**SUPPLEMENTARY INFORMATION**

Article title

# Geographic barriers and Pleistocene climate change shaped patterns of genetic variation in the Eastern Afromontane biodiversity hotspot.

Authors

Mario Mairal[*1], Isabel Sanmartín[1], Alberto Herrero[1], Lisa Pokorny[2], Pablo Vargas[1], Juan J. Aldasoro[†3], and Marisa Alarcón[†3]

[1] *Real Jardín Botánico (RJB-CSIC), 28014 Madrid, Spain*

[2] *Royal Botanic Gardens, Kew (RBGK), Richmond, Surrey, TW9 3DS, UK*

[3] *Instituto Botánico de Barcelona (IBB-CSIC-ICUB), 08038 Barcelona, Spain*

**SUPPORTING INFORMATION**

## Appendix S1 Expanded Material and Methods

### S1.1 DNA amplification, sequencing and alignment DNA

DNA of 72 individuals was extracted using the DNeasy Plant Mini Kit (QIAGEN Inc., California, USA) according to the manufacturer's instructions, from 18-23 mg of silica-gel dried leaves obtained from the fresh plant tissue collected from the field expeditions. PCR amplifications were performed using an Eppendorf Mastercycler Epgradient S (Westbury, NY). Table S3.3 shows the primer sequences for each region. The three intergenic spacers were amplified using the following PCR settings: 1) for *rpl*32-*trn*$L_{UAG}$ : 1 min pretreatment at 95°C, 30 cycles of 1 min at 96°C, 1 min at 52°C, and 2 min at 72°C; 2) for *trn*$S_{GCU}$-*trn*$_{GUCC}$ : 1 min pre-treatment at 96°C, 30 cycles of 45 s at 96°C, 1 min at 54°C, and 1 min 30s at 72°C; and 3) for *pet*B1365–*pet*D738: 1 min pre-treatment at 96°C, 30 cycles of 1 min at 95°C, 2 min at 49°C, and 2 min at 72°C. All these reactions ended with a final extension step for 10 min at 72°C. Amplified products were treated with ExoSAP-IT (USB Corporation, Ohio) and submitted to Macrogen Inc. (Seoul, South Korea) for sequencing. We had difficulties to amplify some of the herbarium specimens, for this we developed internal primers and performed nested-PCRs. The resulting sequence data were assembled and edited using SeqEd v. 1.0.3 software (Applied Biosystems, California, USA). Sequences for each region were aligned using MAFFT software (Katoh *et al*., 2005). Sequences were manually adjusted where necessary by following alignment rules described in Kelchner (2000), and checked through the use of Bioedit software (Hall, 1999).

### S1.2 Divergence time analyses

Model-fit of nucleotide substitution models was assessed via the Bayesian Information Criterion (BIC) as implemented in jModelTest 0.1.1 (Posada, 2008). The higher-level data set was calibrated with fossil-derived secondary age estimates (see Mairal *et al*. 2015), while the tree prior was unlinked to apply a coalescent constant size model to the population-level data set and a stochastic birth–death (Yule) prior to the species-level one (Mairal *et al*. 2015). The clock model was set to an uncorrelated log-normal prior to accommodate the change in mutation rate from species to populations, with a uniform distribution for the ucld.mean ($10^{-4}$– $10^{-1}$) and a default exponential distribution for the ucld.stdev; the substitution model was set to GTR+G. Two MCMC chains were run for 50 million generations, sampling parameters every 1000 generation. We used Tracer v1.6 (Rambaut *et al*. 2007) to verify the following: whether a stationary distribution was attained, whether there was convergence among chains and whether effective sample sizes (ESS values) were >200 for all parameters. A 10% burn-in of the sampled populations was discarded (5 million). Post-burn-in trees were summarized into a maximum clade credibility tree using TREEANNOTATOR v.1.6.1, with mean values and 95% credible intervals for nodal ages, and were visualized in FIGTREE 1.3.1 (Rambaut & Drummond 2009).

### S1.3 Bayesian discrete phylogeographic analyses

This method implements a continuous-time Markov Chain (CTMC) process to model biogeographic evolution, in which the discrete states correspond to geographic locations of the sequences and transition rates between states to migration rates or dispersal between areas (Ronquist & Sanmartín, 2011). Migration rates between areas and the geodispersal rate scaler were modelled using default gamma prior distributions

(Lemey *et al*., 2009). Two replicate searches of 20 million generations each, sampling every 1000th generation, were combined in TreeAnnotator, after removing the 10% burn-in, to produce a maximum clade credibility (MCC) tree. Bayesian stochastic search variable selection (BSSVS, Lemey *et al*., 2009) was used to identify the rates (colonization routes) that are best supported by the data.

