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PRIMER NOTE

MICROSATELLITES IN THE TREE *FOETIDIA MAURITIANA* (LECYTHIDACEAE) AND UTILITY IN OTHER *FOETIDIA* TAXA FROM THE MASCARENE ISLANDS[1]

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- *Premise of the study:* Polymorphic markers were required for a native tree of the Mascarene Islands, *Foetidia mauritiana* (Lecythidaceae), to investigate the effects of fragmentation of lowland tropical habitats on tree mating systems and on gene flow.
- *Methods and Results:* Using microsatellite enrichment and next-generation sequencing, we identified 13 microsatellite loci (dinucleotide repeats). They were highly polymorphic in 121 trees sampled in the largest three populations on Réunion, revealing 2–17 different alleles per locus. Furthermore, they were found to be polymorphic in conspecific populations on Mauritius and in *F. rodriguesiana* from Rodrigues.
- *Conclusions:* These results indicate the utility of these markers to investigate genetic diversity, mating systems, and gene flow in a genus native to the biodiversity hotspot of Madagascar and the Indian Ocean islands.

Key words: ecological restoration; *Foetidia mauritiana*; island biotas; Lecythidaceae; Madagascar; tropical dry forests.

Trees that belong to the family Lecythidaceae are often used as indicators of disturbance in lowland tropical forests, in particular because they are usually among the most common trees in these rich but fragile ecosystems (Mori et al., 2007). In addition to their ecological significance, some species may also be economically important, such as the Brazil nut tree *Bertholletia excelsa* Bonpl. For these reasons, polymorphic genetic markers have been developed for several species of several subfamilies of Lecythidaceae, mostly in taxa occurring as large trees in the Amazon Basin (e.g., *Bertholletia* Bonpl. [Reis et al., 2009], *Cariniana* Casar. [Guidugli et al., 2009, 2010], and *Lecythis* Loefl. [Rodrigues et al., 2015]), but also in a few other taxa found in the Old World (e.g., *Barringtonia* J. R. Forst. & G. Forst. [Xie et al., 2015]). However, to our knowledge, polymorphic markers are not yet available for the representatives of Lecythidaceae in the biodiversity hotspot formed by Madagascar and the Indian Ocean islands.

Out of 18 species that make up the genus *Foetidia* Comm. ex Lam. (subfamily Foetidioideae), 17 are endemic to island biotas in Madagascar, the Comoros, and the Mascarene Islands, while one species is found only on the African continent in Tanzania (Prance, 2008; Labat et al., 2011). The endemic species *F. mauritiana* Lam. was common in drier areas of Mauritius and Réunion where precipitation is low and temperatures are high, relative to the wet conditions generally found on these two tropical islands. However, for this species as for many indigenous taxa adapted to dry tropical habitats, populations have undergone rapid decline in less than 400 years since human settlement, and those few remaining stands are left in highly fragmented landscapes on both islands. This species is considered endangered in Réunion and Mauritius. There is an urgent need to protect and restore natural communities in tropical dry habitats on the Indian Ocean islands as well as worldwide (Miles et al., 2006). A European Union–supported project, Life+ Corexrun, was launched in 2009 on Réunion; it aims at both reintroducing 48 indigenous plant species (including *F. mauritiana*) and controlling invasions by alien plants within and around semi-dry forest stands.

METHODS AND RESULTS

Genomic DNA of *F. mauritiana* was extracted with the DNeasy Plant Mini Kit (QIAGEN, Hilden, North Rhine-Westphalia, Germany). Production of a microsatellite-enriched library was outsourced to the high-throughput platform set up by Genoscreen (Lille, Nord-Pas-de-Calais-Picardie, France). Following the method described in Malausa et al. (2011), 1 µg of genomic DNA was mechanically fragmented, ligated to standard adapters (Adap-F: GTTTAAGGCCTAGCTAGCAGAATC and Adap-R: GATTCTGCTAGCTAGGCCTT), and enriched by addition of eight biotin-labeled oligoprobes corresponding to the following microsatellite motifs: $(TG)_n$, $(TC)_n$, $(AAC)_n$, $(AAG)_n$, $(AGG)_n$, $(ACG)_n$, $(ACAT)_n$, and $(ACTC)_n$. Enriched DNA was isolated using Dynabeads (Invitrogen, Waltham, Massachusetts, USA) and amplified by PCR with primers corresponding to the library adapters (PCR protocol not communicated by Genoscreen). Sequencing was carried out through 454 GS-FLX Titanium pyrosequencing (Roche Applied Science, Penzberg, Bavaria, Germany). Sequences were analyzed using the bioinformatics program QDD (Meglécz et al., 2010), which detects microsatellite sequences and designs primers in flanking regions. We then selected 13 primer pairs among the microsatellite sequences (dinucleotide

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TABLE 1. Characteristics of 13 microsatellite loci developed in *Foetidia mauritiana*.

