) |
| L15_Mainty | | ISB643 | This study | ZooParc de Beauval, France | **Hap_1** | *V. rubra* (C) |
| L4_Marwell_9587h_Volana_var | | ISB4293 | This study | Marwell Zoo, UK | Hap_2 | *V. variegata-EEP* (C) |
| L6_Marwell_9586h_Misangodina_var | | ISB4292 | This study | Marwell Zoo, UK | Hap_2 | *V. variegata-EEP* (C) |
| L5_Howletts_H94043_Ron_var | | ISB1176 | This study | Howletts Wild Animal Park, UK | Hap_2 | *V. variegata-EEP* (C) |
| L7_Howletts_H21294_Aramis_var | | ISB2936 | This study | Howletts Wild Animal Park, UK | Hap_2 | *V. variegata-EEP* (C) |
| L16_NMS_GH199_13_var | Z.2015.176 | ISB5003 | This study | National Museums Scotland | Hap_2 | *V. variegata-EEP* (C) |
| L22_NMS_R33_98_var | Z.2017.160.1 | ISB511 | This study | National Museums Scotland | Hap_2 | *V. variegata-EEP* (C) |
| KJ700503_BET117 | | | Baden et al., 2014 | Wild, Betampona | Hap_2 | *V. v. variegata* (W) |
| KJ700499_BET64 | | | Baden et al., 2014 | Wild, Betampona | Hap_2 | *V. v. variegata* (W) |
| AF173511_VVV163 | | ISB919 | Wyner et al., 1999 | SSP | Hap_2 | *V. variegata-SSP* (C) |
| AF173510_VVV258 | | ISB1120 | Wyner et al., 1999 | SSP | Hap_2 | *V. variegata-SSP* (C) |
| FBC_4 | | | This study | Fenn Bell Conservation Project, UK | Hap_2 | *V. variegata*-FBC (C) |
| L8_PortLympne_Voulcazo_sub | | ISB3653 | This study | Port Lympne Reserve, UK | **Hap_3** | *V. v. subcincta* (C) |
| L10_PortLympne_Ifat_sub | | ISB2220 | This study | Port Lympne Reserve, UK | **Hap_3** | *V. v. subcincta* (C) |
| L12_PortLympne_Sentenza_sub | | ISB2143 | This study | Port Lympne Reserve, UK | **Hap_3** | *V. v. subcincta* (C) |
| L9_Howletts_H20009_Clef_var | | ISB2225 | This study | Howletts Wild Animal Park, UK | Hap_4 | *V. variegata-EEP* (C) |
| L11_Howletts_H20008_Quaver_var | | ISB2224 | This study | Howletts Wild Animal Park, UK | Hap_4 | *V. variegata-EEP* (C) |
| L13_Howletts_H20007_Breve_var | | ISB2223 | This study | Howletts Wild Animal Park, UK | Hap_4 | *V. variegata-EEP* (C) |

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| L14_Howletts_H21424_Akisa_var | | ESB451 | This study | Howletts Wild Animal Park, UK | Hap_4 | *V. variegata-EEP* (C) |
| L17_NMS_PH12_14_var | Z.2014.60 | ISB197 | This study | National Museums Scotland, UK | Hap_4 | *V. variegata-EEP* (C) |
| L18_NMS_PH17_13_var | Z.2017.160.3 | ISB1033 | This study | National Museums Scotland, UK | Hap_4 | *V. variegata-EEP* (C) |
| L19_NMS_PH42_13_var | Z.2019.21 | | This study | National Museums Scotland, UK | Hap_4 | *V. variegata-EEP* (C) |
| L20_NMS_PH21_13_var | Z.2017.161.1 | ISB687 | This study | National Museums Scotland, UK | Hap_4 | *V. variegata-EEP* (C) |
| L21_NMS_RL70_97_var | | | This study | National Museums Scotland, UK | Hap_4 | *V. variegata-EEP* (C) |
| L23_NMS_RL10_97_var | Z.2000.100.1 | ISB230 | This study | National Museums Scotland, UK | Hap_4 | *V. variegata-EEP* (C) |
| L24_NMS_PH18_13_var | Z.2017.158 | ISB373 | This study | National Museums Scotland, UK | Hap_4 | *V. variegata-EEP* (C) |
| KJ700612_ZAHA4_13 | | | Baden et al., 2014 | Wild, Zahamena | Hap_4 | *V. v. variegata* (W) |
| KJ700611_ZAHA4_8 | | | Baden et al., 2014 | Wild, Zahamena | Hap_4 | *V. v. variegata* (W) |
| KJ700610_ZAHA4_4 | | | Baden et al., 2014 | Wild, Zahamena | Hap_4 | *V. v. variegata* (W) |
| KJ700609_ZAHA4_3 | | | Baden et al., 2014 | Wild, Zahamena | Hap_4 | *V. v. variegata* (W) |
| KJ700608_ZAHA4_2 | | | Baden et al., 2014 | Wild, Zahamena | Hap_4 | *V. v. variegata* (W) |
| AF173518_VVV227 | | ISB21 | Wyner et al., 1999 | SSP, Happy Hollow Zoo, California | Hap_4 | *V. variegata-SSP* (C) |
| AF173517_VVV228 | | ISB25 | Wyner et al., 1999 | SSP, Duke Lemur Center, North Carolina | Hap_4 | *V. variegata-SSP* (C) |
| AF173516_VVV225 | | ISB4 | Wyner et al., 1999 | SSP, Fort Worth Zoological Park, Texas | Hap_4 | *V. variegata-SSP* (C) |
| AF173514_VVV257 | | ISB11 | Wyner et al., 1999 | SSP, Duke Lemur Center, North Carolina | Hap_4 | *V. variegata-SSP* (C) |
| AF173512_VVV162 | | ISB32 | Wyner et al., 1999 | SSP, Gladys Porter Zoo, Texas | Hap_4 | *V. variegata-SSP* (C) |
| AF173509_VVV007 | | ISB745 | Wyner et al., 1999 | SSP, Audubon Zoo, Louisiana | Hap_4 | *V. variegata-SSP* (C) |

