PRIMER NOTE

AN EXPANDED NUCLEAR PHYLOGENOMIC PCR TOOLKIT FOR SAPINDALES¹

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• Premise of the study: We tested PCR amplification of 91 low-copy nuclear gene loci in taxa from Sapindales using primers developed for Bursera simaruba (Burseraceae).

• Methods and Results: Cross-amplification of these markers among 10 taxa tested was related to their phylogenetic distance from B. simaruba. On average, each Sapindalean taxon yielded product for 53 gene regions (range: 16–90). Arabidopsis thaliana (Brassicaceae), by contrast, yielded product for two. Single representatives of Anacardiaceae and Rutaceae yielded 34 and 26 products, respectively. Twenty-six primer pairs worked for all Burseraceae species tested if highly divergent Aucoumea klaineana is excluded, and eight of these amplified product in every Sapindalean taxon.

• Conclusions: Our study demonstrates that customized primers for Bursera can amplify product in a range of Sapindalean taxa. This collection of primer pairs, therefore, is a valuable addition to the toolkit for nuclear phylogenomic analyses of Sapindales and warrants further investigation.

Key words: Anacardiaceae; Burseraceae; low-copy nuclear genes; microfluidic PCR; Rutaceae.

Low-copy nuclear gene regions offer increased phylogenetic utility for species- and population-level studies of plants as compared to chloroplast and nuclear ribosomal markers (Zimmer and Wen, 2012), yet sampling these regions remains challenging due to the dearth of universal primers and barriers to sequencing whole or partial nuclear genomes from multiple individuals. Consequently, assessing the phylogenetic limits of custom-designed target sequences or primers for low-copy nuclear gene regions is critical to fully realizing their broader impacts for advancing plant systematics. We report the results of a cross-amplification study incorporating primers for 91 low-copy nuclear gene loci created by Gostel et al. (2015) for species-level phylogenetics of Malagasy Commiphora Jacq. (Burseraceae). Primers for these markers were developed using genomic resources from two rosid orders by mapping sequence data from a transcriptome of Bursera simaruba (L.) Sarg. (Burseraceae; Sapindales) (Matasci et al., 2014) to 950 putative low- or single-copy nuclear gene loci of Arabidopsis thaliana (L.) Heynh. (Brassicaceae; Brassicales) (Duarte et al., 2010). Gostel et al. (2015) further optimized the primers for microfluidic PCR-based target enrichment, a method that allows simultaneous and cost-effective amplification of multiple loci (Blow, 2009; Uribe-Convers et al., 2016).

We tested cross-amplification of these primers using 10 taxa that have varying phylogenetic distances from B. simaruba within Sapindales and included A. thaliana as the outermost limit of the survey. Sapindales is a widespread group that includes ca. 6700 species within nine families (Angiosperm Phylogeny Group, 2016) (Fig. 1). Molecular phylogenies of this order often lack sufficient phylogenetic support along their backbone as well as at the species level (e.g., Fine et al., 2014; Grudinski et al., 2014), thus our understanding of Sapindalean systematics could benefit from an expanded phylogenetic toolkit such as that provided by the Gostel et al. (2015) primers.

METHODS AND RESULTS

Taxonomic sampling and molecular methods—Appendix 1 contains accession information for the 11 taxa sampled; Fig. 1 displays their phylogenetic relationships. Bursera simaruba (Bursera Jacq. ex L. subgenus Bursera) and C. grandifolia Engl. were included as positive controls; prior work has shown that all or most of the custom-designed primers amplify PCR product in these two species (Gostel et al., 2015). For experimental taxa, we included B. tonkinensis Guillaumin, which is sister to Commiphora (Weeks and Simpson, 2007), as well as Aucoumea Pierre, the monotypic genus sister to all other Burseraceae (Weeks et al., 2014). One species from each of Boswellia Roxb. ex Colebr., Canarium L., and Protium Burm. f. were included, as well as Beiselia Forman, the monotypic genus sister to all other Burseraceae (Weeks et al., 2014). We included one species of Anacardiaceae, the family that is sister to Burseraceae (Weeks et al., 2014), and one species of Rutaceae, which represents the Sapindalean clade sister to Burseraceae–Anacardiaceae–Kirkiaaeae (Mueller-Riehl et al., 2016). Arabidopsis thaliana (Brassicaceae) was included because its genomic resources were used in primer design and can test the applicability of these primers to other closely related rosid lineages (Wang et al., 2009).

