

MICROSATELLITE DEVELOPMENT FOR THE GENUS *GUIBOURTIA* (FABACEAE, CAESALPINIOIDEAE) REVEALS DIPLOID AND POLYPLOID SPECIES¹

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- *Premise of the study:* Nuclear microsatellites (nSSRs) were designed for *Guibourtia tessmannii* (Fabaceae, Caesalpinioideae), a highly exploited African timber tree, to study population genetic structure and gene flow.
- *Methods and Results:* We developed 16 polymorphic nSSRs from a genomic library tested in three populations of *G. tessmannii* and two populations of *G. coleosperma*. These nSSRs display three to 14 alleles per locus (mean 8.94) in *G. tessmannii*. Cross-amplification tests in nine congeneric species demonstrated that the genus *Guibourtia* contains diploid and polyploid species. Flow cytometry results combined with nSSR profiles suggest that *G. tessmannii* is octoploid.
- *Conclusions:* nSSRs revealed that African *Guibourtia* species include both diploid and polyploid species. These markers will provide information on the mating system, patterns of gene flow, and genetic structure of African *Guibourtia* species.

Key words: Fabaceae; flow cytometry; *Guibourtia*; microsatellites; next-generation sequencing; polyploidy.

The African tree *Guibourtia tessmannii* (Harms) J. Léonard (Fabaceae, Caesalpinioideae) is a hermaphrodite rainforest species distributed from Cameroon to Gabon (Fougère-Danezan et al., 2007; Tosso et al., 2015). Known as “bubinga” or “kevazingo,” it has high commercial and social value but is under significant threat due to illegal logging. The genus *Guibourtia* Benn. includes 13 African species distributed from Senegal to Mozambique in forest or savannah habitats. The genus was divided by Léonard (1949) into three main subgenera: (i) *Pseudocopaiva*: *G. tessmannii*, *G. pellegriniana* J. Léonard, *G. coleosperma* (Benth.) J. Léonard, *G. leonensis* J. Léonard; (ii) *Guibourtia*: *G. carrisoana* (M. A. Exell) J. Léonard, *G. copallifera* Benn., *G. demeusei* (Harms) J. Léonard, *G. sousae* J. Léonard; and (iii) *Gorskia*: *G. arnoldiana* (De Wild. & T. Durand) J. Léonard, *G. conjugata* (Bolle) J. Léonard, *G. dinklagei* (Harms) J. Léonard, *G. ehie* (A. Chev.) J. Léonard, *G. schliebenii* (Harms) J. Léonard. We developed polymorphic microsatellite

markers for *G. tessmannii* and tested them on nine African congeneric species to verify species delimitation and document population genetic structure and gene flow patterns. Because microsatellite typing suggested that some species were polyploid, we used flow cytometry to compare the ploidy levels of two related species for which appropriate fresh material was available.

METHODS AND RESULTS

Microsatellite development—We extracted total DNA from 30 mg of dry leaf of *G. tessmannii* (FT0001; Appendix 1) using a cetyltrimethylammonium bromide (CTAB) method (Fu et al., 2005). We prepared a nonenriched DNA genomic library, following Mariac et al. (2014), and generated 150-bp-long paired-end reads on an Illumina MiSeq platform (San Diego, California, USA). We assembled the resulting 78,279 reads by pair with PANDaseq (Masella et al., 2012). Using the software QDD (Megléczy et al., 2014), we detected 2483 microsatellite loci. Of these, 149 had at least eight repeats and flanking regions appropriate to define pairs of PCR primers. We developed primers for 48 loci with at least eight di-, tri-, or tetranucleotide repeats and primer regions at least 20 bp distant from the microsatellite region. We added one of four possible linkers (Q1–Q4; Micheneau et al., 2011) to the 5′ end of the forward primer of each locus to label PCR products with fluorochromes FAM, NED, VIC, and PET (Table 1).

