



An Expanded Nuclear Phylogenomic PCR Toolkit for Sapindales

Authors: Collins, Elizabeth S., Gostel, Morgan R., and Weeks, Andrea

Source: Applications in Plant Sciences, 4(12)

Published By: Botanical Society of America

URL: <https://doi.org/10.3732/apps.1600078>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

AN EXPANDED NUCLEAR PHYLOGENOMIC PCR TOOLKIT FOR SAPINDALES¹

ELIZABETH S. COLLINS^{2,4}, MORGAN R. GOSTEL³, AND ANDREA WEEKS²

²George Mason University, 4400 University Drive, MSN 3E1, Fairfax, Virginia 22030-4444 USA; and ³Department of Botany, National Museum of Natural History, Smithsonian Institution, MRC 166, P.O. Box 37012, Washington, D.C. 20013-7012 USA

- **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* (Brassicales), 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 rosoid 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 *Bursera* and *Commiphora* (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–Kirkiaceae (Muellner-Riehl et al., 2016). *Arabidopsis thaliana* (Brassicales) was included because its genomic resources were used in primer design and can test the applicability of these primers to other closely related rosoid lineages (Wang et al., 2009).

¹Manuscript received 25 June 2016; revision accepted 19 September 2016.

The authors thank Cíntia Silva-Luz for providing leaf material of *Schinus* and *Beiselia*. Research was supported in part by the National Science Foundation (grant no. 1403150 to M.R.G. and A.W.) and the Provost/COS/ESP Institutional Graduate Fellowship Research Award to E.S.C. from George Mason University. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation. Publication of this article was funded in part by the George Mason University Libraries Open Access Publishing Fund.

⁴Author for correspondence: ecolli11@masonlive.gmu.edu

doi:10.3732/apps.1600078

Applications in Plant Sciences 2016 4(12): 1600078; <http://www.bioone.org/loi/apps> © 2016 Collins et al. Published by the Botanical Society of America. This work is licensed under a Creative Commons Attribution License (CC-BY-NC-SA).

