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## DEVELOPMENT OF MICROSATELLITE MARKERS IN *CRATYLIA MOLLIS* AND THEIR TRANSFERABILITY TO *C. ARGENTEA* (FABACEAE)<sup>1</sup>

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- **Premise of the study:** This work aimed to develop microsatellite markers for *Cratylia mollis* as tools to assess its genetic diversity and structure and to evaluate their potential cross-amplification in related species.
- **Methods and Results:** Microsatellite markers were developed using a microsatellite-enriched library and an intersimple sequence repeat library. From a set of 19 markers, 12 microsatellite loci were polymorphic and presented considerable variation in allele number (2–11), expected heterozygosity (0.226–0.883), and polymorphism information content per locus (0.212–0.870). Cross-amplification in *C. argentea* was successful in 16 loci, 12 of which were polymorphic (2–10 alleles).
- **Conclusions:** The polymorphism of this set of microsatellite markers for *C. mollis*, as well as their successful cross-amplification in *C. intermedia* and *C. bahiensis* and their transferability to *C. argentea*, supports their use in future comparative studies to understand the mechanism involved in population divergence and speciation in the genus.

**Key words:** *Cratylia*; cross-amplification; Fabaceae; microsatellite; population divergence; transferability.

*Cratylia* (Desv.) Kuntze is a South American genus distributed eastward from the Andes and southward from the Amazon River Basin. Five species are currently accepted: *C. argentea* (Desv.) Kuntze, *C. bahiensis* L. P. Queiroz, *C. hypargyrea* Mart. ex Benth., *C. intermedia* (Hassl.) L. P. Queiroz & R. Monteiro, and *C. mollis* Mart. ex Benth. (Queiroz and Coradin, 1995). *Cratylia argentea* is the most widespread, occurring from western Peru to Bolivia and Brazil in habitats including cerrado and seasonally dry forests. The other species are restricted to a particular type of vegetation: *C. hypargyrea* is found in Brazilian coastal Atlantic Forest, *C. bahiensis* and *C. mollis* grow in seasonally dry forest of northeastern Brazil, and *C. intermedia* occurs to the west of Paraná and to the northeast of Misiones Province in Argentina (Queiroz and Coradin, 1995).

*Cratylia mollis* and *C. argentea* are multipurpose shrub legumes with high potential for animal nutrition due to their nutritive value, particularly for dry season supplementation and silage (Argel and Lascano, 1998), and also show also tolerance to drought and adaptation to acidic soils (Andersson et al., 2006). Recent studies revealed that *C. mollis* seeds are an important lectin source (known as Cramoll) with applications for medicine (Fernandes et al., 2010). In the current study, our aim

was to develop microsatellite markers to assess the genetic variation of *C. mollis*, and to assess their potential transferability to *C. argentea* and cross-amplification in other species of the genus.

### METHODS AND RESULTS

Genomic DNA was isolated from one individual cultivated on the campus of the Universidade Estadual de Feira de Santana (*J. G. Rando 1257*, see Appendix 1) and extracted using the cetyltrimethylammonium bromide (CTAB) 2× protocol. Microsatellite markers were developed using both microsatellite-enriched library and intersimple sequence repeat (ISSR)-based cloning methods. A microsatellite-enriched library was built using the biotin-labeled microsatellite oligoprobes (GT)<sub>8</sub> and (CT)<sub>8</sub> and streptavidin-coated magnetic beads (Promega Corporation, Madison, Wisconsin, USA) according to Billotte et al. (1999). For the ISSR method, we performed PCR reactions with the ISSR primers GGCC(AG)<sub>8</sub>, GGCC(AC)<sub>8</sub>, CCGG(AG)<sub>8</sub>, CCGG(AC)<sub>8</sub>, GCGC(AG)<sub>8</sub>, and GCGC(AC)<sub>8</sub> following closely the protocol of Provan and Wilson (2007). The selected fragments obtained by both methods were linked into pGEM-T Easy Vector (Promega Corporation, São Paulo, Brazil) and then transformed and cloned in TOP10 competent cells (Invitrogen, Life Technologies, Vila Guarani, São Paulo, Brazil).

