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DEVELOPMENT OF MICROSATELLITE LOCI IN *ARTOCARPUS* *ALITILIS* (MORACEAE) AND CROSS-AMPLIFICATION IN CONGENERIC SPECIES¹

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- **Premise of the study:** Microsatellite loci were isolated and characterized from enriched genomic libraries of *Artocarpus altilis* (breadfruit) and tested in four *Artocarpus* species and one hybrid. The microsatellite markers provide new tools for further studies in *Artocarpus*.
- **Methods and Results:** A total of 25 microsatellite loci were evaluated across four *Artocarpus* species and one hybrid. Twenty-one microsatellite loci were evaluated on *A. altilis* (241), *A. camansi* (34), *A. mariannensis* (15), and *A. altilis* × *mariannensis* (64) samples. Nine of those loci plus four additional loci were evaluated on *A. heterophyllus* (jackfruit, 426) samples. All loci are polymorphic for at least one species. The average number of alleles ranges from two to nine within taxa.
- **Conclusions:** These microsatellite primers will facilitate further studies on the genetic structure and evolutionary and domestication history of *Artocarpus* species. They will aid in cultivar identification and establishing germplasm conservation strategies for breadfruit and jackfruit.

Key words: *Artocarpus altilis*; *Artocarpus camansi*; *Artocarpus heterophyllus*; *Artocarpus mariannensis*; breadfruit; jackfruit; Moraceae.

The genus *Artocarpus* J. R. Forst. & G. Forst. (Moraceae) contains several agriculturally significant species, breadfruit (*A. altilis* (Parkinson) Fosberg) and jackfruit (*A. heterophyllus* Lam.) being the most widely cultivated (Zerega et al., 2010). Breadfruit is a traditional staple in Oceania, and its progenitor species are *A. camansi* Blanco and *A. mariannensis* Trécul; hybrids also exist (Zerega et al., 2004). The origin of jackfruit is unclear, but it is likely native to the Indian subcontinent (Jarrett, 1959). Even with morphological descriptors, accurate breadfruit cultivar identification remains difficult (Jones et al., 2013), and little is known about jackfruit genetic diversity (Shyamamma et al., 2008). Despite the fact that breadfruit and jackfruit are in different *Artocarpus* subgenera (Zerega

et al., 2010), several primers cross-amplified well and showed polymorphisms, suggesting they might be useful in additional *Artocarpus* species.

METHODS AND RESULTS

To construct the genomic library, leaf tissue was obtained from an *A. altilis* individual (voucher DR468 deposited at the National Tropical Botanical Garden [NTBG] Herbarium [PTBG], Kalaheo, Hawaii) collected from Fiji (Living Accession 900261.002 at the Breadfruit Institute, NTBG, Hawaii). DNA was extracted using the QIAGEN DNeasy Plant Mini Kit (QIAGEN, Valencia, California, USA) following standard protocol. Microsatellite libraries were developed by Genetic Identification Services (Chatsworth, California, USA), following the methods of Jones et al. (2002). The libraries were enriched for four repeat motifs—(GA)_n, (CA)_n, (ATG)_n, and (TAGA)_n. Thirty-eight clones containing microsatellites were sequenced. Using DesignerPCR version 1.03 (Research Genetics, Huntsville, Alabama, USA), 75 primer pairs were designed (approximately two pairs for each clone) and screened across 25 *A. altilis* and 12 *A. heterophyllus* individuals to test for amplification and polymorphisms. Nineteen primers for breadfruit (*A. altilis*) and related species (*A. camansi*, *A. mariannensis*, and *A. altilis* × *mariannensis*) and 10 primers for *A. heterophyllus* yielded either a single band, or two that were consistently different in size, when separated and visualized under UV light on an agarose gel stained with SYBR Green (Proligo, Hamburg, Germany). Three of these primer pairs amplified two loci of differing lengths (Tables 1 and 2). Loci MAA54a/b have conserved sequences flanking similar microsatellite sequences and may represent historical duplications that have since diverged (Karhu et al., 2000). The same is true for loci MAA178a/b. In loci MAA196a/b the microsatellite flanking sequences are not identical, but share enough similarities to allow for cross

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TABLE 1. Characteristics of 25 microsatellite loci amplified in *Artocarpus* species.

