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Primer Note

Characterization of microsatellite markers in the African tropical tree species *Guibourtia ehie* (Fabaceae, Detarioideae)[1]

Félicien Tosso[2,3,7], Jean-Louis Doucet[2], Jérémy Migliore[3], Kasso Daïnou[2,4,5,6], Esra Kaymak[3], Franck S. Monthe Kameni[3], and Olivier J. Hardy[3]

[2]Central African Forests, TERRA Teaching and Research Centre, Gembloux Agro-Bio Tech, Université de Liège, Passage des Déportés 2, B-5030 Gembloux, Belgium; [3]Evolutionary Biology and Ecology Unit, CP 160/12, Faculté des Sciences, Université Libre de Bruxelles, Av. F. D. Roosevelt 50, B-1050 Brussels, Belgium; [4]Nature+ asbl, Rue Provinciale 62, 1301 Wavre, Belgium; [5]Management of Forest Resources, BIOSE Department, Gembloux Agro-Bio Tech, Université de Liège, B-5030 Gembloux, Belgium; and [6]Université d'Agriculture de Kétou, BP 43 Kétou, Benin

- *Premise of the study:* Microsatellite primers (simple sequence repeats [SSRs]) were developed in *Guibourtia ehie* (Fabaceae, Detarioideae) to study population genetic structure and the history of African vegetation.
- *Methods and Results:* We isolated 18 polymorphic SSRs from a nonenriched genomic library. This set of primer pairs was tested on four populations, and the results showed two to 16 alleles per locus with mean observed and expected heterozygosities of 0.27 ± 0.05 and 0.57 ± 0.05, respectively. Cross-amplification tests in 13 congeneric species were successful for the four taxa belonging to the subgenus *Gorskia*.
- *Conclusions:* This set of microsatellite markers will be useful to investigate the phylogeography and population genetics of *G. ehie*, a key representative of African semideciduous moist forests.

Key words: Fabaceae; *Guibourtia ehie*; microsatellites; next-generation sequencing.

Guibourtia ehie (A. Chev.) J. Léonard (Fabaceae, Detarioideae) is a timber species found in evergreen and semideciduous moist forests from Liberia to Gabon (Tosso et al., 2015). It is distributed on both sides of the Dahomey Gap, a portion of forest–savanna mosaic separating the Upper and Lower Guinean rainforest blocks (Salzmann and Hoelzmann, 2005). *Guibourtia ehie* is an insect-pollinated and wind-dispersed species (Tosso et al., 2015) exhibiting an abundant natural regeneration around the mother plant (Lemmens et al., 2008). Known as ovengkol in Gabon and amazakoué in Ivory Coast, it produces wood of high economic value. The major threat to this species (registered as vulnerable on the IUCN Red List) is logging, which causes local population declines (Hawthorne, 1995). *Guibourtia ehie* is therefore a good candidate to assess the impact of logging on gene flow (pollen and seed dispersal) and to study spatial genetic diversity issues before considering conservation plans. In addition, the wide spatial distribution of this species will likely be useful to better understand the history of African vegetation and the role of the Dahomey Gap in relation to successive past environmental changes. Because only a few of the microsatellites (simple sequence repeats [SSRs]) previously developed for *G. tessmannii* (Harms) J. Léonard (a central African species) cross-amplified in *G. ehie* (Tosso et al., 2016), we developed here a new set of polymorphic SSRs.

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[7]Author for correspondence: dnftosso@ulg.ac.be

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METHODS AND RESULTS

***Development of microsatellites*—**To identify and characterize SSRs, total genomic DNA was extracted (from *G. ehie* dry leaf, voucher FT0272; Appendix 1) following the cetyltrimethylammonium bromide (CTAB) protocol described in Fu et al. (2005). We used the Illumina MiSeq platform (GIGA platform, Liège, Belgium; Illumina, San Diego, California, USA) to construct a nonenriched genomic DNA library following Mariac et al. (2014), generating 255,460 paired-end reads 145 ± 3 bp long, which were pair-assembled with PANDAseq (Masella et al., 2012). The software QDD with the default settings (Meglécz et al., 2014) was used to identify 3597 microsatellite loci following the three classical steps: (i) SSR detection, (ii) elimination of similar sequences, and (iii) primer design. Among the 3597 loci, we selected a subset of 64 loci according to the following criteria: (i) having at least eight di- or trinucleotide repeats, (ii) having primers located at least 20 bp from the SSR motif, and (iii) characterized by PCR products 130–300 bp long. To have a good distribution of loci sizes and to facilitate multiplexing in the next steps, we then selected 48 loci for amplification tests. Each locus was labeled with the fluorochromes FAM, NED, VIC, or PET by adding one of four possible linkers (Q1–Q4; Micheneau et al., 2011) to the 5′ end of the forward primer (Table 1).