Positive clones were amplified with the T7 (5'-TAATACGACTCACTATAGGG-3') and SP6 (5'-ATTAGGTGACTATAGAA-3') primers using the following conditions: denaturation step of 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 15 s, annealing at 55°C for 35 s, and extension at 72°C for 90 s; final extension was at 72°C for 7 min. DNA sequencing was performed with Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, São Paulo, Brazil). The software Imperfect Microsatellite Extractor (Mudunuri and Nagarajaram, 2007) was used for identification of simple sequence repeats in the nonredundant sequences.

Primers were designed using Primer3Plus (Rozen and Skaletsky, 2000) using the following criteria: product size range 100–300 bp, primer melting temperature (*T<sub>m</sub>*) 50–60°C, primer GC 40–60%, maximum *T<sub>m</sub>* difference: 2°C. Genotyping reactions were carried out in 10 µL with the Top Taq Master Mix kit (QIAGEN, Hilden, Germany) with approximately 4 ng of genomic DNA,

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0.15 μM of the forward primer (added M13 sequence: 5'-CACGACGT-TGTAACACGAC-3'), 0.30 μM of the reverse primer, and 0.60 μM of an M13-labeled tail (with one of four fluorescent dyes: 6-FAM, VIC, NED, or PET). For each primer pair, the optimal annealing temperature was established in gradient PCRs from 61–45°C; however, a touchdown program (60–54°C) was needed in some cases. For polymorphism assessment, individuals of two *C. mollis* populations (CMPOP\_1, *N* = 24; CMPOP\_2, *N* = 25) and 20 individuals cultivated from seeds of CMPOP\_2 were genotyped. To test the cross-amplification and potential transferability, we sampled individuals obtained from the Laboratory of Plant Molecular Systematics (LAMOL) DNA bank at the Universidade Estadual de Feira de Santana (see Appendix 1): *C. argentea* (*N* = 9), *C. bahiensis* (*N* = 2), *C. hypargyrea* (*N* = 2), and *C. intermedia* (*N* = 2). Microsatellite profiles were analyzed with GeneMapper 4.0 (Applied Biosystems). The estimates of allelic diversity and deviations from Hardy–Weinberg equilibrium were carried out using GenAlix 6.5 (Peakall and Smouse, 2006).

We amplified and sequenced 180 of 192 positive clones derived from the microsatellite-enriched and ISSR libraries and found 78 nonredundant sequences containing microsatellite loci. Percentage of dinucleotide motifs was higher (62%) than trinucleotide motifs (34%) or tetra-, penta-, and octanucleotide repeats (4% combined). The number of repetitions in dinucleotides varied from three to nine, in trinucleotides from three to five, in tetranucleotides from three to four, and in pentanucleotides from four to five. The motifs found in the sequences of the enriched library had fewer repetitions (3–4) than the ISSR library, and for this reason most of these were not used to design primers. Therefore, 32 primer pairs were designed (30 corresponding to the ISSR library),

from which 19 presented amplification patterns compatible with the expected fragment sizes (Table 1).

Twelve microsatellite loci were polymorphic and presented considerable variation in allele number (2–11), observed heterozygosity (0.040–0.792), expected heterozygosity (0.226–0.883), and polymorphic information content per locus (0.212–0.870). The markers that were monomorphic in CMPOP\_1 (CM\_7, CM\_15, CM\_20) exhibited polymorphism in CMPOP\_2 and vice-versa (CM\_10), reflecting population differentiation in this species. Cross-amplification in *C. argentea* was successful in 16 loci; 12 of these were polymorphic, displaying an allele range of 2–4 in only nine individuals evaluated. Four monomorphic loci in *C. mollis* showed polymorphism in *C. argentea*. Cross-amplification success rates were also high in *C. intermedia* (15 loci) and *C. bahiensis* (12 loci); however, in *C. hypargyrea* only two microsatellite loci were successfully amplified (Table 2). Significant values (*P* < 0.01) of deviation from Hardy–Weinberg equilibrium were found in two loci of CMPOP\_1 and one locus of CMPOP\_2, where levels of observed heterozygosity were lower than expected (Table 2).