We tested 48 primer pairs using two samples of *G. tessmannii* (FT0002 and FT0003; Appendix 1). PCR reactions (total volume of 15 μL) used 1.5 μL of buffer (10×), 0.6 μL MgCl₂ (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 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), 55°C (45 s), and 72°C (1 min); and a final extension at 72°C (10 min). We visualized PCR products stained with SYBR Safe (Invitrogen, Merelbeke, Belgium) on a 1% agarose gel. Forty-two loci amplified consistently.

We assessed polymorphism on seven *G. tessmannii* individuals from Cameroon and Gabon (Appendix 1). We used fluorescent labeling by PCR amplification in

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TABLE 1. Characterization of 16 polymorphic and one monomorphic nuclear microsatellite loci isolated from *Guibourtia tessmannii*.

| Primers ^{a,b} | Primer sequences (5'–3') | Labeled primer ^c | Repeat motif | Allele range size (bp) | GenBank accession no. |
|------------------------|---|-----------------------------|---------------------|------------------------|-----------------------|
| R12-Seq10* | F: AGGACTTAAGAATGGTGTATGCAA R: TTTGGCTTCCCTCTCTTCTT | Q1-6-FAM | (AT) ₁₀ | 150–200 | KX086193 |
| R12-Seq15* | F: CCTGATTGGAGTTACACCACC R: AGGACAAGCTTGAGCGACAT | Q1-6-FAM | (AG) ₁₃ | 98–124 | KX086194 |
| R12-Seq21* | F: TTTCATTCAACAACCGCA R: CTGACACACAACACAGCCA | Q2-NED | (ATA) ₁₁ | 176–218 | KX086197 |
| R12-Seq35* | F: GACACTCCTCAGGTGGTTTCA R: GAGGTTAGATTCCAACATGTGC | Q3-VIC | (AAT) ₂₀ | 123–165 | KX086204 |
| R12-Seq29* | F: CCAAATTGCAGACGATGAAA R: AATTCGGACTTGAAGTTGCAG | Q3-VIC | (TCT) ₁₁ | 205–247 | KX086201 |
| R12-Seq08** | F: AACATGCATACCTTAACCGCAA R: TTTCATCAACACTTATCCTTGG | Q4-PET | (TTTC) ₉ | 148–172 | KX086191 |
| R12-Seq06** | F: ATCTCCGCTGTACTGCGT R: AATCAAGCCTCCGTAAAGCA | Q1-6-FAM | (GA) ₈ | 187–203 | KX086190 |
| R12-Seq26** | F: CACAATACATGAGCTGAAGAAACATGA R: CACGAGAAAGGGAGGAAATG | Q2-NED | (TCT) ₁₃ | 153–186 | KX086200 |
| R12-Seq34** | F: GACACTCCTCAGGTGGTTTCA R: GAGGTTAGATTCCAACATGTGC | Q3-VIC | (TAT) ₁₃ | 150–186 | KX086203 |
| R12-Seq16** | F: CCCATAATCAGCCTACAAACC R: CAGATGAGGTAGACATTGTGGG | Q2-NED | (AG) ₁₁ | 226–262 | KX086195 |
| R12-Seq09*** | F: ACCTACGTTTGTGATTATGAATGG R: TTTGGGTGATCTTTATGCTTTC | Q1-6-FAM | (GA) ₈ | 166–196 | KX086192 |
| R12-Seq20*** | F: AAATCCGGAGGAGGAGGAAGA R: CTGACTCTGGCTTGACCCAT | Q2-NED | (AG) ₈ | 194–218 | KX086196 |
| R12-Seq22*** | F: TTATGATGCGTGTCCCAA R: GAATGAATGCAGGGAGGAC | Q2-NED | (TA) ₂₁ | 157–177 | KX086198 |
| R12-Seq01**** | F: CCTCATCATACAATTCAAGTGC R: GATGCCATGACTCTGGCTAAA | Q1-6-FAM | (AT) ₂₀ | 201–241 | KX086189 |
| R12-Seq25**** | F: CATAGACTTGGAGGGAGCCA R: TTGCTTCCCTGTATCTTTAACAATTT | Q2-NED | (GA) ₉ | 174–196 | KX086199 |
| R12-Seq31**** | F: ATTCCTATCAGATGAACAGATTATCA R: AGCTTGTTGCAAAATGGATTG | Q3-VIC | (AT) ₈ | 221–245 | KX086202 |
| R12-Seq43** | F: GGCAGAATTTCCAGAAGCAA R: ACACAACCTTCTTTCCTGC | Q4-PET | (TA) ₂₃ | 143 | KX086205 |