TABLE 1. Characteristics of 19 nuclear microsatellite markers developed for *Guibourtia ehie.*

Locus[a]	Primer sequences (5′–3′)	Fluorescent label[b]	Repeat motif	Allele size range (bp)	GenBank accession no.
Multiplex 1					
GuiE-ssr39	F: CACTGCTTAGAGCGATGCTCGGTTAGTGAAATGGTTGTTG R: ATTAGTTCCATGATCATTACTTCAAA	Q3-VIC	$(AT)_{14}$	132–156	KY929303
GuiE-ssr34	F: TAGGAGTGCAGCAAGCATGCATTGTTGGAATGATAAATTCAC R: GGATTCAACTATGAAAGGAAACA	Q2-NED	$(AT)_{10}$	152–180	KY929300
GuiE-ssr18	F: TAGGAGTGCAGCAAGCATCAAGTGGGTTCATTGCGTTA R: CGTTGGCTGTGAAGAGAAAGT	Q2-NED	$(AG)_{14}$	180–190	KY929294
GuiE-ssr05	F: TGTAAAACGACGGCCAGTTGACCCAACATATAGAGCATGAG R: CCCTATGGGTGATTGTATGC	Q1-6-FAM	$(TC)_{9}$	262–264	KY929289
GuiE-ssr33	F: TGTAAAACGACGGCCAGTTTAAACCCTAAAGCACAATCAAA R: CCAAGGCATCCACATGAACTA	Q1-6-FAM	$(AG)_{11}$	142–153	KY929299
Multiplex 2					
GuiE-ssr36	F: TAGGAGTGCAGCAAGCATCAAAGGACCTTCCTGCAACT R: TCAAGTACGATCCTCAGAAATCTT	Q2-NED	$(CT)_{13}$	147–163	KY929301
GuiE-ssr03	F: CTAGTTATTGCTCAGCGGTAATGAGGCAGCTTGCATTG R: CGCTAATTAGTTGATACTATGCTCG	Q4-PET	$(TG)_{13}$	219–283	KY929287
GuiE-ssr02	F: CACTGCTTAGAGCGATGCTTAGTAGCTGAATTTCTCATGCAA R: CGCTAATTAGTTGATACTATGCTCG	Q3-VIC	$(ATT)_{10}$	262–294	KY929286
GuiE-ssr06	F: CACTGCTTAGAGCGATGCCCTAAAGCCAAGTGATCCCA R: GAATTGAAGAATGAGATGCCAA	Q3-VIC	$(TA)_{14}$	232–294	KY929290
GuiE-ssr31	F: TGTAAAACGACGGCCAGTTTAAACCCTAAAGCACAATCAAA R: CCAAGGCATCCACATGAAC	Q1-6-FAM	$(AG)_{11}$	143–153	KY929298
Multiplex 3					
GuiE-ssr01	F: TGTAAAACGACGGCCAGTCATGGATCACAAACCCGTTA R: GTGCTAAATTCCTATTTGGTCTTACTG	Q1-6-FAM	$(AG)_{11}$	308–316	KY929285
GuiE-ssr04[c]	F: CTAGTTATTGCTCAGCGGTAATGCCTAATGGGTAAAGCC R: TCCAAGTTTAGAACTTTAAATAGGTTG	Q4-PET	$(CAT)_{8}$	267	KY929288
GuiE-ssr15	F: CACTGCTTAGAGCGATGCTGGACTCAGATGATCCATTGTT R: TGCATACACTAGGAAGGAACGA	Q3-VIC	$(CT)_{14}$	200–230	KY929293
GuiE-ssr21	F: TGTAAAACGACGGCCAGTCCCACGAGTGAGAGTGAAGG R: TCACATTACCAGTCTTCCTTGTC	Q1-6-FAM	$(TC)_{22}$	141–189	KY929295
GuiE-ssr38	F: TAGGAGTGCAGCAAGCATTGTGCCAAAGATACTCCCAA R: TGTGGTAGTCAAGCACCCAA	Q2-NED	$(AG)_{10}$	143–152	KY929302
Multiplex 4					
GuiE-ssr08	F: CTAGTTATTGCTCAGCGGTACATGGCTTCTGGTGACGTT R: GAACGGCCTTACAAACGAAA	Q4-PET	$(TA)_{21}$	222–260	KY929291
GuiE-ssr11	F: CTAGTTATTGCTCAGCGGTTTCTCACGTCTACTTTCAAATCATTG R: CAAATTGCCTGTTGGGTTCT	Q3-VIC	$(AT)_{14}$	205–245	KY929292
GuiE-ssr28	F: CTAGTTATTGCTCAGCGGTTTCTCACGTCTACTTTCAAATCATTG R: AAACAAATTTAGTTAAAGGAAGAGGG	Q4-PET	$(TA)_{10}$	159–167	KY929296
GuiE-ssr30	F: TAGGAGTGCAGCAAGCATATTGATTTCGACAAACACAACA R: CTGAGATATCCTTTGCACATCG	Q2-NED	$(AG)_{14}$	145–157	KY929297