This heterozygote deficiency could reflect patterns of the population distribution and reproductive biology of *C. mollis*. This species presents a facultative breeding system, and experimental tests showed similar success in allogamous and autogamous crosses (Queiroz et al., 1997). It is pollinated by the carpenter bees *Xylocopa griseus* Lepeletier and *X. cearensis* Ducke, which visit flowers that are simultaneously open in the same inflorescence before moving to other inflorescences, thus promoting geitonogamous crosses (L. P. Queiroz, unpublished observations). In addition, the species forms patchy populations on sandy soils (L. P. Queiroz, personal communication), which together with the pollinator behavior favors autogamy and low heterozygosity.

TABLE 1. Characterization of 19 microsatellite markers developed for *Cratylia mollis*.

| Locus  | Primer sequences (5'–3')  | Repeat motif  | Product size (bp) <sup>a</sup> | T <sub>a</sub> (°C) | M13 5' Pigtail | GenBank accession no. |
|--------|---------------------------|---|--------------------------------|---------------------|----------------|-----------------------|
| CM_1F  | CAAGAAAGTGACCTAAATGG      | (TC) <sub>2</sub> AC(TC) <sub>2</sub>                       | 210–224                        | 56                  | PET            | KC351492              |
| CM_1R  | CTGATTCAGCTAAGGTAGACA     |   |                                |                     |                |                       |
| CM_4F  | CGTAGGAAGCACATGGTGTGA     | (GTGCT) <sub>2</sub> TGCT                                   | 121                            | 56                  | 6-FAM          | KC351493              |
| CM_4R  | CCAGAGCCACAAGGATAGAT      |   |                                |                     |                |                       |
| CM_5F  | CCTTGGAAAAGCTGAAGAGAAA    | (AG) <sub>3</sub> AA(AG) <sub>3</sub>                       | 136–142                        | 60                  | VIC            | KC351494              |
| CM_5R  | ATGGCCTCCTTCACATGC        |   |                                |                     |                |                       |
| CM_6F  | GGAGGCCATCTTTGAAGC        | (AGG) <sub>3</sub>  | 130–138                        | 58–60               | 6-FAM          | KC351494              |
| CM_6R  | GATGGTGGCTGTGATGGT        |   |                                |                     |                |                       |
| CM_7F  | GCTCAAGAGATTGGAAATGTG     | (AG) <sub>4</sub> N <sub>12</sub> (AGA) <sub>3</sub> A(AGA) | 112–120                        | 59                  | 6-FAM          | KC351495              |
| CM_7R  | CCTTCTACCTCACTCACTGCT     |   |                                |                     |                |                       |
| CM_8F  | GCAGTGAGTGAGGTAGAAGGAA    | (AATTA) <sub>4</sub> (TAAAAA) <sub>4</sub>                  | 180–228                        | TD 60–54            | NED            | KC351495              |
| CM_8R  | CGAGGAAAACAAAACCCAAA      |   |                                |                     |                |                       |
| CM_9F  | CAATGGAAGACTTGGGGTTT      | (GA) <sub>4</sub>   | 170–180                        | 58–60               | VIC            | KC351496              |
| CM_9R  | GGTTGGGCTCTAATCTCCT       |   |                                |                     |                |                       |
| CM_10F | GTTACTGGTTTCACTTTGGT      | (AGA) <sub>3</sub> GGAAGA                                   | 167–173                        | TD 60–54            | VIC            | KC351497              |
| CM_10R | CTTCAACTTCTGGTTAATGG      |   |                                |                     |                |                       |
| CM_11F | CATCATTAAACATTGGACGGAAAG  | (TG) <sub>9</sub>   | 186–212                        | 59                  | NED            | KC351499              |
| CM_11R | CAACCAACTCTCCACCTG        |   |                                |                     |                |                       |
| CM_15F | CACGGTGCTCTGAAAGTTGT      | (CT) <sub>6</sub>   | 149–183                        | 58                  | VIC            | KC351502              |
| CM_15R | AGAAGAGGGGAATGGGTTGT      |   |                                |                     |                |                       |
| CM_18F | GCGTGAAGTATTGTATCAAG      | (ATA) <sub>3</sub> ATT                                      | 304–336                        | 58–60               | PET            | KC351504              |
| CM_18R | CAGTCACTACAAGTGCAGTAT     |   |                                |                     |                |                       |
| CM_19F | CACGGGAGACAAGAGCGTA       | (GT) <sub>4</sub>   | 226–236                        | 58–60               | PET            | KC351505              |
| CM_19R | GGTCCGCTGTACAATGAACCA     |   |                                |                     |                |                       |
| CM_20F | CCTTGGAAAAGCTGAAGAGAAA    | (AG) <sub>3</sub> AA(AG) <sub>3</sub>                       | 246–258                        | 56                  | PET            | KC351506              |
| CM_20R | CTTCAAGATGGTGGCTGTGA      |   |                                |                     |                |                       |
| CM_21F | CAAGGTATGGGTAGGACAAGG     | (AG) <sub>7</sub>   | 125–139                        | 60–54               | 6-FAM          | KC351507              |
| CM_21R | ACAACACAAGCACACTGCCAA     |   |                                |                     |                |                       |
| CM_22F | TGCAGTGTGCTTGTGTTG        | TCT(TC) <sub>4</sub>  | 134–144                        | 56                  | VIC            | KC351507              |
| CM_22R | TGCCCTTCTTCTCTCTG         |   |                                |                     |                |                       |
| CM_23F | ACACGGAGCGAGAAAAGTGT      | (AG) <sub>4</sub>   | 172–192                        | 56                  | NED            | KC351508              |
| CM_23R | GGAAGCTTCAATCCACCAAT      |   |                                |                     |                |                       |
| CM_25F | CAACATTCGGGAGGCTTTTA      | ATGTG(ATG) <sub>2</sub>                                     | 300–309                        | TD 60–54            | PET            | KC351509              |
| CM_25R | CACCCCAAGACATCAAT         |   |                                |                     |                |                       |
| CM_27F | GTCTCGCGATCAGTTAAGC       | (CT) <sub>9</sub>   | 112–120                        | TD 60–54            | 6-FAM          | KC351510              |
| CM_27R | CATGGCTCGGCTTTACATTT      |   |                                |                     |                |                       |
| CM_31F | TGTGACAAAAGTAATGTGAAAGCAA | (ATG) <sub>3</sub> ATATG                                    | 246                            | 56                  | PET            | KC351513              |
| CM_31R | CCCAGCCACTTCAGTTTTCT      |   |                                |                     |                |                       |