^a* = Multiplex Mix 1, ** = Mix 2, *** = Mix 3, **** = Mix 4.

^bOptimal annealing temperature was 60°C for all loci.

^cQ1 = TGTAACGACGGCCAGT (Schuelke, 2000); Q2 = TAGGAGTGCAGCAAGCAT; Q3 = CACTGCTTAGAGCGATGC; Q4 = CTAGTTATTGCTCAGCGGT (Q2–Q4, after Culley et al., 2008).

a total volume of 15 µL, combining 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), 3 µL of Type-it Microsatellite PCR Kit (QIAGEN), H₂O, and 1 µL of DNA. PCR conditions were: 5-min initial denaturation at 95°C; followed by 30 cycles of (95°C for 30 s, 60°C for 90 s, 72°C for 1 min) and 10 cycles of (95°C for 30s, 55°C for 45 s, 72°C for 60 s, 72°C for 1 min); and a final elongation step at 60°C for 30 min. We mixed 1.1 µL of each PCR product with 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). The preparation was genotyped on an ABI3730 sequencer (Applied Biosystems, Lennik, The Netherlands).

After excluding loci that did not amplify consistently or were unreadable, we combined 16 polymorphic loci (one locus [R12-Seq43] was monomorphic) in four multiplexed reactions (Table 1) using Multiplex Manager 1.0 software (Holleley and Geerts, 2009). Preliminary population genetic analyses were performed on three populations of *G. tessmannii* (35–58 individuals per population; Table 2 and Appendix 1). Multiplexed PCRs were as above except that 3 µL of the 5× Q-solution of the Type-it Microsatellite PCR Kit was added. The individuals of *G. tessmannii* studied revealed a high degree of polymorphism, with more than two alleles per individual, suggesting a polyploid genome (Table 2).

Microsatellite marker data analysis in *G. tessmannii* and *G. coleosperma*—The three populations of *G. tessmannii* (Table 2 and Appendix 1) had three to 14 alleles per locus (mean 8.94 alleles per locus, Table 2). Single-locus genotypes had one to eight alleles (2.35 ± 0.94 alleles per locus) and no fixed heterozygosity, suggesting an autopolyploid.

For *G. coleosperma*, the diploid species in which cross-amplification was the most successful (see below), we considered two populations (Table 2). For each of the 10 amplifiable loci, we calculated allele size range, number of alleles (A)

per locus, observed (H_o) and expected (H_e) heterozygosity, inbreeding coefficient (F), and null allele frequency (r) with INEst 1.0 (Chybicki and Burczyk, 2009). Deviation from Hardy–Weinberg equilibrium (HWE) was tested for each locus with SPAGeDi (Hardy and Vekemans, 2002). Loci exhibited one to 14 alleles (mean 4.5) with H_o (mean ± SE) of 0.28 ± 0.09 and H_e of 0.41 ± 0.11 for the Democratic Republic of Congo (DRC) population and one to 10 alleles (mean 3.67) with H_o of 0.17 ± 0.05 and H_e of 0.36 ± 0.10 for the Namibia population. Significant deviation from HWE was observed in at least one population for four primer pairs. Loci R12-Seq20 and R12-Seq22 for the DRC population exhibited a significant deficit of heterozygotes due to the presence of null alleles (Table 2).