Locus	Primer sequences (5′–3′)	Repeat motif	Allele size range (bp)	T_a (°C)	GenBank accession no.
FmCIR27	F: AAGGAAAAGATGCATGCCAA	$(CA)_{14}$	79–97	57	KU713062
	R: AGACAATTCTAAACAAGATAGGACG				
FmCIR29	F: CATGTGGATTCCAAAATGGC	$(AG)_{12}$	87–93	57	KU713063–70
	R: TTGCAATGATAATTCACCAACC				
FmCIR31	F: CATGAATAGGTCCCAGGCTC	$(CA)_{13}$	86–100	57	KU713071
	R: TATCTATGCTTGCGTGTGCG				
FmCIR32	F: GAAGAGCACAGAAGAACACATCA	$(GA)_{12}$	92–96	57	KU713072
	R: GCCACTTCTATCATCGGGAG				
FmCIR43	F: AGCATGACCCCTAAACCCTAA	$(AC)_{14}$	119–143	57	KU713073
	R: AACATGACTGTGATGGCCTAAG				
FmCIR45	F: GTGACTAGCTCACCAAGAGCC	$(GA)_{12}$	132–146	57	KU713074
	R: TTGTCCCTAACGTTTCCTTCTC				
FmCIR47	F: TTCTTCACTGAGTGTATTTCCATAGG	$(GA)_{12}$	134–156	57	KU713075
	R: TGTAAAATAGTTCCTGGACCGAC				
FmCIR52	F: TGCTACTCTGTGGTGTGAAAGG	$(AC)_{14}$	144–204	57	KU713076
	R: GCATGAACAGGCAGAACATAA				
FmCIR16	F: GAAAAGTCACGGTTCTTCCG	$(AC)_{15}$	164–181	57	KU713061
	R: TTTGGTTCGAGGATGGGTAG				
FmCIR57	F: TAAAATCAACAACCTAAAACACGAA	$(TC)_{12}$	188–196	57	KU713077
	R: TGAGATTACCCAGGAGCAGG				
FmCIR61	F: GAGCACATTGAAGTAGCTGGT	$(GA)_{11}$	196–228	57	KU713078
	R: ATTTGAGCCCTGAACCAATG				
FmCIR11	F: TGAAGCTCAAGCAATTGGAA	$(TC)_{13}$	194–208	57	KU713058–60
	R: GGGTCCGGTAGGGTACTGTT				
FmCIR3	F: CGATTGGCATTGGAGAAAG	$(AG)_{10}$	286–288	57	KU713056–57
	R: GCTCTTGCCCAAGAAGGTC				

Note: T_a = annealing temperature.

repeats), because they had adequate flanking regions for designing primers (see Table 1 for locus information, primers, and GenBank accession numbers). Eight additional microsatellite loci are provided in this paper, although population testing was not conducted for these markers (Appendix 1).

For biological validation, we selected the main wild populations on Réunion. This included 49 individuals sampled near the Lataniers River (mean elevation 330 m), 45 individuals on the southern slope of the Grande-Chaloupe River (545 m), and 27 individuals near the Tamarins River (270 m), for a total of 121 individuals (sampling authorized by the Parc National de La Réunion, the Office National des Forêts, the Département de La Réunion, and the Conservatoire du Littoral). Because the species is considered critically endangered on Réunion, we harvested no more than 1–2 leaves per individual tree, with the exception of three individuals (one per locality) from which voucher specimens were made (see Appendix 2). Plant genomic DNA was isolated with the DNeasy Plant Mini Kit (QIAGEN). Multiplex PCR was performed in a total volume of 15 µL containing 7.5 µL 2× Type-it Multiplex PCR Master Mix (QIAGEN), 1.5 µL 5× Q-Solution, 0.2 µM each primer, and 20–50 ng template DNA. The thermal cycling protocol was as follows: initial denaturation at 95°C for 5 min; 28 cycles of denaturation at 95°C for 30 s, primer annealing at 57°C for 90 s, extension at 72°C for 30 s; and final extension at 60°C for 30 min. PCR products were diluted in HPLC-grade water (1 : 10), denatured in formamide, and separated on a 16-capillary ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, Foster City, California, USA); GeneScan 500 LIZ (Applied Biosystems) was used for sizing alleles in the expected range of 80–300 bp.