**Appendix S2** Supplemental Tables and Figures.

**Table S2.1.** List of the visited localities for this study and description of the sampling.

| Country | Hedberg collections | *C. eminii* collections (X: explored, but not found) | Commentaries (not explored not explored) |
|---|---|---|---|
| **Ethiopia** | Damot near source of Abay river | - | deforested |
| | Burye, road to Lake Tana | Dembecha (north of Debre Marcos), A9979 | deforested, a few scattered trees contain plants of *C. eminii* |
| | | Gifta (north of Debre Marcos), A9982 | |
| | Shoa: Kachise (near Kachissy) | x | deforested |
| | Shoa, Entotto | x | deforested |
| | Shoa: Addis Ababa | x | deforested |
| | Shoa: Little Akaki river | x | deforested |
| | Shoa: Mannaghescia (Mannagascià) | x | deforested |
| | Shoa: Holeta (Oletta), valle di Metcha Coriccia | x | deforested |
| | Borana people, N of Agere Mariam | Agere Maryan, Yirga Chefé Road, A10060 | *C. eminii* abundant |
| | Bale | Harenna Forest, Negele to Goba road, A10317 and A10322 | *C. eminii* abundant |
| | Gallanot exploredSidamo: Welega (Wallega), Kidami | not explored | not explored |
| | Gallanot explored Sidamo: Shisha, Lake Uombo (Wombo) | not explored | not explored |
| | Gallanot explored Sidamo: Cencia | not explored | not explored |
| **Uganda** | Ruwenzori, Kangasaba | x | sparsely forested |
| | Ruwenzori, Mahoma Valley near Nyabitabu (Nyakalengija) | Ruwenzori Mts, Nyabitaba track, Sánchez Meseguer & Aldasoro 18 | *C. eminii* abundant |
| | Mt. Elgon, W. slope between Bulambuli y Butandiga | Mt. Elgon, Sánchez Meseguer & Aldasoro 69 | *C. eminii* abundant |
| | Mt. Elgon, Buginyanya | Mt. Elgon, Sánchez Meseguer & Aldasoro 71 | *C. eminii* present |
| | N of Mt. Elgon at Kyessweri R. Sipi and Kabururon R. Sundet (Sebei) | Mt. Elgon, Sánchez Meseguer & Aldasoro 75 | *C. eminii* present |
| | Mbale dist: Mt Elgon, Bumoni | not explored | not explored |
| | Bugisu distr., Bulago | not explored | not explored |
| | Karamoja Distr: Rom Mts | not explored | not explored, entry forbidden |
| | Karamoja Distr: Langia Mts | not explored | not explored, entry forbidden |

| | | | |
|---|---|---|---|
| | Karamoja Distr: Monte Kadam | not explored | not explored, entry forbidden |
| | Karamoja Distr: Monte Moroto | not explored | not explored, entry forbidden |
| **South Sudan** | Imatong Mts. | not explored | not explored, entry forbidden |
| | Lomwaga Mts. | not explored | not explored, entry forbidden |
| **Kenya** | Kapolet (Kaboret) forest | not explored | not explored |
| | Cherangani Hills | x | sparsely forested |
| | Elgeyo Escarpment | x | sparsely forested |
| | Kapsabet, Yala river | x | sparsely forested |
| | Nyanza Province | x | sparsely forested |
| | Aberdare Range | Aberdare Mts, Sánchez Meseguer & Aldasoro 103 | *C. eminii* abundant |
| | Aberdare (east), Tuso | x | sparsely forested |
| | Dimbilil river, SW Mau | not explored | not explored |
| | Mt. Kenya, Embu distr., Thiba camp | x | sparsely forested |
| | Mt. Kenya, Kirinyaga distr. | x | sparsely forested |

Despite intensive sampling effort (two field expeditions in 2009, 2015 to Ethiopia and one in 2010 to Kenya-Uganda), we failed to find *C. eminii* in many of the historically recorded localities (Hedberg, 1961). During the present field work, the number of localities where *C. eminii* grow in the northern Ethiopian Plateaus, and the Elgon Mt. was much smaller than that reported by Hedberg (1961). The highlands of northern Ethiopia as well as many parts of Kenya, now given over to cultivation or pasture, are largely devoid of forest vegetation. We noted that much of the forests in these localities were highly degraded, having either completely disappeared or being threatened by the advancement of extensive agriculture, cattle rising, forestry and other human activities. Although that species appeared there in previous studies, currently the absence of forested patches and adequate phorophytes likely impeded this species to survive there. All these difficulties hampered a deeper sampling of *C. eminii*. Moreover, some of the localities could not be visited due to security concerns, such as: South Sudan, eastern Congo, Burundi, South Sudan, and eastern Congo borders were not allowed to be visited during our sampling period. Besides, there are some places in north of Uganda where the authorities prohibit any entry.

**Table S2.2.** Plastid sequences of *C. eminii* newly generated for this study: localities and geographical coordinates, sample collector, and GenBank accessions number. Voucher specimens of all sampled species and populations were deposited in the herbarium of the *Instituto Botánico de Barcelona*- CSIC (BC).

| **Voucher specimen** *Canarina eminii* Asch. ex Schweinf. | **trnS–trnG accession number** | | ***pet*B–*pet*D accession number** | | ***rpl*32-*trn*L$^{UAG}$ accession number** |
|---|---|---|---|---|---|
| Ethiopia, Gifta, Debre Markos to Bahir Da Road, N of Bure, 09/08/2009, 2478m, 10º 50' 29" N, 37º 02' 56" E, *Aldasoro & Alarcón 9982* (BC) | KF028817 | KM189335 | KF028856 | KM189234 | KM189267 |
| | KF028818 | KM189336 | KF028857 | KM189235 | KM189268 |
| | KF028819 | KM189337 | KF028858 | KM189236 | KM189269 |
| | KM189334 | KM189338 | KM189233 | KM189237 | KM189270 |
| Ethiopia, between Dembecha and Bure, Debre Markos to Bahir Dar Road, 08/08/2009, 2493m, 10°40'50" N, 37°14'53" E, *Aldasoro & Alarcón 9979* (BC) | KF028814 | KM189331 | KF028853 | KM189230 | KM189260 |
| | KF028815 | KM189332 | KF028854 | KM189231 | KM189261 |
| | KF028816 | KM189333 | KF028855 | KM189232 | KM189262 |
| | KM189330 | | KM189229 | | KM189263 |
| Ethiopia, Agere Maryan, Yirga Chefé Road, 14/08/2009, 2434m, 6º 04' 54" N, 38º 14' 33" E, *Aldasoro & Alarcón 10060* (BC) | KF028820 | KF028825 | KF028859 | KF028863 | KM189275 |
| | KF028821 | KF028826 | KF028860 | KF028864 | KM189276 |
| | KF028822 | KM189339 | KF028861 | KF028865 | KM189277 |
| | KF028823 | KM189340 | KF028862 | KM189238 | KM189278 |
| | KF028824 | | | KM189239 | KM189279 |