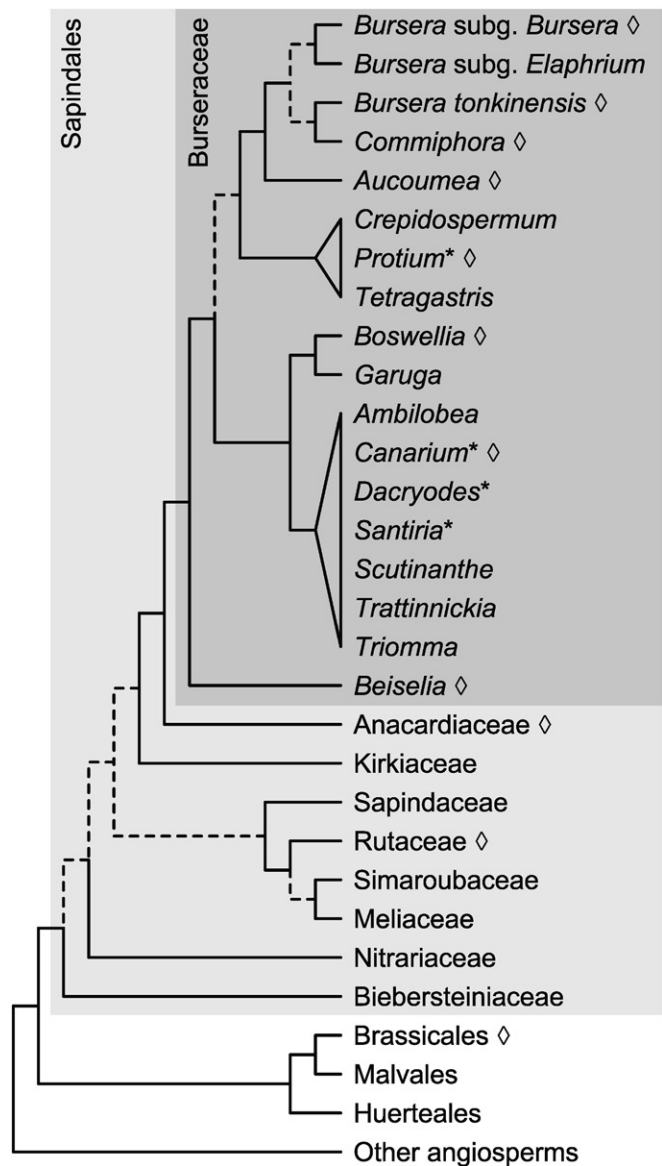


Fig. 1. Phylogeny of Sapindalean lineages condensed from Wang et al. (2009), Weeks et al. (2014), and Muellner-Riehl et al. (2016); nodes having low or conflicting support are indicated by dashed branches. Lineages sampled by the current study are noted by open diamonds. Generalized generic phylogeny of Burseraceae does not depict *Rosselia* or *Pseudodacryodes*, which have not been included in any molecular phylogenetic analysis; paraphyletic genera are indicated by asterisks.

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 91 markers is detailed by Gostel et al. (2015); primer sequences are listed in Table 1. Markers were amplified via PCR in 15-μL reactions including: 0.15 μL of forward and reverse primers (50 μM), 0.75 μL spermidine (4 mM), 7.5 μL GoTaq Green Master Mix (Promega Corporation, Madison, Wisconsin, USA), 5.6 μL nuclease-free water, and 1 μL genomic DNA (0.1–25.8 ng/μL). Markers that failed to amplify for *B. simaruba* and *C. grandifolia* were then trialed using reaction chemistry based on that recommended for microfluidic PCR-based target enrichment including: 0.15 μL of forward and reverse primers (50 μM); FastStart High Fidelity PCR System reagents (Roche Diagnostics, Mannheim, Germany), composed of 1.5 μL FastStart High Fidelity Reaction Buffer without MgCl₂ (10× concentration), 2.7 μL MgCl₂ (25 mM), 0.75 μL DMSO, 1.2 μL Nucleotide Mix (10 mM), 0.15 μL FastStart High Fidelity Enzyme Blend

(5 U/μL); 0.75 μL Loading Reagent (Fluidigm Corporation, San Francisco, California, USA); 6.8 μL nuclease-free water; and 1 μL genomic DNA.

The PCR thermocycler protocol followed that of Gostel et al. (2015) and included three alternating standard and C₀t cycles (Mathieu-Daude et al., 1996), beginning with 2 min at 50°C, 20 min at 70°C, and 10 min at 95°C. The first set of 10 standard cycles included a denaturation step at 95°C for 15 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min. Two C₀t cycles followed, including four steps consisting of 95°C for 15 s, 80°C for 30 s, 60°C for 30 s, and 72°C for 1 min. Standard and C₀t cycles alternated two more times with eight, two, eight, and five cycles, respectively. After 35 cycles, samples were held at 4°C prior to being visually verified via agarose gel electrophoresis (1% agarose; 94 V for 40 min). Low DNA mass ladder (Invitrogen, Carlsbad, California, USA) was included in the first and last wells of each gel to guide length estimation of PCR products.

Marker amplification results—Table 1 contains amplification results for the low-copy nuclear loci, including the range of amplicon lengths for all taxa and GenBank numbers for markers sequenced by Gostel 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 Gostel 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

ANGIOSPERM PHYLOGENY GROUP. 2016. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society* 181: 1–20.

BLOW, N. 2009. Microfluidics: The great divide. *Nature Methods* 6: 683–686.

DUARTE, J. M., P. K. WALL, P. P. EDGER, L. L. LANDERR, H. MA, J. C. PIRES, J. LEEBENS-MACK, AND C. W. DEPAMPHILIS. 2010. Identification of shared single copy nuclear genes in *Arabidopsis*, *Populus*, *Vitis*, and *Oryza* and their phylogenetic utility across various taxonomic levels. *BMC Evolutionary Biology* 10: 61.