Locus	Primer sequences (5'–3')	Repeat motif ^a	T _a (°C) ^b	GenBank accession no.
MAA3	F: TGTTCTAGCTGCACGAATTATG R: CTTGAATCAAACAGGCCAATTA	(TA) ₅	59.8/55.0	JX415243
MAA9	F: AACAGGGTTAAAAATCCCTTCAC R: GTTCCCGTTTTGTTCAAAGAG	(CA) ₁₅	59.8/55.0	JX415244
MAA26	F: CATGAATGAAACAACATCAGAC R: ATAGTCATAAAGCCCTGCC	(GT) ₉	59.8/55.0	JQ952762
MAA40	F: AGCATTTTCAGGTTGGTGAC R: GTTGTCTGTTCCTCATC	(TG) ₁₆	59.8/55.0	JX415245
*MAA54a	F: AACCTCCAACACTAGGACAAC R: AGCTACTCCAAAACGTGACA	(CA) ₅ (AT) ₄	59.8/55.0	JQ952763
*MAA54b	F: AACCTCCAACACTAGGACAAC R: AGCTACTCCAAAACGTGACA	(AT) ₉ (CA) ₆ (AT) ₄	59.8/55.0	JQ952764
MAA71	F: TTCTATTCTTGAGATCTCTC R: AGTGGTGGTAAGATTCAAAGTG	(CT) ₁₁ (CA) ₁₉	59.8/55.0	JX415246
MAA85	F: TCAGGGTGTAGCGAAGACA R: AGGGCTCCTTTGATGGAA	(CA) ₁₁	59.8/55.0	JX415247
MAA96	F: GGACCTCAAGGATGTGATCTC R: ACACGGTCTTCTTTGGATAGC	(CA) ₁₄ (TA) ₇ (TG) ₃	59.8/55.0	JX415248
MAA105	F: TTGGGACACTGTGAACATTTTC R: AAAAGCTAGTGGATTAGATGCA	(GT) ₁₁	59.8/55.0	JQ952765
MAA122	F: CTGGCCTTCAGTTTTGTCAAC R: CACCAGGCTTCAAGATGAAA	(GT) ₁₁ (GA) ₄ (GA) ₁₁	59.8/55.0	JQ952766
MAA135	F: TGCCATATAAGGTTGCTCTG R: TGGGCTTTTTCTGGAAAC	(AG) ₂₂	59.8/55.0	JX415249
MAA140	F: CCATCCCCATCTTTCTCT R: TCCTCGTTTGGCCACAGTG	(CT) ₂₅	59.8/55.0	JQ952767
MAA145	F: CCAACGCATAGCCAAATC R: AAATCCCAAACCAACGT	(CTT) ₉ (GA) ₁₄ (GA) ₈	59.8/55.0	JQ952768
MAA156	F: CTGGTGCTTCAGCCTAATG R: TCAGCGTCAAAGATAAATCG	(GA) ₃ (GA) ₅ (GA) ₈ (GA) ₁₃	59.8/55.0	JQ952769
*MAA178a	F: GATGGAGACACTTTGAAC TAGC R: CACCAGGGTTTAAAGATGAAAC	(GT) ₃ (GT) ₆ (GT) ₃ (GA) ₃ (GA) ₁₀	59.8/55.0	JQ952770
*MAA178b	F: GATGGAGACACTTTGAAC TAGC R: CACCAGGGTTTAAAGATGAAAC	(GT) ₃ (GT) ₃ (GA) ₃ (GA) ₁₁	59.8/55.0	JQ952771
MAA182	F: TACTGGGCTCTGAAAAGATGTCT R: CGTTTGCCTTTGGATAAAT	(CT) ₁₉	59.8/55.0	JQ952772
*MAA196a	F: GGAATGTGGTAGATGAAACTCC R: CGACAAAAAACAAGGAAGAC	(CT) ₁₁ (GA) ₄	59.8/55.0	JQ952773
*MAA196b	F: GAATGTGAGAGATAAATCTCC R: CGACAAAAAACAAGGAAGAC	(CT) ₁₂	59.8/55.0	JQ952774
MAA201	F: GGTTC AATTCACACATACAGG R: TTGAGGCTAAAAGAAATATGAGG	(GA) ₁₅	59.8/55.0	JX415250
MAA219	F: ATTTGCATCATGTAGGACA R: GGACACAACGACATTGAC	(CAT) ₈	59.8/55.0	JX415251
MAA251	F: ATCGTCTTTGTCCACCACCAC R: ATAGCCGAGTAACTGGATGGA	(ATC) ₁₀	59.8/55.0	JX415252
MAA287	F: CTTCCCACTAAATGTAAACG R: TCTCAAACAATGGAGTGATC	(TCTA) ₅	59.8/55.0	JX415253
MAA293	F: TCCCCTTCACTTTCCGGAT R: CGATTTGACCCACCATTTC	(CTAT) ₆	59.8/55.0	JX415254

Note: T_a = annealing temperature.

^a Commas indicate presence of nonrepeating nucleotides between repeats.

^b PCR was performed using a two-step process with varying annealing temperatures (see Methods and Results section).

* Primers amplified two separate loci.

amplification. For all three primers, the two loci amplified were straightforward to score and consistently amplified in all samples. Loci 178a and 196a do not demonstrate linkage to 178b and 196b, respectively; loci 54a/b did deviate from the null hypothesis that the loci were not linked ($P = 0.04$), as tested in GENEPOP (Raymond and Rousset, 1995). Nineteen primers (amplifying 21 loci) were then used to evaluate 241 *A. altilis*, 34 *A. camansi*, 15 *A. mariannensis*, and 64 *A. altilis* × *mariannensis* samples collected from trees growing in private and public collections (Table 2, Appendix 1), and representing original collections from throughout Oceania as well as Indonesia, the Philippines, and the Seychelle Islands. Ten primers (amplifying 13 loci) were used to evaluate 426 *A. heterophyllus* samples collected from public collections with provenance from Thailand, Indonesia, Malaysia, Jamaica, Singapore, Australia, India, and

Miami, and from private holdings in Bangladesh (Table 2, Appendices 1 and 2). Herbarium vouchers were made from representative sites in Bangladesh; accession numbers are given for samples from public living collections (Appendix 2).