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Whole genomic DNA was extracted from taxa using the FastPrep FastDNA Spin Kit (Bio101 Systems, La Jolla, California, USA), or the cetyltrimethylammonium bromide (CTAB) method (Weeks et al., 2005). Primer development for the low-copy nuclear loci, including the range of amplicon lengths for all taxa and GenBank numbers for markers sequenced by Gestel et al. (2015) for B. simaruba and C. grandifolia that had ≥15 sequence reads mapped. Table 2 summarizes marker amplification success for each taxon. Ninety primer pairs amplified product in B. simaruba and, on average, 54 primer pairs worked for other Burseraceae taxa. The low number of markers amplified in Aucoumea (16) was unexpected given its close relationship to Bursera. This result may have been caused by primer mismatch due to increased genetic change within this monotypic genus, as evidenced by its long branch within Burseraceae phylogeny (Weeks et al., 2014). In total, nine primer pairs worked for every Burseraceae taxon tested, and if Aucoumea is excluded as an outlier, the panel of family-universal primer pairs increases to 26. Thirty-four and 26 primer pairs generated product in Anacardiaceae and Rutaceae, respectively, while only two primer pairs worked in Arabidopsis. Comparing the Burseraceae panel to that of Anacardiaceae and Rutaceae reveals 16 and 12 successfully amplified regions in common, respectively, with eight shared among the three families. PCR chemistry may have suppressed amplification of markers, as high-fidelity PCR reagents were not used due to their high cost. Among the positive controls, high fidelity as compared to standard PCR reagents increased amplification success by 8% (Bursera, 83 to 90 primer pairs) and 85% (Commiphora, 39 to 72 primer pairs). Thus, our experimental results report a conservative baseline for the cross-amplification success of these primer pairs.

**CONCLUSIONS**

Our study demonstrates that 90 of 91 primer pairs for novel low-copy nuclear loci developed by Gestel et al. (2015) for B. simaruba successfully amplify product in a broad range of Sapindalean taxa and effectively expand the phylogenomic toolkit for this order. Twenty-six markers amplify all Burseraceae taxa (excluding Aucoumea) and eight amplify all Sapindalean groups tested. Our results present a new source for universal targets or primers for phylogenetic reconstruction of taxa within Sapindales. Future efforts will include sequencing amplicons to determine the number of phylogenetically informative characters for each locus.

**LITERATURE CITED**

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### Table 1. Primer pair sequences and validation results by taxon.

