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Author(s): Félicien Tosso, Jean-Louis Doucet, Jérémie Migliore, Kasso Daïnou, Esra Kaymak, Franck S. Monthe Kameni, and Olivier J. Hardy

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CHARACTERIZATION OF MICROSATELITE 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 Dainou**\(^2,4,5,6\), **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 Kérou, BP 43 Kérou, 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.

**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 Mariaec 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 (Miglécz 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).

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\(^2\)Author for correspondence: dnftosso@ulg.ac.be

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**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 MgCl₂ (25 mM), 0.45 μL of dNTPs (10 mM each), 0.5 μL of each primer (0.2 μM), 0.08 μL of Top Taq DNA Polymerase (5 U/μL), 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: 5-min initial de- naturation at 94 °C (10 min); 30 cycles of 94 °C for 45 s, and 72 °C for 60 s; and a final extension at 72 °C (10 min). Amplification products stained with 9 μL of Hi-Di Formamide (Life Technologies, Carlsbad, California, USA), and 0.3 μL of Map Marker 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.

**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 an ABI3730 sequencer (Applied Biosystems, Lennox, 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 Map Marker 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.

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

| Locus  | Primer sequences (5′–3′) | Fluorescent label | Repeat motif | Allele size range (bp) | GenBank accession no. |
|--------|------------------------|-----------------|--------------|-----------------------|----------------------|
| **Multiplex 1** | | | | | |
| GuiE-ssr39 | F: CACTGCTTAGAGCGATGCTTGGTGTTAAAGTTGTTGGTTG | Q3-VIC | (AT)₄⁺ | 132–156 | KY929303 |
| GuiE-ssr34 | R: ATTAGTTCTATGCTTATCTTCAA | Q2-NED | (AT)₁₀ | 152–180 | KY929300 |
| GuiE-ssr18 | R: GAGGTCAAAGCAGGGAACAA | Q2-NED | (AG)₁₄ | 180–190 | KY929294 |
| GuiE-ssr05 | F: GTGAAAAAGACTGATGTTGCG | Q1-6-FAM | (TC)₆ | 262–264 | KY929289 |
| GuiE-ssr33 | R: CCAAGGCTCCATGCAATGCA | Q1-6-FAM | (AG)₁₁ | 142–153 | KY929299 |
| **Multiplex 2** | | | | | |
| GuiE-ssr36 | F: TTAGGGATGACCAAGCATCAAAAGACCCCTCCCGAATCT | Q2-NED | (CT)₁₃ | 147–163 | KY929301 |
| GuiE-ssr03 | R: TCAAGTACGATCTAAAGAACCTTT | Q4-PET | (TG)₃ | 219–283 | KY929287 |
| GuiE-ssr02 | R: GCCAATGTAGTAGACTATGCAG | Q3-VIC | (ATT)₁₀ | 262–294 | KY929286 |
| GuiE-ssr06 | R: CAGCTCTTAGAGGATGCTCCTAAGAAGCTAACC | Q3-VIC | (TA)₁₄ | 232–294 | KY929290 |
| GuiE-ssr31 | F: GTGAAAAACAGGAGCCAGTATTAAACCTAAGCACAATC | Q1-6-FAM | (AG)₁₁ | 143–153 | KY929298 |
| **Multiplex 3** | | | | | |
| GuiE-ssr01 | F: TTAGAAACAGGAGCCAGTATGAGTAGCAAAACCGGTA | Q1-6-FAM | (AG)₁₁ | 308–316 | KY929285 |
| GuiE-ssr04′ | R: TGCCTAAATGCAGGTTGGAGTTTCAATC | Q4-PET | (CT)₈ | 267 | KY929288 |
| GuiE-ssr15 | R: TCAAGTACGATCTAAAGAACCTTT | Q3-VIC | (TA)₁₀ | 200–230 | KY929293 |
| GuiE-ssr21 | F: TCGAAACAGGAGCCAGTATGAGTAGCAAAACCGGTA | Q1-6-FAM | (TC)₁₂ | 141–189 | KY929295 |
| GuiE-ssr38 | R: CCAAGGCTCCATGCAATGCA | Q2-NED | (AG)₁₀ | 143–152 | KY929302 |
| **Multiplex 4** | | | | | |
| GuiE-ssr30 | F: CACTGCTTAGAGCGATGCTTGGTGTTAAAGTTGTTGGTTG | Q4-PET | (TA)₁₀ | 222–260 | KY929291 |
| GuiE-ssr11 | R: GAGGTCAAAGCAGGGAACAA | Q3-VIC | (AT)₁₀ | 205–245 | KY929292 |
| GuiE-ssr28 | R: CCAAGGCTCCATGCAATGCA | Q4-PET | (TA)₁₀ | 159–167 | KY929296 |
| GuiE-ssr30 | F: TTAGGGATGACCAAGCATCAAAAGACCCCTCCCGAATCT | Q2-NED | (AG)₁₁ | 145–157 | KY929297 |

* Optimal annealing temperature was 57°C and 53°C, respectively, for PCR cycles 1 and 2.

* The linkers (Q1, Q2, Q3, Q4) attached to the forward primers are underlined in the forward primer sequences.

* Monomorphic locus.
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 Gorskaia J. Léonard (to which G. ehie belongs), whereas two to six amplified for subgenus Pseudopomatia 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.

LITERATURE CITED

Chybicki, J. J., and J. Burczyk. 2009. Simultaneous estimation of null alleles and inbreeding coefficients. Journal of Heredity 100: 106–113.