Note: T<sub>a</sub> = annealing temperature; TD = touchdown program.

<sup>a</sup>Expected size based on the cloned fragment, excluding the 5' M13 universal sequence.

TABLE 2. Results of initial microsatellite marker screening in two populations of *Cratylia mollis* and cross-amplification in other species.

| Locus | <i>C. mollis</i> |                |                |       |         |     |                  |                |       |         |    |                | Cross-amplification and transferability |     |         |                     |     |    |                      |                       |                       |                       |  |  |                       |  |  |
|-------|------------------|----------------|----------------|-------|---------|-----|------------------|----------------|-------|---------|----|----------------|---|-----|---------|---------------------|-----|----|----------------------|-----------------------|-----------------------|-----------------------|--|--|-----------------------|--|--|
|       | CMPOP_1 (N = 24) |                |                |       |         |     | CMPOP_2 (N = 25) |                |       |         |    |                | CMPOP_2 (seeds) (N = 20)                |     |         | C. argentea (N = 9) |     |    | C. bahiensis (N = 2) |                       |                       | C. hypargyrea (N = 2) |  |  | C. intermedia (N = 2) |  |  |
|       | A                | H <sub>o</sub> | H <sub>e</sub> | PIC   | P (HWE) | A   | H <sub>o</sub>   | H <sub>e</sub> | PIC   | P (HWE) | A  | H <sub>o</sub> | H <sub>e</sub>                          | PIC | P (HWE) | A                   | A   | A  | C. bahiensis (N = 2) | C. hypargyrea (N = 2) | C. intermedia (N = 2) |                       |  |  |                       |  |  |
| CM_1  | 1                | —              | —              | —     | —       | 3   | 0.176            | 0.420          | 0.377 | 0.580   | 2  | —              | —                                       | —   | 2       | no                  | no  | no | no                   | no                    | yes                   |                       |  |  |                       |  |  |
| CM_4  | 1                | —              | —              | —     | —       | 1   | —                | —              | —     | —       | 2  | —              | —                                       | —   | —       | 2                   | yes | no | no                   | no                    | yes                   |                       |  |  |                       |  |  |
| CM_5  | 5                | 0.458          | 0.671          | 0.629 | 0.317   | 3   | 0.042            | 0.515          | 0.403 | 0.919   | 3  | —              | —                                       | —   | 3       | 1                   | 1   | 1  | yes                  | no                    | yes                   |                       |  |  |                       |  |  |
| CM_6  | 1                | —              | —              | —     | —       | 1   | —                | —              | —     | —       | 1  | —              | —                                       | —   | —       | 1                   | 1   | 1  | yes                  | yes                   | yes                   |                       |  |  |                       |  |  |
| CM_7  | 1                | —              | —              | —     | —       | 5   | 0.542            | 0.603          | 0.552 | 0.102   | 1  | —              | —                                       | —   | 1       | 2                   | 2   | 2  | no                   | no                    | no                    |                       |  |  |                       |  |  |
| CM_8  | 8                | 0.261          | 0.526          | 0.507 | 0.504   | 5   | 0.500            | 0.548          | 0.518 | 0.087   | 3  | —              | —                                       | —   | 3       | 3                   | 3   | 3  | yes                  | no                    | yes                   |                       |  |  |                       |  |  |
| CM_9  | 1                | —              | —              | —     | —       | 1   | —                | —              | —     | —       | 1  | —              | —                                       | —   | —       | 2                   | 2   | 2  | yes                  | no                    | yes                   |                       |  |  |                       |  |  |
