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Primer Note

Characterization of microsatellite markers in two exploited African trees, *Entandrophragma candollei* and *E. utile* (Meliaceae)[1]

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- *Premise of the study:* Multiplexes of nuclear microsatellite primers were developed to investigate population genetic structure and diversity in two exploited African rainforest trees: *Entandrophragma candollei* and *E. utile* (Meliaceae).
- *Methods and Results:* Microsatellite isolation was performed simultaneously on two nonenriched genomic libraries after next-generation sequencing. We developed 16 and 22 polymorphic markers for *E. candollei* and *E. utile* in three and four multiplexes, respectively. The number of alleles ranged from two to 17 for *E. candollei* and from three to 19 for *E. utile*. Mean expected and observed heterozygosity ranged between 0.75 ± 0.13 and 0.55 ± 0.23 for *E. candollei* and between 0.73 ± 0.10 and 0.49 ± 0.2 for *E. utile*.
- *Conclusions:* These sets of nuclear microsatellite markers constitute useful tools for exploring gene flow patterns in these two *Entandrophragma* species.

Key words: *Entandrophragma*; gene flow; Meliaceae; microsatellites; next-generation sequencing.

The genus *Entandrophragma* C. DC. (Meliaceae) includes emblematic African trees, growing in both humid and dry African forests. It is one of the most economically important African genera, comprising 11 species among which five are intensively exploited for their wood. Known under the commercial names kosipo and sipo, *E. candollei* Harms and *E. utile* (Dawe & Sprague) Sprague are distributed from Sierra Leone to Uganda and from the Democratic Republic of Congo to Angola. They are pollinated by insects, and their seeds are dispersed by wind. They have undergone extreme logging in many African countries since 1970 and are now registered as vulnerable species on the IUCN Red List (Hawthorne, 1998). The sustainable management of these timber species is therefore urgent. To this end, we developed for each species highly polymorphic nuclear microsatellite markers (nSSRs), which will be used to study patterns of spatial genetic diversity and gene flow (mating system, pollen and seed dispersal).

METHODS AND RESULTS

***Microsatellite development*—**Next-generation sequencing is a rapid method for acquiring a large quantity of genomic data, allowing the identification of nSSRs. Due to the low transferability of *E. cylindricum* (Sprague) Sprague nSSRs in other *Entandrophragma* species (Garcia et al., 2004), we isolated new nSSRs for *E. candollei* and *E. utile* separately. The total genomic DNA was extracted from leaf tissue of one specimen from each species (GEM09 and GEM11, Appendix 1) using the cetyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle, 1987). Following Rohland and Reich (2012) and Mariac et al. (2014), nonenriched genomic libraries were constructed after shearing, sizing, DNA end-repair, tagging by blunt ligation, real-time PCR, and Illumina MiSeq sequencing (GIGA platform, Liège, Belgium). The resulting 144 ± 2-bp-long paired-end reads were aligned using PANDAseq (Masella et al., 2012), providing 419,184 and 528,740 reads, respectively, for *E. candollei* and *E. utile*. Microsatellite motifs were identified using a QDD pipeline (Meglécz et al., 2009). We obtained 58,113 (13.86%) and 52,216 (10.99%) sequences containing at least one nSSR motif, including 1234/671 di- and 201/53 trinucleotide repeats for *E. candollei* and *E. utile*, respectively. We then selected 112 candidate loci for *E. candollei* and 67 for *E. utile* from QDD output files using the following criteria: (i) a minimum 20-bp distance between the primers and the microsatellite motif, (ii) a minimum of seven microsatellite repetitions, (iii) only one microsatellite motif present in the fragment, (iv) GC content of the fragment between 20% and 60%, and (v) expected PCR product size between 100 and 300 bp. For each species, amplification tests were then done on 48 loci, using the distribution size of the candidate loci as a subselection criterion to facilitate multiplex definition in the next steps.

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Table 1. Characterization of 16 polymorphic nuclear microsatellite loci isolated from *Entandrophragma candollei*.

