

ISOLATION, CHARACTERISATION AND CROSS-SPECIES AMPLIFICATION OF NUCLEAR MICROSATELLITES IN THE AFRICAN TREE GENUS *GREENWAYODENDRON* (ANNONACEAE)

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PIÑEIRO R, MICHENEAU C, DAUBY G & HARDY OJ. 2016. Isolation, characterisation and cross-species amplification of nuclear microsatellites in the African tree genus *Greenwayodendron* (Annonaceae). *Greenwayodendron* is a genus of rainforest trees endemic to tropical Africa. Eight nuclear microsatellite loci, amplifiable in two multiplexed reactions, were developed in *G. suaveolens* and cross-amplified in the second species of the genus, *G. oliveri*, as well as in all subspecies and varieties recognised by current taxonomic treatments. Characterisation of the microsatellite markers in one population of *G. suaveolens* from Cameroon revealed polymorphism levels suitable for characterisation of the spatial genetic variation (7 to 16 alleles per locus and expected heterozygosity ranging from 0.57 to 0.89). The eight microsatellite loci optimised could help estimate genetic diversity levels in populations of *G. suaveolens*, *G. oliveri* and new local endemic species of *Greenwayodendron* in rainforests from western and central Africa.

Keywords: Microsatellite-enriched library, next-generation sequencing, tropical trees, universal fluorescent-labelled primer

INTRODUCTION

Greenwayodendron (Annonaceae) is a monophyletic tree genus from tropical Africa (Mols et al. 2004). Current taxonomic treatments recognise two species, namely, *G. oliveri* in western Africa, and *G. suaveolens* with two disjoint subspecies in central and eastern Africa: *G. suaveolens* ssp. *suaveolens* and *G. suaveolens* ssp. *usambaricum* respectively (Aubréville 1959, Le Thomas 1969, Verdcourt 1971). In rainforests of central Africa, two taxa, currently considered as varieties of *G. suaveolens* ssp. *suaveolens*, are recognised, i.e. a widespread taxon, var. *suaveolens*, and a narrowly-distributed variety restricted to Gabon, var. *gabonica*. These two varieties may grow in sympatry, have exclusive plastid haplotypes (Dauby et al. 2010) and display clear morphological differences in terms of leaf and floral dimensions and pubescence of leaves (Le Thomas 1969), suggesting that they may deserve the status of species. In addition, based on their divergent morphology, two putative new local species may exist, although they have not been formally described yet (G Dauby, personal observation). The first occurs in São

Tomé Island, in the Gulf of Guinea, hereafter referred to as *G. cf. "São Tomé"*. The second is distributed along the south-west coast of Gabon (hereafter referred to as *G. cf. "littoral"*). In this study we intended to isolate and characterise the first set of nuclear microsatellite markers for *G. suaveolens* ssp. *suaveolens* var. *suaveolens*, and examine cross-amplification in all taxa within *Greenwayodendron*.

MATERIALS AND METHODS

A microsatellite library was generated by pooling 1 µg of genomic DNA from a single individual of *G. suaveolens* ssp. *suaveolens* var. *suaveolens*, and two individuals of two unrelated species with a sequencer at the Genoscreen genomic platform in Lille, France, following the methods by Malausa et al. (2011) and Micheneau et al. (2011). From a total of 23,901 reads, 7789 primers for 846 loci containing _nSSR for the three pooled species were designed. After discarding short reads and reads containing short microsatellites,

primers were designed automatically by Genoscreen genomic platform using Primer3 in the software QDD, version 2 (Megléczy et al. 2010). Seventy-two primer pairs were tested for robust amplification and polymorphism on seven individuals from different populations of *G. suaveolens* ssp. *suaveolens* var. *suaveolens* according to Piñeiro et al. (2012). For 29 loci, where faint amplifications were observed, new primer pairs were redesigned manually using Primer3 (Rozen & Skaletsky 1999). Eight loci were finally optimised in two multiplex reactions, which consistently amplified either five (GMF2) or three loci (GMG3) (Table 1). PCR reactions were carried out in a total volume of 15 µL using Qiagen multiplex kit. Fluorescent labelling was performed via amplification with (1) reverse primer, (2) forward primer with a Q1–Q4 universal sequence at the 5' end and (3) Q1 labelled with 6-FAM, Q2 with NED, Q3 with VIC and Q4 with PET (Schuelke 2000, Micheneau et al. 2011). The PCR protocol was: 7.5 µL multiplex PCR master mix, 0.1 µL (0.07 µM) of forward primers, and 0.15 µL (0.1 µM) of reverse primers, 0.15 µL (0.1 µM) of labelled primers Q1, Q2, Q3 and Q4, 1.5 µL DNA (10–100 ng µL⁻¹), and H₂O. Multiplex PCR programs consisted of 95 °C (15 min), followed by 20 cycles of 94 °C (30 s), 57 °C (90 s) for GMF2 or 55 °C (180 s) for GMG3, 72 °C (60 s), followed by 10 cycles of 94 °C (30 s), 53 °C (45 s), 72 °C (45 s), and a final extension at 60 °C (30 min). PCR products were run on DNA sequencer with 12 µL HiDi and 0.3 µL of size standard LIZ®500. The resulting electropherograms were automatically scored with Genemapper 3.7 and manually corrected.