All Bangladeshi jackfruit samples were processed at the Chicago Botanic Garden (CBG), while all breadfruit and some jackfruit samples were processed at U.S. Department of Agriculture (USDA) laboratories. A subset of the jackfruit samples were processed at both laboratories, yielding identical results. For samples processed at CBG, DNA was extracted as described above. For PCR reactions, forward primers had an M13 tail (5'-CAGCACGTTGTAAAA-3'), and M13 primer labeled with WellRED Dye D2, D3, or D4 (Beckman Coulter, Brea, California, USA) was added to each reaction (Schuelke, 2000). PCR reactions used a two-step process. First, 10-μL reactions contained 5 μL of Master Mix (Promega Corporation,

TABLE 2. Genetic diversity results for 25 microsatellite loci in *Artocarpus* species.^{a,b}

Locus	<i>A. altilis</i> (diploid, n = 79)			<i>A. altilis</i> (triploid, n = 162)			<i>A. a × m</i> (diploid, n = 31)			<i>A. a × m</i> (triploid, n = 33)			<i>A. camansi</i> (n = 34)			<i>A. mariannensis</i> (n = 15)			<i>A. heterophyllus</i> (n = 426) ^c						
	ASR	H _o	H _e	A	ASR	% het.	A	ASR	H _o	H _e	A	ASR	% het.	A	ASR	H _o	H _e	A	ASR	H _o	H _e				
	(bp)				(bp)			(bp)				(bp)			(bp)				(bp)			(bp)			
MAA3	1	216	0.000	0.000	1	216	0.000	1	216	0.000	0.000	1	216	0.000	0.000	0.000	0.000	1	216	0.000	0.000	0.000	nd	nd	
MAA9	5	153-173	0.104	0.113	5	153-171	0.185	3	163-171	0.323	0.332	5	161-171	0.758	2 ^d	161-171	0.088	0.086	2	164-168	0.400	0.405	nd	nd	nd
MAA26	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
MAA40	8	170-188	0.897	0.795	7	180-192	0.932	2	182-186	0.032	0.032	3	180-190	0.697	2 ^d	180-182	0.000	0.059	1	182	0.000	0.000	nd	nd	nd
*MAA54a	10	167-195	0.709	0.783	10	173-195	0.994	2	173-187	0.194	0.229	4	173-187	1.000	6 ^d	173-185	0.412	0.790	1	173	0.000	0.000	3	181-185	0.187
*MAA54b	3	205-215	0.308	0.313	2	207-215	0.914	2	205-207	0.032	0.032	3	205-207	0.606	1	207	0.000	0.000	1	207	0.000	0.000	9	211-239	0.724
MAA71	10	154-184	0.846	0.816	9	152-178	0.988	4	152-182	0.290	0.264	5	152-182	1.000	4 ^d	154-160	0.088	0.294	1	152	0.000	0.000	nd	nd	nd
MAA85	7 ^d	154-178	0.468	0.777	6	154-164	0.938	3 ^d	154-164	0.065	0.485	4	158-164	0.394	1	156	0.000	0.000	1	162	0.000	0.000	nd	nd	nd
MAA96	8	176-214	0.772	0.791	6	204-214	0.975	4 ^d	204-220	0.387	0.547	6	204-220	0.939	4 ^d	208-218	0.441	0.682	1	204	0.000	0.000	nd	nd	nd
MAA105	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
MAA122	10	241-293	0.747	0.698	7	277-295	0.988	3	285-291	0.323	0.405	6	277-291	0.939	1	279	0.000	0.000	2	289-291	0.267	0.333	15	254-312	0.482
MAA135	12	258-300	0.823	0.809	11	268-322	0.994	7	270-320	0.419	0.501	14	268-328	0.970	8 ^d	278-302	0.324	0.808	4	280-326	0.400	0.643	nd	nd	nd
MAA140	10 ^d	131-161	0.532	0.695	9	129-163	0.957	4	145-165	0.097	0.184	9	137-163	0.909	7 ^d	139-157	0.265	0.812	2	147-149	0.067	0.190	9	142-170	0.748
MAA145	10	256-304	0.608	0.659	9	262-328	0.895	6 ^d	282-328	0.516	0.703	8	268-304	0.970	5 ^d	268-320	0.176	0.514	3 ^d	282-304	0.067	0.195	9 ^d	275-303	0.307
MAA156	11 ^d	273-307	0.316	0.533	6	273-307	0.988	4	279-307	0.355	0.406	6	277-309	0.970	5 ^d	257-279	0.324	0.715	2	281-307	0.133	0.243	6 ^d	283-307	0.732
*MAA178a	9 ^d	207-235	0.414	0.793	8	209-229	0.963	5	209-229	0.452	0.578	8	209-229	0.848	3 ^d	211-245	0.029	0.115	2	223-245	0.357	0.302	4	230-258	0.179
*MAA178b	9 ^d	241-259	0.846	0.729	8	241-259	0.981	5	241-253	0.645	0.686	6	241-253	0.879	6 ^d	241-257	0.242	0.612	3	251-255	0.786	0.566	7	268-284	0.625
MAA182	6	182-214	0.494	0.536	7	182-212	0.944	5 ^d	200-210	0.484	0.720	6	182-210	0.970	6 ^d	182-210	0.412	0.797	2 ^d	202-204	0.067	0.295	12	186-216	0.578
*MAA196a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
*MAA196b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
MAA201	10 ^d	238-288	0.291	0.503	11	262-294	0.975	4 ^d	262-278	0.419	0.588	10	262-296	0.939	15 ^d	268-312	0.382	0.930	3	266-276	0.267	0.388	nd	nd	nd
MAA219	7	247-277	0.705	0.718	5	259-277	0.944	3	259-271	0.290	0.262	4	259-277	0.545	4 ^d	256-277	0.029	0.584	1	260	0.000	0.000	nd	nd	nd
MAA251	6 ^d	173-200	0.911	0.682	6	173-200	0.988	7 ^d	173-212	0.516	0.717	7	173-209	0.970	2 ^d	191-197	0.030	0.326	4 ^d	199-209	0.333	0.721	nd	nd	nd
MAA287	10 ^d	179-223	0.397	0.695	7	179-215	0.975	3	183-211	0.097	0.154	5	179-199	0.970	1	179	0.000	0.000	1	183	0.000	0.000	nd	nd	nd
MAA293	3 ^d	158-166	0.449	0.593	5	154-174	0.957	3	160-166	0.300	0.372	3	160-166	0.879	2 ^d	162-166	0.147	0.316	1	160	0.000	0.000	nd	nd	nd
Average	7.86		0.55	0.62	6.90		0.88	3.8		0.30	0.31	5.86		0.82	4.1		0.17	0.41	1.9		0.15	0.20	8.9		0.42