FINE, P. V. A., F. ZAPATA, AND D. C. DALY. 2014. Investigating processes of Neotropical rain forest tree diversification by examining the evolution and historical biogeography of the Protieae (Burseraceae). *Evolution* 68: 1988–2004.

TABLE 1. Primer pair sequences and validation results by taxon.

Locus ID ^a	Primer sequences (5'–3') ^a	GenBank accession no. ^b		Amplicon length range among all taxa															
		<i>B. sinaruba</i>	<i>C. grandifolia</i>		<i>Arbidoopsis thaliana</i>	<i>Aucoumea klatieana</i>	<i>Beilschia mexicana</i>	<i>Boswellia neglecta</i>	<i>Bursera sinaruba</i>	<i>Bursera tonkinensis</i>	<i>Canarium pilosum</i>	<i>Commiphora grandifolia</i>	<i>Phellodendron amurense</i>	<i>Protium guianense</i>	<i>Schinus fasciculatus</i>				
AT3G54460 ^c	F: GGACACACCTTGGCTCTAG R: CTCCCATGACTTTGGTCTGTCT	KX767982	KX767983	270–290	X	X	X	X	X	X	X	X	X	X	X				
AT2G04620	F: TCCACCATATTTGATGAGAGAA R: AATGGAGTGGGAATGAGAATGTG	KX76792	KX767929	420–520	X			X	X	X	X	X	X	X	X	X			
AT4G37510 ^c	F: TTCAATTTGAGACCTCCATTAGATGAC R: GCTTAGCCGGATTATCGTCTCC		KX768000	280	X	X	X	X	X	X	X	X	X	X	X	X			
AT3G22660 ^c	F: AGATGAAAGATGTGAAATTGGTTGAACC R: TTTCTGCTTAGTCTCTCTCTTTCACT	KX767974	KX767975	450		X	X	X	X	X	X	X	X	X	X				
AT1G21840 ^c	F: TGTGGAGAGAGTTGAAGAGAGAGG R: CACCAATTTATCCCAACCTCTCTGAA			630–640	X	X	X	X	X	X	X	X	X	X	X				
AT2G04740 ^d	F: CAAACTCCAAAACCCTAAACCGG R: TCAAAAGCCTTCAAAAGCTTCCTC	KX767930	KX767931	460–590	X		X	X	X	X	X	X	X	X	X				
AT4G14605 ^d	F: CTTCTCACTCATAGCAGGACAGAG R: CTTTCTCACAGCCTTATCAAAGTCA	KX767986	KX767987	510–580		X	X	X	X	X	X	X	X	X	X	X			
AT4G19900 ^c	F: GTTCTCTGAGACGATTGAGCTTGA R: CTTGTAGAGAGCAGCAAGTCGG	KX767990	KX767991	350–420		X	X	X	X	X	X	X	X	X	X				