Locus[a]	Primer sequences (5′–3′)	Fluorescent label[b]	Repeat motif	Allele size range (bp)	GenBank accession no.
Multiplex mix 1					
EnC-ssr4	F: AAAGAATTCCAAGCTGGCCT R: ATGAACTTCCGGTGCAGATT	Q1-6-FAM	$(AC)_{21}$	194–222	KY048359
EnC-ssr13	F: TCCATGCCCATAAATTCACA R: GGCATAAAGCTTGCAACACA	Q2-NED	$(AG)_{14}$	118–156	KY048362
EnC-ssr26	F: TCTGCAGAAACGGACACTTG R: CCAATAAACATATGCGTTCCC	Q3-VIC	$(AG)_{21}$	132–166	KY048365
EnC-ssr32	F: TGAAGCTAAGCGTTTGCTGA R: TGAAGAACCTAGAAAGCCGAA	Q3-VIC	$(AC)_{13}$	204–212	KY048368
EnC-ssr36	F: GCGACCATCATGATACCACT R: TCCGGTGCTCTTAACTTTGG	Q3-VIC	$(AG)_{12}$	306–334	KY048370
EnC-ssr42	F: TAGGCTCGGTTCTTTCTCCC R: CACACGTAGCTTTCCCACAA	Q4-PET	$(AG)_{13}$	220–255	KY048372
EnC-ssr48	F: TCCTCAACATTAACAGCTCTCAC R: GAGGTGCGATGGATTGAGAT	Q4-PET	$(AG)_{10}$	167–175	KY048374
Multiplex mix 2					
EnC-ssr9	F: AGATCGCGTTCTCCTCCAC R: GTCCCAATTTGCCTGAAGAG	Q1-6-FAM	$(AG)_{15}$	210–238	KY048360
EnC-ssr24	F: TGCCGTGAGCTAATGATGTC R: AGTGTTAAATGTGCGCGATG	Q2-NED	$(ATC)_{10}$	182–210	KY048364
EnC-ssr29	F: CTTGTTGAACCAAATGATCCC R: GTGTTTCATCGAAATTGCCG	Q3-VIC	$(AG)_{23}$	136–186	KY048367
EnC-ssr33	F: TCCACTCGGTCTACAAGTATAACA R: ATTCGATGGAGAAGCCACAT	Q3-VIC	$(AG)_{16}$	235–269	KY048369
EnC-ssr38	F: AGGAGTCGGCTTCTATGCTG R: CAATCACTGATGGATGCAAA	Q4-PET	$(AG)_{15}$	188–218	KY048371
Multiplex mix 3					
EnC-ssr12	F: TGCCACTGTGGTTGGTTT R: TTAAGCATAAATGCGTCGGG	Q1-6-FAM	$(AC)_{10}$	144–182	KY048361
EnC-ssr16	F: ATTGCGTTCCAGACGTTCTT R: ACATTTCGTTTCATGCTCCC	Q2-NED	$(AG)_{14}$	158–202	KY048363
EnC-ssr27	F: ACAATTGCCTGCACCTTCTT R: CTGGTCCCTACTGAGGGTCA	Q3-VIC	$(AG)_{16}$	180–212	KY048366
EnC-ssr43	F: AATGCTCACATCACAAGCCA R: CGGCAGGGATGTTGTAGTTC	Q4-PET	$(AG)_{16}$	193–229	KY048373

[a] Optimal annealing temperature was 57°C for all loci.
[b] Q1 = TGTAAAACGACGGCCAGT; Q2 = TAGGAGTGCAGCAAGCAT; Q3 = CACTGCTTAGAGCGATGC; Q4 = CTAGTTATTGCTCAGCGGT (Q1 after Schuelke [2000]; Q2–Q4 after Culley et al. [2008]).

Microsatellite marker selection and simplex reactions—The amplification of these 48 loci was tested on two individuals per species (GEM09 and FM1355 for *E. candollei*, GEM11 and FM1818 for *E. utile*) using the following PCR conditions: 1.5 µL buffer (10×), 0.6 µL $MgCl_2$ (25 mM), 0.45 µL dNTPs (10 mM each), 0.3 µL of each primer (0.2 µM), 0.08 µL TopTaq DNA Polymerase 5 U/µL (QIAGEN, Venlo, The Netherlands), 1.5 µL of template DNA (of ca. 10–50 ng/µL), and brought to a total volume of 15 µL with purified water. Thermal cycler conditions were: 94°C for 3 min; 30 PCR cycles of 94°C for 30 s, 57°C and 55°C for 45 s (respectively for *E. candollei* and *E. utile*), and 72°C for 1 min; and a final extension at 72°C for 10 min. PCR products were mixed with 9 µL of TE 1× and visualized using the QIAxcel DNA Screening Kit (method AL420; alignment markers 15–5000 bp; size marker 100–2500 bp; QIAGEN). We obtained 34 positive amplifications for *E. candollei* and 44 for *E. utile*. This set of markers was tested individually (using the above-described PCR conditions) to exclude all unreadable and bad amplification loci. Finally, 16 and 22 (respectively for *E. candollei* and *E. utile*) readable loci were retained to define nSSR multiplexes and test polymorphism. In this aim, we added one of the four fluorochrome linkers (Q1–Q4; Micheneau et al., 2011; Tables 1, 2) to the 5′ end of the forward primer of each locus.