Note: A = number of alleles; A. a × m = A. altilis × mariannensis; ASR = allele size range; H_e = expected heterozygosity; H_o = observed heterozygosity; n = sample size for each species; nd = no data.

^aH_o and H_e are shown for diploids.

^bThe percent heterozygosity (% het.) is shown for triploids.

^cAllele sizes for A. heterophyllus include tag used in PCR (see Methods and Results section).

^dLocus deviated significantly from Hardy-Weinberg equilibrium for indicated taxon.

*Primer amplified two separate loci.

Madison, Wisconsin, USA), 0.5 μ L of 10 mg/mL bovine serum albumin (BSA), 0.25 μ L of 10 μ M forward primer with the M13 tail, 0.25 μ L of 10 μ M reverse primer, 3 μ L of H₂O, and 1 μ L of template DNA. PCR conditions for the first step were 94°C for 3 min; 13 cycles at 94°C for 30 s, 59.8°C for 30 s, and 72°C for 1 min; and a final extension of 72°C for 10 min. To each 10- μ L reaction was added 2.5 μ L Master Mix (Promega Corporation), 0.25 μ L of 10 mg/mL BSA, 0.125 μ L of 2.5 μ M MgCl₂, 0.25 μ L of 10 μ M labeled M13 primer, and 1.875 μ L of H₂O. PCR conditions for the second step were 94°C for 3 min; 27 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min; and a final extension of 72°C for 10 min. PCR product (0.5 μ L of WellRED Dye D4-labeled product, 1 μ L of WellRED Dye D3-labeled product, or 2.5 μ L of WellRED Dye D2-labeled product [Beckman Coulter]) was added to 30 μ L of HiDi formamide (Azco Biotech, San Diego, California, USA) and 3.3 μ L of 400-bp size standard ladder (Beckman Coulter) and analyzed on a Beckman Coulter CEQ 8000 Genetic Analysis System. Alleles were scored using the CEQ 8000 software version 9.0 (Beckman Coulter). For samples processed at USDA laboratories, DNA was extracted at the USDA–Agricultural Research Service (ARS) Tropical Agriculture Research Station (TARS) following manufacturer’s protocol using MP Biomedicals FastDNA Spin Kit (MP Biomedicals, Solon, Ohio, USA). DNA samples were then shipped to the USDA–ARS Genomics and Bioinformatics Research Unit in Stoneville, Mississippi. PCR reactions were carried out with 10 ng of DNA using the QIAGEN Multiplex PCR Kit (QIAGEN) in 5.0- μ L reactions. Reactions contained 2.5 μ L of 2 \times QIAGEN Multiplex PCR Master Mix, 0.1 μ L each of a 10 mM forward and reverse primer labeled with HEX fluorescent dye, 0.1 μ L each of a 10 mM forward and reverse primer labeled with FAM fluorescent dye, 10 ng of DNA, and water to equal 5 μ L. PCR conditions were 95°C for 15 min; 40 cycles of 94°C for 30 s, 55°C for 90 s, and 72°C for 1 min; and a final extension of 60°C for 30 min. PCR fragments were analyzed on an ABI 3730xl DNA Analyzer and data processed using GeneMapper version 3.7 (Applied Biosystems, Foster City, California, USA).

Many breadfruit cultivars are known to be triploid based on chromosome counts (Ragone, 2001; Zerega et al., 2004), and this was confirmed with microsatellite data. The data revealed additional cultivars, which were thought to be triploid due to sterile fruits, to indeed be triploid. GenoDive (Meirmans and Van Tienderen, 2004) was used for diversity statistics. The number of alleles was evaluated for all primers. Methods for assessing allele dosage in triploids were only partially successful (Esselink et al., 2004), so observed and expected heterozygosities and deviation from Hardy–Weinberg equilibrium (HWE) were only analyzed in diploids, while percent heterozygosity was evaluated in triploids. All loci are polymorphic for at least one species. The allele numbers across loci and species range from one to 15, with the average number within a species ranging from two to nine (Table 2). GenoDive also assigned clones to determine if individuals could be differentiated. Genotypes across all loci were unique for *A. heterophyllus* and diploid *A. altilis* \times *mariannensis* samples. In the remaining species, multiple samples share genotypes (number of unique genotypes/total samples): triploid *A. altilis* (53/162), triploid *A. altilis* \times *mariannensis* (27/33), diploid *A. altilis* (46/79), *A. mariannensis* (14/15), and *A. camansi* (33/34). Several loci showed significant departure from HWE, although the loci varied between taxa (Table 2). Given that these are cultivated species and they violate several assumptions for HWE, this is not a surprising result and could be due to selection, nonrandom mating, or asexual reproduction.