| | | | | | |
|---|---|---|---|---|---|
| Ethiopia, Harenna Forest, Negele to Goba Road, 19/08/2009, 2182m, 6º 42' 03" N, 39º 43' 35" E, *Aldasoro & Alarcón 10322* (BC) | KF028827 | KF028831 | KF028866 | KF028870 | KM189284 |
| | KF028828 | KF028832 | KF028867 | KF028871 | KM189285 |
| | KF028829 | KM189341 | KF028868 | KM189240 | KM189286 |
| | KF028830 | | KF028869 | | KM189287 |
| Ethiopia, Harenna Forest, Negele to Goba Road, 19/08/2009, 1939m, 6º 39' 3"N, 39º 43' 57"E, *Aldasoro & Alarcón 10317* (BC) | KF028833 | | KF028872 | | KM189290 |
| Uganda, Mt. Elgon 31/08/2010, 2230 m, 1º 19' 44" N, 34º, 24' 58" E, *Sánchez-Meseguer & Aldasoro 69* (BC) | KF028834 | KM189345 | KF028873 | KM189244 | KM189292 |
| | KF028835 | KM189346 | KF028874 | KM189245 | KM189293 |
| | KF028836 | KM189347 | KF028875 | KM189246 | KM189294 |
| | KF028837 | KM189348 | KF028876 | KM189247 | KM189295 |
| | KF028838 | KM189349 | KF028877 | KM189248 | KM189296 |
| | KF028839 | KM189350 | KF028878 | KM189249 | KM189297 |
| | KM189342 | KM189351 | KM189241 | KM189250 | KM189298 |
| | KM189343 | KM189352 | KM189242 | KM189251 | KM189299 |
| | KM189344 | | KM189243 | | KM189300 |
| Uganda, Ruwenzori Mts, 27/08/2010, 2599 m, 0º 21' 35" N, 29º 58' 20" E, *Sánchez-Meseguer & Aldasoro18* (BC) | KF028840 | KF028846 | KF028879 | KF028885 | KM189309 |
| | KF028841 | KM189353 | KF028880 | KM189252 | KM189310 |
| | KF028842 | KM189354 | KF028881 | KM189253 | KM189311 |
| | KF028843 | KM189355 | KF028882 | KM189254 | KM189312 |
| | KF028844 | KM189356 | KF028883 | KM189255 | KM189313 |
| | KF028845 | KM189357 | KF028884 | KM189256 | KM189314 |
| Kenya, Aberdare Mts, 03/09/2010, 2467 m, 0º 45' 52" N, 36º 44' 35" E, *Sánchez-Meseguer & Aldasoro103* (BC) | KF028847 | KF028852 | KF028886 | KF028891 | KM189321 |
| | KF028848 | KM189358 | KF028887 | KM189257 | KM189322 |
| | KF028849 | KM189359 | KF028888 | KM189258 | KM189323 |
| | KF028850 | KM189360 | KF028889 | KM189259 | KM189324 |
| | KF028851 | | KF028890 | | KM189325 |
| Kenya, K2 N. Cherangani Hills Kaibwibich. IS377 | XXX | | XXX | | XXX |
| Tanzania, Mbeya: Rungwe District. Livingstone Mountains; foot trail above Bumbigi. IS379 | XXX | | XXX | | XXX |
| Tanzania, Mbeya: Rungwe District. Road between Igoma and Kitulo on south slope of Mporoto Mountains. IS378 | XXX | | XXX | | XXX |
| Tanzania, Tukuyu district. IS382 | XXX | | XXX | | XXX |

| | | | |
|---|---|---|---|
| Tanzania, Rungwe District. Mwakeleli, vincinity of Mwatesi River. IS381 | XXX | XXX | XXX |
| Uganda, Mount Elgon, Sasa trail. IS376 | XXX | XXX | XXX |
| Rwanda, Gikongoro Prefecture: Route Butare-Cyangugu, vers km 60. IS384 | XXX | XXX | XXX |
| Burundi, Teza. IS386 | XXX | XXX | XXX |
| Malawi, Misuku District, Sllindi Forest, Misuku Hills. IS 383 | XXX | XXX | XXX |

**Table S2.3.** Specific primer pairs used in the amplification and sequencing of plastid markers in *C. eminii*.

| Primer name's | Primer Sequence (5'–3') | Author primer's |
|---|---|---|
| ***trn*S$^{GCU}$ -F** | GCC GCT TTA GTC CAC TCA GC | Hamilton, 1999 |
| ***trn*G$^{UCC}$ -R** | GAA CGA ATC ACA CTT TTA CCA C | Hamilton, 1999 |
| ***pet*B$^{1365}$-F** | TTG ACY CGT TTT TAT AGT TTA | Löhne and Borsch, 2005 |
| ***pet*D$^{738}$-R** | AAT TTA GCY CTT AAT ACA GG | Löhne and Borsch, 2005 |
| ***rpl*32-F** | CAG TTC CAA AAA AAC GTA CTT C | Shaw *et al*., 2007 |
| ***trnL*$^{UAG}$-R** | CTG CTT CCT AAG AGC AGC GT | Shaw *et al*., 2007 |

**Table S2.4.** Hierarchical analysis of molecular variance (AMOVA) for *C. eminii* based on AFLP data at different levels.