[a] Optimal annealing temperature was 57°C and 53°C, respectively, for PCR cycles 1 and 2.
[b] The linkers (Q1, Q2, Q3, Q4) attached to the forward primers are underlined in the forward primer sequences.
[c] Monomorphic locus.

***Microsatellite screening*—**Amplification tests of 48 primer pairs were performed using two individuals of *G. ehie* (FT0288 and FT0478; Appendix 1) in 15-µL PCR reactions with the following conditions: 1.5 µL of buffer (10×), 0.6 µL of $MgCl_2$ (25 mM), 0.45 µL of dNTPs (10 mM each), 0.3 µL of each primer (0.2 µM), 0.08 µL of TopTaq DNA Polymerase (5 U/µL; QIAGEN, Venlo, The Netherlands), 1.5 µL of Coral Load, 1 µL of template DNA (of ca. 10–50 ng/µL), and 9.27 µL of water. PCR conditions were: 94°C (4 min); 30 cycles of 94°C (30 s), 57°C (45 s), and 72°C (1 min); and a final extension at 72°C (10 min). Amplification products stained with 9 µL of TE 1× were examined using the QIAxcel DNA Screening Kit (method AL420; alignment marker 15–5000 bp; size marker 100–2500 bp; QIAGEN). Thirty loci amplified the expected target fragments out of the 48 primer pairs selected for the initial trial.

These 30 loci were further tested in eight individuals from Ghana and Cameroon (Appendix 1). PCR reactions were performed for each of the 30 loci in 15-µL total volumes: 0.15 µL of the reverse and 0.1 µL of the forward (0.2 µM for both) microsatellite primers, 0.15 µL of Q1–Q4 labeled primers (0.2 µM each), 7.5 µL of Type-it Microsatellite PCR Kit (QIAGEN), 3 µL of 5× Q-solution, 3.1 µL of H_2O, and 1 µL of DNA. PCR conditions were: 5-min initial denaturation at 95°C; followed by 25 cycles of 95°C for 30 s, 57°C for 90 s, and 72°C for 1 min; 10 cycles of 94°C for 30 s, 53°C for 45 s, and 72°C for 60 s; and a final elongation step at 60°C for 30 min. All individuals were genotyped on an ABI3730 sequencer (Applied Biosystems, Lennik, The Netherlands) at the Department of Evolutionary Biology and Ecology, Université Libre de Bruxelles (Brussels, Belgium) using 1.1 µL of each 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). We selected 19 primer pairs exhibiting clear chromatograms with no ambiguity in allele size determination. Eighteen primer pairs were polymorphic, and one locus (GuiE-ssr04) was monomorphic.

These loci were included in four multiplexed reactions (Table 1) using Multiplex Manager 1.0 software (Holleley and Geerts, 2009). To assess their polymorphism level, we genotyped between 15 and 23 individuals in each of four populations from Ghana, Ivory Coast, Liberia, and Cameroon, totaling 78 samples (Table 2, Appendix 1). We conducted multiplexed PCR reactions with the conditions as previously described, except that we readjusted the quantity of H_2O to obtain a total volume of 15 µL.

***Data analysis*—**INEst 1.0 (Chybicki and Burczyk, 2009) was used to calculate the following indices on each of the four populations: number of alleles per locus, observed and expected heterozygosities, and inbreeding coefficient. We also tested deviation from Hardy–Weinberg equilibrium for each locus with SPAGeDi (Hardy and Vekemans, 2002).