AT4G29590	F: GAGCAATTCCTCTCAAAGAGGA R: GTGCTTGTAATCTTTTGGGTAATGG	KX767994, KX767996	KX767995, KX767997	490				X	X	X	X	X	X	X	X				
AT5G04910	F: TAAAGATCCAAACAGACATGAGTG R: TAAAGAAATGATGTCACACGCTTCG	KX768005	KX768006	260		X		X	X	X	X	X	X	X					
AT3G15110 ^c	F: CTCACCTGGTCCATATCTGTCTTC R: ATTCTCTGTAACCTTTGCTCTCTGGA			1560				X											
AT1G18060 ^d	F: AACAAAGAAAGTTGCAGTAGAGGA R: GCTTGGCTCTCTGTCACTCTTTTGG	KX767902	KX767903	740–930		X	X	X	X	X	X	X	X	X	X				
AT2G03667 ^c	F: CTAGTTGGCTCTGGTCTGATG R: CACAAAGGAATATCAAGCAAAAGTCTT	KX767926	KX767927	590		X	X	X	X	X	X	X	X	X	X				
AT2G40760	F: GGTGTATCATCTGGAAGGGG R: CGCTCTCGCCCTCTCTTTTC	KX768007	KX768008	400		X		X	X	X	X	X	X	X	X				
AT2G20790 ^{c,g}	F: CCAATTGTCAATGGTCTCTGAAGATG R: CCATGGTGCAAAATTTAACTGTCTCC	KX767940	KX767941	320–350		X	X	X	X	X	X	X	X	X	X	X			
AT2G36740	F: AGTCCACAAGAACTGCAGTGTAT R: CATCTTTGAGAAATACCGTATCTGT			640–810		X	X	X	X	X	X	X	X	X	X				
AT3G01380 ^d	F: AATCATCATATAGGGGACGCCG R: CCAAGAAATATAGAAATAGTTCGGAC	KX767958, KX767960	KX767959, KX767961	530–930		X	X	X	X	X	X	X	X	X	X				
AT3G10400	F: AGAAGAAAAAGACTAACAGTGACAGC R: CGGCTTTGAGCACGCTGA	KX767966	KX767967	340			X	X	X	X	X	X	X	X	X				
AT1G59990 ^d	F: GTACTTGGTTCTCTTAATTGATAAGC R: TGACACACGAATAAATCCAAAGC	KX767908, KX767910	KX767909, KX767911	450–510		X	X	X	X	X	X	X	X	X	X	X			
AT2G22370B ^c	F: AACCCACATGGACTGTTAAACATG R: CATCAGACATAAGAGATGCAGCAG	KX767944	KX767945	610–780		X	X	X	X	X	X	X	X	X	X	X			
AT1G31780 ^{c,g}	F: CTTGTCTTGGGTTACTTGATCCA R: TTGTGGGTCTCAATGATTTCAAGC	KX767904, KX767906	KX767905, KX767907	520–570		X	X	X	X	X	X	X	X	X	X	X			
AT1G31780 (INT) ^{c,g}	F: CTTGTCTTGGGTTACTTGATCCA R: GGACCCAAAGTGTACTACAGAGAG			380–832		X	X ^h	X	X	X	X	X	X	X	X	X			

