**MICROSATELLITES IN THE TREE Foetidia mauritiana (Lecythidaceae) AND UTILITY IN OTHER Foetidia taxa FROM THE MASCARENE ISLANDS**

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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 (France, 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 Malaua et al. (2011), 1 μg of genomic DNA was mechanically fragmented, ligated to standard adapters (Adap-F: GTTAAAGGCCCTAGC-TAGCAGAATC and Adap-R: GATTCTGCTAGCTAGGCTT), 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, (AC)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...
seven individuals (one per locality) from which voucher specimens were made.

We harvested no more than 1–2 leaves per individual tree, with the exception of

Littoral). Because the species is considered critically endangered on Réunion,

National des Forêts, the Département de La Réunion, and the Conservatoire du

individuals (sampling authorized by the Parc National de La Réunion, the Office

(545 m), and 27 individuals near the Tamarins River (270 m), for a total of 121

This included 49 individuals sampled near the Lataniers River (mean elevation

Table 1. Characteristics of 13 microsatellite loci developed in Foetidia mauritiana.

| Locus    | Primer sequences (5′–3′) | Repeat motif | Allele size range (bp) | T<sub>e</sub> (°C) | GenBank accession no. |
|----------|--------------------------|--------------|------------------------|-------------------|----------------------|
| FmCIR27  | F: AAGGAAAAGATGCATGCCAA | (CA)<sub>14</sub> | 79–97                  | 57                | KU713062             |
|          | R: AGCAAATCTTAAACAGATAGGACG | (AG)<sub>12</sub> |                        |                   |                      |
| FmCIR29  | F: CATGTAATGTTCCCAATGACG | (CA)<sub>15</sub> | 86–100                 | 57                | KU713071             |
|          | R: TTGCAATGTTACACACACACACC | (CA)<sub>15</sub> |                        |                   |                      |
| FmCIR31  | F: CATGTAATGTTCCCAATGACG | (CA)<sub>15</sub> | 132–146                | 57                | KU713074             |
|          | R: TATCTATGTTCCGTTGTCGTCG | (CA)<sub>15</sub> |                        |                   |                      |
| FmCIR32  | F: GAAGGCAAGGAGAAGAGACTCA | (GA)<sub>12</sub> | 92–96                  | 57                | KU713072             |
|          | R: GCACCTTCTATCTGAGGAGGAGG | (AC)<sub>14</sub> |                        |                   |                      |
| FmCIR43  | F: AGACTGAGAATCTAAGCTAAG | (AC)<sub>15</sub> | 148–400                | 57                | KU713061             |
|          | R: AATACGTTTTGCTGTTTGAAGG | (AC)<sub>15</sub> |                        |                   |                      |
| FmCIR45  | F: TTCTCTCACTGAGTTATTTCTCCAGG | (TC)<sub>12</sub> | 188–196                | 57                | KU713077             |
|          | R: TGTTAATTTCTTCTCTGAGCAGGCA | (TC)<sub>12</sub> |                        |                   |                      |
| FmCIR47  | F: TGGACTGCTGCCAGGAGGGCC | (TC)<sub>12</sub> | 194–208                | 57                | KU713058–60          |
|          | R: TTTGGTTCGAGGATGGGTAG | (TC)<sub>12</sub> |                        |                   |                      |
| FmCIR52  | F: TGCTTATCTTCTTTGTTGGAAGG | (AC)<sub>14</sub> | 144–200                | 57                | KU713061             |
|          | R: GCACTGACGGAGGAGGACGAA | (AC)<sub>14</sub> |                        |                   |                      |
| FmCIR57  | F: TAAAATCTACAACTTAAAACACGAA | (TC)<sub>12</sub> | 186–199                | 57                | KU713077             |
|          | R: TGATAGTACCCAGAGGACGACG | (TC)<sub>12</sub> |                        |                   |                      |
| FmCIR61  | F: GGAGCAGAATGAGTTGCTGCTG | (GA)<sub>12</sub> | 196–228                | 57                | KU713078             |
|          | R: ATTTGAGGCTGACGAAATGTTG | (GA)<sub>12</sub> |                        |                   |                      |
| FmCIR11  | F: TGAACAGTGCTACAGAATGTTG | (TC)<sub>12</sub> | 194–208                | 57                | KU713058–60          |
|          | R: GGGCTGTGAGGATGTGGTTT | (TC)<sub>12</sub> |                        |                   |                      |
| FmCIR3   | F: CGATTGCATTAGAGGAAGAAG | (AG)<sub>10</sub> | 286–288                | 57                | KU713056–57          |
|          | R: GTCTTCGCCACAGGAAACGAC | (AG)<sub>10</sub> |                        |                   |                      |

Note: T<sub>e</sub> = annealing temperature.