TABLE 2. Genetic characterization of 19 newly developed microsatellite markers in four populations of *Guibourtia ehie*.[a]

	Ghana (N = 20)				Ivory Coast (N = 23)				Cameroon (N = 15)				Liberia (N = 20)			
Locus	A	H_o	H_e	F[b]	A	H_o	H_e	F[b]	A	H_o	H_e	F[b]	A	H_o	H_e	F[b]
Multiplex 1																
GuiE-ssr39	2	0.10	0.19	0.47	2	0.04	0.33	0.87	2	0.07	0.30	0.78	7	0.65	0.77	0.16
GuiE-ssr34	3	0.45	0.66	0.32	3	0.09	0.64	0.86***	5	0.07	0.74	0.91***	3	0.30	0.55	0.46**
GuiE-ssr18	3	0.00	0.68	1.00***	3	0.39	0.75	0.48	3	0.67	0.69	0.03	2	0.05	0.50	0.90***
GuiE-ssr05	1	0.00	0.00	1.00	1	0.00	0.23	1.00	1	0.00	0.24	1.00	2	0.05	0.14	0.65
GuiE-ssr33	1	0.00	0.00	1.00	1	0.00	0.09	1.00	1	0.00	0.00	1.00	1	0.00	0.00	1.00
Multiplex 2																
GuiE-ssr36	3	0.10	0.53	0.81***	7	0.22	0.72	0.70***	8	0.67	0.71	0.07	4	0.10	0.38	0.74
GuiE-ssr03	3	0.50	0.47	–0.07	7	0.26	0.65	0.60**	8	0.67	0.87	0.23	3	0.10	0.41	0.75
GuiE-ssr02	5	0.15	0.49	0.69***	2	0.00	0.54	1.00***	0	0.00	0.00	1.00	1	0.00	0.43	1.00
GuiE-ssr06	9	0.60	0.88	0.32***	7	0.26	0.70	0.63**	7	0.33	0.86	0.61***	7	0.50	0.84	0.41
GuiE-ssr31	1	0.00	0.00	1.00	3	0.09	0.44	0.80**	1	0.00	0.00	1.00	1	0.00	0.18	1.00
Multiplex 3																
GuiE-ssr01	2	0.05	0.05	0.00	1	0.00	0.43	1.00	1	0.00	0.24	1.00	1	0.00	0.26	1.00
GuiE-ssr04	1	0.00	0.00	1.00	1	0.00	0.50	1.00	1	0.00	0.24	1.00	1	0.00	0.26	1.00
GuiE-ssr15	1	0.00	0.00	1.00	2	0.00	0.51	1.00	4	0.20	0.31	0.35	2	0.00	0.19	1.00*
GuiE-ssr21	5	0.85	0.78	–0.09	8	0.48	0.86	0.45	4	0.13	0.36	0.63*	4	0.40	0.57	0.30
GuiE-ssr38	2	0.25	0.22	–0.11	2	0.26	0.65	0.60	3	0.00	0.68	1.00***	2	0.40	0.58	0.31
Multiplex 4																
GuiE-ssr08	11	0.75	0.92	0.18*	11	0.39	0.88	0.55***	2	0.00	0.57	1.00**	7	0.50	0.82	0.39
GuiE-ssr11	8	0.40	0.72	0.44***	10	0.43	0.84	0.48	10	0.27	0.88	0.69***	3	0.20	0.72	0.72*
GuiE-ssr28	4	0.50	0.48	–0.03	3	0.26	0.68	0.61	1	0.00	0.24	1.00	2	0.45	0.67	0.33
GuiE-ssr30	3	0.65	0.66	0.01	3	0.26	0.75	0.65**	5	0.53	0.77	0.31	4	0.45	0.77	0.41

Note: A = number of alleles; F = fixation index; H_e = expected heterozygosity; H_o = observed heterozygosity; N = number of individuals sampled.

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

[b] Significance of deviation from Hardy–Weinberg equilibrium: *P < 0.05; **P < 0.01; ***P < 0.001.

The mean number of alleles per locus among the four populations was seven (range 1–11). The observed heterozygosity (mean ± SE) was 0.28 ± 0.10 (range 0–0.85), 0.18 ± 0.17 (range 0–0.48), 0.19 ± 0.09 (range 0–0.67), and 0.22 ± 0.07 (range 0–0.65) for the Ghana, Ivory Coast, Cameroon, and Liberia populations, respectively. The expected heterozygosity was 0.41 ± 0.11 (range 0–0.92), 0.59 ± 0.07 (range 0–0.88), 0.46 ± 0.10 (range 0–0.88), and 0.48 ± 0.08 (range 0–0.84) for the Ghana, Ivory Coast, Cameroon, and Liberia populations, respectively. Significant deviation from Hardy–Weinberg equilibrium was observed for 13 loci at least in one population, in part due to the presence of null alleles (Table 2). All these SSR sequences have been deposited in GenBank (Table 1).