Multiplex reactions and polymorphism tests—PCR amplification was performed using the QIAGEN Multiplex PCR Kit in a 15-µL volume of 0.3 µL of the reverse (0.2 µM) and 0.1 µL of the forward (0.07 µM) primers with a Q1–Q4 universal sequence at the 5′ end, 0.15 µL of Q1–Q4 labeled primer (0.2 µM each), 7.5 µL of Type-it Microsatellite PCR Kit, H_2O, and 1.5 µL of DNA. PCR program conditions were: initial denaturation at 95°C for 3 min; followed by 30 PCR cycles of 95°C for 30 s, with 57°C or 55°C for 90 s (respectively for *E. candollei* and *E. utile*), and 72°C for 1 min; and a final elongation at 60°C for 30 min. The nSSR polymorphism was investigated in three populations of *E. candollei* and four populations of *E. utile* (Appendix 1). The 16 polymorphic loci for *E. candollei* and the 22 polymorphic loci for *E. utile* were combined in three and four multiplexes, respectively (Tables 1, 2) using Multiplex Manager 1.0 software (Holleley and Geerts, 2009). We added 3 µL of 5× Q-solution and adjusted the volume of the reverse primer labeled by Q-tailed fluorescent Q1 to Q4 based on the number of loci containing the corresponding tail in the final multiplex.

Using 1.5 µL of PCR product, 12 µL of Hi-Di Formamide (Life Technologies, Carlsbad, California, USA), and 0.3 µL of MapMarker 500 labeled with DY-632 (Eurogentec, Seraing, Belgium), all individuals were genotyped using an ABI3730 sequencer (Applied Biosystems, Lennik, The Netherlands; ULB-EBE platform). Microsatellite profiles of each individual were analyzed with Peak Scanner software version 1.0 (Applied Biosystems). One or two alleles per individual and per locus were found, suggesting that *E. candollei* and *E. utile* are diploids. For each locus, we estimated the number of alleles (A), observed heterozygosity (H_o), expected heterozygosity (H_e), inbreeding coefficient (F), and null allele frequency (r) using INEst 1.0 (Chybicki and Burczyk, 2008). Deviations from Hardy–Weinberg equilibrium (HWE) were measured with SPAGeDi (Hardy and Vekemans, 2002).

***Microsatellite marker data analysis in* E. candollei *and* E. utile**—In *E. candollei*, the 16 polymorphic loci exhibited up to 17 alleles per locus and population, with mean A and H_e per population ranging from 7.3 to 8.9 and 0.67 to 0.75, respectively (Table 3). Significant deviation from HWE was observed in all populations for two loci (EnC-ssr9, EnC-ssr13), and in at least one population

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Table 2. Characterization of 22 polymorphic nuclear microsatellite loci isolated from *Entandrophragma utile*.

Locus[a]	Primer sequences (5′–3′)	Fluorescent label[b]	Repeat motif	Allele size range (bp)	GenBank accession no.
Multiplex mix 1					
EnU- ssr1	F: AGATAGGAAGACGGCAGCAG R: TGTCATGTGATTGTGAGCCA	Q1-6-FAM	$(AAG)_9$	218–266	KY048375
EnU- ssr6	F: AAATCCACAATTTGACCGGA R: TCATTGATTGATGCATTGCC	Q1-6-FAM	$(AC)_{12}$	161–209	KY048377
EnU- ssr13	F: TTAAGGCATGTGGAAGAGGG R: AATGCACCCTTACTTGACGG	Q2-NED	$(AG)_{10}$	223–271	KY048380
EnU- ssr19	F: GCCATTAGGCAGCAAATATGA R: TCTGCAGTAACTGTGGAGCTTT	Q2-NED	$(AG)_{15}$	138–186	KY048384
EnU- ssr35	F: CTTTAACCCAGATCGCCAAA R: GGTTCTGATCACCATTGCAG	Q3-VIC	$(AG)_{15}$	114–143	KY048390
EnU- ssr39	F: ATGACACAAGCATATGCCCA R: TCTTCTTGTTTGTGGTAGCGAA	Q4-PET	$(AC)_{10}$	239–287	KY048392
EnU- ssr42	F: GGCCAACCAGCTAAACCCTA R: CACGTGTAAACGTTTGTGGG	Q4-PET	$(AG)_{12}$	138–186	KY048394
Multiplex mix 2					
EnU- ssr10	F: TCGAATACTAGCTCCTTGGCA R: GGAAGAGCTTCTCAACTAAGCC	Q1-6-FAM	$(AG)_{10}$	130–178	KY048379
EnU- ssr17	F: CGACTTGCCACTTACCACTTT R: GAAATCGGTTTGAGACGCAT	Q2-NED	$(AG)_{12}$	155–203	KY048383
EnU- ssr23	F: CAGGCGCTGTCAGTTGATTA R: TGTGTTGATGGGTTGTCACC	Q2-NED	$(AG)_9$	200–248	KY048386
EnU- ssr31	F: GCATGTAAAGGATGAACGTGG R: TCAAAGAAAGGGTTCAAGACC	Q3-VIC	$(AG)_{10}$	159–207	KY048388
EnU- ssr43	F: ACATTCACTCGCCAATCACA R: GTATTGGCTTAGGCGGCAT	Q4-PET	$(AG)_{11}$	142–190	KY048395
Multiplex mix 3					
EnU- ssr9	F: AATGCATCTCCCTGCAAGTT R: CACCTTCACTGACTAACCCG	Q1-6-FAM	$(AC)_{17}$	111–159	KY048378
EnU- ssr14	F: AGCTGAAAGGAGTTCTGCCA R: TGGTCGACCTAAATGGCTTC	Q2-NED	$(AT)_{12}$	238–286	KY048381
EnU- ssr27	F: TCGAGGAAATATTTGGACAGC R: TCAGCCGTAGCCTTAACTTGA	Q3-VIC	$(AC)_{10}$	179–227	KY048387
EnU- ssr41	F: AGGGCTGAGAGTCCTTGTCA R: TAGGTCTGGGAATTGGAGCA	Q4-PET	$(AAG)_9$	149–197	KY048393
Multiplex mix 4					
EnU- ssr2	F: ATTCGCATGCATACACCGTA R: CAAGTTGCTTGCTGCTGTTC	Q1-6-FAM	$(AAG)_9$	210–258	KY048376
EnU- ssr16	F: ATTCTCCCACTGCCAATCAC R: ACCGATTATGAGCGCCTTG	Q2-NED	$(AC)_{12}$	192–240	KY048382
EnU- ssr22	F: GCATCTGAAGGGAATTGAGG R: TCCCTGAGTCACTCCTCAGC	Q2-NED	$(AG)_{14}$	118–148	KY048385
EnU- ssr34	F: GAGGAGTCACGACACCTTCA R: TGCAAGGTCAGAAAGCAGAA	Q3-VIC	$(AC)_{10}$	124–172	KY048389
EnU- ssr38	F: TTGCTGATATGGATCGGATG R: ACCTGAAACAGCCAAAGCTC	Q4-PET	$(AC)_{11}$	197–245	KY048391
EnU-ssr45	F: GGGTATGGATGACCTAGAAGAAA R: CACCAAATATGAACCCTAGATCC	Q4-PET	$(AG)_{10}$	116–164	KY048396