Sixty-three individuals from a single population of *G. suaveolens* ssp. *suaveolens* var. *suaveolens* from south-east Cameroon were genotyped (see Appendix). The quality of microsatellite markers isolated was evaluated by calculating the allele size range, number of alleles per locus, observed (H_o) and expected (H_e) heterozygosities and inbreeding coefficient (F_{is}). Hardy–Weinberg equilibrium (HWE) tests were performed for each locus using SPAGeDi 1.4 (Hardy & Vekemans 2002). Null alleles were coded differently from missing data. The frequency of null alleles was estimated by taking into account average inbreeding of the population using the software INEst 1.0 (Chybicki

& Burczyk 2009). Linkage disequilibrium tests among pairs of loci were performed using the software GENEPOP 4.1.4 (Raymond & Rousset 1995, Rousset 2008).

RESULTS AND DISCUSSION

All eight microsatellite loci were shown to be polymorphic within *G. suaveolens* ssp. *suaveolens* var. *suaveolens* (Table 1). The number of alleles per locus ranged from 7 to 16, with an average of 11.2. Observed heterozygosity varied between 0.55 and 0.95 (average 0.71) and expected heterozygosity between 0.57 and 0.90 (average 0.72). The microsatellite loci in the multiplex reaction, namely, PIPE3-67B and, to a lesser extent, PIPE3-40B, exhibited slightly high null allele frequencies (9.8 and 4.2% respectively) and departure from HWE due to homozygote excess. Linkage disequilibrium was detected between pairs of loci PIPE3-21/PIPE3-65, PIPE3-21/PIPE3-72B and PIPE3-18/PIPE3-72B.

Amplification of the two multiplexed PCR reactions was tested on all taxa of the genus *Greenwayodendron* (Table 2, Appendix), i.e. the western African species *G. oliveri* (13 individuals from three locations in Ivory Coast), the narrowly-distributed taxon from Gabon *G. suaveolens* ssp. *suaveolens* var. *gabonica* (63 individuals largely distributed across Gabon), the subspecies from east Africa *G. suaveolens* ssp. *usambaricum* (1 individual), the putative new local species from São Tomé island (3 individuals from one location) and from the south-west coast of Gabon (1 individual). All eight loci were successfully genotyped in var. *gabonica* (seven of them polymorphic), and seven loci amplified in *G. oliveri* (six of them polymorphic) (Table 2). *Greenwayodendron suaveolens* ssp. *usambaricum* was successfully genotyped for six loci and the putative local species from São Tomé, *G. cf.* "São Tomé", and from coastal Gabon, *G. cf.* "littoral", worked for seven and five loci respectively.

The eight microsatellite loci constituted significant contribution to the genetic resources available for the family Annonaceae. This is one of the major families of tropical trees in the Neotropics and in the Old World (Couvreur et al. 2011, Chatrou et al. 2012). Since abundance and species richness of Annonaceae correlate well with temperature and precipitation, this family provide a good proxy for tropical rainforests. To

Table 1 Characterisation of 8 nuclear microsatellite loci isolated from *Greenwayodendron suaveolens* ssp. *suaveolens* var. *suaveolens* and characterisation of polymorphisms in 63 individuals from a population in south-east Cameroon