TABLE 1. Continued.

Locus ID ^a	Primer sequences (5'–3') ^a	GenBank accession no. ^b		Amplicon length range among all taxa															
		<i>B. simaruba</i>	<i>C. grandifolia</i>		<i>Arbidoopsis thaliana</i>	<i>Aucoumea klatiueana</i>	<i>Beiselia mexicana</i>	<i>Boswellia neglecta</i>	<i>Bursaria simaruba</i>	<i>Bursaria tonkinensis</i>	<i>Canarium pilosum</i>	<i>Commiphora grandifolia</i>	<i>Phellodendron amurense</i>	<i>Protium guianense</i>	<i>Schinus fasciculatus</i>				
AT2G27760	F: GAACCTTAAACCCTAACATGGAGAA R: GGCGTTCCGTGACCATAT			930					X										
AT2G27760 (INT)	F: GAACCTTAAACCCTAACATGGAGAA R: CGAAATCCTTAGCAGTGAACCTC		X	160–470		X		X	X	X	X	X	X	X	X	X	X		
AT1G63160 (INT)	F: GACGCTGTATCTAGGCTCCAAG R: AAAATGTTGCATGTGAAGTTTGGC			220–640					X	X	X		X	X	X		X		
AT1G63160	F: GACGCTGTATCTAGGCTCCAAG R: CACCATGAGACGCCCAAGTAT			1070–1490			X	X	X	X	X						X		
AT1G65030	F: CGGTTTCTGTAACCTCGGTACAG R: CGGGGAAAGAGAGGTTTGTG	KX767912	KX767913	340					X	X	X	X	X						
AT5G52180	F: CTCGAGAAATTGGTTGGAATGT R: CATAcAGAAAGCCGCTCGATA	KX768003	KX768004	460				X	X		X	X							
AT2G44760 ^d	F: CAGCATGGAATACGTTTGTAGTA R: TATCAACTGGACCCCTGGAATAAG	KX767954	KX767955	530–900		X			X	X	X	X	X	X	X	X	X		
AT2G05320A ^c	F: TGCCAAGTGAACAGATATTGCT R: TCTCCAAACAGCTCTGGTTAAAGGA	KX767934	KX767935	440			X	X	X	X	X	X	X		X	X	X		
AT4G31770 ^e	F: GCGGTGAGAAATGAGAATGACATG R: AACAAgTCTTCCAAATCCCAA	KX767998	KX767999	580–780			X	X	X	X	X	X	X		X	X	X		
AT2G20330 ^{c,g}	F: TCATTGAAGGTTTGGGATTTACGC R: ACGACTTGGCTGATCTCTGAATAA	KX767938	KX767939	610–750	X	X	X	X	X	X	X	X	X	X	X	X	X		
AT1G66080	F: CCTTCTCTTCCATAGTGTGCT R: CCCACAAAACGACTGCATAAAGTT			900					X										
AT2G05170B ^c	F: GCACAGTACATTAACACCATTTGCT R: TGGCTTGTGGTCTATGAGAACTTT			430–480		X	X	X	X	X	X			X	X	X	X		
AT1G65070	F: CCTAATACTGGAGGAAACTGCT R: CAGTACTTCCCAGAGAAATTCGAA	KX767914	KX767915	510–600				X	X	X		X		X					
AT5G67220	F: CGGTTAAAATGCTCTCAGGATCC R: CATCTGCCGAATGAGTAACCTTCT			690					X										
AT2G17265 ^{c,d,g}	F: TTATGGAGGTTTCGTTTGTATTGG R: CTAGCACCAACTCTATCCAACTC	KX767936	KX767937	470–1690			X	X	X	X	X ^h	X ^h	X	X	X	X	X		
AT2G46890B ^d	F: TTCTTTGCTGTACCTCTCTCAG R: CGATGTCGTCTTCTGATATAGCCT			570–780			X	X	X	X	X	X		X					
AT2G31890B ^{c,g}	F: CTCTCCAGTGCAGTTTAAcAG R: CTTGAGAAATGTTGGTCCATCA	KX767946	KX767947	410			X	X	X	X	X	X	X	X	X	X	X		
AT2G46100 ^e	F: TTTAAAGCACTCGCGTTTCAAA R: GGCAGAAAGAAATAGGCCTCCAG	KX767956	KX767957	310–370		X	X	X	X	X	X	X	X	X	X	X	X		
AT3G26580 ^{c,g}	F: AGTGACGGTGTGGATTATGATG R: GTGACGGTTAFTTGCCTCGTAAG	KX767976, KX767978	KX767977, KX767979	660–920		X	X	X	X	X	X	X	X	X	X	X	X		
AT2G44660B	F: GTTTTTCGAGGAAGGATGATTT R: TGAAGGTTTGTCTGGAGTTATCT	KX767952	KX767953	590–1130			X	X	X	X	X	X	X	X	X	X	X		
AT2G44660B (INT) ^c	F: GTTTTTCGAGGAAGGATGATTT R: TGCCCTGAATCTTGAACCTAGTTT	KX767952	KX767953	520–900			X	X	X	X	X	X	X	X	X	X	X		
AT3G49730	F: CCGAAACTGGAGATGGCTTTG R: AATCAACTCAGGCCTTCTTTTCTC			140					X										

TABLE 1. Continued.