[a] Optimal annealing temperature was 55°C for all loci.
[b] Q1 = TGTAAAACGACGGCCAGT; Q2 = TAGGAGTGCAGCAAGCAT; Q3 = CACTGCTTAGAGCGATGC; Q4 = CTAGTTATTGCTCAGCGGT (Q1 after Schuelke [2000]; Q2–Q4 after Culley et al. [2008]).

for five other loci, in part due to the presence of null alleles (Table 3). Nevertheless, null allele frequencies were always below 0.20 for 14 loci.

For *E. utile*, the 22 loci showed up to 19 alleles per locus and population, with mean A and H_e per population ranging from 7.09 to 8.5 and 0.69 to 0.73, respectively (Table 4). Significant deviation from HWE was observed in all populations for six loci, here also in part due to null alleles (Table 4). Nevertheless, null allele frequencies were always below 0.20 for 15 loci.

***Cross-amplification in* E. congoense *and* E. angolense**—These sets of markers were also tested with the same PCR conditions on *E. congoense* (Pierre & De Wild.) A. Chev. and *E. angolense* (Welw. ex C. DC.) C. DC. (Appendix 1). A total of eight and six loci developed on *E. utile* amplified on *E. angolense* and *E. congoense*, respectively; at least five of these loci were monomorphic (Table 5). For primers developed in *E. candollei*, four and three successfully amplified on *E. angolense* and *E. congoense*, respectively, and two were monomorphic (Table 5). The developed markers have been deposited in GenBank (Tables 1, 2), and the physical specimens from which markers were developed have been deposited in BioSample (submission ID SAMN06009795 [*E. candollei*] and SAMN06009796 [*E. utile*]).

CONCLUSIONS

In this paper, we developed 16 and 22 polymorphic nSSR markers for *E. candollei* and *E. utile*, respectively. These markers will be useful to study intraspecific diversity and gene flow within both species, allowing the implementation of sustainable conservation programs for populations of these vulnerable species (Hawthorne, 1998).