| **AMOVA for all populations** | | | | | | |
|---|---|---|---|---|---|---|
| **Groupings** | **Source of variation** | **d.f.** | **Sum of squares** | **Variance components** | **Percentage of variation** | **Φ Statistics** |
| **1 group** | Among populations | 5 | 2966.483 | 51.70776 | 40.42 | - |
| | Within populations | 55 | 4192.042 | 76.21894 | 59.58 | $\Phi_{ST}$= 0.4042 |
| | **Total** | **60** | **7158.525** | **127.92670** | | |
| **2 groups: Eastern Rift-Western Rift:** | Among groups | 1 | 712.698 | 5.17812 | 3.98 | $\Phi_{CT}$= 0.0398 |
| 1) **Western Rift**: Gifta, Dembecha, Mt. Elgon and Ruwenzori Mts. | Among populations | 4 | 2253.785 | 48.69257 | 37.43 | $\Phi_{SC}$= 0.3898 |
| 2) **Eastern Rift**: Agere Maryan, Harenna Forest and Aberdare Mts. | Within populations | 55 | 4192.042 | 76.21894 | 58.59 | $\Phi_{ST}$= 0.4141 |
| | **Total** | **60** | **7158.525** | **130.08963** | | |
| **3 groups:** | Among groups | 2 | 1443.203 | 3.30884 | 2.57 | $\Phi_{CT}$= |

| Groupings | Source of variation | d.f. | Sum of squares | Variance components | Percentage of variation | Φ Statistics |
|---|---|---|---|---|---|---|
| | | | | | | 0.0257 |
| 1. Abyssinian Plateau (Gifta and Dembecha) | Among populations | 3 | 1523.280 | 49.05.202 | 38.15 | $\Phi_{SC}$= 0.3916 |
| 2. Agere Maryan, Harenna Forest and Aberdare Mts | Within populations | 55 | 4192.042 | 76.21894 | 59.28 | $\Phi_{ST}$= 0.4072 |
| 3. Elgon and Rwenzori Mts. | **Total** | **60** | **7158.525** | **128.57979** | | |
| **4 groups:** | | | | | | |
| 1. Abyssinian Plateau (Gifta and Dembecha) | Among groups | 3 | 2187.769 | 14.13032 | 10.89 | $\Phi_{CT}$= 0.1089 |
| 2. Agere Maryan, Harenna Forest and Aberdare Mts. | Among populations | 2 | 778.714 | 39.34721 | 30.34 | $\Phi_{SC}$= 0.3405 |
| 3. Elgon Mts. | Within populations | 55 | 4192.042 | 76.21894 | 58.77 | $\Phi_{ST}$= 0.4123 |
| 4. Rwenzori Mts | **Total** | **60** | **7158.525** | **129.69647** | | |

| **AMOVA for Eastern Rift populations** | | | | | | |
|---|---|---|---|---|---|---|
| **Groupings** | **Source of variation** | **d.f.** | **Sum of squares** | **Variance components** | **Percentage of variation** | **Φ Statistics** |
| **1 group in the Eastern Rift:** | Among populations | 2 | 778.714 | 38.93272 | 32.87 | |
| Harar massif (Agere Maryan, Harenna Forest) and Aberdare Mts. | Within populations | 21 | 1669.869 | 79.51757 | 67.13 | $\Phi_{ST}$= 0.3287 |
| | **Total** | **23** | **2448.583** | **118.45029** | | |
| **2 groups in the Eastern Rift** | Among groups | 1 | 395.356 | 4.91574 | 4.09 | $\Phi_{CT}$= 0.0409 |
| 1. Harar massif: Agere Maryan and Harenna Forest | Among populations | 1 | 383.358 | 35.87003 | 29.82 | $\Phi_{SC}$= 0.3109 |
| 2. Aberdare Mts. | Within populations | 21 | 1669.869 | 79.51757 | 66.10 | $\Phi_{ST}$= 0.3390 |
| | **Total** | **23** | **2448.583** | **120.30335** | | |

| **AMOVA for Western Rift populations** | | | | | | |
|---|---|---|---|---|---|---|
| **Groupings** | **Source of variation** | **d.f.** | **Sum of squares** | **Variance components** | **Percentage of variation** | **Φ Statistics** |
| **1 group Western:** | Among populations | 2 | 1475.071 | 55.03159 | 42.59 | |
| Debre Markos, Elgon and Rwenzori Mts. | Within populations | 34 | 2522.173 | 74.18155 | 57.41 | $\Phi_{ST}$= 0.42590 |
| | **Total** | **36** | **3997.243** | **129.21314** | | |
| **2 groups in the Western Rift** | Among groups | 1 | 730.505 | -11.89280 | -9.42 | $\Phi_{CT}$= -0.0942 |
| 1. Abyssinian plateau: Debre Markos | Among populations | 1 | 744.565 | 63.99118 | 50.67 | $\Phi_{SC}$= 0.4631 |
| 2. Elgon and Rwenzori Mts. | Within populations | 34 | 2522.173 | 74.18155 | 58.74 | $\Phi_{ST}$= 0.4126 |
| | **Total** | **36** | **3997.243** | **126.27993** | | |

| **AMOVA for Ethiopian plateaus populations** | | | | | | |
|---|---|---|---|---|---|---|
| **Groupings** | **Source of variation** | **d.f.** | **Sum of squares** | **Variance components** | **Percentage of variation** | **Φ Statistics** |
| **2 groups Eastern Plateau - Western Plateau:** | Among groups | 1 | 726.589 | 12.54985 | 9.28 | $\Phi_{CT}$= 0.0928 |
| 1. Abyssinian Plateau: Gifta and Dembecha | Among populations | 1 | 383.358 | 34.89585 | 25.81 | $\Phi_{SC}$= 0.2845 |
| 2. Harar Plateau: Agere Maryan and Harenna Forest | Within populations | 30 | 2633.083 | 87.76944 | 64.91 | $\Phi_{ST}$= 0.3509 |
| | **Total** | **32** | **3743.030** | **135.21515** | | |

**Table S2.5.** Migration routes supported by Bayes Factor comparisons with a cut off value >3 using Bayesian stochastic search variable selection (BSSVS, Lemey *et al*., 2009).

| Migration routes | Bayes Factor |
|---|---|
| Harenna Forest - Aberdare Mts. | 7.6760 |
| Rwenzori - Mt. Elgon | 5.3979 |
| Mt. Elgon - Rwenzori | 4.6847 |
| Aberdare Mts - Harenna Forest | 4.0291 |
| Rwenzori - Southern Mountain sky-islands | 3.8762 |
| Rwenzori - Abyssinian massif | 3.6395 |
| Mt.Elgon - Southern Mountain sky-islands | 3.3288 |
| Southern Mountain sky-islands - Rwenzori | 3.1858 |
| Southern Mountain sky-islands - Mt. Elgon | 3.0084 |