Locus ID ^a	Primer sequences (5'–3') ^a	GenBank accession no. ^b		Amplicon length range among all taxa	Species														
		<i>B. simaruba</i>	<i>C. grandifolia</i>		<i>Arbidiopsis thaliana</i>	<i>Aucoumea klaineana</i>	<i>Betisella mexicana</i>	<i>Boswellia neglecta</i>	<i>Bursera simaruba</i>	<i>Bursera tonkinensis</i>	<i>Canarium pilosum</i>	<i>Commiphora grandifolia</i>	<i>Phellodendron amurense</i>	<i>Protium guianense</i>	<i>Schinus fasciculatus</i>				
AT2G44660A ^f	F: ATCGTATCAGACAGACACTTTGA R: GCAAAAACAAAACCAACCCATCAAA	KX767950	KX767951	790						X									
AT2G21710 ^e	F: TTTCCTCCTTTACTAAATACAGCCT R: CTTGCTGCAACCTTCTGATTGAA	KX767942 (5' only)	KX767943 (5' only)	1040–1360					X	X	X	X	X	X	X				
AT2G21710 (INT) ^d	F: TTTCCTCCTTTACTAAATACAGCCT R: GCTGCATCCCAAGAGCTCTGG			750–860					X	X	X	X	X	X	X	X			
AT2G22370A ^e	F: ATGTTGAGGCCCTTGAGATTCTTC R: TAGGTGCTGTACTTTCAACCAAGTT			980–1320					X	X	X	X	X	X	X	X			
AT1G77930A ^d	F: ACCCTAATCTGTTCTGCGATTGG R: GAGCAGTTCAATAAGCAGCTTGAAT	KX767924	KX767925	580–740					X	X	X	X	X	X	X				
AT1G77930A (INT)	F: ACCCTAATCTGTTCTGCGATTGG R: GCATCCCTCTCACTCTGCAATT			410–460					X	X	X	X	X	X	X				
AT5G02250 ^d	F: CACTTATCCCATGTTTCCAGAGAAC R: GGATCTGCCTGGTTTCAACATAT			1240–1680					X	X	X	X	X	X	X	X			
AT2G31440	F: GTATGGAGGGCTTTTCTTCCCTTTG R: ATTCCTGCAGCAAGATGAACATACA			1000–1350					X	X	X	X	X	X	X				
AT1G77550A ^d	F: TGTGAGCTTTTCTATATTGGGCC R: TGATGCTTCATGACCAGACAAGA	KX767920	KX767921	740–860				X	X	X	X	X	X	X	X	X			
AT3G15290 ^e	F: GATGTTGTAGTCGAGGCTATTGTG R: ATCTGCAAGTTCTAAAGGACCCAT			1090						X									
AT5G11980	F: TTCAACCATGCATCCCAAAATTACC R: GACAGAGATCCGCCCTTCAGTTATC			N/A															
AT5G14580 ^e	F: TATACGTATTGGCAGAAATTCGGG R: TCTTGTGCAATCTTATCTAAGGCCCT			1030–1750				X	X										
AT2G31840 ^d	F: AGTGAATTGATTTGGTCTCTGATGT R: CATCTTGGTGAAGGTAGCTACAG			480–1220				X	X	X	X	X	X	X	X	X			
AT5G57655	F: TTGGTTATGCTCAGTGTAAATCCGA R: CTACAGTGCAGATTGAAAAGCAT			340–1340				X ^h	X ^h	X	X ^h	X ^h	X ^h	X ^h	X ^h	X ^h			
AT2G47760	F: CAGCATGGAATACGTTTGTCTAGTA R: TATCAACTGGACCCCTGGAATAAG			620–1480					X								X		
AT3G29130 ^d	F: TTGCGGAGGTTTCTGGTGATT R: AAGTACTTCTTTGTGATTCATCCG		KX767980, KX767981	980–1720					X	X		X ^h	X						
AT3G13200 ^e	F: AACTCATCGGCTTTTCTCTCTCT R: GAATCATCAGCATCTACATTGCGT			1970					X										
AT4G33030 ^d	F: GATGGTGTCTTTGGTACTGCTTTG R: CCAAGAAACAGTGGGCATTATCTG			770–1340					X	X	X	X	X	X	X				
AT1G73180 ^d	F: AACTCCTGCCAGTGTCCAAATATA R: AGAATGCCATATCACCAAGTAAAT			810–1000					X	X	X	X	X	X	X	X			
AT2G31890A	F: AGATTGGAGGGAGCTACTTTATT R: CCTCCCTATACTGCTCTGAAATCC	KX767948	KX767949	450–620					X	X	X	X	X	X	X	X			
AT3G46220 ^d	F: CAATTGAGGAATGAAATGGTGGCT R: TCCATTCTTGCAAAAGCTTCTGT			330–570					X	X		X	X	X	X				
AT2G05120 ^{e-g}	F: TGTCAAAGCTCTGGTCTCATGAAA R: CGAAGGAAGAACTGAAGCATCTAG	KX767932	KX767933	370–570				X	X	X	X	X	X	X	X	X			
AT1G73740 ^d	F: TTGATATTGGAGGCTCTTTGGG R: CACCAGCTCTTGAACAACGAG			870–1230					X ^h	X		X ^h	X ^h	X ^h	X ^h	X ^h			