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| AF173508_VVV191 | | ISB252 | Wyner et al., 1999 | SSP, Detroit Zoological Society, Michigan | Hap_4 | *V. variegata-SSP* (C) |
| IVO1 | | | This study | Parc Ivoloina, Madagascar | Hap_4 | *V. variegata*-Madagascar (C) |
| FBC_1 | | | This study | Fenn Bell Conservation Project | Hap_4 | *V. variegata*-FBC (C) |
| FBC_2 | | | This study | Fenn Bell Conservation Project | Hap_4 | *V. variegata*-FBC (C) |
| FBC_3 | | | This study | Fenn Bell Conservation Project | Hap_4 | *V. variegata*-FBC (C) |
| KJ700626_RANO5_13 | | | Baden et al., 2014 | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| KJ700625_RANO5_12 | | | Baden et al., 2014 | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| KJ700624_RANO5_11 | | | Baden et al., 2014 | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| KJ700623_RANO5_10 | | | Baden et al., 2014 | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| KJ700622_RANO5_9 | | | Baden et al., 2014 | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| KJ700621_RANO5_8 | | | Baden et al., 2014 | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| KJ700620_RANO5_7 | | | Baden et al., 2014 | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| KJ700619_RANO5_6 | | | Baden et al., 2014 | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| KJ700618_RANO5_5 | | | Baden et al., 2014 | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| KJ700617_RANO5_4 | | | Baden et al., 2014 | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| KJ700616_RANO5_3 | | | Baden et al., 2014 | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| KJ700615_RANO5_2 | | | Baden et al., 2014 | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| KJ700614_RANO2_43 | | | Baden et al., 2014 | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| KJ700613_RANO322B | | | Baden et al., 2014 | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| KJ700601_VVAT2_10 | | | Baden et al., 2014 | Wild, Vatovavy | Hap_5 | *V. v. editorum*-S (W) |

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| KJ700586_TOL9 | | | Baden et al., 2014 | Wild, Tolongoina | Hap_5 | *V. v. editorum*-S (W) |
| KJ700585_TOL8 | | | Baden et al., 2014 | Wild, Tolongoina | Hap_5 | *V. v. editorum*-S (W) |
| KJ700584_TOL7 | | | Baden et al., 2014 | Wild, Tolongoina | Hap_5 | *V. v. editorum*-S (W) |
| KJ700583_TOL6 | | | Baden et al., 2014 | Wild, Tolongoina | Hap_5 | *V. v. editorum*-S (W) |
| KJ700582_TOL3 | | | Baden et al., 2014 | Wild, Tolongoina | Hap_5 | *V. v. editorum*-S (W) |
| KJ700581_TOL1 | | | Baden et al., 2014 | Wild, Tolongoina | Hap_5 | *V. v. editorum*-S (W) |
| KJ700549_LAKI5_16 | | | Baden et al., 2014 | Wild, Lakia | Hap_5 | *V. v. editorum*-S (W) |
| KJ700548_LAKI5_9 | | | Baden et al., 2014 | Wild, Lakia | Hap_5 | *V. v. editorum*-S (W) |
| KJ700547_LAKI5_8 | | | Baden et al., 2014 | Wild, Lakia | Hap_5 | *V. v. editorum*-S (W) |
| KJ700546_LAKI5_7 | | | Baden et al., 2014 | Wild, Lakia | Hap_5 | *V. v. editorum*-S (W) |
| KJ700545_LAKI5_6 | | | Baden et al., 2014 | Wild, Lakia | Hap_5 | *V. v. editorum*-S (W) |
| KJ700544_LAKI5_5 | | | Baden et al., 2014 | Wild, Lakia | Hap_5 | *V. v. editorum*-S (W) |
| KJ700543_LAKI5_4 | | | Baden et al., 2014 | Wild, Lakia | Hap_5 | *V. v. editorum*-S (W) |
| KJ700542_LAKI5_3 | | | Baden et al., 2014 | Wild, Lakia | Hap_5 | *V. v. editorum*-S (W) |
| KJ700541_LAKI5_2 | | | Baden et al., 2014 | Wild, Lakia | Hap_5 | *V. v. editorum*-S (W) |
| KJ700540_LAKI5_1 | | | Baden et al., 2014 | Wild, Lakia | Hap_5 | *V. v. editorum*-S (W) |
| KJ700534_KIAN2_28 | | | Baden et al., 2014 | Wild, Kianjavato | Hap_5 | *V. v. editorum*-S (W) |
| KJ700533_KIAN2_27 | | | Baden et al., 2014 | Wild, Kianjavato | Hap_5 | *V. v. editorum*-S (W) |
| KJ700532_KIAN138 | | | Baden et al., 2014 | Wild, Kianjavato | Hap_5 | *V. v. editorum*-S (W) |