CONCLUSIONS

The newly developed microsatellite loci show high levels of polymorphism in several *Artocarpus* species, and the success in

cross-amplification is promising for broad use of these markers in congeners. These markers provide an excellent resource to quantify levels of genetic variation, patterns of population structure, and evolutionary and domestication history. They should also prove useful in future studies for cultivar identification, establishing crop germplasm conservation strategies, helping to determine ploidy level, and understanding origins and dispersal of breadfruit and jackfruit.

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APPENDIX 1. Location data for *Artocarpus* samples used to characterize microsatellite loci.

Below is the list of samples from public germplasm collections. For each sample, the following information is given: germplasm accession number, the collection provenance or cultivar name, and the name of the germplasm collection, in that order. Abbreviations: CHIC = Nancy Poole Rich Herbarium at the Chicago Botanic Garden; FTG-H = Fairchild Tropical Botanic Garden, Homestead, Florida; FTG-M = Fairchild Tropical Botanic Garden, Miami, Florida (<http://www.fairchildgarden.org/livingcollections/tropicalfruitprogram/jackfruit/>); NTBG = National Tropical Botanic Garden, Kalaheo, Hawaii (<http://ntbg.org/breadfruit/collection/>); TARS = Tropical Agriculture Research Station; USDA-NGR-H = U.S. Department of Agriculture–National Germplasm Repository, Hilo, Hawaii (http://www.ars-grin.gov/cgi-bin/npgs/html/site_holding.pl?HILO); USDA-NGR-M = U.S. Department of Agriculture–National Germplasm Repository, Mayagüez, Puerto Rico (http://www.ars-grin.gov/cgi-bin/npgs/html/site_holding.pl?MAY).

***Artocarpus altilis* (Parkinson) Fosberg, diploid samples:** HART 26, unknown, USDA-NGR-H; HART 1, Samoa, USDA-NGR-H; HART 21, Marquesas Islands, USDA-NGR-H; HART 50, Solomon Islands, USDA-NGR-H; 020354.001, Samoa, NTBG; 910278.001, Vanuatu, NTBG; 890470.002, Rotuma, NTBG; 890470.001, Rotuma, NTBG; 900248.001, Society Islands, NTBG; 890457.001, Rotuma, NTBG; 900265.001, Fiji, NTBG; 890469.002, Solomon Islands, NTBG; 890469.001, Solomon Islands, NTBG; 70659.021, Samoa, NTBG; 70659.022, Samoa, NTBG; 770517.001, Samoa, NTBG; 890259.001, Vanuatu, NTBG; 890473.001, Vanuatu, NTBG; 900263.001, Samoa, NTBG; 890461.001, Marquesas Islands, NTBG; 890461.002, Marquesas Islands, NTBG; 070246.002, 'Meiuhpw en Samoa', NTBG; 770519.001, Samoa, NYBG; 960575.001, 'Mos en Samoa', NTBG; 890454.001, Cook Islands, NTBG; 900231.001, Cook Islands, NTBG; 890471.002, Fiji, NTBG; 890153.001, Cook Islands, NTBG; 910266.001, Society Islands, NTBG; 790492.001, Society Islands, NTBG; 900233.002, Rotuma, NTBG; 900233.001, Rotuma, NTBG; 030039.001, 'Puou', NTBG; 770520.001, Samoa, NTBG; 890474.001, Samoa, NTBG; 910275.001, Vanuatu, NTBG; 880691.001, Tonga, NTBG; 900257.001, Rotuma, NTBG; 890475.002, Samoa, NTBG; 900234.001, Fiji, NTBG; 900261.001, Fiji, NTBG; 900261.002, Fiji, NTBG; 910279.001, Vanuatu, NTBG; 910279.002, Vanuatu, NTBG; 910276.001, Vanuatu, NTBG; 890156.002, Cook Islands, NTBG; 890156.001, Cook Islands, NTBG; 910277.001, Vanuatu, NTBG; 900281.001, Solomon Islands, NTBG; 900281.002, Solomon Islands, NTBG; 890456.001, Solomon Islands, NTBG; 770521.001, Samoa, NTBG; 040063.001, 'Ulu Fiti', NTBG; 890258.001, Rotuma, NTBG; 020356.001, 'Ulu Fiti', NTBG; 890458.001, Rotuma, NTBG; 890458.002, Rotuma, NTBG; 900368.001, Rotuma, NTBG; 900260.002, Fiji, NTBG; 900260.001, Fiji, NTBG; 890155.001, Samoa, NTBG; 890155.002, Samoa, NTBG; 900228.001, Samoa, NTBG; 900235.001, Solomon Islands, NTBG; 020498.001, Solomon Islands, NTBG; 020500.001, Solomon Islands, NTBG; 890471.001, Fiji, NTBG; 900264.001, Fiji, NTBG; 890477.001, Fiji, NTBG; 970236.001, Rotuma, NTBG; 910289.001, unknown, NTBG; 900226.001, unknown, NTBG; 900224.001, unknown, NTBG; 020347.001, unknown, NTBG; 020348.001, unknown, NTBG; 020352.001, unknown, NTBG; 020353.002, unknown, NTBG; 020353.001, unknown, NTBG; 020355.001, unknown, NTBG