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TABLE 3. Genetic characterization of the 16 polymorphic microsatellite loci for four populations of *Entandrophragma candollei*.[a]

	Campo Ma'an (n = 18)					Loundougou (n = 47)					Yangambi (n = 17)					Mindourou (n = 18)				
Locus	A	H_e	H_o	F[b]	r	A	H_e	H_o	F[b]	r	A	H_e	H_o	F[b]	r	A	H_e	H_o	F[b]	r
EnC-ssr4	6	0.57	0.41	0.28	0.11 ± 0.07	4	0.40	0.28	0.30**	0.10 ± 0.05	4	0.40	0.27	0.35	0.12 ± 0.08	3	0.53	0.31	0.43*	0.13 ± 0.08
EnC-ssr13	12	0.82	0.50	0.40***	0.14 ± 0.06	9	0.73	0.37	0.49***	0.19 ± 0.05	9	0.84	0.73	0.13	0.06 ± 0.05	7	0.74	0.43	0.43**	0.04 ± 0.03
EnC-ssr26	14	0.89	0.94	–0.06	0.03 ± 0.02	11	0.76	0.72	0.05	0.04 ± 0.03	8	0.89	0.67	0.26*	0.10 ± 0.06	11	0.85	0.81	0.05	0.05 ± 0.04
EnC-ssr32	3	0.52	0.53	–0.01	0.07 ± 0.06	3	0.56	0.40	0.28	0.12 ± 0.05	3	0.54	0.46	0.15	0.09 ± 0.07	4	0.48	0.63	–0.32	0.05 ± 0.04
EnC-ssr36	2	0.67	0.00	1.00	0.89 ± 0.07	2	0.53	0.00	1.00**	0.85 ± 0.21	1	0.00	0.00	—	0.94 ± 0.05	1	0.00	0.00	—	0.87 ± 0.08
EnC-ssr42	7	0.73	0.94	–0.30*	0.03 ± 0.03	12	0.85	0.87	–0.03	0.02 ± 0.01	9	0.84	0.93	–0.12	0.03 ± 0.03	10	0.84	0.86	–0.02	0.04 ± 0.04
EnC-ssr48	2	0.23	0.00	1.00**	0.23 ± 0.11	3	0.15	0.11	0.30	0.10 ± 0.06	2	0.14	0.14	–0.04	0.11 ± 0.09	2	0.08	0.08	0.00	0.12 ± 0.1
EnC-ssr9	12	0.89	0.56	0.38**	0.12 ± 0.06	11	0.88	0.66	0.25***	0.10 ± 0.04	10	0.81	0.53	0.35**	0.11 ± 0.06	12	0.91	0.69	0.25*	0.08 ± 0.05
EnC-ssr24	7	0.76	0.71	0.07	0.06 ± 0.05	7	0.65	0.37	0.44***	0.17 ± 0.05	8	0.78	0.43	0.46**	0.15 ± 0.08	7	0.75	0.41	0.46**	0.16 ± 0.07
EnC-ssr29	17	0.95	0.94	0.01	0.02 ± 0.02	17	0.92	0.87	0.05	0.03 ± 0.02	12	0.91	0.80	0.13	0.06 ± 0.04	17	0.95	0.88	0.08	0.03 ± 0.03
EnC-ssr33	12	0.83	0.67	0.20	0.06 ± 0.04	7	0.68	0.63	0.08	0.04 ± 0.03	7	0.62	0.63	–0.01	0.05 ± 0.04	8	0.75	0.69	0.09	0.06 ± 0.05
EnC-ssr38	7	0.83	0.83	–0.01	0.04 ± 0.04	10	0.83	0.79	0.05	0.03 ± 0.02	7	0.64	0.38	0.42***	0.13 ± 0.07	8	0.85	0.64	0.25	0.10 ± 0.06
EnC-ssr12	10	0.83	0.89	–0.07	0.03 ± 0.03	9	0.82	0.71	0.14	0.05 ± 0.03	9	0.84	0.67	0.21	0.08 ± 0.06	6	0.81	0.54	0.34*	0.13 ± 0.07
EnC-ssr16	12	0.88	0.89	–0.01	0.04 ± 0.03	16	0.92	0.79	0.14**	0.05 ± 0.03	13	0.92	0.93	–0.02	0.03 ± 0.03	14	0.93	0.75	0.20*	0.06 ± 0.04
EnC-ssr27	9	0.85	0.71	0.17	0.08 ± 0.05	11	0.85	0.44	0.49***	0.19 ± 0.05	7	0.83	0.63	0.26**	0.10 ± 0.06	7	0.83	0.44	0.48***	0.18 ± 0.07
EnC-ssr43	11	0.85	0.78	0.09	0.04 ± 0.04	11	0.74	0.81	–0.09	0.02 ± 0.01	7	0.78	0.77	0.02	0.05 ± 0.04	10	0.83	0.81	0.02	0.04 ± 0.04
Multilocus average	8.94	0.75	0.64	0.174	0.02 ± 0.1	8.94	0.7	0.55	0.23	0.02 ± 0.01	7.25	0.67	0.56	0.56	0.03 ± 0.02	7.94	0.69	0.56	0.2	0.04 ± 0.03

Note: A = number of alleles; F = fixation index; H_e = expected heterozygosity; H_o = observed heterozygosity; n = number of individuals; r = frequency of null alleles.

[a] Locality and voucher information are provided in Appendix 1.