Locus name	GenBank nr	Primer sequence (5'–3')	Label F-primer*	Repeat motif	Ta (°C)	Allele size range	Allele nr	H _o	H _e	F _{is}	Null
Multiplex GMF2											
PIPE3-18	KP172239	F:ACAAAAATTTTCATTACAGAGCCAG R:AAAGTGGAAATCTGCTCACAAA	Q2-NED	(gt) ₁₀	57	154–182	7	0.612	0.581	-0.067	0
PIPE3-40B	KP172234	F:AAAAACTAAATTTACAAAGAATGCAGA R:ACCGAGCCAAACTGAGTAGC	Q4-PET	(ga) ₁₇	57	161–181	8	0.645	0.736	0.124**	0.042
PIPE3-65	KP172235	F:GACAATGCTAAGCGTGTGGA R:AGCTGCACCCAGAGAAGGATT	Q1-6-FAM	(tct) ₁₈	57	238–313	10	0.556	0.568	0.023	0.002
PIPE3-67B	KP172236	F:GGTTTAGATTGGGCATCTTCA R:CCAACATGAATTTTTCGACG	Q3-VIC	(tct) ₁₃	57	191–251	15	0.694	0.9	0.229**	0.098
PIPE3-72B	KP172237	F:GAAGGTCAAACCGAGCAGTC R:AACGCCAGCTATTGGTAACGC	Q2-NED	(ctt) ₁₁	57	194–227	11	0.746	0.705	-0.059	0
Multiplex GMG3											
PIPE3-03B	KP172238	F:GGGATTATTCTTCATGGATTCCG R:TGCATCATGATTGAACCTTACCA	Q3-VIC	(tc) ₁₁	55	191–217	12	0.746	0.697	-0.071	0
PIPE3-21	KP172240	F:TTGGCCATTTCTACTTTGGG R:CCAGTGAACCAATTC AACCCAG	Q1-6-FAM	(ct) ₁₁	55	152–197	16	0.952	0.893	-0.066	0
PIPE3-38	KP172233	F:ACATCTTGCCATTCCTTIGATTG R:AGTTATAGGGACAAATGTGATAGTTGG	Q2-NED	(ca) ₁₂	55	212–239	11	0.714	0.724	0.013	0

*Q1 = TGTA AAAAGGAGCCAGT (Schuelke 2000), Q2 = TAGGAGTGCAGCAAGCAT, Q3 = CACTGCTTAGAGCGATGC, Q4 = CTAGTTATTGCTCAGCGGT (Q2-Q4) (Culley et al. 2008); Ta = annealing temperature, H_o = observed heterozygosity, H_e = expected heterozygosity, F_{is} = inbreeding coefficient, ** significant deviation from Hardy-Weinberg equilibrium, Null = frequency of null alleles; nr = number

Table 2 Cross-amplification of eight microsatellite markers developed in all taxa of the genus *Greenwayodendron*

Taxon	PIPE3-18		PIPE3-40B		PIPE3-65		PIPE3-67B		PIPE3-72B		PIPE3-03B		PIPE3-21		PIPE3-38	
	Allele nr	Allele size range	Allele nr	Allele size range	Allele nr	Allele size range	Allele nr	Allele size range	Allele nr	Allele size range	Allele nr	Allele size range	Allele nr	Allele size range	Allele nr	Allele size range
<i>G. suaaveolens</i> ssp. <i>suaaveolens</i> var. <i>suaaveolens</i> (n = 63)	7	154–182	8	161–181	10	238–313	15	191–251	11	194–227	12	191–217	16	152–197	11	212–239
<i>G. suaaveolens</i> ssp. <i>suaaveolens</i> var. <i>gabonica</i> (n = 63)	3	150–171	9	156–182	8	273–310	6	191–221	13	206–238	10	193–248	1	196	17	212–248
<i>G. oliveri</i> (n = 13)	–	–	6	156–171	1	270	4	191–209	4	199–218	8	188–205	3	166–174	2	210–212
<i>G. cf. "São Tomé"</i> (n = 3)	2	160–161	2	163–165	3	260–298	5	191–233	1	218	2	189–191	–	–	2	207–212
<i>G. suaaveolens</i> ssp. <i>usambaricum</i> (n = 1)	2	170–176	2	165–167	–	–	1	192	2	221–227	2	188–189	–	–	1	212
<i>G. cf. "littoral"</i> (n = 1)	2	185–187	1	147	2	265–282	2	221–227	2	230–233	–	–	–	–	–	–

nr = number

date, microsatellite markers have been developed only for a few of the 109 validly-described genera, mostly those containing cultivated species such as cherimoya (Escribano et al. 2008, Ribeiro et al. 2014) or pawpaw (Pomper et al. 2010).