| Locus ID | Primer sequences (5′−3′) | GenBank accession no. | Amplion length range among all taxa | Arabidopsis thaliana | Alocasia macrorrhiza | Bacopa monniera | Banyan macroura | Barosera simulans | Banisteriopsis pseudotropica | Caesalpinia pulcherrima | Commiphora grandiflora | Fallopia amurensis | Phellodendron x frangula | Sinapis arvensis |
|----------|---------------------------|----------------------|-------------------------------------|----------------------|---------------------|-----------------|-----------------|-------------------|-----------------------------|------------------------|------------------------|-------------------|---------------------|----------------------|-------------------------|----------------------|
| AT3G54460 | F: GGACACACCTTGGCTCTTAG R: CTTCCTGAGTTTGGTGTCGTC | KX767982 KX767983 | 270−290 | X | X | X | X | X | X | X | X | X | X | X | X | X |
| AT2G04620 | F: TCCACCATTTTTGAGAGGAGGA R: AATTGAGAAGGAAAATGATCTTG | KX76792 KX767929 | 420−520 | X | X | X | X | X | X | X | X | X | X | X | X | X |
| AT4G37510 | F: TCTTTTGGAGACCCTTTAGATGAC R: GCTTAGCCGAGTTATCGTCTCGC | KX768000 | 280 | X | X | X | X | X | X | X | X | X | X | X | X | X |
| AT3G22660 | F: AGATGAGAGTGGAAATTGGTGAACCC R: TTTCTGCTTGCTCTCCTCTCTCT | KX767974 KX767975 | 450 | X | X | X | X | X | X | X | X | X | X | X | X | X |
| AT1G21840 | F: TGGTGGAAGATGGAGAGAGGAGG R: CACATTTTCCTCGACCCTCCTGA | 630−640 | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| AT2G04740 | F: CAAACCTCGAAACCCCTAAAGGG R: TCACAAAGCTCTTTCCTCCT | KX767930 KX767931 | 460−590 | X | X | X | X | X | X | X | X | X | X | X | X | X |
| AT4G14605 | F: CTTCTCTCACTTATCAAGGACGAGA R: CTCTCTCTACCTTTGCTTTC | KX767986 KX767987 | 510−580 | X | X | X | X | X | X | X | X | X | X | X | X | X |
| AT4G19900 | F: CTTCTCACTCAGCTATACAGGACGAGA R: CTCTCTCTACCTTTGCTTTC | KX767990 KX767991 | 350−420 | X | X | X | X | X | X | X | X | X | X | X | X | X |
| AT4G29590 | F: GACGAATCTCCCTTCAAGAGAGA R: GITGCTGATTTGGTAAATTCG | KX767994 KX767995 | 490 | X | X | X | X | X | X | X | X | X | X | X | X | X |
| AT5G04910 | F: TAAAGCTTCACGAGACAGATGAGT R: TAAAGAGATGAGATGACAGTGCTC | KX768005 KX768006 | 260 | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| AT3G15110 | F: CTCCTCTGACCTTTTGGCTCTCG | 1560 | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| AT1G18060 | F: AACAGGAAAAGTTGCGATAGAGGA R: GCTCTCTCTTGTGCTCTCCTCTT | KX767902 KX767903 | 740−930 | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| AT2G33667 | F: CTATGCTGTCTGTCTGTACTTTCG R: CACAAAGAAATACAGGCAAGATTCCTC | KX767926 KX767927 | 590 | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| AT2G40760 | F: CGCTGATCATCTCTTGAGGGG R: GCGCTCTCGCCCTCTCTCTCTC | KX768007 KX768008 | 400 | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| AT2G20790 | F: CAAATTCATGACAGACCATCTCTTC R: CCACTGTTCAATTTATGCTGTTC | KX767940 KX767941 | 320−350 | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| AT2G36740 | F: AGTCCAGAAAGACGGCGAGTATGAG | 640−810 | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| AT3G01380 | F: AACTCATTAGATAGGAGGAGGAGG R: CAGCATGACGTCATACTCTTGT | KX767958 KX767959 | 530−930 | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| AT3G10400 | F: AGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG
| Locus ID | Primer sequences (5′−3′) | GenBank accession no. | B. simaruba | C. grandifolia | Amplicon length range among all taxa | Arabisopsis thaliana | Alocasia clavatula | Batocalon macrophyllum | Buxus microphylla | Buxus sarmentosa | Canarium pilosum | Cinnamomum grandifolium | Phyllodendron amurense | Pimelea guianensis | Schinus terebinthifolius |
|----------|--------------------------|----------------------|-------------|---------------|-------------------------------------|---------------------|-------------------|-------------------|------------------|----------------|----------------|-------------------|-------------------|-------------------|-------------------|
| AT2G27760 | GAACCTTAAACCAACTAACATGGGAGAA | AT2G27760 | 930          | X             | 160–470                             | X                   | X     | X     | X     | X     | X     | X     | X     | X     | X     | X     |
| AT2G27760 (INT) | GAACCTTAAACCAACTAACATGGGAGAA | AT2G27760 (INT) | 160–470 | X             | 220–640                             | X                   | X     | X     | X     | X     | X     | X     | X     | X     | X     | X     |
| AT1G63160 | GAACCTTAAACCAACTAACATGGGAGAA | AT1G63160 | 1070–1490 | X             | 160–470                             | X                   | X     | X     | X     | X     | X     | X     | X     | X     | X     | X     |
| AT1G65030 | CCGTTTCTCTGCTAGCTGGTAGAAGGAA | AT1G65030 | 340          | X             | 340                                  | X                   | X     | X     | X     | X     | X     | X     | X     | X     | X     | X     |
| AT5G2180 | CTCGAGAATTTGCTGGGAAATGT     | AT1G66080 | 460          | X             | 460                                  | X                   | X     | X     | X     | X     | X     | X     | X     | X     | X     | X     |
| AT2G44760 | CACATGGAATTTGCTGGGAAATGT     | AT2G44760 | 530–900      | X             | 530–900                              | X                   | X     | X     | X     | X     | X     | X     | X     | X     | X     | X     |
| AT2G05320 | TCGGAAATTTGCTGGGAAATGT     | AT2G05320 | 440          | X             | 440                                  | X                   | X     | X     | X     | X     | X     | X     | X     | X     | X     | X     |
| AT4G31770 | GCCTGGAAATTTGCTGGGAAATGT     | AT4G31770 | 580–780      | X             | 580–780                              | X                   | X     | X     | X     | X     | X     | X     | X     | X     | X     | X     |