Allele sizes were estimated using the Microsatellite Plugin version 1.4 implemented in Geneious version 8.1.7 (Biomatters, Auckland, New Zealand). The number of different alleles and observed and expected heterozygosity were calculated for each locus and population in GenAlEx (Peakall and Smouse, 2006, 2012). Hardy–Weinberg exact tests (9999 iterations) and linkage disequilibrium were analyzed in GENEPOP version 4.2 (Rousset, 2008). The presence of null alleles was estimated with MICRO-CHECKER version 2.2.3 (van Oosterhout et al., 2004). The minimum number of microsatellite loci necessary to discriminate

TABLE 2. Genetic properties of the 13 newly developed microsatellites of *Foetidia mauritiana*.[a]

	Grande-Chaloupe (n = 45)			Lataniers (n = 49)			Tamarins (n = 27)		
Locus	A	H_o	H_e[b]	A	H_o	H_e[b]	A	H_o	H_e[b]
FmCIR27	8	0.163	0.678***	7	0.408	0.695***	6	0.480	0.762***
FmCIR29	4	0.644	0.621	4	0.521	0.683	4	0.741	0.735
FmCIR31	4	0.489	0.539	4	0.592	0.586	5	0.741	0.651
FmCIR32	3	0.578	0.560	3	0.510	0.574	3	0.667	0.592
FmCIR43	10	0.778	0.752	10	0.918	0.831	10	0.926	0.783
FmCIR45	5	0.727	0.624	4	0.59	0.526	3	0.64	0.506
FmCIR47	2	0.001	0.044*	7	0.167	0.299***	4	0.037	0.372***
FmCIR52	17	0.867	0.886	15	0.857	0.874	11	0.889	0.845
FmCIR16	10	0.432	0.767***	10	0.479	0.808***	11	0.667	0.804*
FmCIR57	5	0.511	0.720	5	0.653	0.690	4	0.593	0.536
FmCIR61	11	0.644	0.675	11	0.714	0.653	9	0.778	0.696
FmCIR11	6	0.578	0.548	6	0.408	0.394	5	0.481	0.514
FmCIR3	2	0.023	0.107***	2	0.020	0.230***	2	0.037	0.324***

Note: A = number of alleles; H_e = expected heterozygosity; H_o = observed heterozygosity; n = number of individuals sampled.

[a]All three populations are located on Réunion; see Appendix 2 for locality and voucher information.

[b]Asterisks refer to significant deviations from Hardy–Weinberg equilibrium (*$P < 0.05$, ***$P < 0.01$).

Table 3. Cross-species amplification (showing number of different alleles and size range) of the 13 newly developed microsatellites of *Foetidia mauritiana*.[a]

	F. mauritiana		*F. rodriguesiana*
Locus	Réunion (n = 121)	Mauritius (n = 28)	Rodrigues (n = 30)
FmCIR27	8 (79–97)	1 (91)	3 (97–101)
FmCIR29	4 (87–93)	6 (85–95)	3 (79–89)
FmCIR31	5 (86–100)	3 (94–98)	—
FmCIR32	3 (92–96)	3 (92–96)	2 (98–102)
FmCIR43	13 (119–143)	5 (121–135)	6 (121–133)
FmCIR45	5 (132–146)	6 (130–146)	5 (122–134)
FmCIR47	7 (134–156)	5 (132–152)	1 (134)
FmCIR52	21 (144–204)	6 (154–168)	10 (146–184)
FmCIR16	14 (164–181)	8 (164–180)	9 (164–182)
FmCIR57	5 (188–196)	2 (190–192)	9 (192–210)
FmCIR61	13 (196–228)	9 (192–222)	17 (200–264)
FmCIR11	7 (194–208)	6 (196–218)	1 (200)
FmCIR3	2 (286–288)	4 (278–288)	1 (286)

Note: — = no amplification; n = number of individuals sampled.
[a] See Appendix 2 for locality and voucher information.

individuals of *F. mauritiana* was assessed using the package *poppr* in R software (Kamvar et al., 2015).

All microsatellite loci revealed polymorphisms in *F. mauritiana* populations on Réunion. The number of different alleles per locus ranged from two to 17 (Table 2). Four loci showed significant deviation from Hardy–Weinberg equilibrium: FmCIR27, FmCIR47, FmCIR16, and FmCIR3. No significant linkage disequilibrium was detected between pairs of loci. We found that the minimum number of loci necessary to discriminate individuals in the data set was eight (data not shown).

Transferability of the microsatellite loci was tested on 28 individuals of *F. mauritiana* and 30 individuals of *F. rodriguesiana* F. Friedmann sampled across Mauritius and Rodrigues, respectively (sampling authorized by the National Parks and Conservation Service of Mauritius). Conspecific populations on different islands were tested because they are expected to experience strong genetic isolation. *Foetidia rodriguesiana* is morphologically similar to *F. mauritiana* (Prance, 2008). Using the above-mentioned protocol, we found that all loci amplified in *Foetidia* populations found on other islands, with the exception of FmCIR31, which did not amplify in *F. rodriguesiana* (Table 3). Moreover, most loci were polymorphic in the Mauritius and Rodrigues populations.