Flow cytometry—We used flow cytometry to confirm the ploidy level of *G. tessmannii* and compare its genome size with *G. coleosperma*. We used fresh material from seeds collected in central Gabon (*G. tessmannii*) and northern Namibia (*G. coleosperma*) (Appendix 1). From 1 cm² pieces of fresh leaves, we obtained suspensions of leaf cell nuclei by chopping them in a buffer solution using the CyStain UV Precise P Kit (Partec GmbH, Münster, Germany) with DAPI (4',6-diamidino-2-phenylindole, diacetate). We ran samples with Ploidy Analyser equipment (Partec GmbH). We used tomato as an internal standard (*Solanum lycopersicum* L. “Montfavet 63-5” [2C = 1.99 pg, 40.0% GC; Marie and Brown, 1993]). Under the assumption that the GC content of our samples and the standard were similar, the genome size of *G. coleosperma* ranged from 3.20 to 3.70 pg ($N = 3$) and *G. tessmannii* from 11.87 to 15.78 pg ($N = 3$). Although these estimates should be considered with caution in the absence of information on the GC content, the genome size of *G. tessmannii* is nearly four times larger than that of *G. coleosperma*. Because the latter species displays microsatellite profiles typical of diploids, the flow cytometry results confirm that *G. tessmannii* is an octoploid species.

TABLE 2. Results of initial primer screening of 17 nuclear microsatellite loci developed in *Guibourtia tessmannii* (three populations) and 10 that cross-amplified in *G. coleosperma* (two populations).

| Locus | <i>G. tessmannii</i> (octoploid) | | | | | | <i>G. coleosperma</i> (diploid) | | | | | | | | | | |
|-----------|----------------------------------|--------------------|--------------------------|--------------------|--------------------------|--------------------|---------------------------------|----|----------------|-------------------------|---------|----|----|----------------|----------------|---------|-------------|
| | Gabon (Makokou; N = 35) | | Gabon (Bambidie; N = 58) | | Cameroon (Ma'an; N = 38) | | DRC (Dilolo; N = 20) | | | Namibia (Rundu; N = 13) | | | | | | | |