**Table S2.6.** Pairwise $F_{ST}$ between populations of *C. eminii* with nuclear data (below) and geographic distances in km (above).

| | Gifta | Dembecha | Agere Maryan | Harenna Forest | Mt. Elgon | Rwenzori Mts. | Aberdare Mts. |
|---|---|---|---|---|---|---|---|
| **Gifta** | - | 28 | 546 | 545.9 | 1091.84 | 1398.95 | 1115.13 |
| **Dembecha** | 0.0801 | - | 520.37 | 517.78 | 1080.55 | 1396.78 | 1098 |
| **Agere Maryan** | 0.2157 | 0.1593 | - | 177.87 | 675.86 | 1112.88 | 611.1 |
| **Harenna Forest** | 0.3028 | 0.2352 | 0.1706 | - | 836.87 | 1287.61 | 735.28 |
| **Mt. Elgon** | 0.3555 | 0.2877 | 0.2985 | 0.3698 | - | 503.09 | 266.4 |
| **Rwenzori Mts.** | 0.3306 | 0.2627 | 0.2887 | 0.2174 | 0.3779 | - | 752 |
| **Aberdare Mts.** | 0.3041 | 0.2390 | 0.2058 | 0.2247 | 0.3302 | 0.3142 | - |

**Figure S2.1.**

Nested analysis of the Platycodoneae dataset. Numbers above branches indicate mean ages and numbers below branches indicate Bayesian PP. For more details see Mairal *et al.* 2015a.

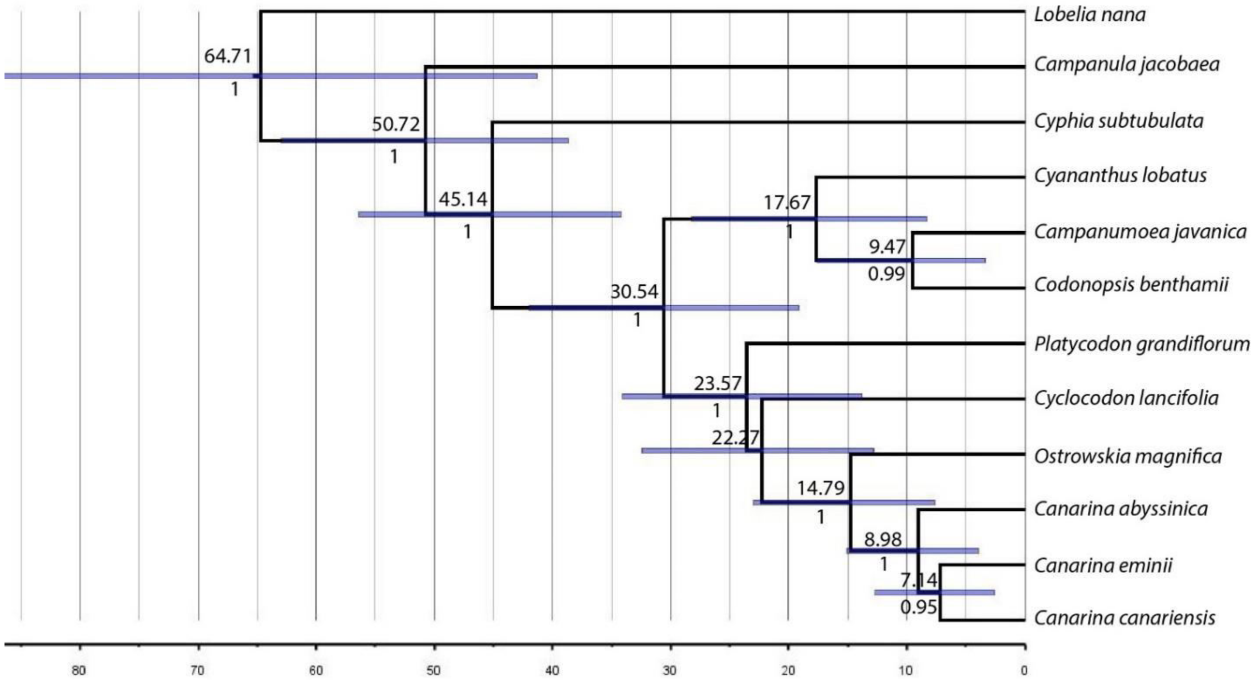
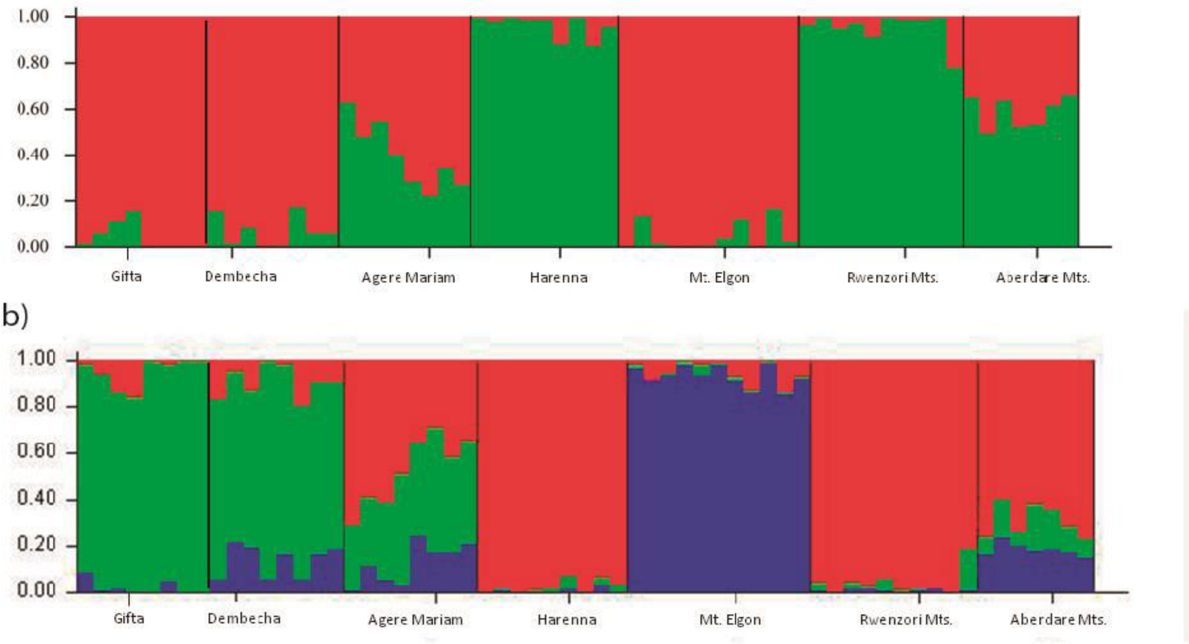
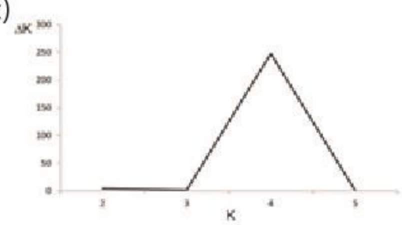