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| KJ700531_KIAN115 | | | Baden et al., 2014 | Wild, Kianjavato | Hap_5 | *V. v. editorum*-S (W) |
| KJ700530_KIAN101A | | | Baden et al., 2014 | Wild, Kianjavato | Hap_5 | *V. v. editorum*-S (W) |
| AF493671_RANO2-43 | | | Louis et al., unpublished | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF493670_RANO2-42 | | | Louis et al., unpublished | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF493669_RANO2-40 | | | Louis et al., unpublished | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF493668_RANO2-39 | | | Louis et al., unpublished | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF173547_VVV207 | | | Wyner et al., 1999 | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF173546_VVV206 | | | Wyner et al., 1999 | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF173545_VVV201 | | | Wyner et al., 1999 | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF173544_VVV204 | | | Wyner et al., 1999 | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF173543_VVV202 | | | Wyner et al., 1999 | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF173542_VVV205 | | | Wyner et al., 1999 | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF173541_VVV198 | | | Wyner et al., 1999 | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF173540_VVV199 | | | Wyner et al., 1999 | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF173539_VVV157 | | | Wyner et al., 1999 | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF173538_VVV155 | | | Wyner et al., 1999 | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF173537_VVV152 | | | Wyner et al., 1999 | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF173536_VVV154 | | | Wyner et al., 1999 | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF173535_VVV156 | | | Wyner et al., 1999 | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |

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| AF173534_VVV153 | | | Wyner et al., 1999 | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF475904_GG | | | Louis et al., unpublished | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF475903_Vangavanga | | | Louis et al., unpublished | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF475902_Tursiops | | | Louis et al., unpublished | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF475901_AR | | | Louis et al., unpublished | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF475900_CALYPSO | | | Louis et al., unpublished | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF475899_NEONCOWBOY | | | Louis et al., unpublished | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF475898_CARL | | | Louis et al., unpublished | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF475897_SHAVETAIL | | | Louis et al., unpublished | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF475896_84COWBOY | | | Louis et al., unpublished | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF475895_RANO322B | | | Louis et al., unpublished | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF475894_RANO321B | | | Louis et al., unpublished | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF475893_RANO317 | | | Louis et al., unpublished | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF475891_RANO313 | | | Louis et al., unpublished | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF475890_RANO195 | | | Louis et al., unpublished | Wild, Ranomafana | Hap_5 | *V. v. editorum*-S (W) |
| AF475881_VVAT64 | | | Louis et al., unpublished | Wild, Vatovavy | Hap_5 | *V. v. editorum*-S (W) |
| AF475880_VVAT58 | | | Louis et al., unpublished | Wild, Vatovavy | Hap_5 | *V. v. editorum*-S (W) |
| AF475879_VVAT57 | | | Louis et al., unpublished | Wild, Vatovavy | Hap_5 | *V. v. editorum*-S (W) |
| AF475878_VVAT55 | | | Louis et al., unpublished | Wild, Vatovavy | Hap_5 | *V. v. editorum*-S (W) |