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Appendix Genotype of 63 individuals from a single population of *G. suaveolens* ssp. *suaveolens* var. *suaveolens* from south-east Cameroon

Voucher	Locality	Elevation (m asl)	Latitude (N)	Longitude (E)	Collector	Identification	Date (m/d/y)
<i>G. suaveolens</i> ssp. <i>suaveolens</i> var. <i>suaveolens</i>							
JW0210	Pallisco Forestry Concession, Cameroon	650	14.13	3.35	Vleminckx J	Vleminckx J	2/1/2012
JW0211	Pallisco Forestry Concession, Cameroon	650	14.14	3.34	Vleminckx J	Vleminckx J	2/1/2012
JW0212	Pallisco Forestry Concession, Cameroon	650	14.14	3.34	Vleminckx J	Vleminckx J	2/1/2012
JW0213	Pallisco Forestry Concession, Cameroon	650	14.14	3.34	Vleminckx J	Vleminckx J	2/1/2012
JW0214	Pallisco Forestry Concession, Cameroon	650	14.14	3.34	Vleminckx J	Vleminckx J	2/1/2012
JW0215	Pallisco Forestry Concession, Cameroon	650	14.14	3.34	Vleminckx J	Vleminckx J	2/1/2012
JW0216	Pallisco Forestry Concession, Cameroon	650	14.14	3.34	Vleminckx J	Vleminckx J	2/1/2012
JW0217	Pallisco Forestry Concession, Cameroon	650	14.15	3.34	Vleminckx J	Vleminckx J	2/1/2012
JW0218	Pallisco Forestry Concession, Cameroon	650	14.15	3.34	Vleminckx J	Vleminckx J	2/1/2012
JW0219	Pallisco Forestry Concession, Cameroon	650	14.15	3.34	Vleminckx J	Vleminckx J	2/1/2012
JW0220	Pallisco Forestry Concession, Cameroon	650	14.15	3.34	Vleminckx J	Vleminckx J	2/1/2012
JW0221	Pallisco Forestry Concession, Cameroon	650	14.16	3.34	Vleminckx J	Vleminckx J	2/1/2012
JW0222	Pallisco Forestry Concession, Cameroon	650	14.16	3.34	Vleminckx J	Vleminckx J	2/1/2012
JW0223	Pallisco Forestry Concession, Cameroon	650	14.16	3.34	Vleminckx J	Vleminckx J	2/1/2012
JW0224	Pallisco Forestry Concession, Cameroon	650	14.16	3.34	Vleminckx J	Vleminckx J	2/1/2012
JW0225	Pallisco Forestry Concession, Cameroon	650	14.16	3.34	Vleminckx J	Vleminckx J	2/1/2012
JW0226	Pallisco Forestry Concession, Cameroon	650	14.16	3.34	Vleminckx J	Vleminckx J	2/1/2012
JW0227	Pallisco Forestry Concession, Cameroon	650	14.16	3.34	Vleminckx J	Vleminckx J	2/1/2012
JW0228	Pallisco Forestry Concession, Cameroon	650	14.16	3.34	Vleminckx J	Vleminckx J	2/1/2012
JW0229	Pallisco Forestry Concession, Cameroon	650	14.16	3.34	Vleminckx J	Vleminckx J	2/1/2012
JW0230	Pallisco Forestry Concession, Cameroon	650	14.16	3.34	Vleminckx J	Vleminckx J	2/1/2012
JW0231	Pallisco Forestry Concession, Cameroon	650	14.16	3.34	Vleminckx J	Vleminckx J	2/1/2012
JW0232	Pallisco Forestry Concession, Cameroon	650	14.16	3.34	Vleminckx J	Vleminckx J	2/1/2012
JW0233	Pallisco Forestry Concession, Cameroon	650	14.16	3.34	Vleminckx J	Vleminckx J	2/1/2012
JW0234	Pallisco Forestry Concession, Cameroon	650	14.17	3.34	Vleminckx J	Vleminckx J	2/1/2012
JW0235	Pallisco Forestry Concession, Cameroon	650	14.17	3.34	Vleminckx J	Vleminckx J	2/1/2012
JW0236	Pallisco Forestry Concession, Cameroon	650	14.17	3.34	Vleminckx J	Vleminckx J	2/1/2012
JW0237	Pallisco Forestry Concession, Cameroon	650	14.17	3.34	Vleminckx J	Vleminckx J	2/1/2012

(continued)

Appendix (continued)