| CM_10 | 3                | 0.368          | 0.481          | 0.406 | 0.233   | 1   | —                | —              | —     | —       | 2  | —              | —                                       | —   | —       | no                  | no  | no | no                   | no                    | yes                   |                       |  |  |                       |  |  |
| CM_11 | 9                | 0.450          | 0.836          | 0.817 | 0.462   | 11  | 0.261            | 0.883          | 0.870 | 0.704   | 10 | —              | —                                       | —   | 10      | 3                   | 3   | 3  | yes                  | no                    | yes                   |                       |  |  |                       |  |  |
| CM_15 | 1                | —              | —              | —     | —       | 5   | 0.040            | 0.498          | 0.462 | 0.920   | 2  | —              | —                                       | —   | 2       | 4                   | 4   | 4  | yes                  | no                    | yes                   |                       |  |  |                       |  |  |
| CM_18 | 2                | 0.545          | 0.496          | 0.373 | -0.100* | 3   | 0.458            | 0.478          | 0.410 | 0.042   | 1  | —              | —                                       | —   | 1       | 2                   | 2   | 2  | no                   | no                    | yes                   |                       |  |  |                       |  |  |
| CM_19 | 1                | —              | —              | —     | —       | 1   | —                | —              | —     | —       | 1  | —              | —                                       | —   | —       | 3                   | 3   | 3  | no                   | no                    | yes                   |                       |  |  |                       |  |  |
| CM_20 | 1                | —              | —              | —     | —       | 3   | 0.250            | 0.226          | 0.212 | -0.108* | 3  | —              | —                                       | —   | 3       | 2                   | 2   | 2  | yes                  | yes                   | yes                   |                       |  |  |                       |  |  |
| CM_21 | 4                | 0.792          | 0.601          | 0.524 | -0.318* | 8   | 0.458            | 0.666          | 0.644 | 0.312   | 5  | —              | —                                       | —   | 5       | 2                   | 2   | 2  | yes                  | no                    | yes                   |                       |  |  |                       |  |  |
| CM_22 | 1                | —              | —              | —     | —       | 1   | —                | —              | —     | —       | 4  | —              | —                                       | —   | —       | 2                   | 2   | 2  | yes                  | no                    | no                    |                       |  |  |                       |  |  |
| CM_23 | 1                | —              | —              | —     | —       | 1   | —                | —              | —     | —       | 1  | —              | —                                       | —   | —       | 3                   | 3   | 3  | yes                  | no                    | yes                   |                       |  |  |                       |  |  |
| CM_25 | 1                | —              | —              | —     | —       | 1   | —                | —              | —     | —       | 3  | —              | —                                       | —   | —       | no                  | no  | no | no                   | no                    | no                    |                       |  |  |                       |  |  |
| CM_27 | n/a              | —              | —              | —     | —       | n/a | —                | —              | —     | —       | 5  | —              | —                                       | —   | —       | 1                   | 1   | 1  | no                   | no                    | no                    |                       |  |  |                       |  |  |
| CM_31 | 1                | —              | —              | —     | —       | 1   | —                | —              | —     | —       | 1  | —              | —                                       | —   | —       | 1                   | 1   | 1  | yes                  | no                    | yes                   |                       |  |  |                       |  |  |