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| AF475877_VVAT44 | | | Louis et al., unpublished | Wild, Vatovavy | Hap_5 | *V. v. editorum*-S (W) |
| AF475876_VVAT35 | | | Louis et al., unpublished | Wild, Vatovavy | Hap_5 | *V. v. editorum*-S (W) |
| AF475875_VVAT30 | | | Louis et al., unpublished | Wild, Vatovavy | Hap_5 | *V. v. editorum*-S (W) |
| AF475874_VVAT23 | | | Louis et al., unpublished | Wild, Vatovavy | Hap_5 | *V. v. editorum*-S (W) |
| AF475873_VVAT01 | | | Louis et al., unpublished | Wild, Vatovavy | Hap_5 | *V. v. editorum*-S (W) |
| AF475872_KIAN10 | | | Louis et al., unpublished | Wild, Kianjavato | Hap_5 | *V. v. editorum*-S (W) |
| AF475871_KIAN19 | | | Louis et al., unpublished | Wild, Kianjavato | Hap_5 | *V. v. editorum*-S (W) |
| AF475869_KIAN45 | | | Louis et al., unpublished | Wild, Kianjavato | Hap_5 | *V. v. editorum*-S (W) |
| AF475868_KIAN46 | | | Louis et al., unpublished | Wild, Kianjavato | Hap_5 | *V. v. editorum*-S (W) |
| AF475867_KIAN100 | | | Louis et al., unpublished | Wild, Kianjavato | Hap_5 | *V. v. editorum*-S (W) |
| AF475866_KIAN115 | | | Louis et al., unpublished | Wild, Kianjavato | Hap_5 | *V. v. editorum*-S (W) |
| AF475865_KIAN137 | | | Louis et al., unpublished | Wild, Kianjavato | Hap_5 | *V. v. editorum*-S (W) |
| KJ700607_ZAH293 | | | Baden et al., 2014* | Wild, Zahamena | Hap_6 | *V. v. variegata* (W) |
| KJ700606_ZAH291 | | | Baden et al., 2014* | Wild, Zahamena | Hap_6 | *V. v. variegata* (W) |
| KJ700605_ZAH280 | | | Baden et al., 2014* | Wild, Zahamena | Hap_6 | *V. v. variegata* (W) |
| KJ700603_ZAH266 | | | Baden et al., 2014* | Wild, Zahamena | Hap_6 | *V. v. variegata* (W) |
| KJ700602_ZAH112 | | | Baden et al., 2014* | Wild, Zahamena | Hap_6 | *V. v. variegata* (W) |
| AF173524_VVV123 | | ISB1078 | Wyner et al., 1999 | SSP - Parc Ivoloina, Madagascar | Hap_6 | *V. variegata-SSP* (C) |
| AF173520_VVV129 | | ISB1436 | Wyner et al., 1999 | SSP - Parc Ivoloina, Madagascar | Hap_6 | *V. variegata-SSP* (C) |

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| VAIKO4 | | | This study | Vakona Lodge, Madagascar | Hap_6 | *V. variegata*-Madagascar (C) |
| KJ700604_ZAH267 | | | Baden et al., 2014* | Wild, Zahamena | Hap_7 | *V. v. variegata* (W) |
| KJ700600_VOLA7 | | | Baden et al., 2014* | Wild, Mangerivola | Hap_8 | *V. v. variegata* (W) |
| KJ700599_VOLA3 | | | Baden et al., 2014* | Wild, Mangerivola | Hap_8 | *V. v. variegata* (W) |
| KJ700598_VOLA1 | | | Baden et al., 2014* | Wild, Mangerivola | Hap_8 | *V. v. variegata* (W) |
| KJ700597_VAK23 | | | Baden et al., 2014* | Wild, Ambatovaky | Hap_9 | *V. v. variegata* (W) |
| KJ700595_VAK18 | | | Baden et al., 2014* | Wild, Ambatovaky | Hap_9 | *V. v. variegata* (W) |
| KJ700594_VAK17 | | | Baden et al., 2014* | Wild, Ambatovaky | Hap_9 | *V. v. variegata* (W) |
| KJ700593_VAK16 | | | Baden et al., 2014* | Wild, Ambatovaky | Hap_9 | *V. v. variegata* (W) |
| KJ700592_VAK15 | | | Baden et al., 2014* | Wild, Ambatovaky | Hap_9 | *V. v. variegata* (W) |
| KJ700591_VAK07 | | | Baden et al., 2014* | Wild, Ambatovaky | Hap_9 | *V. v. variegata* (W) |
| KJ700596_VAK22 | | | Baden et al., 2014* | Wild, Ambatovaky | Hap_10 | *V. v. variegata* (W) |
| KJ700590_VAK05 | | | Baden et al., 2014* | Wild, Ambatovaky | Hap_11 | *V. v. variegata* (W) |
| KJ700589_TORO8_15 | | | Baden et al., 2014* | Wild, Torotorofotsy | Hap_12 | *V. v. editorum*-N (W) |
| KJ700516_TAD4_50 | | | Baden et al., 2014* | Wild, Mantadia | Hap_12 | *V. v. editorum*-N (W) |
| KJ700510_VVE6_4 | | | Baden et al., 2014* | Wild, Andasibe | Hap_12 | *V. v. editorum*-N (W) |
| KJ700508_VVE6_2 | | | Baden et al., 2014* | Wild, Andasibe | Hap_12 | *V. v. editorum*-N (W) |
| KJ700507_VVE6_1 | | | Baden et al., 2014* | Wild, Andasibe | Hap_12 | *V. v. editorum*-N (W) |
| KJ700506_DAS7_2V | | | Baden et al., 2014* | Wild, Andasibe | Hap_12 | *V. v. editorum*-N (W) |