Table 2. Genetic properties of the 13 newly developed microsatellites of Foetidia mauritiana.*

| Locus    | Grande-Chaloupe (n = 45) | Lataniers (n = 49) | Tamarins (n = 27) |
|----------|--------------------------|--------------------|------------------|
|          | A | H<sub>e</sub>  | H<sub>e</sub>b | A | H<sub>e</sub>  | H<sub>e</sub>b | A | H<sub>e</sub>  | H<sub>e</sub>b |
| FmCIR27  | 8 | 0.163 | 0.678*** | 4 | 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 |
| FmCIR16  | 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<sub>e</sub> = expected heterozygosity; H<sub>e</sub>b = observed heterozygosity; n = number of individuals sampled.

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

Asterisks refer to significant deviations from Hardy–Weinberg equilibrium (*P < 0.05, ***P < 0.01).
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, FmCIR31, FmCIR47, and FmCIR53. 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 on Mauritius and Rodrigues populations found on other islands, with the exception of FmCIR31, which did not amplify in *F. rodriguesiana* (data not shown).

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

| 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.

See Appendix 2 for locality and voucher information.

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

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.*

| Locus     | Primer sequences (5′–3′) | Repeat motif | Allele size (bp) |
|-----------|--------------------------|--------------|-----------------|
| FmCIR7    | F: GGTAAAACAGCTCAAGCCCAA | (AAC)$_{12}$ | 148             |
|           | R: TATATTCCGCACATTCATTC  | (CT)$_{12}$  | 113             |
| FmCIR37   | F: AAGAAAAATCTGGCCGAGGTTG | (AC)$_{12}$  | 144             |
|           | R: CACTGTGGACAGTGAGCTTG   | (TG)$_{12}$  | 116             |
| FmCIR38   | F: TTTGGGAGATCTATGTTGACA | (AG)$_{11}$  | 190             |
|           | R: AAATTTGCCAATTAACCCA   | (TG)$_{12}$  | 127             |
| FmCIR41   | F: CTCCTCAGGATGGTTGCTTTC | (CA)$_{12}$  | 144             |
|           | R: TATGGCAAGGTTTGGATGTT  | (CT)$_{12}$  | 127             |
| FmCIR48   | F: AAGGATAAATCTATCCAGAGCA | (AC)$_{12}$  | 144             |
|           | R: ACCCTCAGGTATGTGTCAGTT | (AC)$_{12}$  | 144             |
| FmCIR49   | F: CCAATGGTGGCCCATGAC    | (CA)$_{12}$  | 144             |
|           | R: TGGCCGAGATGCAATAATGT  | (CA)$_{12}$  | 144             |
| FmCIR58   | F: TTGTCTCTGTTCAAAGTTGAG | (GC)$_{11}$  | 190             |
|           | R: TGCGGAATCTTGAGCAC     | (GC)$_{11}$  | 190             |
| FmCIR68   | F: AAGTCAATGCTGACATCGAGC | (GA)$_{11}$  | 173             |
|           | R: CCAAGAAAATCTATGATCTTC  | (GA)$_{11}$  | 173             |

Polymorphism has not been assessed in these markers.

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

| Species        | Voucher specimen no. | 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′56.85″E | 49 |
| *F. mauritiana* | Cir 936              | Ravine des Tamarins, Saint-Denis, Réunion | 20°53′50.798″S, 55°23′31.33″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′44.83″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°25′76.699″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 Provoit, Rodrigues | 19°40′26.4″S, 63°26′25.399″E | 1  |
| *F. rodriguesiana* | —                    | Cèvre-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.

*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.*