| | A | A _{range} | A _{ind} | A _{range} | A | A _{range} | A _{ind} | A | H _e | F _a | r | N | A | H _e | F _a | r | |
| R12-Seq15 | 4 | 1-3 | 1.13 | 1-3 | 11 | 1-3 | 1.5 | 6 | 0.11 | 0.26 | 0.31 | 20 | 4 | 0.11 | 0.26 | 0.31 | 0.09 ± 0.08 |
| R12-Seq10 | 14 | 1-5 | 2.34 | 1-4 | 18 | 1-6 | 2.13 | 20 | 0.67 | 0.91 | 0.23 | 18 | 14 | 0.67 | 0.91 | 0.23 | 0.07 ± 0.05 |
| R12-Seq35 | 6 | 1-4 | 1.85 | 1-5 | 13 | 1-3 | 2.69 | 8 | 0.5 | 0.71 | 0.12 | 20 | 4 | 0.5 | 0.71 | 0.12 | 0.06 ± 0.05 |
| R12-Seq08 | 6 | 1-4 | 2.45 | 1-3 | 5 | 1-3 | 1.89 | 5 | 0 | 0.09 | 1* | 20 | 2 | 0 | 0.09 | 1* | 0.11 ± 0.09 |
| R12-Seq26 | 7 | 1-5 | 2.56 | 1-4 | 11 | 1-4 | 1.91 | 9 | — | — | — | 20 | 1 | — | — | — | 0.12 ± 0.09 |
| R12-Seq34 | 7 | 1-6 | 4.48 | 1-4 | 5 | 1-4 | 2 | 5 | 0.67 | 0.67 | 0 | 20 | 7 | 0.67 | 0.67 | 0 | 0.05 ± 0.04 |
| R12-Seq16 | 14 | 1-8 | 5.38 | 1-6 | 12 | 2-8 | 4.72 | 13 | 0.5 | 0.39 | -0.07 | 20 | 3 | 0.5 | 0.39 | -0.07 | 0.05 ± 0.04 |
| R12-Seq09 | 10 | 1-5 | 2.95 | 1-2 | 16 | 2-6 | 3.55 | 12 | 0.06 | 0.11 | 0.49 | 19 | 2 | 0.06 | 0.11 | 0.49 | 0.29 ± 0.09 |
| R12-Seq20 | 9 | 1-5 | 2.63 | 1-3 | 5 | 1-3 | 1.51 | 2 | 0 | 0.3 | 1** | 20 | 3 | 0 | 0.3 | 1** | 0.20 ± 0.36 |
| R12-Seq22 | 3 | 1-3 | 1.42 | 1-3 | 9 | 1-3 | 1.22 | 4 | 0.34 | 0.77 | 0.49*** | 20 | 6 | 0.34 | 0.77 | 0.49*** | 0.16 ± 0.07 |
| R12-Seq01 | 11 | 1-6 | 2.83 | 1-4 | 14 | 1-7 | 2.98 | 17 | — | — | — | — | — | — | — | — | — |
| R12-Seq25 | 7 | 1-4 | 2.03 | 1-4 | 7 | 1-4 | 2.25 | 7 | — | — | — | — | — | — | — | — | — |
| R12-Seq31 | 13 | 1-5 | 2.31 | 1-4 | 10 | 1-4 | 2.26 | 7 | — | — | — | — | — | — | — | — | — |
| R12-Seq21 | 10 | 1-4 | 2.44 | 1-3 | 12 | 1-3 | 2.07 | 11 | — | — | — | — | — | — | — | — | — |
| R12-Seq29 | 6 | 1-4 | 2.16 | 1-4 | 4 | 1-3 | 1.76 | 10 | — | — | — | — | — | — | — | — | — |
| R12-Seq06 | 5 | 1-3 | 2.03 | 1-4 | 4 | 1-4 | 2.14 | 5 | — | — | — | — | — | — | — | — | — |
| R12-Seq43 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | — | — | — | — | — | — | — | — | — |

Note: — = not applicable; A = number of alleles; A_{ind} = mean number of alleles per individual; A_{range} = range of number of alleles per individual; DRC = Democratic Republic of Congo; F = fixation index; H_e = expected heterozygosity; H_o = observed heterozygosity; N = number of individuals sampled; r = null allele frequency.
*Significance of deviation from Hardy-Weinberg equilibrium: *P < 0.05, **P < 0.01, ***P < 0.001.

Cross-amplification in congeneric species and ploidy determination—
Among the 17 loci selected from *G. tessmannii*, a majority successfully amplified in two other species from the subgenus *Pseudocopaiva* (Table 3). Less than six loci amplified in the other species, most of which belong to other subgenera (Table 3). In *G. pellegriniana*, all loci were polymorphic and the genotypes showed up to eight alleles per individual and locus, suggesting an octoploid genome. By contrast, in the other species individuals did not display more than two alleles per locus, suggesting diploid genomes.

CONCLUSIONS

We developed 16 polymorphic microsatellite markers in *G. tessmannii* that amplified to varying degrees in nine congeneric species. The microsatellites and flow cytometry results showed for the first time that the genus *Guibourtia* includes diploid and polyploid species. These markers will be useful to assess the mating system and genetic structure of *Guibourtia* species.

LITERATURE CITED

CHYBICKI, I. J., AND J. BURCZYK. 2009. Simultaneous estimation of null alleles and inbreeding coefficients. *Journal of Heredity* 100: 106–113.
 CULLEY, T. M., S. G. WELLER, A. K. SAKAI, AND K. A. 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.
 FOUGÈRE-DANEZAN, M., S. MAUMONT, AND A. BRUNEAU. 2007. Relationships among resin-producing Detarieae s.l. (Leguminosae) as inferred by molecular data. *Systematic Botany* 32: 748–761.
 FU, X., Y. HUANG, S. DENG, R. ZHOU, G. YANG, X. NI, W. LI, AND S. SHI. 2005. Construction of a SSH library of *Aegiceras corniculatum* under salt stress and expression analysis of four transcripts. *Plant Science* 169: 147–154.
 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.
 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.
 LÉONARD, J. 1949. Notulae systematicae IV (Caesalpiniaceae-Amherstieae africanae americanae). *Bulletin du Jardin Botanique de l'État, Bruxelles/Bulletin van den Rijksplantentuin, Brussel* 19: 383–408.
 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.
 MARIE, D., AND S. C. BROWN. 1993. A cytometric exercise in plant DNA histograms, with 2C values for 70 species. *Biology of the Cell* 78: 41–51.
 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., N. PECH, A. GILLES, V. DUBUT, P. HINGAMP, A. TRILLES, R. GRENIER, AND J. F. MARTIN. 2014. QDD version 3.1: A user-friendly computer program for microsatellite selection and primer design revisited: Experimental validation of variables determining genotyping success rate. *Molecular Ecology Resources* 14: 1302–1313.
 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.
 SCHUELKE, M. 2000. An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology* 18: 233–234.
 TOSSE, F., K. DAINOU, O. J. HARDY, B. SINSIN, AND J.-L. DOUCET. 2015. Le genre *Guibourtia* Benn., un taxon à haute valeur commerciale et sociétale (synthèse bibliographique). *Biotechnologie, Agronomie, Société et Environnement* 19: 71–88.

TABLE 3. Results of cross-amplification (allele size ranges) of microsatellite loci isolated from *Guibourtia tessmannii* and tested in nine additional taxa.

| Species | Subg. <i>Pseudocopaiva</i> | | | Subg. <i>Guibourtia</i> | | | Subg. <i>Gorskia</i> | | |
|-----------|-------------------------------------|--------------------------------|-----------------------------------|---------------------------------|----------------------------------|-------------------------------|---------------------------------|----------------------------|--------------------------------|
| | <i>G. pellegriniana</i> (N = 14) | <i>G. leonensis</i> (N = 3) | <i>G. coleosperma</i> (N = 33) | <i>G. carrisoana</i> (N = 2) | <i>G. copallifera</i> (N = 7) | <i>G. demousei</i> (N = 9) | <i>G. arnoldiana</i> (N = 2) | <i>G. ehie</i> (N = 20) | <i>G. dinklagei</i> (N = 1) |
| R12-Seq10 | 156–170 | 148–150 | 148–186 | — | — | — | — | — | — |
| R12-Seq15 | 108–124 | — | 108–122 | — | — | — | — | — | — |
| R12-Seq21 | 182–212 | — | — | — | — | — | — | — | — |
| R12-Seq35 | 129–159 | 141–150 | 136–154 | — | — | — | — | — | — |
| R12-Seq29 | 199–223 | — | — | — | — | 217 | — | — | — |
| R12-Seq08 | 136–168 | — | 152–156 | 142–148 | — | — | — | 136–208 | — |
| R12-Seq06 | 194–198 | 194 | — | — | — | — | — | — | — |
| R12-Seq26 | 156–180 | 158–160 | 158* | — | — | 158 | — | 138–198 | — |
| R12-Seq34 | 150–174 | 150 | 150–160 | 150 | — | — | — | 150–174 | 154 |
| R12-Seq16 | 226–250 | 202 | 224–266 | 236 | 226 | 228–252 | 232 | 232–252 | — |
| R12-Seq09 | 200 | — | 168–170 | — | — | — | — | — | — |
| R12-Seq20 | 205 | — | 203–223 | — | 203–205 | 203–207 | — | — | — |
| R12-Seq22 | 169–173 | — | 168–172 | — | 164–172 | 162–166 | — | — | — |
| R12-Seq01 | 205–231 | — | — | — | — | — | — | — | — |
| R12-Seq25 | 180–190 | — | — | — | — | — | — | 180–192 | — |
| R12-Seq31 | 221–231 | — | — | — | — | — | — | — | — |
| R12-Seq43 | 143* | 143* | — | — | — | — | — | 143* | — |

*Monomorphic locus.