| AT2G20330 | TTCATTGAAGGTTGTTGGGTATTGACG | AT2G20330 | 610–750      | X             | 610–750                              | X                   | X     | X     | X     | X     | X     | X     | X     | X     | X     | X     |
| AT1G66080 | ACCGCTTGGCTGATCCTGCATGATCA   | AT1G66080 | 900          | X             | 900                                  | X                   | X     | X     | X     | X     | X     | X     | X     | X     | X     | X     |
| AT2G05170 | CTCGAGAATTTGCTGGGAAATGT     | AT2G05170 | 430–480      | X             | 430–480                              | X                   | X     | X     | X     | X     | X     | X     | X     | X     | X     | X     |
| AT1G65070 | CTGCAATGAAATTTGCTGGGAAATGT     | AT1G65070 | 510–600      | X             | 510–600                              | X                   | X     | X     | X     | X     | X     | X     | X     | X     | X     | X     |
| AT5G67220 | CCGTAAAATAAGCTCAGGATACCTGGATGAGAA | AT5G67220 | 690          | X             | 690                                  | X                   | X     | X     | X     | X     | X     | X     | X     | X     | X     | X     |
| AT2G17265 | TTTAGGAGGCTTGGCTGGGAAATGT     | AT2G17265 | 470–1690     | X             | 470–1690                             | X                   | X     | X     | X     | X     | X     | X     | X     | X     | X     | X     |
| AT2G46890 | TCTTTCTCTCTCTCTCTCTCTAAAGAAGAA | AT2G46890 | 570–780      | X             | 570–780                              | X                   | X     | X     | X     | X     | X     | X     | X     | X     | X     | X     |
| AT2G31890 | CTCTCGAGAATTTGCTGGGAAATGT     | AT2G31890 | 410          | X             | 410                                  | X                   | X     | X     | X     | X     | X     | X     | X     | X     | X     | X     |
| AT2G46100 | CTGAAGGACACCTGACCTTGGCAAAAGAA | AT2G46100 | 310–370      | X             | 310–370                              | X                   | X     | X     | X     | X     | X     | X     | X     | X     | X     | X     |
| AT3G26580 | AGGGCATGGGCTTGGGAAATGT     | AT3G26580 | 660–920      | X             | 660–920                              | X                   | X     | X     | X     | X     | X     | X     | X     | X     | X     | X     |
| AT2G44660 | GTGTTTCTGAGGAGGGATGGGATACCTGGAGG | AT2G44660 | 590–1130     | X             | 590–1130                             | X                   | X     | X     | X     | X     | X     | X     | X     | X     | X     | X     |
| AT2G44660 (INT) | GTGTTTCTGAGGAGGGATGGGATACCTGGAGG | AT2G44660 (INT) | 520–900      | X             | 520–900                              | X                   | X     | X     | X     | X     | X     | X     | X     | X     | X     | X     |
| AT3G49730 | CCGAACTGGGAGAGGAGGAGGATGGGATACCTGGAGG | AT3G49730 | 140          | X             | 140                                  | X                   | X     | X     | X     | X     | X     | X     | X     | X     | X     | X     |
| Locus ID | Primer sequences (5′−3′) | GenBank accession no. | Apomictic length range among all taxa | Arabian oplodsp | A. korupensis | B. mitriformis | B. nefrecta | B. sinuata | C. pallidicaulis | C. graminifolia | C. pulcherrima | E. pauciflora | G. latifolia |
|----------|--------------------------|-----------------------|--------------------------------------|----------------|-------------|-------------|-------------|-------------|----------------|----------------|----------------|----------|----------|
| AT2G4460A | F: ATGCTATCAACACAGACGCTTGA | KX767950 | KX767951 | 790 | X | X | X | X | X | X | X | X | X |
| AT2G2110 | F: TGTCTCTCCACACGCTTGA | KX767942 (5′ only) | KX767943 (5′ only) | 1040−1360 | X | X | X | X | X | X | X | X | X |
| AT2G2110 (INT) | F: TTTCTCCTTTACCTATACGACGCTTGA | R: GCAGCAGACGCTTGA | 750−860 | X | X | X | X | X | X | X | X | X | X |
| AT2G22370A | F: ATTTGAGAGGGCTTCTATACGAG | 980−1320 | X | X | X | X | X | X | X | X | X | X | X |
| AT1G77930A | F: ACCCTAATCTTGCTTCGGATTGG | R: GCAGCAGACGCTTGA | 410−460 | X | X | X | X | X | X | X | X | X | X |
| AT1G77930A (INT) | F: GCAGCAGACGCTTGA | 1240−1680 | X | X | X | X | X | X | X | X | X | X | X |
| AT1G77930A (INT) | F: GCAGCAGACGCTTGA | 1000−1330 | X | X | X | X | X | X | X | X | X | X | X |
| AT3G15290 | F: ATCTGTTGAAGCAGCAGATTGG | R: GCAGCAGACGCTTGA | 740−860 | X | X | X | X | X | X | X | X | X | X | X |
| AT5G11980 | F: GCAGCAGACGCTTGA | 1090 | X | X | X | X | X | X | X | X | X | X | X | X |
| AT5G14580 | F: TTGGCTAGAGGGCTTCTATACGAG | 1030−1750 | X | X | X | X | X | X | X | X | X | X | X | X |
| AT2G31440 | F: ATGAGAAGGGCTTCTATACGAG | 480−1220 | X | X | X | X | X | X | X | X | X | X | X | X |
| AT1G77550A | F: ATCTGTTGAAGCAGCAGATTGG | R: GCAGCAGACGCTTGA | 340−1340 | X0 | X0 | X | X0 | X | X0 | X | X | X | X | X |
| AT2G47760 | F: ATGAGAAGGGCTTCTATACGAG | 620−1480 | X | X | X | X | X | X | X | X | X | X | X | X |
| AT3G29130 | F: TTGGCTAGAGGGCTTCTATACGAG | 980−1720 | X | X | X | X | X | X | X | X | X | X | X | X |
| AT3G3200 | F: ATCAGCTACCTTTCTTGTATCC | KX767980 | KX767981 | 1970 | X | X | X | X | X | X | X | X | X | X |
| AT1G73180 | F: TTGGCTAGAGGGCTTCTATACGAG | 770−1340 | X | X | X | X | X | X | X | X | X | X | X | X |
| AT4G33030A | F: ATGAGAAGGGCTTCTATACGAG | 810−1000 | X | X | X | X | X | X | X | X | X | X | X | X |
| AT1G73180 | F: TTGGCTAGAGGGCTTCTATACGAG | 450−620 | X | X | X | X | X | X | X | X | X | X | X | X |
| AT3G46220A | F: ATGAGAAGGGCTTCTATACGAG | 330−570 | X | X | X | X | X | X | X | X | X | X | X | X |
| AT2G50120 | F: CGAAGGAGAACAGCGACGCTTGA | 370−570 | X | X | X | X | X | X | X | X | X | X | X | X |
| AT1G73740 | F: TTGGCTAGAGGGCTTCTATACGAG | 870−1230 | X0 | X | X | X | X | X | X | X | X | X | X | X | X |
Table 1. Continued.