**Figure S2.2.** Histograms showing the Bayesian clustering of individuals within populations (STRUCTURE) for a) K=2 and b) K=3. Colours represent the proportion of individual membership to each inferred Bayesian group. c) The estimated probability of the likelihood function according to the Evanno method for STRUCTURE analyses of *C. eminii*. Maximum Δ$K$ values correspond to the presumed true number of K clusters**.**

a)




c)




| K groups | L(K) | stdev | L'(K) | L"(K) | [L"K] | delta K |
|---|---|---|---|---|---|---|
| 1 | -22408,89 | 1,319 | | | | |
| 2 | -20282,01 | 153,241 | 2126,88 | -664,81 | 664,81 | 4,338 |
| 3 | -18819,94 | 112,03 | 1462,07 | -146,942 | 146,94 | 1,311 |
| 4 | -17504,812 | 5,574 | 1315,127 | -1380,602 | 1380,6 | 247,661 |
| 5 | -17570,287 | 636,51 | -65,475 | 488,148 | 488,15 | 0,766 |

**Figure S2.3.** Neighbor tree (A) and neighbor-net (B) for the AFLP data for individuals of *C. eminii*. Numbers are bootstrap values from neighbor-joining analysis using Nei-Li distances over 1000 bootstrap replicates. To root the neighbor tree, we used a population of *C. abyssinica* collected in Ethiopia (ab).