[b] Significant deviation from Hardy–Weinberg equilibrium at $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

LITERATURE CITED

CHYBICKI, I. J., AND J. BURCZYK. 2008. Simultaneous estimation of null alleles and inbreeding coefficients. *Journal of Heredity* 100: 106–113.

CULLEY, T., S. WELLER, A. SAKAI, AND K. PUTNAM. 2008. Characterization of microsatellite loci in the Hawaiian endemic shrub *Schiedea adamantis* (Caryophyllaceae) and amplification in related species and genera. *Molecular Ecology Resources* 8: 1081–1084.

DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

GARCIA, F., J. L. NOYER, A. M. RISTERUCCI, AND M. H. CHEVALLIER. 2004. Genotyping of mature trees of *Entandrophragma cylindricum* with microsatellites. *Journal of Heredity* 95: 454–457.

HARDY, O. J., AND X. VEKEMANS. 2002. SPAGeDi: A versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes* 2: 618–620.

HAWTHORNE, W. 1998. *Entandrophragma candollei.* The IUCN Red List of Threatened Species 1998: e.T33050A9753509. Website: http://dx.doi.org/10.2305/IUCN.UK.1998.RLTS.T33050A9753509.en [accessed 10 January 2017].

HOLLELEY, C. E., AND P. G. GEERTS. 2009. Multiplex Manager 1.0: A cross-platform computer program that plans and optimizes multiplex PCR. *BioTechniques* 46: 511–517.

MARIAC, C., N. SCARCELLI, J. POUZADOU, A. BARNAUD, C. BILLOT, A. FAYE, A. KOUGBEADJO, ET AL. 2014. Cost-effective enrichment hybridization capture of chloroplast genomes at deep multiplexing levels for population genetics and phylogeography studies. *Molecular Ecology Resources* 14: 1103–1113.

MASELLA, A. P., A. K. BARTRAM, J. M. TRUSZKOWSKI, D. G. BROWN, AND J. D. NEUFELD. 2012. PANDAseq: Paired-end assembler for Illumina sequences. *BMC Bioinformatics* 13: 31.

MEGLÉCZ, E., C. COSTEDOAT, V. DUBUT, A. GILLES, T. MALAUSA, N. PECH, AND J.-F. MARTIN. 2009. QDD: A user-friendly program to select microsatellite markers and design primers from large sequencing projects. *Bioinformatics (Oxford, England)* 26: 403–404.

MICHENEAU, C., G. DAUBY, N. BOURLAND, J. L. DOUCET, AND O. J. HARDY. 2011. Development and characterization of microsatellite loci in *Pericopsis elata* (Fabaceae) using a cost-efficient approach. *American Journal of Botany* 98: e268–e270.

ROHLAND, N., AND D. REICH. 2012. Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture. *Genome Research* 22: 939–946.

SCHUELKE, M. 2000. An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology* 18: 233–234.

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Table 4. Genetic characterization of the 22 polymorphic microsatellite loci for three populations of *Entandrophragma utile*.[a]