Note: — = value not calculated; A = number of alleles; H<sub>e</sub> = expected heterozygosity; H<sub>o</sub> = observed heterozygosity; HW = Hardy–Weinberg equilibrium; N = number of individuals; n/a = no amplification; PIC = polymorphism information content.  
 \* Significant values (P < 0.01).

## CONCLUSIONS

This is the first report of microsatellite markers in *C. mollis* and the genus as a whole. The observed polymorphism in 12 of 19 microsatellite loci in *C. mollis*, their successful cross-amplification in *C. intermedia* and *C. bahiensis*, and their trans-ability to *C. argentea* indicate promising applications for the study of genetic diversity and structure, gene flow, and mating system in the species. These studies can be followed by comparative studies to understand the mechanisms involved in population divergence and speciation in this neotropical genus.

## LITERATURE CITED

- ANDERSSON, M. S., M. PETERS, R. SCHULTZE-KRAFT, L. H. FRANCO, AND C. E. LASCANO. 2006. Phenological, agronomic and forage quality diversity among germplasm accessions of the tropical legume shrub *Cratylia argentea*. *Journal of Agricultural Science* 144: 237–248.
- ARGEL, P. J., AND C. E. LASCANO. 1998. *Cratylia argentea* (Desvaux) O. Kuntze: Una nueva leguminosa arbustiva para suelos ácidos en zonas subhúmedas tropicales. *Pasturas Tropicales* 20: 37–43.
- BILLOTTE, N. P., P. J. L. LAGODA, A. M. RISTERUCCI, AND F. C. BAURENS. 1999. Microsatellite-enriched libraries: Applied methodology for

the development of SSR markers in tropical crops. *Fruits* 54: 277–288.