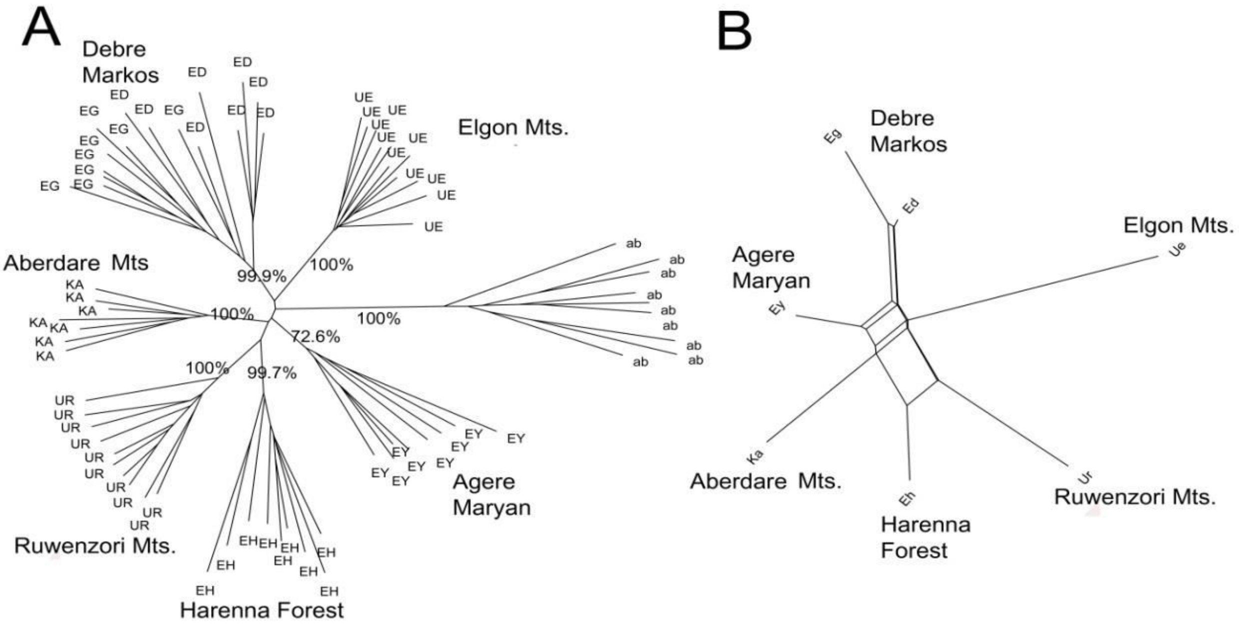



**Figure S2.4.** Principal coordinates analysis of AFLP data for individuals of *C. eminii.*

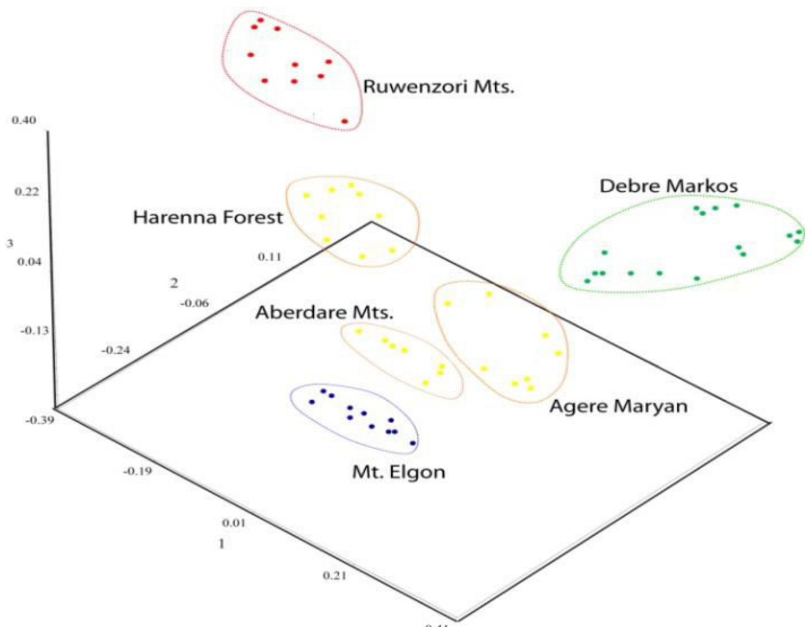


**Figure S2.5.** Regression plot among pairwise $F_{ST}$ and logarithms of geographical distances. Acronyms are E: Elgon Mt., R: Rwenzori, D: Debre Markos (Gifta and Dembecha), Y: Agere Maryan, A: Abredare Mts., H: Harenna Forest.

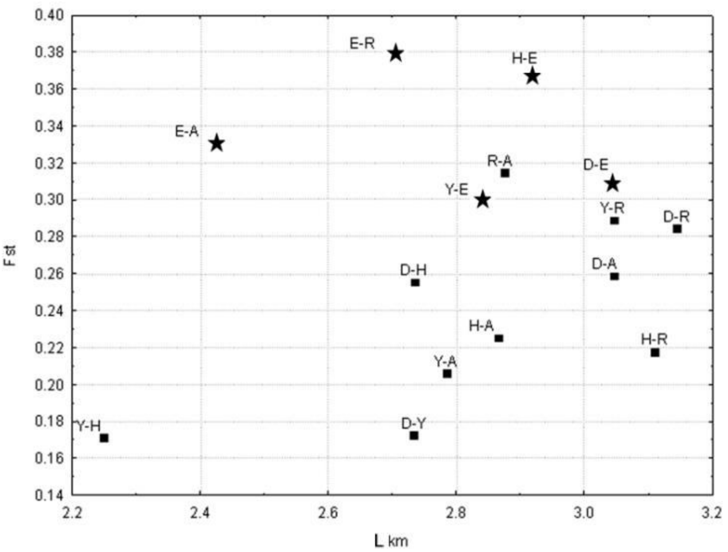
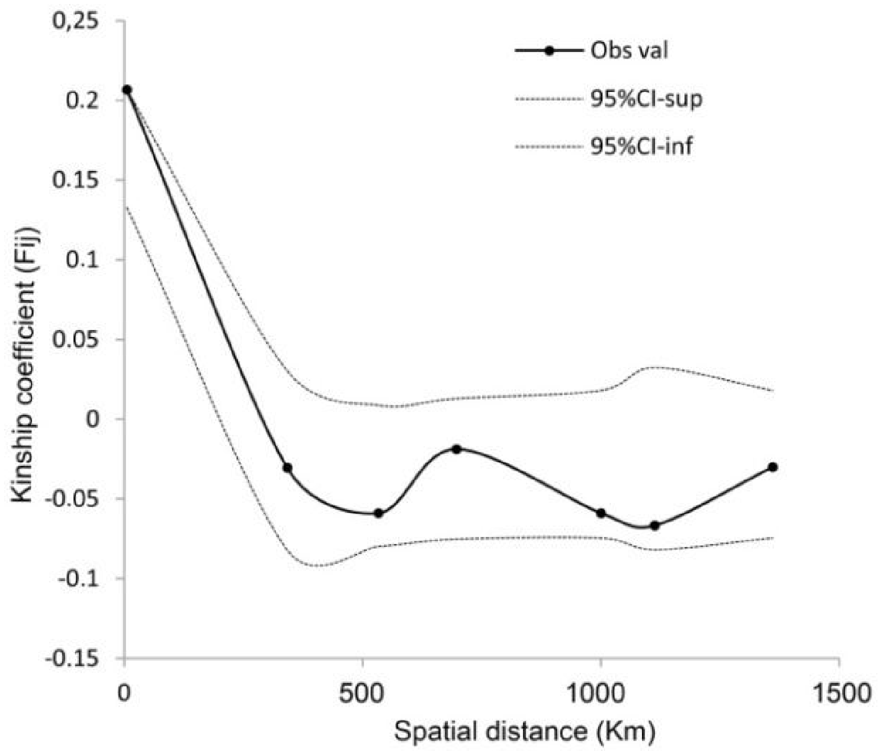



**Figure S2.6.** Distances intervals for *C. eminii* populations in SPAGeDi analysis (dotted lines show the 95% confidence level).



**Appendix S3** Supplemental references


Arctander, P., Johansen, C. & Coutellec-Vreto, M.A. Phylogeography of three closely related African bovids (tribe Alcelaphini). *Molecular Biology and Evolution.* **16**, 1724-1739 (1999).

Belay, G. & Mori, A. Intraspecific phylogeographic mitochondrial DNA (D-loop) variation of Gelada baboon, *Theropithecus gelada*, in Ethiopia. *Biochemical Systematics and Ecology*. **34**, 554-561 (2006).