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| KJ700505_DAS7_1V | | | Baden et al., 2014* | Wild, Andasibe | Hap_12 | *V. v. editorum*-N (W) |
| AF173507_VVV192 | | ISB23 | Wyner et al., 1999 | SSP, Detroit Zoological Society, Michigan | Hap_12 | *V. variegata-SSP* (C) |
| LPA5 | | | This study | Lemurs Park, Madagascar | Hap_12 | *V. variegata*-Madagascar (C) |
| LPA6 | | | This study | Lemurs Park, Madagascar | Hap_12 | *V. variegata*-Madagascar (C) |
| KJ700588_TORO8_14 | | | Baden et al., 2014 | Wild, Torotorofotsy | Hap_13 | *V. v. editorum*-N (W) |
| KJ700587_TORO8_13 | | | Baden et al., 2014 | Wild, Torotorofotsy | Hap_13 | *V. v. editorum*-N (W) |
| KJ700553_MIZA5_12 | | | Baden et al., 2014 | Wild, Maromizaha | Hap_13 | *V. v. editorum*-N (W) |
| KJ700552_MIZA5_5 | | | Baden et al., 2014 | Wild, Maromizaha | Hap_13 | *V. v. editorum*-N (W) |
| KJ700551_MIZA4_2 | | | Baden et al., 2014 | Wild, Maromizaha | Hap_13 | *V. v. editorum*-N (W) |
| KJ700550_MIZA4_1 | | | Baden et al., 2014 | Wild, Maromizaha | Hap_13 | *V. v. editorum*-N (W) |
| KJ700509_VVE6_3 | | | Baden et al., 2014 | Wild, Andasibe | Hap_13 | *V. v. editorum*-N (W) |
| KJ700580_TANDRA4_14 | | | Baden et al., 2014 | Wild, Marotandrano | Hap_14 | *V. v. subcincta* (W) |
| KJ700579_TANDRA4_13 | | | Baden et al., 2014 | Wild, Marotandrano | Hap_14 | *V. v. subcincta* (W) |
| KJ700578_TANDRA4_12 | | | Baden et al., 2014 | Wild, Marotandrano | Hap_14 | *V. v. subcincta* (W) |
| KJ700577_TANDRA4_11 | | | Baden et al., 2014 | Wild, Marotandrano | Hap_14 | *V. v. subcincta* (W) |
| KJ700576_TANDRA4_5 | | | Baden et al., 2014 | Wild, Marotandrano | Hap_14 | *V. v. subcincta* (W) |
| KJ700575_TANDRA4_4 | | | Baden et al., 2014 | Wild, Marotandrano | Hap_14 | *V. v. subcincta* (W) |
| KJ700574_TAND39 | | | Baden et al., 2014 | Wild, Marotandrano | Hap_14 | *V. v. subcincta* (W) |
| KJ700573_TAND27 | | | Baden et al., 2014 | Wild, Marotandrano | Hap_15 | *V. v. subcincta* (W) |

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| KJ700572_TAND9 | | | Baden et al., 2014 | Wild, Marotandrano | Hap_15 | *V. v. subcincta* (W) |
| KJ700571_NOSY79 | | | Baden et al., 2014 | Wild, Nosy Mangabe | Hap_16 | *V. v. subcincta* (W) |
| KJ700570_NOSY75 | | | Baden et al., 2014 | Wild, Nosy Mangabe | Hap_16 | *V. v. subcincta* (W) |
| KJ700568_NOSY61 | | | Baden et al., 2014 | Wild, Nosy Mangabe | Hap_16 | *V. v. subcincta* (W) |
| KJ700567_NOSY60 | | | Baden et al., 2014 | Wild, Nosy Mangabe | Hap_16 | *V. v. subcincta* (W) |
| KJ700566_NOSY40 | | | Baden et al., 2014 | Wild, Nosy Mangabe | Hap_16 | *V. v. subcincta* (W) |
| KJ700565_NOSY30 | | | Baden et al., 2014 | Wild, Nosy Mangabe | Hap_16 | *V. v. subcincta* (W) |
| KJ700564_NOSY18 | | | Baden et al., 2014 | Wild, Nosy Mangabe | Hap_16 | *V. v. subcincta* (W) |
| KJ700563_NOSY17 | | | Baden et al., 2014 | Wild, Nosy Mangabe | Hap_16 | *V. v. subcincta* (W) |
| KJ700562_NOSY16 | | | Baden et al., 2014 | Wild, Nosy Mangabe | Hap_16 | *V. v. subcincta* (W) |
| KJ700569_NOSY67 | | | Baden et al., 2014 | Wild, Nosy Mangabe | Hap_17 | *V. v. subcincta* (W) |
| KJ700561_NARA5_18 | | | Baden et al., 2014 | Wild, Mananara-Nord | Hap_18 | *V. v. subcincta* (W) |
| KJ700560_NARA5_17 | | | Baden et al., 2014 | Wild, Mananara-Nord | Hap_18 | *V. v. subcincta* (W) |
| KJ700559_NARA5_16 | | | Baden et al., 2014 | Wild, Mananara-Nord | Hap_18 | *V. v. subcincta* (W) |
| KJ700558_NARA5_15 | | | Baden et al., 2014 | Wild, Mananara-Nord | Hap_18 | *V. v. subcincta* (W) |
| KJ700557_NARA5_14 | | | Baden et al., 2014 | Wild, Mananara-Nord | Hap_18 | *V. v. subcincta* (W) |
| KJ700555_NARA5_08 | | | Baden et al., 2014 | Wild, Mananara-Nord | Hap_18 | *V. v. subcincta* (W) |
| KJ700554_NARA5_07 | | | Baden et al., 2014 | Wild, Mananara-Nord | Hap_18 | *V. v. subcincta* (W) |
| KJ700556_NARA5_09 | | | Baden et al., 2014 | Wild, Mananara-Nord | Hap_19 | *V. v. subcincta* (W) |