- FERNANDES, M. P., N. M. INADA, M. R. CHIARATTI, F. F. B. ARAUJO, F. V. MEIRELLES, M. T. S. CORREIA, L. C. B. B. COELHO, ET AL. 2010. *Trypanosoma cruzi* death induced by *Cratylia mollis* seed lectin. *Journal of Bioenergetics and Biomembranes* 42: 69–78.
- MUDUNURI, S., AND H. NAGARAJARAM. 2007. IMEX: Imperfect microsatellite extractor. *Bioinformatics (Oxford, England)* 23: 1181–1187.
- PEAKALL, R., AND P. E. SMOUSE. 2006. GenAIEx 6: Genetic analysis in Excel: Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288–295.
- PROVAN, J., AND P. J. WILSON. 2007. Development of microsatellites for the peat moss *Sphagnum capillifolium* using ISSR cloning. *Molecular Ecology Notes* 7: 254–256.
- QUEIROZ, L. P., AND L. CORADIN. 1995. Biogeografia de *Cratylia* e áreas prioritárias para coleta. In E. A. Pizarro and L. Coradin [eds.], “Potencial del género *Cratylia* como leguminosa forrajera”: Memories of Workshop about *Cratylia* realized from 19 to 20 of July 1995, 11–15. EMBRAPA/CENARGEN/CPAC/CIAT, Brasília, Brazil.
- QUEIROZ, L. P., M. M. DA SILVA, A. K. B. RAMOS, AND E. A. PIZARRO. 1997. Estudos reprodutivos em *Cratylia argentea* (Desv.) O. Kuntze e *Cratylia mollis* Mart. ex Benth. (Leguminosae-Papilionoideae). *Pasturas Tropicales* 19: 20–23.
- ROZEN, S., AND H. SKALETSKY. 2000. Primer3 on the WWW for general users and for biologist programmers. In S. Misener and S. A. Krawetz [eds.], *Methods in molecular biology*, vol. 132: Bioinformatics methods and protocols, 365–386. Humana Press, Totowa, New Jersey, USA.

APPENDIX 1. Voucher specimens used in this study. All specimens were collected in Brazil and are deposited at the Herbarium of the Universidade Estadual de Feira de Santana (HUEFS). Information shown: taxon, voucher specimen, collection locale/geographic coordinates.

*Cratylia mollis* Mart. ex Benth.: *J. G. Rando 1257*. Individual cultivated at the Universidade Estadual de Feira de Santana (Feira de Santana, Bahia).

*Cratylia mollis* Mart. ex Benth. (Population 1): *G. Pereira-Silva 8450* (Barra, Bahia: 10°47'27"S, 042°50'05"W).

*Cratylia mollis* Mart. ex Benth. (Population 2): *G. Pereira-Silva 9165* (Casa Nova, Bahia: 09°24'55"S, 41°08'22"W).

*Cratylia argentea* (Desv.) Kuntze: *C. Snak 878* (Rio Verde, Mato Grosso do Sul: 18°58'14.8"S, 54°49'17.6"W), *902* (Pedro Gomes, Mato Grosso do Sul: 18°10'53.2"S, 54°38'30.6"W), *965* (Loreto, Maranhão: 07°19'07.1"S, 45°08'56.1"W), *989* (Carolina, Maranhão: 07°13'18.9"S, 47°25'50.7"W), *L. C. P. Lima 614* (Cáceres, Mato Grosso), *L. P. Queiroz 13975* (Posse,

Goiás: 14°17'32.999"S, 46°24'14.000"W), *10570* (Barra do Bugres, Mato Grosso: 15°11'27.001"S, 57°6'28.001"W), *10281* (Posse, Goiás: 14°17'43.000"S, 46°24'19.001"W), *10405* (Barra do Garças, Mato Grosso: 15°49'44.002"S, 52°29'59.999"W).

*Cratylia bahiensis* L. P. Queiroz: *G. Pereira-Silva 9081* (Caetitê, Bahia: 14°07'51"S, 42°23'35"W), *9129* (Abaíra, Bahia: 13°16'06"S, 41°41'27"W).

*Cratylia intermedia* (Hassl.) L. P. Queiroz: *C. Snak 592* (Diamante do Oeste, Paraná), *1052* (Formosa do Oeste, Paraná: 24°12'52.86"S, 53°19'39.14"W).

*Cratylia hypargyrea* Mart. ex Benth.: *J. Carvalho-Sobrinho 2948* (Oliveira, Bahia: 15°6'56.002"S, 39°1'12.001"W), *L. P. Queiroz 2847* (Ilhéus, Bahia: 14°56'00.001"S, 39°2'00.001"W).