| Locus ID a | Primer sequences (5′−3′) | GenBank accession no. b | B. simaruba | C. grandifolia | Amplicon length range among all taxa | Anthracnose blisternut | Anthracnose brown spot | Botrytis botryosphaeria | Botrytis gray mold | Buxus | Bursera simaruba | Bursera obtusifolia | Canarium platanoides | Camptotheca acuminata | Phellodendron amurense | Pisonia guianensis | Schima |
|------------|--------------------------|------------------------|--------------|---------------|-------------------------------------|------------------------|----------------------|------------------------|------------------------|--------|----------------|------------------|-------------------|-------------------|----------------|------------------|
| AT4G31790 d | F: TTTGTTGTTCAGGCAAGAAA R: TGCACCAATTTAGCTTGTGCTTT | AT4G31790 | 1620–2180 | X | X | X | X |
| AT5G10460 | F: GCGTGTATAGCTTGTGCTTT | AT5G10460 | 1320–1800 | X | X | X | X |
| AT4G26980 | F: TTTACACAGTTAAGCTGCTTC | AT4G26980 | 940–1170 | X | X | X | X |
| AT5G48790 | F: GAGGGATTTTGTGTTGCTGCTTC | AT5G48790 | 680–1250 | X | X | X | X | X |
| AT5G15680 | F: GTTCTTATTTTGTGCTTCATC | AT5G15680 | 560 | X | X | X | X | X |
| AT3G04650 | F: CAAAGCTTCTCTGCTGCTT | AT3G04650 | 490–660 | X | X | X | X | X |
| AT2G25570 | F: GCAATATACTACATGCTGCTTC | AT2G25570 | 660–1160 | X | X | X | X |
| AT2G31040 | F: AGGAAATTTGCTGAGCCAA | AT2G31040 | 1230–1690 | X | X | X | X |
| AT4G04955 | F: AGGAAATTTGCTGAGCCAA | AT4G04955 | 440–1170 | X | X | X | X |
| AT3G21540 | F: GTGCTCATTGCTTGTGCTTCATC | AT3G21540 | 730–1020 | X | X | X | X | X |
| AT2G05170 | F: AGGAAATTTGCTGAGCCAA | AT2G05170 | 650–880 | X | X | X | X |
| AT2G28450 | F: TTTTCAAGTCACTTTTTGTCATTC | AT2G28450 | 1330 | X | X | X | X |
| AT3G07750 | F: GTATATTTATGCTGCTGCTTCATC | AT3G07750 | 940–1330 | X | X | X | X | X |
| AT1G76450 | F: GTGCTCATTGCTTGTGCTTCATC | AT1G76450 | 1330–1570 | X | X | X | X |
| AT3G10530 | F: TTTTCAAGTCACTTTTTGTCATTC | AT3G10530 | 700–960 | X | X | X | X | X |
| AT3G61620 | F: TTTTCAAGTCACTTTTTGTCATTC | AT3G61620 | 1330 | X | X | X | X |
| AT4G17700 | F: TTTTCAAGTCACTTTTTGTCATTC | AT4G17700 | 730–1130 | X | X | X | X | X |
| AT3G22990 | F: TTTTCAAGTCACTTTTTGTCATTC | AT3G22990 | 900–1130 | X | X | X | X | X | X |
| AT4G18810B | F: TTTTCAAGTCACTTTTTGTCATTC | AT4G18810B | 540–640 | X | X | X | X | X | X |
| AT1G77550 | F: TTTTCAAGTCACTTTTTGTCATTC | AT1G77550 | 760–820 | X | X | X |
| AT5G16690 | F: TTTTCAAGTCACTTTTTGTCATTC | AT5G16690 | 760–820 | X | X | X |
| AT4G00560 | F: TTTTCAAGTCACTTTTTGTCATTC | AT4G00560 | 900–1180 | X | X | X | X | X | X | X |