***Artocarpus altilis* (Parkinson) Fosberg, triploid samples:** TARS 6732 plot 22, Barbados, USDA-NGR-M; TARS plot 11, unknown, USDA-NGR-M; TARS 7990 plot 5, Samoa, USDA-NGR-M; TARS plot 18, unknown, USDA-NGR-M; HART 11, Marquesas Islands, USDA-NGR-H; HART 16, Society Islands, USDA-NGR-H; HART 18, Society Islands, USDA-NGR-H; HART 23, Marquesas Islands, USDA-NGR-H; HART 28, Marquesas Islands, USDA-NGR-H; HART 29, Society Islands, USDA-NGR-H; HART 30, Society Islands, USDA-NGR-H; HART 32, Society Islands, USDA-NGR-H; HART 33, Society Islands, USDA-NGR-H; HART 34, Society Islands, USDA-NGR-H; HART 35, Palau, USDA-NGR-H; HART 33, Chuuk, USDA-NGR-H; HART 38, Northern Mariana Islands, USDA-NGR-H; HART 40, Society Islands, USDA-NGR-H; HART 42, Society Islands, USDA-NGR-H; HART 44, Society Islands, USDA-NGR-H; HART 45, Society Islands, USDA-NGR-H; HART 33, Palau, USDA-NGR-H; HART 49, Pohnpei, USDA-NGR-H; HART 51, Chuuk, USDA-NGR-H; HART 52, Pohnpei, USDA-NGR-H; HART 53, Pohnpei, USDA-NGR-H; HART 54, Society Islands, USDA-NGR-H; HART 56, unknown, USDA-NGR-H; HART 57, unknown, USDA-

NGR-H; 900232.001, Cook Islands, NTBG; 890153.002, Cook Islands, NTBG; 900256.001, Cook Islands, NTBG; 890472.002, Cook Islands, NTBG; 890472.001, Cook Islands, NTBG; 890476.002, Fiji, NTBG; 890162.002, Northern Mariana Islands, NTBG; 890162.001, Northern Mariana Islands, NTBG; 900241.001, Marquesas Islands, NTBG; 900240.001, Marquesas Islands, NTBG; 900238.001, Marquesas Islands, NTBG; 900242.001, Marquesas Islands, NTBG; 900239.001, Marquesas Islands, NTBG; 890462.001, Marquesas Islands, NTBG; 900237.001, Marquesas Islands, NTBG; 890159.001, Palau, NTBG; 890159.002, Palau, NTBG; 890183.003, Palau, NTBG; 900266.002, Pohnpei, NTBG; 900266.001, Pohnpei, NTBG; 890478.001, Pohnpei, NTBG; 890478.002, Pohnpei, NTBG; 000534.001, Pohnpei, NTBG; 790497.002, Pohnpei, NTBG; 000531.001, Pohnpei, NTBG; 890167.001, Pohnpei, NTBG; 890167.002, Pohnpei, NTBG; 890479.002, Pohnpei, NTBG; 890479.001, Pohnpei, NTBG; 790493.001, Pohnpei, NTBG; 910273002, Pohnpei, NTBG; 910271.001, Pohnpei, NTBG; 910274.001, Pohnpei, NTBG; 900262.001, Samoa, NTBG; 770524.001, Samoa, NTBG; 890455.001, Samoa, NTBG; 810290.001, Seychelles, NTBG; 810290.002, Seychelles, NTBG; 810290.003, Seychelles, NTBG; 810289.002, Seychelles, NTBG; 780332.001, Society Islands, NTBG; 40051.001, Society Islands, NTBG; 780325.001, Society Islands, NTBG; 910267.001, Society Islands, NTBG; 780333.001, Society Islands, NTBG; 900249.001, Society Islands, NTBG; 900249.002, Society Islands, NTBG; 890157.001, Society Islands, NTBG; 890158.001, Society Islands, NTBG; 890158.002, Society Islands, NTBG; 900243.001, Society Islands, NTBG; 890147.001, Society Islands, NTBG; 890147.002, Society Islands, NTBG; 780335.001, Society Islands, NTBG; 780330.002, Society Islands, NTBG; 890151.001, Society Islands, NTBG; 890151.002, Society Islands, NTBG; 780291.001, Society Islands, NTBG; 790487.001, Society Islands, NTBG; 900245.001, Society Islands, NTBG; 890150.001, Society Islands, NTBG; 800269.001, Society Islands, NTBG; 890459.001, Society Islands, NTBG; 890459.002, Society Islands, NTBG; 890149.001, Society Islands, NTBG; 890154.001, Society Islands, NTBG; 30035.001, Society Islands, NTBG; 780327.001, Society Islands, NTBG; 890464.001, Society Islands, NTBG; 890463.002, Society Islands, NTBG; 890463.001, Society Islands, NTBG; 790489.001, Society Islands, NTBG; 910266.002, Society Islands, NTBG; 030037.001, Society Islands, NTBG; 900244.001, Society Islands, NTBG; 780328.001, Society Islands, NTBG; 890460.001, Society Islands, NTBG; 790485.001, Society Islands, NTBG; 890152.002, Society Islands, NTBG; 890152.001, Society Islands, NTBG; 780329.001, Society Islands, NTBG; 790486.001, Society Islands, NTBG; 780338.001, Society Islands, NTBG; 890465.001, Society Islands, NTBG; 790488.001, Society Islands, NTBG; 790491.001, Society Islands, NTBG; 900246.001, Society Islands, NTBG; 900247.001, Society Islands, NTBG; 890186.001, Society Islands, NTBG; 890186.002, Society Islands, NTBG; 890148.001, Society Islands, NTBG; 900236.001, Solomon Islands, NTBG; 880690.001, Tonga, NTBG; 890165.001, Chuuk, NTBG; 070883.001, unknown, NTBG; 30033.001, 'Mei Tehid', NTBG; 40518.001, 'Rare Autia', NTBG; 780345.001, 'Raumae', NTBG; 030042.001, 'Toneno', NTBG; 070882.001, 'Ulu', NTBG; 070882.002, 'Ulu', NTBG; 090739.001, 'Ulu', NTBG; 100346.001, 'Ulu', NTBG; 100347.001, 'Ulu', NTBG; 100348.001, 'Ulu', NTBG; 100349.001, 'Ulu', NTBG; 100369.001, 'Ulu', NTBG; 100370.001, 'Ulu', NTBG; 970274.001, 'Ulu', NTBG; 070101.001, 'Ulu', NTBG; 070132.001, 'Ulu', NTBG; 080439.001, 'Ulu', NTBG; 080440.001, 'Ulu', NTBG; 080858.001, 'Ulu', NTBG; 080859.001, 'Ulu', NTBG; 080863.001, 'Ulu', NTBG; 080864.001, 'Ulu', NTBG; 080864.002, 'Ulu', NTBG; 080881.001, 'Ulu', NTBG; 030044.001, 'Ulu Tala', NTBG; 900268.001, unknown, NTBG; 910290.001, unknown, NTBG; 900267.001, unknown, NTBG; 910288.001, unknown, NTBG; 910287.001, unknown, NTBG; 900225.001, unknown, NTBG; 910286.001, unknown, NTBG; 030028.001, 'White', NTBG; 810290.004, 'White', NTBG; 810289.001, 'White', NTBG