Voucher	Locality	Elevation (m asl)	Latitude (N)	Longitude (E)	Collector	Identification	Date (m/d/y)
JV0238	Pallisco Forestry Concession, Cameroon	650	14.17	3.34	Vleminckx J	Vleminckx J	2/1/2012
RP0001	Pallisco Forestry Concession, Cameroon	538	14.27	3.22	Piñeiro R	Théofile AYOL	7/21/2012
RP0008	Pallisco Forestry Concession, Cameroon	551	14.27	3.21	Piñeiro R	Théofile AYOL	7/21/2012
RP0058	Pallisco Forestry Concession, Cameroon	560	14.27	3.21	Piñeiro R	Théofile AYOL	7/21/2012
RP0060	Pallisco Forestry Concession, Cameroon	560	14.27	3.21	Piñeiro R	Théofile AYOL	7/21/2012
RP0075	Pallisco Forestry Concession, Cameroon	572	14.27	3.22	Piñeiro R	Théofile AYOL	7/22/2012
RP0077	Pallisco Forestry Concession, Cameroon	574	14.27	3.22	Piñeiro R	Théofile AYOL	7/22/2012
RP0133	Pallisco Forestry Concession, Cameroon	571	14.25	3.19	Piñeiro R	Théofile AYOL	7/23/2012
RP0175	Pallisco Forestry Concession, Cameroon	560	14.22	3.16	Piñeiro R	Théofile AYOL	7/24/2012
RP0225	Pallisco Forestry Concession, Cameroon	605	14.22	3.16	Piñeiro R	Théofile AYOL	7/25/2012
RP0234	Pallisco Forestry Concession, Cameroon	587	14.22	3.17	Piñeiro R	Théofile AYOL	7/25/2012
RP0271	Pallisco Forestry Concession, Cameroon	552	14.22	3.16	Piñeiro R	Théofile AYOL	7/25/2012
RP0285	Pallisco Forestry Concession, Cameroon	567	14.22	3.16	Piñeiro R	Théofile AYOL	7/25/2012
RP0286	Pallisco Forestry Concession, Cameroon	567	14.22	3.16	Piñeiro R	Théofile AYOL	7/25/2012
RP0293	Pallisco Forestry Concession, Cameroon	561	14.27	3.21	Piñeiro R	Théofile AYOL	7/26/2012
RP0294	Pallisco Forestry Concession, Cameroon	565	14.27	3.21	Piñeiro R	Théofile AYOL	7/26/2012
RP0301	Pallisco Forestry Concession, Cameroon	569	14.27	3.21	Piñeiro R	Théofile AYOL	7/26/2012
RP0320	Pallisco Forestry Concession, Cameroon	569	14.27	3.21	Piñeiro R	Théofile AYOL	7/26/2012
RP0322	Pallisco Forestry Concession, Cameroon	569	14.27	3.21	Piñeiro R	Théofile AYOL	7/26/2012
RP0326	Pallisco Forestry Concession, Cameroon	564	14.27	3.21	Piñeiro R	Théofile AYOL	7/26/2012
RP0329	Pallisco Forestry Concession, Cameroon	560	14.27	3.21	Piñeiro R	Théofile AYOL	7/26/2012
RP0331	Pallisco Forestry Concession, Cameroon	563	14.27	3.21	Piñeiro R	Théofile AYOL	7/26/2012
RP0337	Pallisco Forestry Concession, Cameroon	562	14.27	3.21	Piñeiro R	Théofile AYOL	7/26/2012
RP0345	Pallisco Forestry Concession, Cameroon	584	14.11	3.17	Piñeiro R	Théofile AYOL	7/30/2012
RP0368	Pallisco Forestry Concession, Cameroon	581	14.12	3.18	Piñeiro R	Théofile AYOL	7/30/2012
RP0369	Pallisco Forestry Concession, Cameroon	579	14.12	3.18	Piñeiro R	Théofile AYOL	7/30/2012
RP0382	Pallisco Forestry Concession, Cameroon	596	14.11	3.18	Piñeiro R	Théofile AYOL	7/30/2012
RP0401	Pallisco Forestry Concession, Cameroon	584	14.11	3.17	Piñeiro R	Théofile AYOL	7/31/2012
RP0413	Pallisco Forestry Concession, Cameroon	598	14.11	3.17	Piñeiro R	Théofile AYOL	7/31/2012
RP0414	Pallisco Forestry Concession, Cameroon	596	14.11	3.17	Piñeiro R	Théofile AYOL	7/31/2012
RP0441	Pallisco Forestry Concession, Cameroon	595	14.11	3.17	Piñeiro R	Théofile AYOL	7/31/2012