***Cross-amplification in other* Guibourtia *species*—**We tested the 19 loci on 13 congeneric species using the PCR conditions described above. Three to eight of the 19 loci successfully amplified in four species from subgenus *Gorskia* J. Léonard (to which *G. ehie* belongs), whereas two to six amplified for subgenus *Pseudocopaiva* J. Léonard and two to three amplified for subgenus *Guibourtia* (Table 3). The locus GuiE-ssr15 amplified in all species. The limited transferability of *G. ehie* SSRs, which was also observed for *G. tessmannii* SSRs (Tosso et al., 2016), indicates a rather deep molecular divergence among *Guibourtia* species.

CONCLUSIONS

In this study, we developed 18 polymorphic microsatellite markers in *G. ehie*. These microsatellite markers will be useful to study intraspecific diversity and gene flow. They are also suitable to study the demographic history of *G. ehie* and provide insights into the past changes in African moist forest cover.

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TABLE 3. Cross-amplification results of 19 microsatellite markers isolated from *Guibourtia ehie* and tested in 13 congeneric species belonging to three *Guibourtia* subgenera.[a]

	Subgenus *Gorskia*				Subgenus *Pseudocopaiva*					Subgenus *Guibourtia*			
Locus	*G. arnoldiana* (*N* = 3)	*G. schliebenii* (*N* = 3)	*G. conjugata* (*N* = 1)	*G. dinklagei* (*N* = 1)	*G. tessmannii* (*N* = 10)	*G. pellegriniana* (*N* = 7)	*G. coleosperma* (*N* = 6)	*G. leonensis* (*N* = 1)	*G. hymenaefolia* (*N* = 1)	*G. carrissoana* (*N* = 2)	*G. copallifera* (*N* = 5)	*G. demeusei* (*N* = 6)	*G. sousae* (*N* = 1)
Multiplex 1													
GuiE-ssr39	122–136	130	154–156	—	130	130–136	—	—	—	118	—	118	132
GuiE-ssr34	—	—	—	—	—	—	—	—	—	—	—	—	—
GuiE-ssr18	180	—	196–198	—	—	—	—	—	—	—	—	—	—
GuiE-ssr05	268–274	—	—	248–266	—	262–270	—	—	—	—	—	—	—
GuiE-ssr33	—	—	—	—	—	—	—	—	—	—	—	—	—
Multiplex 2													
GuiE-ssr36	153–155	136–154	—	—	148–172	154–156	—	—	—	—	144–156	182–206	—
GuiE-ssr03	—	—	—	—	—	—	—	—	—	—	—	—	—
GuiE-ssr02	278–280	—	—	—	—	—	—	—	—	—	—	—	—
GuiE-ssr06	200	—	—	—	—	—	—	—	—	—	—	—	—
GuiE-ssr31	—	—	—	—	—	—	—	—	—	—	—	—	—
Multiplex 3													
GuiE-ssr01	314	—	266–272	—	—	—	—	—	—	—	—	—	—
GuiE-ssr04	—	—	—	—	—	—	—	—	—	—	—	—	—
GuiE-ssr15	242	200–266	200	210–224	204	194–206	174–206	204	214	208	160	208–240	206
GuiE-ssr21	—	146	148	156–168	141–146	141	—	—	144	—	—	—	—
GuiE-ssr38	—	—	—	—	—	—	—	—	—	—	—	—	—
Multiplex 4													
GuiE-ssr08	—	—	—	—	—	—	—	—	—	—	—	—	—
GuiE-ssr11	—	—	—	—	—	174–182	182	182	—	—	—	—	—
GuiE-ssr28	—	—	—	—	—	—	—	—	—	—	—	—	—
GuiE-ssr30	—	150–160	—	—	—	—	—	—	—	—	157	—	—

Note: — = not applicable; *N* = number of individuals sampled.