a Locus ID:

b GenBank accession no.:
Table 1. Continued

| GenBank accession no. | Primer sequences (5′−3′) | Amplification range among all taxa | Total no. of primers amplified | Amplification success for Commiphora grandifolia | Amplification success for Bursera simaruba | Amplification success for Bursera grandifolia | Amplification success for Canarium pilosum | Amplification success for Canarium neglecta | Amplification success for Boswellia neglecta | Amplification success for Protium guianense | Amplification success for Phellodendron amurense | Amplification success for Phellodendron thaliana |
|-----------------------|---------------------------|----------------------------------|-------------------------------|-----------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|
| R. simaruba            | F: GATGATGAACTATTTTTCCCTGAGGC | 630–900                          | 2                             |                                |                                          |                                          |                                          |                                          |                                          |                                          |                                          |                                          |
|                       | R: AAAGCAATATACGACCAAGAGAATCTG | 630–900                          | 2                             |                                |                                          |                                          |                                          |                                          |                                          |                                          |                                          |                                          |
| C. grandifolia         | F: GTCGACTTCACTCAGACACACC    | 360–840                           | 2                             |                                |                                          |                                          |                                          |                                          |                                          |                                          |                                          |                                          |
|                       | R: TGACCCCTCTCTACTCTACTC     | 360–840                           | 2                             |                                |                                          |                                          |                                          |                                          |                                          |                                          |                                          |                                          |

Note: INT = reverse primer is an internal primer for the locus.