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.

Lemmens, R. H. M. J., D. Louppe, and A. A. Oteng-Amoako. 2008. Bois d’œuvre, vol. 2. PROTA, Wageningen, The Netherlands.

Litt, S. M., C. A. Reitz, K. L. Pechmann, J. A. Rzedowski, and R. B. Moden. 2011. Development and characterization of microsatellite loci in Periplocas lota (Fabaceae) using a cost-efficient approach. American Journal of Botany 98: e268–e270.

Miegélec, E., N. Pich, 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.
### Table 3. Cross-amplification results of 19 microsatellite markers isolated from *Guibourtia ehie* and tested in 13 congeneric species belonging to three *Guibourtia* subgenera.\(^a\)

| Locus   | Subgenus Gorskia                      | Subgenus Pseudocopaiva                  | Subgenus Guibourtia                     |
|---------|--------------------------------------|----------------------------------------|----------------------------------------|
|         | 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. carrissomana \((N = 2)\) | 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 | — | — | — | — | — | — | — | — | — | — | — | — | — |
|          | 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.
## Voucher information for the *Guibourtia* samples used in this study.

| Species                          | Number | Voucher no.                                                                 | Country         | Latitude     | Longitude    |
|---------------------------------|--------|-----------------------------------------------------------------------------|-----------------|--------------|--------------|
| *Guibourtia ehie* (A. Chev.)    | 1      | FT0272                                                                      | Ghana           | 7.09241      | -2.11953     |
| *Guibourtia ehie*               | 1      | FT0288                                                                      | Ghana           | 7.08999      | -2.11845     |
| *Guibourtia ehie*               | 1      | FT0478                                                                      | Ivory Coast     | 6.30892      | -5.28866     |
| *Guibourtia ehie*               | 5      | FT0497, FT0491, FT0515, FT0510, FT0521                                      | Ivory Coast     | 6.21          | -3.41        |
| *Guibourtia ehie*               | 3      | FT0241, FT0261, FT0241                                                     | Ghana           | 7.07          | -2.08        |
| *Guibourtia ehie*               | 8      | OH4661-OH4668                                                               | Cameroon        | 2.31          | 9.96         |
| *Guibourtia ehie*               | 20     | FT0029, FT0038, FT0059, FT0078, FT0087, FT0095, FT0102, FT0104, FT0115, FT0125, FT0137, FT0146, FT0158, FT0163, FT0169, FT0180, FT0192, FT0192a, FT0193, FT0197 | Cameroon        | 7.06          | -2.08        |
| *Guibourtia ehie*               | 23     | FT0398-FT0400, FT0336, FT0355, FT0373, FT0382, FT0384, FT0389, FT0411, FT0430, FT0465, FT0489, FT0491, FT0497, FT0497, FT0498, FT0510, FT0515, FT0519, FT0521, FT0858, FT0859 | Ivory Coast     | 6.21          | -2.42        |
| *Guibourtia ehie*               | 15     | FT0398, FT0336, FT0355, FT0373, FT0382, FT0384, FT0389, FT0411, FT0430, FT0465, FT0489, FT0491, FT0497, FT0497, FT0498, FT0510, FT0515, FT0519, FT0521, FT0858, FT0859 | Cameroon        | 2.44          | 9.92         |
| *Guibourtia ehie*               | 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 | 3      | HB00527556                                                                  | Gabon           | -1.3465      | 9.7232       |
| *Guibourtia schliebenii* (Harms) J. Léonard | 3      | B23-HB10151                                                                 | Mozambique      | -11.1529     | 39.7343      |
| *Guibourtia conjugata* (Bolle) J. Léonard | 1      | B33-HB3499528                                                               | Mozambique      | -23.6548     | 32.1746      |
| *Guibourtia dinklagei* (Harms) J. Léonard | 1      | B21-HB11235                                                                 | Liberia         | 6.279        | -10.7603     |
| *Guibourtia tessmannii* (Harms) J. Léonard | 10     | FT0607–FT0613, FT0635–FT0636                                                | Cameroon        | 2.2236       | 10.3793      |
| *Guibourtia pellegriniana* J. Léonard | 7      | B11-HB1578                                                                  | Gabon           | 1.4286       | 11.5886      |
| *Guibourtia coleosperma* (Benth.) J. Léonard | 6      | FT0021–FT0025, FT0028                                                      | Namibia         | -17.85       | 19.67        |
| *Guibourtia leonensis* J. Léonard | 1      | B45-HB3015140                                                               | Sierra Leone    | 8.9852       | -11.7169     |
| *Guibourtia hymenaefolia* (Moric.) J. Léonard | 1      | B44-HB252852                                                                | Cuba            | 22.1315      | -80.3382     |
| *Guibourtia carrissoma* (M. A. Exell) J. Léonard | 2      | B19-HB10458                                                                 | Angola          | -8.9341      | 13.1864      |
| *Guibourtia copallifera* Benn. | 5      | FT0880–FT0884                                                              | Angola          | -8.836       | 13.2593      |
| *Guibourtia demeusei* (Harms) J. Léonard | 6      | FT0873–FT0875, OH3245                                                       | Burkina-Faso   | 9.95         | 4.67         |
| *Guibourtia sousae* J. Léonard | 1      | B52-HB892206                                                                | Gabon           | -2.2487      | 9.5929       |

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

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

*Individual used for genomic library.*

*Individuals used for amplification tests.*

*Individuals used for polymorphism tests.*

*Individuals used for cross-amplification tests.*