(continued)

Appendix (continued)

Voucher	Locality	Elevation (m asl)	Latitude (N)	Longitude (E)	Collector	Identification	Date (m/d/y)
RP0447	Pallisco Forestry Concession, Cameroon	591	14.05	3.20	Piñeiro R	Théofile AYOL	9/1/2012
RP0449	Pallisco Forestry Concession, Cameroon	605	14.05	3.20	Piñeiro R	Théofile AYOL	9/1/2012
RP0454	Pallisco Forestry Concession, Cameroon	598	14.05	3.20	Piñeiro R	Théofile AYOL	9/1/2012
RP0479	Pallisco Forestry Concession, Cameroon	568	14.05	3.21	Piñeiro R	Théofile AYOL	9/1/2012
<i>G. suaveolens</i> ssp. <i>suaevolens</i> var. <i>gabonica</i>							
CD0161	Gabon		9.96	-1.15	Doumange C	Doumange C	
CD0175	Gabon		9.76	-0.64	Doumange C	Doumange C	
CD0184	Gabon		9.34	-1.00	Doumange C	Doumange C	
CD0186	Gabon		9.34	-1.00	Doumange C	Doumange C	
CD0200	Gabon		9.43	-1.14	Doumange C	Doumange C	
CD0207	Gabon		9.76	-0.94	Doumange C	Doumange C	
CD0267	Gabon		9.94	-1.72	Doumange C	Doumange C	
CD0295	Gabon		10.48	-2.01	Doumange C	Doumange C	
CD0527	Versant Est du Mayombe, région du mont Pelé et du village de Bikamba, province de la Nyanga, Gabon		11.14	-3.29	Doumange C	Doumange C	6/25/2008
CD0676	PN Moukalaba-Doudou, région de Doussala, province de l'Ogooué Maritime, Gabon		10.53	-2.42	Doumange C	Doumange C	6/29/2008
CD0746	Pied des monts Koumounabouali, région de Bikourou, province de la Ngounié, Gabon		10.58	-1.41	Doumange C	Doumange C	7/3/2008
CD0771	Monts Koumounabouali, région de Bikourou, province de la Ngounié, Gabon		10.57	-1.40	Doumange C	Doumange C	7/3/2008
GiD0090	Waka East, Gabon	573	11.29	-1.23	Missouri Botanical Garden	Dauby G	2/20/2008
GiD0151	Concession Rougier du Haut-Abanga, Sud-Est de Mikongo, partie Nord des montagnes Mekié, Gabon	400	11.22	0.40	Missouri Botanical Garden	Dauby G	7/13/2008
GiD0185	Concession Rougier du Haut-Abanga, Sud-Est de Mikongo, partie Nord des montagnes Mekié, Gabon	700	11.23	0.40	Missouri Botanical Garden	Dauby G	7/16/2008
GiD0202	Concession Rougier du Haut-Abanga, Sud-Est de Mikongo, partie Nord des montagnes Mekié, Gabon	940	11.23	0.40	Missouri Botanical Garden	Dauby G	7/17/2008
GiD0673	CFAD de Rimbunan Hijau, au Sud-Ouest du Parc National de la Lopé, Gabon	669	11.22	-0.68	Dauby G	Dauby G	2/26/2009

(continued)

Appendix (continued)