***Artocarpus altilis* × *marianensis*, diploid samples:** 890452.001, Kiribati, NTBG; 890177.003, Tokelau, NTBG; 890178.001, Tokelau, NTBG; 890177.001, Tokelau, NTBG; 890172.001, Kiribati, NTBG; 890179.001, Tokelau, NTBG; 890257.001, Tokelau, NTBG; 890171.001, Tokelau, NTBG; 890176.001, Tokelau, NTBG; 890174.001, Tokelau, NTBG; 890172.002, Tokelau, NTBG; 890176.002, Tokelau, NTBG; 890168.002, Tokelau, NTBG; 890177.002, Tokelau, NTBG; 890171.002, Tokelau, NTBG; 890181.002, Tokelau, NTBG; 890181.001, Tokelau, NTBG; 890173.001, Tokelau, NTBG; 890173.002,

Tokelau, NTBG; 900230.001, Tokelau, NTBG; 890175.001, Tokelau, NTBG; 890182.001, Tokelau, NTBG; 890182.002, Tokelau, NTBG; 890180.001, Tokelau, NTBG; 890170.002, Tokelau, NTBG; 900253.002, Chuuk, NTBG; 900253.001, Chuuk, NTBG; 890166.001, Chuuk, NTBG; 890466.002, Chuuk, NTBG; 890184.001, Yap, NTBG

***Artocarpus altilis* × *marianensis*, triploid samples:** 890468.002, Kiribati, NTBG; 890163.002, Northern Mariana Islands, NTBG; 890163.001, Northern Mariana Islands, NTBG; 980210.001, Palau, NTBG; 890160.001, Palau, NTBG; 910652.001, Palau, NTBG; 890182.001, Tokelau, NTBG; 890183.001, Palau, NTBG; 890183.002, Palau, NTBG; 900250.001, Palau, NTBG; 890480.002, Pohnpei, NTBG; 890480.001, Pohnpei, NTBG; 910270.001, Pohnpei, NTBG; 890480.003, Pohnpei, NTBG; 790494.001, Pohnpei, NTBG; 910272.002, Pohnpei, NTBG; 910272.001, Pohnpei, NTBG; 900255.001, Pohnpei, NTBG; 890467.001, Pohnpei, NTBG; 790490.001, Society Islands, NTBG; 910265.001, Society Islands, NTBG; 890453.001, Tokelau, NTBG; 890185.001, Tokelau, NTBG; 910269.001, Chuuk, NTBG; 910268.001, Chuuk, NTBG; 890164.001, Chuuk, NTBG; 890164.002, Chuuk, NTBG; 890161.001, Yap, NTBG; 030034.001, 'Pohnpei, NTBG; 030034.002, Pohnpei, NTBG; 030041.001, 'Rotuma', NTBG; 990781.001, unknown, NTBG; 30045.001, 'Yap', NTBG

***Artocarpus camansi* Blanco, all diploid:** TARS 18009 plot 14, unknown, USDA-NGR-M; HART 63 N90-148, unknown, USDA-NGR-H; NTBG50, 910283.001, Indonesia, NTBG; 980212.001, Palau, NTBG; 000398.001, Papua New Guinea, NTBG; 000501.001, Papua New Guinea, NTBG; 000398.003, Papua New Guinea, NTBG; 000399.001, Papua New Guinea, NTBG; 000398.002, Papua New Guinea, NTBG; 000502.001, Papua New Guinea, NTBG; 000501.001, Papua New Guinea, NTBG; 000499.001, Papua New Guinea, NTBG; 000503.001, Papua New Guinea, NTBG; 000499.002, Papua New Guinea, NTBG; 000395.001, Papua New Guinea, NTBG; 000390.001, Papua New Guinea, NTBG; 000503.002, Papua New Guinea, NTBG; 000389.001, Papua New Guinea, NTBG;