	Loundougou (n = 43)					Yangambi (n = 26)					Mindourou (n = 40)				
Locus	A	H_e	H_o	F^b	r	A	H_e	H_o	F^b	r	A	H_e	H_o	F^b	r
EnU-ssr1	7	0.74	0.54	0.27**	0.11 ± 0.05	5	0.69	0.68	0.01	0.04 ± 0.04	5	0.71	0.66	0.07	0.02 ± 0.05
EnU-ssr6	7	0.73	0.60	0.18	0.06 ± 0.05	7	0.81	0.65	0.20	0.06 ± 0.04	8	0.82	0.74	0.09	0.04 ± 0.05
EnU-ssr13	5	0.42	0.10	0.77***	0.25 ± 0.04	4	0.58	0.32	0.47**	0.12 ± 0.08	7	0.65	0.31	0.53**	0.20 ± 0.05
EnU-ssr19	10	0.56	0.51	0.08	0.04 ± 0.05	9	0.61	0.46	0.26	0.05 ± 0.04	13	0.47	0.50	−0.06	0 ± 0
EnU-ssr35	11	0.82	0.85	−0.04	0 ± 0	8	0.84	0.64	0.25	0.06 ± 0.05	11	0.82	0.51	0.38***	0.14 ± 0.06
EnU-ssr39	8	0.66	0.66	0.00	0.03 ± 0.03	5	0.66	0.50	0.25	0.10 ± 0.06	7	0.71	0.21	0.71***	0.28 ± 0.05
EnU-ssr42	6	0.65	0.42	0.37***	0.14 ± 0.05	5	0.74	0.57	0.24*	0.07 ± 0.05	5	0.71	0.23	0.69***	0.27 ± 0.04
EnU-ssr10	8	0.68	0.19	0.72***	0.29 ± 0.04	8	0.84	0.65	0.23	0.06 ± 0.05	14	0.76	0.62	0.20*	0.07 ± 0.05
EnU-ssr17	13	0.88	0.83	0.07	0.03 ± 0.04	9	0.90	0.40	0.56***	0.17 ± 0.07	9	0.75	0.26	0.65**	0.26 ± 0.04
EnU-ssr23	6	0.52	0.48	0.08	0.05 ± 0.05	4	0.66	0.50	0.25	0.09 ± 0.06	4	0.66	0.35	0.48**	0.17 ± 0.05
EnU-ssr31	14	0.87	0.85	0.02	0 ± 0	12	0.89	0.86	0.04	0.03 ± 0.03	13	0.89	0.77	0.13*	0.04 ± 0.04
EnU-ssr43	19	0.93	0.83	0.11*	0.05 ± 0.03	13	0.92	0.55	0.41***	0.10 ± 0.05	14	0.91	0.65	0.29**	0.12 ± 0.04
EnU-ssr9	14	0.86	0.75	0.13	0.04 ± 0.04	9	0.85	0.47	0.45***	0.11 ± 0.07	12	0.84	0.80	0.05	0.02 ± 0.04
EnU-ssr14	5	0.18	0.15	0.17	0 ± 0	7	0.35	0.17	0.51**	0.09 ± 0.07	3	0.14	0.14	−0.04	0 ± 0
EnU-ssr27	7	0.79	0.57	0.29**	0.12 ± 0.05	8	0.80	0.38	0.53***	0.14 ± 0.07	8	0.82	0.51	0.37***	0.15 ± 0.05
EnU-ssr41	5	0.68	0.60	0.12	0.05 ± 0.05	5	0.71	0.57	0.20	0.06 ± 0.05	8	0.79	0.59	0.26**	0.10 ± 0.05
EnU-ssr2	6	0.71	0.17	0.76***	0.60 ± 0.16	3	0.62	0.00	1.00**	0.79 ± 0.09	6	0.78	0.12	0.85**	0.63 ± 0.05
EnU-ssr16	6	0.70	0.63	0.10	0.05 ± 0.05	8	0.73	0.48	0.35***	0.06 ± 0.05	4	0.58	0.50	0.14	0.07 ± 0.06
EnU-ssr22	14	0.84	0.74	0.12	0.06 ± 0.04	8	0.76	0.62	0.19	0.05 ± 0.04	13	0.85	0.82	0.05	0 ± 0.04
EnU-ssr34	7	0.83	0.95	−0.15*	0 ± 0	9	0.80	0.70	0.14	0.04 ± 0.03	7	0.72	0.67	0.08	0.01 ± 0.05
EnU-ssr38	4	0.57	1.00	−0.76***	0 ± 0	6	0.65	0.53	0.20	0.07 ± 0.05	8	0.74	0.73	0.02	0 ± 0
EnU-ssr45	5	0.61	0.39	0.36*	0.18 ± 0.11	4	0.64	0.28	0.57***	0.43 ± 0.08	5	0.63	0.03	0.95**	0.58 ± 0.05
Multilocus average	8.5	0.69	0.58	0.16	0.01 ± 0.01	7.09	0.73	0.49	0.325	0.15 ± 0.06	8.36	0.71	0.49	0.323	0.07 ± 0.04

Note: A = number of alleles; F = fixation index; H_e = expected heterozygosity; H_o = observed heterozygosity; n = numbers of individuals; r = frequency of null alleles.
[a] Locality and voucher information are provided in Appendix 1.
[b] Significant deviation from Hardy–Weinberg equilibrium at *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

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Table 5. Cross-amplification results (allele size ranges) of microsatellite loci isolated from *Entandrophragma utile* and *E. candollei* tested in two congeneric species.

Primers	*E. angolense*	*E. congoense*
E. utile		
EnU-ssr1	234	246
EnU-ssr6	—	—
EnU-ssr13	—	—
EnU-ssr19	—	—
EnU-ssr35	—	—
EnU-ssr39	—	—
EnU-ssr42	—	—
EnU-ssr10	—	—
EnU-ssr17	—	—
EnU-ssr23	220	226
EnU-ssr31	—	—
EnU-ssr43	140–152	146–159
EnU-ssr9	—	—
EnU-ssr14	236	236
EnU-ssr27	—	—
EnU-ssr41	176	192
EnU-ssr2	—	—
EnU-ssr16	223	—
EnU-ssr22	—	—
EnU-ssr34	131	136
EnU-ssr38	196–202	—
EnU-ssr45	—	—
E. candollei		
EnC-ssr4	—	—
EnC-ssr13	—	—
EnC-ssr26	—	—
EnC-ssr32	—	—
EnC-ssr36	—	—
EnC-ssr42	—	—
EnC-ssr48	—	—
EnC-ssr9	—	—
EnC-ssr24	—	—
EnC-ssr29	—	—
EnC-ssr33	242	—
EnC-ssr38	194–202	198–212
EnC-ssr12	—	—
EnC-ssr16	—	—
EnC-ssr27	188	188
EnC-ssr43	202–232	216

Note: — = no amplification.