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| KJ700539_MAB4_4 | | | Baden et al., 2014 | Wild, Manombo | Hap_20 | *V. v. editorum*-S (W) |
| KJ700538_MAB4_3 | | | Baden et al., 2014 | Wild, Manombo | Hap_20 | *V. v. editorum*-S (W) |
| KJ700537_MAB4_2 | | | Baden et al., 2014 | Wild, Manombo | Hap_20 | *V. v. editorum*-S (W) |
| KJ700536_MAB4_1 | | | Baden et al., 2014 | Wild, Manombo | Hap_20 | *V. v. editorum*-S (W) |
| KJ700535_L8 | | | Baden et al., 2014 | Wild, Manombo | Hap_20 | *V. v. editorum*-S (W) |
| KJ700529_FAN4_19 | | | Baden et al., 2014 | Wild, Fandriana | Hap_20 | *V. v. editorum*-S (W) |
| KJ700528_FAN4_18 | | | Baden et al., 2014 | Wild, Fandriana | Hap_20 | *V. v. editorum*-S (W) |
| KJ700527_FAN4_15 | | | Baden et al., 2014 | Wild, Fandriana | Hap_20 | *V. v. editorum*-S (W) |
| KJ700526_FAN4_13 | | | Baden et al., 2014 | Wild, Fandriana | Hap_20 | *V. v. editorum*-S (W) |
| KJ700525_FAN4_12 | | | Baden et al., 2014 | Wild, Fandriana | Hap_20 | *V. v. editorum*-S (W) |
| KJ700524_FAN4_10 | | | Baden et al., 2014 | Wild, Fandriana | Hap_20 | *V. v. editorum*-S (W) |
| KJ700523_FAN4_9 | | | Baden et al., 2014 | Wild, Fandriana | Hap_20 | *V. v. editorum*-S (W) |
| KJ700522_FAN2_9 | | | Baden et al., 2014 | Wild, Fandriana | Hap_20 | *V. v. editorum*-S (W) |
| KJ700521_FAN15 | | | Baden et al., 2014 | Wild, Fandriana | Hap_20 | *V. v. editorum*-S (W) |
| KJ700520_FAN9 | | | Baden et al., 2014 | Wild, Fandriana | Hap_20 | *V. v. editorum*-S (W) |
| AF173533_VVV165 | | | Wyner et al., 1999 | Wild, Manombo | Hap_20 | *V. v. editorum*-S (W) |
| AF173532_VVV167 | | | Wyner et al., 1999 | Wild, Manombo | Hap_20 | *V. v. editorum*-S (W) |
| AF173531_VVV168 | | | Wyner et al., 1999 | Wild, Manombo | Hap_20 | *V. v. editorum*-S (W) |
| AF173529_VVV151 | | | Wyner et al., 1999 | Wild, Manombo | Hap_20 | *V. v. editorum*-S (W) |