a Primer originally developed by Gostel et al. (2015).
b GenBank accession numbers from loci used in phylogenetic analysis in Gostel et al. (2015). GenBank numbers were only created for loci of Bursera simaruba and those that were used in the phylogenetic analysis in Gostel et al. (2015). Some loci have two GenBank numbers for a species because sequence reads did not cover the full length of the locus. The first GenBank number corresponds to the read from the 5′ end of the locus; the second GenBank number corresponds to the read from the 3′ end of the locus.
c Universal Burseraceae primer (excluding Aucoumea).
d Primer for which high-fidelity TAQ increased amplification success for Commiphora grandifolia.
e Primer for which high-fidelity TAQ increased amplification success for Bursera simaruba.
f Primer for which high-fidelity TAQ increased amplification success for Bursera grandifolia.
g Universal Sapindales primer (excluding Aucoumea).
h Faint double band observed.

Table 2. Number of primer pairs amplified of the 91 primer pairs tested for each of the 11 taxa.

| Species tested (Order; Family) | Primer pairs amplified/tested (%) |
|--------------------------------|----------------------------------|
| Arabidopsis thaliana (Brassicaceae; Brassicaceae) | 2/9 (0.22) |
| Acrocomia klaineana (Sapindales; Burseraceae) | 16/91 (17) |
| Beiselia mexicana (Sapindales; Burseraceae) | 47/91 (52) |
| Boswellia neglecta (Sapindales; Burseraceae) | 68/91 (75) |
| Bursera simaruba (Sapindales; Burseraceae) | 90/91 (99) |
| Bursera tonkinensis (Sapindales; Burseraceae) | 53/91 (58) |
| Canarium pilosum (Sapindales; Burseraceae) | 71/91 (78) |
| Commiphora grandifolia (Sapindales; Burseraceae) | 72/91 (79) |
| Phellodendron amurense (Sapindales; Rutaceae) | 26/91 (28) |
| Protium guianense (Sapindales; Burseraceae) | 54/91 (59) |
| Schinus fasciculatus (Sapindales; Anacardiaceae) | 34/91 (37) |

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Appendix 1. Accession information for taxa used in this study, including voucher information, country of origin, and latitude and longitude coordinate data, if available, and DNA extraction method.

| Species | Voucher (Herbarium) | Country of origin | Geographic coordinates | DNA extraction method |
|---------|---------------------|-------------------|------------------------|----------------------|
| **Sapindales** | | | | |
| Burseraceae | | | | |
| *Aucoumea klaineana* Pierre | Walters et al. 466 (MO) | Gabon | 00°07′12″S, 11°42′57″E | 1 |
| *McPherson 16293* (MO) | Gabon | 00°27′S, 11°45′E | 1 |
| *Beiselia mexicana* Forman | Pell s.n. (TEX) | Mexico | NA | 1, 2 |
| *Boswellia neglecta* S. Moore | Weeks 00-VII-29-1 (TEX) | Ethiopia | NA | 2 |
| *Bursera simaruba* (L.) Sarg. | Weeks 16-VI-16-01 (GMUF) | USA | NA | 1 |
| *Goldman s.n.* (BH) | USA | NA | 2 |
| *Bursera tonkinensis* Guillain | Daly et al. 13929 (NY) | Vietnam | 20°15′12.6″N, 105°43′2.5″E | 1 |
| *Canarium pilosum* A. W. Benn. | Bogler s.n. (TEX) | Malaysia | NA | 2 |
| *Commiphora grandifolia* Engl. | Gostel 121 (GMUF) | Madagascar | 23°39′19.64″S, 44°37′44.36″E | 1 |
| *Protium guianense* (Aubl.) Marchand | Miller and Hauk 9391 (MO) | Suriname | 04°45′22″N, 056°52′30″W | 1 |
| **Anacardiaceae** | | | | |
| *Schinus fasciculatus* (Griseb.) I. M. Johnst. | Silva-Luz 287 (NY) | Argentina | 24°52′05.4″S, 65°32′41.4″W | 1 |
| **Rutaceae** | | | | |
| *Phellodendron amurense* Rupr. | Weeks 15-VII-13-01 (GMUF) | USA | 38°49′53.76″N, 77°18′32.04″W | 1 |
| **Brassicaceae** | | | | |
| *Arabidopsis thaliana* (L.) Heynh. | Gostel s.n. (GMUF) | USA | NA | 1 |

Note: NA = not available.  
1 = FastDNA, 2 = CTAB.