APPENDIX 1. Voucher and locality information for the samples used in this study.^a

| Species | n | Voucher no. | Country | Latitude | Longitude |
|--|----|---|--------------|----------|-----------|
| <i>Guibourtia tessmannii</i> (Harms) J. Léonard ^b | 1 | FT0001 | Gabon | 1.4286 | 11.5886 |
| <i>Guibourtia tessmannii</i> ^c | 3 | FT0002, FT0635–FT0636 | Cameroon | 2.2236 | 10.3793 |
| <i>Guibourtia tessmannii</i> ^c | 4 | FT0003, FT0800–FT0802 | Gabon | –0.3802 | 12.5649 |
| <i>Guibourtia tessmannii</i> ^d | 35 | FT0540–FT0545, FT0572–FT0600 | Gabon | 0.36 | 13.10 |
| <i>Guibourtia tessmannii</i> ^d | 58 | FT0800–FT0849, FT0851–FT0856, FT0900–FT0902 | Gabon | 0.76 | 12.9 |
| <i>Guibourtia tessmannii</i> ^d | 38 | FT0605–FT0636, OH4675, OH4679, OH4682, OH4683, OH4684, OH4685 | Cameroon | 2.37 | 10.63 |
| <i>Guibourtia pellegriniana</i> J. Léonard ^d | 14 | FT0641–FT0654 | Gabon | –2.53 | 9.77 |
| <i>Guibourtia coleosperma</i> (Benth.) J. Léonard ^d | 20 | FT0698–FT0717 | DRC | –10.48 | 22.45 |
| <i>Guibourtia coleosperma</i> ^d | 13 | FT0021–FT0024, FT0028–FT0031, FT0722–FT0726 | Namibia | –18.05 | 19.62 |
| <i>Guibourtia leonensis</i> J. Léonard ^d | 3 | BR0000013186371 ^f , BR0000013186401 ^f , BR0000013186388 ^f | Liberia | 7.66 | –10.02 |
| <i>Guibourtia demousei</i> (Harms) J. Léonard ^d | 9 | FT0873–FT0879, OH3245, BR0000009459977 ^f | DRC | –0.88 | 18.12 |
| <i>Guibourtia ehie</i> (A. Chev.) J. Léonard ^d | 10 | FT0335–FT0344 | Ivory Coast | 6.28 | –3.68 |
| <i>Guibourtia ehie</i> ^d | 10 | FT0163–FT0172 | Ghana | 7.02 | –2.05 |
| <i>Guibourtia carrisoana</i> (M. A. Exell) J. Léonard ^d | 2 | BR0000013186210 ^f , BR0000013186418 ^f | Angola | –8.83 | 13.25 |
| <i>Guibourtia copallifera</i> Benn. ^d | 7 | FT0880–FT0886 | Burkina-Faso | 9.95 | –4.67 |
| <i>Guibourtia arnoldiana</i> (De Wild. & T. Durand) J. Léonard ^d | 2 | FT0638, GID2040 | Gabon | –3.4098 | 11.4185 |
| <i>Guibourtia dinklagei</i> (Harms) J. Léonard ^d | 1 | BR0000013186265 ^f | Liberia | 6.23084 | –9.81249 |
| <i>Guibourtia tessmannii</i> ^e | 3 | FT0007, FT006, FT008 | Gabon | –0.42 | 12.58 |
| <i>Guibourtia coleosperma</i> ^e | 3 | FT0020, FT0024, FT0028 | Namibia | –17.99 | 24.09 |

Note: DRC = Democratic Republic of Congo; n = number of individuals.

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

^b Individual used for DNA bank.

^c Individual used for tests of amplification and polymorphism.

^d Individuals used for cross-amplification.

^e Individuals used for flow cytometry (code for the mother tree).

^f Codes of specimens from which samples were collected in Botanic Garden Meise (BR), Belgium.