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| AF173528_VVV150 | | | Wyner et al., 1999 | Wild, Manombo | Hap_20 | *V. v. editorum*-S (W) |
| AF475889_L8 | | | Louis et al., unpublished | Wild, Manombo | Hap_20 | *V. v. editorum*-S (W) |
| AF475888_L4 | | | Louis et al., unpublished | Wild, Manombo | Hap_20 | *V. v. editorum*-S (W) |
| AF475887_L3 | | | Louis et al., unpublished | Wild, Manombo | Hap_20 | *V. v. editorum*-S (W) |
| AF475886_L2 | | | Louis et al., unpublished | Wild, Manombo | Hap_20 | *V. v. editorum*-S (W) |
| AF475885_L1 | | | Louis et al., unpublished | Wild, Manombo | Hap_20 | *V. v. editorum*-S (W) |
| AF475884_M127 | | | Louis et al., unpublished | Wild, Manombo | Hap_20 | *V. v. editorum*-S (W) |
| AF475883_M123B | | | Louis et al., unpublished | Wild, Manombo | Hap_20 | *V. v. editorum*-S (W) |
| AF475882_M92B | | | Louis et al., unpublished | Wild, Manombo | Hap_20 | *V. v. editorum*-S (W) |
| AY584494_FAN21 | | | Andriantompohavana et al., 2006 | Wild, Manombo | Hap_20 | *V. v. editorum*-S (W) |
| KJ700519_TAD4_57 | | | Baden et al., 2014 | Wild, Mantadia | Hap_21 | *V. v. editorum*-N (W) |
| KJ700518_TAD4_54 | | | Baden et al., 2014 | Wild, Mantadia | Hap_21 | *V. v. editorum*-N (W) |
| KJ700517_TAD4_53 | | | Baden et al., 2014 | Wild, Mantadia | Hap_21 | *V. v. editorum*-N (W) |
| KJ700515_TAD4_49 | | | Baden et al., 2014 | Wild, Mantadia | Hap_21 | *V. v. editorum*-N (W) |
| KJ700514_TAD4_48 | | | Baden et al., 2014 | Wild, Mantadia | Hap_21 | *V. v. editorum*-N (W) |
| KJ700513_TAD4_13 | | | Baden et al., 2014 | Wild, Mantadia | Hap_21 | *V. v. editorum*-N (W) |
| KJ700512_TAD27 | | | Baden et al., 2014 | Wild, Mantadia | Hap_21 | *V. v. editorum*-N (W) |
| KJ700511_TAD26 | | | Baden et al., 2014 | Wild, Mantadia | Hap_21 | *V. v. editorum*-N (W) |

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| KJ700504_BET118 | | | Baden et al., 2014 | Wild, Betampona | Hap_22 | *V. v. variegata* (W) |
| KJ700502_BET102 | | | Baden et al., 2014 | Wild, Betampona | Hap_22 | *V. v. variegata* (W) |
| KJ700501_BET67 | | | Baden et al., 2014 | Wild, Betampona | Hap_22 | *V. v. variegata* (W) |
| KJ700500_BET65 | | | Baden et al., 2014 | Wild, Betampona | Hap_22 | *V. v. variegata* (W) |
| KJ700498_BET52 | | | Baden et al., 2014 | Wild, Betampona | Hap_22 | *V. v. variegata* (W) |
| KJ700497_BET51 | | | Baden et al., 2014 | Wild, Betampona | Hap_22 | *V. v. variegata* (W) |
| KJ700496_BET18 | | | Baden et al., 2014 | Wild, Betampona | Hap_22 | *V. v. variegata* (W) |
| AF173527_VVV160 | | | Wyner et al., 1999 | Wild, Betampona | Hap_22 | *V. v. variegata* (W) |
| AF173526_VVV159 | | | Wyner et al., 1999 | Wild, Betampona | Hap_22 | *V. v. variegata* (W) |
| AF173525_VVV158 | | | Wyner et al., 1999 | Wild, Betampona | Hap_22 | *V. v. variegata* (W) |
| AF173523_VVV131 | | ISB1006 | Wyner et al., 1999 | SSP, Parc Ivoloina, Madagascar | Hap_22 | *V. variegata-SSP* (C) |
| LPA1 | | | This study | Lemurs Park, Madagascar | Hap_22 | *V. variegata*-Madagascar (C) |
| LPA2 | | | This study | Lemurs Park, Madagascar | Hap_22 | *V. variegata*-Madagascar (C) |
| LPA7 | | | This study | Lemurs Park, Madagascar | Hap_22 | *V. variegata*-Madagascar (C) |
| KJ700495_SIB7_10 | | | Baden et al., 2014 | Wild, Anosibe an'ala | Hap_23 | *V. v. editorum*-N (W) |
| KJ700494_SIB7_9 | | | Baden et al., 2014 | Wild, Anosibe an'ala | Hap_23 | *V. v. editorum*-N (W) |
| KJ700493_SIB7_5 | | | Baden et al., 2014 | Wild, Anosibe an'ala | Hap_23 | *V. v. editorum*-N (W) |
| KJ700492_SIB7_4 | | | Baden et al., 2014 | Wild, Anosibe an'ala | Hap_23 | *V. v. editorum*-N (W) |
| KJ700491_SIB7_3 | | | Baden et al., 2014 | Wild, Anosibe an'ala | Hap_23 | *V. v. editorum*-N (W) |
| KJ700490_SIB7_2 | | | Baden et al., 2014 | Wild, Anosibe an'ala | Hap_23 | *V. v. editorum*-N (W) |