000398004, Papua New Guinea, NTBG; 000500.001, Papua New Guinea, NTBG; 910281.001, Philippines, NTBG; 910280.001, Pohnpei, NTBG; 770444.001, Society Islands, NTBG; 960576.001, unknown, NTBG; 000390.002, Papua New Guinea, NTBG; 000394.002, Papua New Guinea, NTBG; 000395.002, Papua New Guinea, NTBG; 000398.005, Papua New Guinea, NTBG; 000499.003, Papua New Guinea, NTBG; 000500.002, Papua New Guinea, NTBG; 000501.004, Papua New Guinea, NTBG; 000501.005, Papua New Guinea, NTBG; 000502.002, Papua New Guinea, NTBG; 000503.004, Papua New Guinea, NTBG

***Artocarpus heterophyllus* Lam., all diploid:** TARS 1566 plot 5, Indonesia, USDA-NGR-M; TARS 1566 plot 9, Indonesia, USDA-NGR-M; TARS 1566 plot 10, Indonesia, USDA-NGR-M; TARS 1566 plot 19, Indonesia, USDA-NGR-M; TARS 18002 plot 16, Malaysia, USDA-NGR-M; HART 27, Thailand, USDA-NGR-H; WB2-FTBG-F-21-A, unknown, FTG-M; WB2-FTBG-F-05-A, unknown, FTG-M; S2-01-07-07, unknown, FTG-M; WB2-FTBG-F-16-A, unknown, FTG-M; T#10 R4, 'Black Gold', FTG-H; T#5 R5, 'Chenna', FTG-H; T#15 R4, 'Cochin', FTG-H; T#5 R3, 'Dang Rasimi', FTG-H; T#11 R4, 'J31', FTG-H; T#6 R4, 'L.', FTG-H; T#2 R4, 'L.', FTG-H; T#12 R4, 'NS1', FTG-H; T#5 R4, 'Pani Varaka', FTG-H; WB2-FTBG-F-15-A, 'Golden Nugget', FTG-M; WB2-FTBG-F-01-A, 'Kwai Mok', FTG-M

***Artocarpus mariannensis* Trécul, all diploid:** HART 67 N05-8, unknown, USDA-NGR-H; 900252.001, Northern Mariana Islands, NTBG; 900252.003, Northern Mariana Islands, NTBG; 900252.002, Northern Mariana Islands, NTBG; 000522.002, Northern Mariana Islands, NTBG; 000523.001, Northern Mariana Islands, NTBG; 000521.002, Northern Mariana Islands, NTBG; 000521.001, Northern Mariana Islands, NTBG; 000522.001, Northern Mariana Islands, NTBG; 000521.003, Northern Mariana Islands, NTBG; 970306.001, Pohnpei, NTBG; 970306.002, Pohnpei, NTBG; 000528.001, Pohnpei, NTBG; 000523.002, Northern Mariana Islands, NTBG; 940010.002, Northern Mariana Islands, NTBG

APPENDIX 2. *Artocarpus heterophyllus* samples collected in Bangladesh by authors Witherup, Zuberi, and Zerega. A representative herbarium voucher was made at most sites, and a picture voucher exists for all samples.

N	Collection date	Collection site	District	GPS Latitude	GPS Longitude	Collection no.	Voucher
12	5 July 2010	Madhupur village	Tangail	N24.61475	E090.03149	CW1–12	CHIC
21	6 July 2010	Mohismara village	Tangail	N24.59060	E90.12699	CW13–33	CHIC
30	6 July 2010	GachaBari village	Tangail	N24.67237	E090.07662	CW34–64	
27	8 July 2010	Bangladeshi Tea Research Institute, Srymangal	Sylhet	N24.29532	E091.74686	CW65–91	CHIC
29	9 July 2010	Ashidu orchard and village	Sylhet	N24.28276	E091.76509	CW92–119, 101b	CHIC
36	9 July 2010	Lawachara National Park	Sylhet	N24.31972	E091.78361	CW120–155	CHIC
23	10 July 2010	Bangladesh Agricultural Research Institute	Sylhet			CW160–182	CHIC
13	11/19 July 2010	National Botanic Garden of Bangladesh	Dhaka	N23.81300	E90.34690	CW183–191, 285–288	CHIC
13	12 July 2010	Bangladesh Agricultural Research Institute	Gazipur	N23.99420	E090.41130	CW192–204	
16	12 July 2010	Bagabazar village	Gazipur	N24.16302	E090.43024	CW205–220	CHIC
19	14 July 2010	Leather Research Institute	Savar	N23.91534	E090.23549	CW221–239	CHIC
28	16 July 2010	Madan Hati village	Rajshahi	N24.48321	E088.59244	CW240–267	CHIC
17	17 July 2010	Nimtolli village	Jessore	N23.16448	E089.29896	CW268–284	CHIC
31	20 July 2010	Jahangirnagar University	Savar	N23.88113	E090.26915	CW289–319	CHIC
11	20 July 2010	Gono University	Savar	N23.91812	E90.24538	CW320–330	
30	21 July 2010	Khula Pater village	Comilla	N23.67553	E091.17191	CW331–360	CHIC
20	23 July 2010	Council of Scientific and Industrial Research	Dhaka	N23.74027	E090.38531	CW361–380	CHIC
18	20 July 2010	Hortus Nursery	Savar			Hortus1–18	

Note: CHIC = Nancy Poole Rich Herbarium at the Chicago Botanic Garden; N = number of samples.