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

Appendix 1. Voucher information for the *Guibourtia* samples used in this study.[a]

Species	*N*	Voucher no.	Country	Latitude	Longitude
Guibourtia ehie (A. Chev.) J. Léonard[b]	1	FT0272	Ghana	7.09241	−2.11953
Guibourtia ehie[c]	1	FT0288	Ghana	7.08999	−2.11845
Guibourtia ehie[c]	1	FT0478	Ivory Coast	6.30892	−5.28866
Guibourtia ehie[d]	5	FT0497, FT0491, FT0515, FT0510, FT0521	Ivory Coast	6.21	−3.41
Guibourtia ehie[d]	3	FT0241, FT0261, FT0241	Ghana	7.07	−2.08
Guibourtia ehie[d]	8	OH4661–OH4668	Cameroon	2.31	9.96
Guibourtia ehie[d]	20	FT0029, FT0038, FT0059, FT0078, FT0087, FT0095, FT0102, FT0104, FT0115, FT0125, FT0137, FT0146, FT0158, FT0163, FT0169, FT0180, FT0192, FT0192a, FT0193, FT0197	Ghana	7.06	−2.08
Guibourtia ehie[d]	23	FT0398–FT0400, FT0336, FT0355, FT0363, FT0373, FT0382, FT0384, FT0389, FT0411, FT0430, FT0465, FT0489, FT0491, FT0497, FT0498, FT0510, FT0515, FT0519, FT0521, FT0858, FT0859	Ivory Coast	6.21	−2.42
Guibourtia ehie[d]	15	FT0398, FT0336, FT0355, FT0363, FT0373, FT0382, FT0384, FT0389, FT0411, FT0430, FT0465, FT0489, FT0491, FT0497, FT0498, FT0510, FT0515, FT0519, FT0521, FT0858, FT0859	Cameroon	2.44	9.92
Guibourtia ehie[d]	20	NB116, NB389, NB391, NB395, NB399, NB401, NB402, NB403, NB405, NB408, NB413, NB414, NB415, NB417, NB418, NB419, NB423, NB424, NB425, NB91	Liberia	7.56	−8.64
Guibourtia arnoldiana (De Wild. & T. Durand) J. Léonard[e]	3	HB00527556	Gabon	−1.3465	9.7232
		HB00253056	Congo	−4.5	12.23
		GiD2040	Gabon	−3.4098	11.4185
Guibourtia schliebenii (Harms) J. Léonard[e]	3	B23-HB10151	Mozambique	−11.1529	39.7343
		B50-HB3015138	Mozambique	−11.8175	40.34167
		B51-HB6893814	Mozambique	−11.0005	39.7358
Guibourtia conjugata (Bolle) J. Léonard[e]	1	B33-HB3499528	Mozambique	−23.6548	32.1746
Guibourtia dinklagei (Harms) J. Léonard[e]	1	B21-HB11235	Liberia	6.279	−10.7603
Guibourtia tessmannii (Harms) J. Léonard[e]	10	FT0607–FT0613, FT0635–FT0636	Cameroon	2.2236	10.3793
		FT0001	Gabon	1.4286	11.5886
Guibourtia pellegriniana J. Léonard[e]	7	B11-HB1578	Congo	−1.94472	9.86578
		FT0641–FT0646	Gabon	−2.53	9.77
Guibourtia coleosperma (Benth.) Léonard[e]	6	FT0021–FT0025, FT0028	Namibia	−17.85	19.67
Guibourtia leonensis J. Léonard[e]	1	B45-HB3015140	Sierra Leone	8.9852	−11.7169
Guibourtia hymenaefolia (Moric.) J. Léonard[e]	1	B44-HB252852	Cuba	22.1315	−80.3382
Guibourtia carrissoana (M. A. Exell) J. Léonard[e]	2	B19-HB10458	Angola	−8.9341	13.1864
		B20-HB11322	Angola	−8.836	13.2593
Guibourtia copallifera Benn.[e]	5	FT0880–FT0884	Burkina-Faso	9.95	−4.67
Guibourtia demeusei (Harms) J. Léonard[e]	6	FT0873–FT0875, OH3245	Congo	−0.8831	18.123
		B15-HB0069	Gabon	−2.2487	9.5929
		B38-HB527577	DRC	0.807	24.4529
Guibourtia sousae J. Léonard[e]	1	B52-HB892206	Mozambique	−24.6254	33.9579

Note: DRC = Democratic Republic of the Congo; *N* = number of individuals.

[a] Vouchers are deposited at the Herbarium of the Université Libre de Bruxelles, Brussels, Belgium (BRLU), silica gel collection of Dr. Olivier Hardy.

[b] Individual used for genomic library.

[c] Individuals used for amplification tests.

[d] Individuals used for polymorphism tests.

[e] Individuals used for cross-amplification tests.