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| KJ700489_ANOSIB17 | | | Baden et al., 2014 | Wild, Anosibe an'ala | Hap_23 | *V. v. editorum*-N (W) |
| KJ700488_ANOSIB10 | | | Baden et al., 2014 | Wild, Anosibe an'ala | Hap_23 | *V. v. editorum*-N (W) |
| KJ700487_ANOSIB8 | | | Baden et al., 2014 | Wild, Anosibe an'ala | Hap_23 | *V. v. editorum*-N (W) |
| KJ700486_ANOSIB7 | | | Baden et al., 2014 | Wild, Anosibe an'ala | Hap_23 | *V. v. editorum*-N (W) |
| AF173506_VVR197 | | | Wyner et al., 1999 | Wild | Hap_24 | V_rubra_W |
| AF173504_VVR138 | | | Wyner et al., 1999 | Wild | Hap_24 | V_rubra_W |
| AF173515_VVV226 | | ISB16 | Wyner et al., 1999 | SSP, San Antonio Zoological Gardens, Texas | Hap_25 | *V. variegata-SSP* (C) |
| AF173513_VVV196 | | ISB437 | Wyner et al., 1999 | SSP, Duke Lemur Center, North Carolina | Hap_26 | *V. variegata-SSP* (C) |
| AF475892_RANO314 | | | Louis et al., unpublished | Wild, Ranomafana | Hap_27 | *V. v. editorum*-S (W) |
| AF475870_KIAN27 | | | Louis et al., unpublished | Wild, Kianjavato | Hap_28 | *V. v. editorum*-S (W) |
| IVO2sub | | | This study | Parc Ivoloina, Madagascar | **Hap_29** | *V. v. subcincta* (C) |
| IVO3 | | | This study | Parc Ivoloina, Madagascar | **Hap_30** | *V. variegata*-Madagascar (C) |
| VAIKO1 | | | This study | Vakona Lodge, Madagascar | **Hap_30** | *V. variegata*-Madagascar (C) |
| LEMULA3 | | | This study | Lemurialand, Madagascar | **Hap_30** | *V. variegata*-Madagascar (C) |
| editorum_Tzimbazaza_female | | | This study | Tsimbazaza Zoo, Madagascar | **Hap_30** | *V. variegata*-Madagascar (C) |
| TIMBA1sub | | | This study | Tsimbazaza Zoo, Madagascar | **Hap_31** | *V. v. subcincta* (C) |
| LEMULA4sub | | | This study | Lemurialand, Madagascar | **Hap_31** | *V. v. subcincta* (C) |
| VAIKO2 | | | This study | Vakona Lodge, Madagascar | **Hap_32** | *V. variegata*-Madagascar (C) |
| VAIKO3 | | | This study | Vakona Lodge, Madagascar | **Hap_33** | *V. variegata*-Madagascar (C) |
| VAIKO5 | | | This study | Vakona Lodge, Madagascar | **Hap_33** | *V. variegata*-Madagascar (C) |
| VAIKO6 | | | This study | Vakona Lodge, Madagascar | **Hap_33** | *V. variegata*-Madagascar (C) |
| LPA3 | | | This study | Lemurs Park, Madagascar | **Hap_33** | *V. variegata*-Madagascar (C) |
| LEMULA1 | | | This study | Lemurialand, Madagascar | **Hap_34** | *V. variegata*-Madagascar (C) |

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| LPA4 | | | This study | Lemurs Park, Madagascar | **Hap_34** | *V. variegata*-Madagascar (C) |
| LEMULA2 | | | This study | Lemurialand, Madagascar | **Hap_35** | *V. variegata*-Madagascar (C) |
| editorum_Tzimbazaza_male | | | This study | Tsimbazaza Zoo, Madagascar | **Hap_36** | *V. variegata*-Madagascar (C) |
| [1]Haplotypes in bold are newly described in this study. | | | | | | |
| EEP = European Endangered Species Programme, SSP = Species Survival Plan Programmes, FBC = Fenn Bell Conservation Project, W = Wild, C = Captive, S/N = south or north of the Mangoro River. | | | | | | |
| Andriantompohavana, R. Zaonarivelo, J.R., Engberg, S.E., Randriamampionona, R., McGuire, S.M., Shore, G.D., et al. (2006) Mouse lemurs of northwestern Madagascar with a description of a new species at Lokobe Special Reserve. *Occasional Papers, Museum Texas Tech University*, 259, 1–23. | | | | | | |

SUPPLEMENTARY FIG. 1 Mismatch distributions of selected groups of ruffed lemurs *Varecia variegata* showing observed and expected nucleotide differences in pairwise sequence comparisons of D-loop mtDNA sequences. Expected distributions obtained under a neutral model of evolution. EEP = European Endangered Species Programme, SSP = Species Survival Plan Programmes, W = wild, C = captive, S/N = south or north of the Mangoro River.