CONCLUSIONS

We developed 13 polymorphic genetic markers for *Foetidia*, a widespread genus in the Indian Ocean islands biodiversity hotspot. They will aid in designing priority populations for conservation and implementing adaptive conservation plans for the genus. They may also be used to study mating systems and pollen and seed flow between lowland forest fragments.

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Appendix 1. Eight additional microsatellite loci identified in *Foetidia mauritiana*.[a]

Locus	Primer sequences (5′–3′)	Repeat motif	Allele size (bp)
FmCIR7	F: GGTAAACAGCTCAAGCCCAA	$(AAC)_{12}$	148
	R: TTATTCCGGCCAAACAACTC		
FmCIR37	F: AAGAAAATTCTGCCCGATTG	$(CT)_{12}$	113
	R: CACTGTTGCAAGAGGGTGAG		
FmCIR38	F: TTTGGAGATCCTATGTTGAGCA	$(TG)_{12}$	116
	R: AAATTTTCCCAAATTAACCCAA		
FmCIR41	F: CTTCCTCCCACTGTTTCTCG	$(CT)_{12}$	127
	R: TATGGCAAGGGTTTGGATGT		
FmCIR48	F: AAGGATAACTATCAACCTCAAGCA	$(AC)_{12}$	144
	R: ACCCTCAGGTATGTGTCAGTTT		
FmCIR49	F: CCATGTTTGCCCATGCAC	$(CA)_{12}$	144
	R: TGGCCGAGATGCATAATGT		
FmCIR58	F: TTGTCTCTGTCTAAAGTTTGTGAGG	$(AC)_{11}$	190
	R: TCGCGAAATCTTGACCATC		
FmCIR68	F: AGGTCAGTGCTCACCAATACAG	$(GA)_{11}$	173
	R: CCAGAAATCTCCTATCCTCTTGC		

[a]Polymorphism has not been assessed in these markers.

Appendix 2. Voucher information for *Foetidia* populations used in this study.

Species	Voucher specimen no.[a]	Collection locality	Geographic coordinates	*N*
F. mauritiana	Cir 919	Grande-Chaloupe/Cap Francis, La Possession, Réunion	20°55′24.532″S, 55°23′16.63″E	45
F. mauritiana	Cir 875	Ravine des Lataniers, La Possession, Réunion	20°56′41.467″S, 55°20′56.857″E	49
F. mauritiana	Cir 936	Ravine des Tamarins, Saint-Denis, Réunion	20°53′50.798″S, 55°23′1.33″E	27
F. mauritiana	—	Black River Gorges National Park, Mauritius	20°23′53.467″S, 57°25′32.564″E	11
F. mauritiana	—	Chamarel, Black River, Mauritius	20°25′4.843″S, 57°23′10.731″E	8
F. mauritiana	—	Domaine du Chasseur National Park, Anse Jonchée, Mauritius	20°20′46.128″S, 57°45′23.91″E	7
F. mauritiana	—	Bras d'Eau National Park, Mauritius	20°8′42.781″S, 57°43′35.767″E	2
F. rodriguesiana	—	Anse Quitor Nature Reserve, Rodrigues	19°45′18.9″S, 63°22′10.599″E	5
F. rodriguesiana	—	Graviers, Rodrigues	19°43′57″S, 63°28′38.099″E	5
F. rodriguesiana	—	Mourouk, Rodrigues	19°44′13.7″S, 63°27′40.499″E	5
F. rodriguesiana	—	Mont Malgache, Rodrigues	19°43′40.598″S, 63°27′21.099″E	5
F. rodriguesiana	—	Grande Montagne, Rodrigues	19°42′14.501″S, 63°27′56.699″E	4
F. rodriguesiana	—	Baie Malgache, Rodrigues	19°43′39.299″S, 63°23′24.399″E	1
F. rodriguesiana	—	Rivière Cascade Victoire, Rodrigues	19°43′49.4″S, 63°26′59.798″E	1
F. rodriguesiana	—	Caverne Provert, Rodrigues	19°40′26.4″S, 63°26′25.299″E	1
F. rodriguesiana	—	Crève-coeur, Rodrigues	19°40′43.201″S, 63°25′47.499″E	1
F. rodriguesiana	—	Grande Baie, Rodrigues	19°40′57.202″S, 63°26′57.001″E	1
F. rodriguesiana	—	Solitude, Rodrigues	19°41′37″S, 63°26′15.399″E	1

Note: *N* = number of individuals.

[a]Vouchers for the *F. mauritiana* populations collected in Réunion were deposited at Université de la Réunion (REU). Vouchers were not collected for the *F. mauritiana* populations sampled in Mauritius and the *F. rodriguesiana* populations sampled in Rodrigues per agreements with local authorities to collect DNA samples only.