Voucher	Locality	Elevation (m asl)	Latitude (N)	Longitude (E)	Collector	Identification	Date (m/d/y)
GiD0684	CEAD de Rimbunan Hijau, au Sud-Ouest du Parc National de la Lopé, Gabon	644	11.22	-0.68	Dauby G	Dauby G	2/26/2009
GiD0687	CEAD de Rimbunan Hijau, au Sud-Ouest du Parc National de la Lopé, Gabon	347	11.26	-0.85	Dauby G	Dauby G	1/29/2009
GiD0688	CEAD de Rimbunan Hijau, au Sud-Ouest du Parc National de la Lopé, Gabon	425	11.28	-0.86	Dauby G	Dauby G	2/1/2009
GiD0689	CEAD de Rimbunan Hijau, au Sud-Ouest du Parc National de la Lopé, Gabon	423	11.26	-0.87	Dauby G	Dauby G	2/2/2009
GiD0861	Zone d'exploration de Sogademin, au Nord de Andok Foula, à l'est de la route, Gabon	168	10.19	0.45	Missouri Botanical Garden	Dauby G	7/12/2009
GiD0873	Région de Tchibanga, Gabon	591	11.12	-3.28	Missouri Botanical Garden	Nguema D	4/6/2009
GiD0875	Région de Tchibanga, Gabon	530	11.12	-3.28	Missouri Botanical Garden	Nguema D	4/6/2009
GiD1120	Gabon	130	10.87	-1.37	Missouri Botanical Garden	Stévant T	
GiD1122	Gabon	230	10.88	-1.38	Missouri Botanical Garden	Stévant T	
GiD1123	Gabon	183	10.88	-1.39	Missouri Botanical Garden	Stévant T	
GiD1127	Gabon		9.89	-1.81	Missouri Botanical Garden	Nguema D	
GiD1128	Gabon		9.85	-1.97	Missouri Botanical Garden	Nguema D	
GiD1131	Gabon		9.88	-1.83	Missouri Botanical Garden	Nguema D	
GiD1136	Gabon		9.84	-1.93	Missouri Botanical Garden	Nguema D	9/16/2009
GiD1172	Gabon	168	10.86	-1.39	Missouri Botanical Garden	Stévant T	11/11/2009
GiD1194	Concession de Rabi-Shell, Gabon	33	9.89	-1.79	Missouri Botanical Garden	Dauby G	1/27/2010
GiD1195	Concession de Rabi-Shell, Gabon	61	9.87	-1.83	Missouri Botanical Garden	Dauby G	1/25/2010
GiD1199	Concession de la CEB-zone milolé, Gabon	389	12.74	-0.24	Missouri Botanical Garden	Dauby G	2/14/2010
GiD1202	Concession de CEB, Nord de la zone de Milolé, Sud du Parc National de l'Ivindo, Gabon	387	12.74	-0.24	Missouri Botanical Garden	Dauby G	2/13/2010
GiD1208	Sud-Est de Koulamoutou, concession SFIK, Gabon	562	12.71	-1.34	Missouri Botanical Garden	Dauby G	2/22/2010
GiD1209	Sud-Est de Koulamoutou, concession SFIK, Gabon	562	12.71	-1.34	Missouri Botanical Garden	Dauby G	2/22/2010
GiD1307	Concession de la CEB-zone milolé, Gabon	387	12.73	-0.24	Missouri Botanical Garden	Dauby G	2/15/2010
GiD1721	Zone de Mabounié, à environ 45 km au sud-est de Lambaréné, rive est de la Ngounié, Gabon	26	10.47	-0.77	Dauby G	Dauby G	5/9/2012

(continued)

Appendix (continued)

Voucher	Locality	Elevation (m asl)	Latitude (N)	Longitude (E)	Collector	Identification	Date (m/d/y)
GiD1742	Zone de Mabounié, à environ 45 km au sud-est de Lambaréné, rive est de la Ngounié, Gabon	118	10.52	-0.75	Dauby G	Dauby G	5/13/2012
GiD1916	Zone de Mabounié, à environ 45 km au sud-est de Lambaréné, rive est de la Ngounié, Gabon	29	10.47	-0.77	Dauby G	Dauby G	5/9/2012
GiD2180	Zone de Mabounié, à environ 45 km au sud-est de Lambaréné, rive est de la Ngounié, Gabon		10.53	-0.80	Dauby G	Dauby G	
GK0034	Kinguélé, Gabon	410	10.27	0.48	Issembé Y	Koffi-Hardy	10/25/2007
MH0772	Popa, près du village Michimba et du PN Mont Birougou, Gabon	722	12.29	-1.65	Heuertz M	Heuertz M	9/2/2006
MH0831	La Lopé, sentier phénologique I, Gabon	331	11.58	-0.22	Heuertz M	Heuertz M	9/8/2006
MH0836	La Lopé, sentier phénologique I, Gabon	315	11.58	-0.22	Heuertz M	Heuertz M	9/8/2006
MH0843	La Lopé, sentier phénologique I, Gabon	374	11.58	-0.22	Heuertz M	Heuertz M	9/8/2006
MH0848	Koulamoutou-Banati, 20 km à l'ouest de Koulamoutou, Gabon	311	12.31	-1.09	Heuertz M	Heuertz M	8/28/2006
MH0904	La Lopé, sentier phénologique I, Gabon	343	11.58	-0.23	Heuertz M	Heuertz M	9/8/2006
MH0924	La Lopé, sentier phénologique "Crête", Gabon	294	11.58	-0.17	Heuertz M	Heuertz M	9/9/2006
OH2577	Mt de Cristal, Gabon	59	10.17	0.49	Niangadouma R	Hardy O	5/10/2011
OH2641	Mt de Cristal, Gabon	598	10.35	0.70	Niangadouma R	Hardy O	5/11/2011
OH2870	Woleu Niem, Gabon	330	11.47	0.53	Niangadouma R	Hardy O	5/16/2011
OH2963	Sud Mt Cristal (Rougier-Ht Abanga), Gabon	509	11.11	0.57	Niangadouma R	Hardy O	5/17/2011
OH2975	Sud Mt Cristal (Rougier-Ht Abanga), Gabon	468	11.11	0.56	Niangadouma R	Hardy O	5/17/2011
OH3013	Sud Mt Cristal (Rougier-Ht Abanga), Gabon	477	11.11	0.56	Niangadouma R	Hardy O	5/18/2011
RM0009	SEEF/Milolé, Gabon		13.14	-0.29	Mboma R	Mboma R	5/27/2009
TS0095	Concession SEEF, Monts de Cristal, Gabon	605	10.50	0.47	Niangadouma R	Stévant T	10/23/2010
TS0097	Concession SEEF, Monts de Cristal, Gabon	565	10.50	0.46	Niangadouma R	Stévant T	10/23/2010
TS0101	Concession SEEF, Monts de Cristal, Gabon	565	10.51	0.46	Niangadouma R	Stévant T	10/21/2010
TS0134	Mt Birougou, Gabon	750	12.20	-2.04	Issembé Y	Stévant T	2/1/2011
TS0137	Mt Birougou, Gabon	780	12.22	-2.04	Issembé Y	Stévant T	2/1/2011