Braginets, O.P., Minakawa, N., Mbogo, C.M. & Ya, G. Population genetic structure of the African malaria mosquito *Anopheles funestus* in Kenya. *The American journal of tropical medicine and hygiene*. **69**, 303-308 (2003).

Colangelo, P. *et al*. A mitochondrial phylogeographic scenario for the most widespread African rodent, *Mastomys natalensis*. *Biological Journal of the Linnean Society*. **108**, 901-916 (2013).

Derero, A., Gailing, O. & Finkeldey. R. Maintenance of genetic diversity in *Cordia africana* Lam., a declining forest tree species in Ethiopia. *Tree Genetics & Genomes*. **7**, 1-9 (2010).

Dubach, J. *et al*. Molecular genetic variation across the southern and eastern geographic ranges of the African lion, *Panthera leo*. *Conservation Genetics*. **6**, 15-24 (2005).

Evanno, G., Regnaut, S. & Goudet, J. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*. **14**, 2611-2620 (2005).

Evans, B.J., Bliss, S.M., Mendel, S.A. & Tinsley, R.C. The Rift Valley is a major barrier to dispersal of African clawed frogs (*Xenopus*) in Ethiopia. *Molecular Ecology*. **20**, 4216-4230 (2011).

Field, L.M. *et al*. Analysis of genetic variability in *Anopheles arabiensis* and *Anopheles gambiae* using microsatellite loci. *Insect Molecular Biology*. **8**, 287-297 (1999).

Girman, D.J. *et al*. Patterns of population subdivision, gene flow and genetic variability in the African wild dog (*Lycaon pictus*). *Molecular Ecology*. **10**, 1703-1723 (2001).

Hall, T.A. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic acids symposium series*. **41**, 95-98 (1999).

Hamilton, M. Four primers pairs for the amplification of chloroplast intergenic regions with intraspecific variation. *Molecular Ecology*. **8**, 521-523 (1999).

Kadu, C.A.C., *et al*. Divergent pattern of nuclear genetic diversity across the range of the Afromontane *Prunus africana* mirrors variable climate of African highlands. *Annals of Botany*. **111**, 47-60 (2013).

Katoh, K., Kuma, K., Toh, H. & Miyata, T. MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Research*. **33**, 511-518 (2005).

Kelchner, S.A. The Evolution of Non-Coding Chloroplast DNA and Its Application in Plant Systematics. *Annals of the Missouri Botanical Garden*. **87**, 482 (2000).

Krafsur, E.S. Population structure of the tsetse fly *Glossina pallidipes* estimated by allozyme, microsatellite and mitochondrial gene diversities. *Insect Molecular Biology*. **11**, 37-45 (2002).

Lehmann, T. *et al*. The Rift Valley complex as a barrier to gene flow for *Anopheles gambia*e in Kenya. *Journal of Heredity*. **90**, 613-621 (1999).

Lehmann, T. *et al*. Brief communication. The Rift Valley complex as a barrier to gene flow for *Anopheles gambiae* in Kenya: the mtDNA perspective. *Journal of Heredity*. **91**, 165-168 (2000).

Lehmann, T. *et al*. Population Structure of *Anopheles gambiae* in Africa. *Journal of Heredity*. **94**, 133-147 (2003).

Löhne, C. & Borsch, T. Molecular evolution and phylogenetic utility of the *petD* group II intron: a case study in basal angiosperms. *Molecular Biology and Evolution*, **22**. 317-332 (2005).

Matthee, C.A. & Robinson, T.J. Molecular phylogeny of the springhare, *Pedetes capensis*, based on mitochondrial DNA sequences. *Molecular Biology and Evolution*- **14**, 20-29 (1997).

Muchugi, A. *et al*. Genetic structuring of important medicinal species of genus *Warburgia* as revealed by AFLP analysis. *Tree Genetics & Genomes*. **4**, 787-795 (2008).

Nicolas, V. *et al*. Phylogeographic structure and regional history of *Lemniscomys striatus* (Rodentia: Muridae) in tropical Africa. *Journal of Biogeography*. **35**, 2074-2089 (2008).

Omondi, S.F. *et al*. Genetic Diversity and Population Structure of *Acacia senegal* (L) Willd. in Kenya. *Tropical Plant Biology*. **3**, 59-70 (2010).

Pitra, C., Hansen, A.J., Lieckfeldt, D. & Arctander, P. An exceptional case of historical outbreeding in African sable antelope populations. *Molecular Ecology*. **11**, 1197-1208 (2002).

Pokorny, L., Oliván, G., Shaw, A. Phylogeographic patterns in two southern hemisphere species of *Calyptrochaeta* (Daltoniaceae, Bryophyta). *Systematic Botany*. **36**, 542-553 (2011).

Posada, D. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*. **25**, 1253-1256 (2008).

Rambaut A., Drummond A.J. & Suchard M. Tracer v1. 6. http://tree.bio.ed.ac.uk/software/tracer/ (2007)

Rambaut, A. & Drummond, A. FigTree v1.3.1. http://tree. bio. ed. ac. uk/software/figtree (2009).

Ronquist, F. & Sanmartín, I. Phylogenetic methods in biogeography. *Annual Review of Ecology, Evolution, and Systematics*. **42**, 441 (2011).

Sertse, D., Gailing, O., Eliades, N.G. & Finkeldey, R. Anthropogenic and natural causes influencing population genetic structure of *Juniperus procera* Hochst. ex Endl. in the Ethiopian highlands. *Genetic Resources and Crop Evolution*. **58**, 849-859 (2011).

Shaw, J., Lickey, E.B., Schilling, E.E. & Small, R.L. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. *American Journal of Botany*. **94**, 275-288 (2007).

Silvestrini, M., *et al*. Genetic diversity and structure of Ethiopian, Yemen and Brazilian *Coffea arabica* L. accessions using microsatellites markers. *Genetic Resources and Crop Evolution*. **54**, 1367-1379 (2007).