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Appendix 1. Voucher information for *Entandrophragma* individuals used in this study.

Species	*n*	Collection samples[a]	Collection locality	Country	Latitude	Longitude	Collector
Entandrophragma candollei Harms[d]	1	GEM09[b,c]	Mindourou	Cameroon	3.18	12.81	Armel Donkpegan
	18	FM2265, FM2278, FM2288, FM2309, FM2349, FM2355, FM2361, FM2466, FM2472, FM2494, FM2504, FM2520, FM2522, FM2570, FM2587, FM2602, FM2604, FM2608	Campo Ma'an	Cameroon	2.44	10.79	Franck Monthe
	17	FM2647, FM2648, FM2649, FM2650, FM2651, FM2652, FM2653, FM2654, FM2655, FM2656, FM2657, FM2658, FM2659, OH3895, OH4357, OH4358, OH4388	Yangambi	DRC	0.81	24.47	Emmanuel Kasongo
	17	Senterre B. et al., 1177 (BRLU)[e], JM490, JM495, JM500, JM502, JM516, JM536, JM551, JM574, JM650, JM656, JM679, JM681, JM682, JM684, JM685, JM689	Mindourou	Cameroon	3.18	13.62	Jérémy Migliore
	47	FM3018–FM3020, FM3023, FM3026–FM3029, FM3032, FM3033, FM3038–FM3042, FM3057, FM3036, FM3044–FM3047, FM3049–FM3055, FM3061–FM3064, FM3066, FM3069, FM3071–FM3073, FM3077, FM3084, FM3086, FM3088, FM3075	Loundougou	Republic of Congo	2.38	17.1	Franck Monthe
Entandrophragma utile (Dawe & Sprague) Sprague[d]	1	GEM11[b,c]	Mindourou	Cameroon	3.18	12.81	Armel Donkpegan
	26	OH4398, OH4400, OH4410, OH4455, OH4457, OH4458, OH4461, OH4462, OH4463, OH4466, OH4467, OH4469, OH4471, OH4468, OH4493, OH4516, OH4530, FM2663, FM2664, FM2665, FM2666, FM2667, FM2668, FM2669, FM2670, FM2671	Yangambi	DRC	0.8	24.52	Emmanuel Kasongo
	39	QE0539–QE0570, QE823, JM478, JM483, JM626, JM627, JM668, JM783, JM837	Mindourou	Cameroon	2.38	13.62	Quentin Evrard & Jérémy Migliore
	43	FM3144, FM3169, FM3190, FM3194, FM3197, FM3199, FM3205, FM3230, FM3235, FM3239, FM3240, FM3252, FM3318, FM3322, FM3332, FM3347, FM3361, FM3396, FM3402, FM3425, FM3427, FM3436, FM3451, FM3485, FM3490, FM3495, FM3496, FM3500, FM3502, FM3513, FM3518, FM3520, FM3530, FM3534, FM3545, FM3565, FM3566, FM3571, FM3589, FM3591, FM3595, FM3601, FM3611	Loundougou	Republic of Congo	2.38	17.1	Franck Monthe
Entandrophragma angolense (Welw. ex C. DC.) C. DC.[f]	6	FM1358, FM1371, FM1373, FM1388	Ngambé Tikar	Cameroon	5.74	11.51	Franck Monthe
		WAG1096874[e]	Ashanti kokote	Ghana	6.57	−1.81	Franck Monthe
		WAG1096876[e], WAG1096878[e]	Bipinidi	Cameroon	3.07	10.41	Franck Monthe
Entandrophragma congoense (Pierre & De Wild.) A. Chev.	6	GID1142	Loango	Gabon	−1.88	9.83	Gilles Dauby
		WAG1096930[e], WAG1096931[e], WAG1096932[e]	Kasai weka Bena-longo	Republic of Congo	−6.29	22.6	Franck Monthe
		FM1329, MH1560	Campo Ma'an	Cameroon	2.39	10.62	Franck Monthe & Myriam Heuertz

Note: DRC = Democratic Republic of Congo; *n* = number of individuals.
[a] Unless stated otherwise, codes refer to the silica gel collection of Dr. Olivier Hardy (ULB, EBE team). Samples are available on request.
[b] Vouchers deposited at the Herbarium of the Université Libre de Bruxelles, Belgium (BRLU).
[c] Individuals used for sequencing genomic libraries.
[d] Individuals used for amplification and polymorphism tests.
[e] Codes of specimens from the Herbarium of the Université Libre de Bruxelles, Belgium (BRLU) or the National Herbarium of The Netherlands (WAG).
[f] Individual used for cross-amplification tests.

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