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Appendix (continued)

Voucher	Locality	Elevation (m asl)	Latitude (N)	Longitude (E)	Collector	Identification	Date (m/d/y)
<i>G. oliveri</i>							
GK0521	Haute Dodo, Ivory Coast	391	-7.05	5.05	Koffi G	Assi Yapo J	11/22/2008
GK0527	Haute Dodo, Ivory Coast	409	-7.05	5.05	Koffi G	Assi Yapo J	11/22/2008
GK0626	Haute Dodo, Ivory Coast	404	-7.06	5.05	Koffi G	Assi Yapo J	11/23/2008
GK0661	Haute Dodo, Ivory Coast	384	-7.06	5.04	Koffi G	Assi Yapo J	11/24/2008
GK0688	Haute Dodo, Ivory Coast	404	-7.05	5.04	Koffi G	Assi Yapo J	11/25/2008
GK0703	Haute Dodo, Ivory Coast	403	-7.05	5.04	Koffi G	Assi Yapo J	11/25/2008
GK0918	Mont Nimba, Ivory Coast	379	-8.27	7.34	Koffi G	Assi Yapo J	1/24/2009
GK0931	Mont Nimba, Ivory Coast	402	-8.27	7.34	Koffi G	Assi Yapo J	1/24/2009
GK0943	Mont Nimba, Ivory Coast	404	-8.27	7.34	Koffi G	Assi Yapo J	1/24/2009
GK0951	Mont Nimba, Ivory Coast	402	-8.27	7.34	Koffi G	Assi Yapo J	1/24/2009
GK0964	Mont Nimba, Ivory Coast	372	-8.27	7.34	Koffi G	Assi Yapo J	1/24/2009
GK1053	Scio, Ivory Coast	460	-7.37	6.41	Koffi G	Assi Yapo J	1/28/2009
GK1072	Scio, Ivory Coast	408	-7.37	6.41	Koffi G	Assi Yapo J	1/28/2009
<i>G. cf. "São Tomé"</i>							
GiD0620	Lago Amélia, Sao Tomé	1395	6.59	0.29	Dauby G	Dauby G	2/13/2009
GiD0623	Lago Amélia, Sao Tomé	1476	6.59	0.29	Dauby G	Dauby G	2/13/2009
GiD1531	Lago Amélia, Sao Tomé		6.59	0.28	Dauby G	Dauby G	2/11/2009
<i>G. suaevelens</i> ssp. <i>usambaricum</i>							
GiD1102	Monts Usambara, Tanzania				Rasplus JY	Rasplus J. Y.	2000
<i>G. cf. "littoral"</i>							
RP0634	Ogooué-Maritime/Gamba, Gabon	5	9.98	-2.72	Wieringa J		3/15/1994

asl = above sea level