TABLE 1. Continued.

Locus ID ^a	Primer sequences (5'–3') ^a	GenBank accession no. ^b		Amplicon length range among all taxa															
		<i>B. simaruba</i>	<i>C. grandifolia</i>		<i>Arabidopsis thaliana</i>	<i>Aucoumea klatieana</i>	<i>Beiselia mexicana</i>	<i>Boswellia neglecta</i>	<i>Bursera sinaruba</i>	<i>Bursera tonkinensis</i>	<i>Canarium pilosum</i>	<i>Commiphora grandifolia</i>	<i>Phellodendron amurense</i>	<i>Protium guianense</i>	<i>Schinus fasciculatus</i>				
AT4G31790 ^d	F: ATTTGGTTGTTTCAGCCAGAAA R: GTCCAAAATCAACCATCTGCAGTT			1620–2180					X	X	X		X						
AT5G10460	F: TGGTCATCATAGCAATTCTTCAG R: GCTTCTCAACATCTCCACAAC			1320–1800			X	X	X	X				X					
AT4G26980 ^d	F: CTGCTAGTGGTGTTCGAAATTGG R: ACTTCTCAGCATTCGACAACTCAT			940–1170				X	X	X	X			X					
AT5G48790 ^d	F: GAGGATTTTGGTTTCACTGAAAAG R: TCGCGACCTTTAAAATTGTGAATGT			680–1250			X	X	X	X	X	X	X		X				
AT5G15680A	F: TTCTCAATCAAACATCATTTGGGCC R: GAGGAATTGCATCAGATTCTCGTC	KX768001	KX768002	560			X	X	X	X	X	X	X		X				
AT3G04650	F: CAAATCGCTTGTGTTGTTCAATCA R: CTGTGCAGTTGGGATGTTTTC	KX767962, KX767964	KX767963, KX767965	490–660			X	X	X	X	X			X	X				
AT2G25570 ^d	F: GACAACCTCAAATACACATGCCAGG R: GTCCCTTCTCTGATGCCCTATG			660–1160			X	X	X	X	X			X	X				
AT2G31040 ^d	F: AAGTACTGGGTGGAGAAAAAGAG R: CCAAGTGTGAGGATTTGCAACTTC			1230–1690			X	X	X	X	X	X	X						
AT4G04955	F: GAACAGATACGGTACAGAGCCAG R: TGCAGCTTTAGTCCCTGAAGG			440–1170				X	X										
AT3G21540 ^c	F: GTTGCTATTAGTCTCTGATGCCAAA R: AATGGTTCTTCAGTACGATCCCAA	KX767970, KX767972	KX767971, KX767973	730–1020			X	X	X	X	X			X					
AT2G05170A ^c	F: GAAAGGAAATGTACCAAGTGGAGGA R: TGAGAAGAAATGGTGGAGCTTCTT			650–880			X	X	X	X	X			X	X				
AT2G28450 ^f	F: TTTCTGAGATCATGCTTATAGTTCAGG R: CGCCCAATTGTTCCAGTACCA			1330					X		X								
AT3G07750 ^d	F: GCTATAATTGTTGATTGCGCCCT R: TGGTTGCTCATCGTTTAAATGCATC			940–1330			X	X	X	X	X			X					
AT1G76450 ^d	F: TGGTCCGGATTTTACAAGATGGA R: CGTCCGACAAAAATCAAAATAGG	KX767916, KX767918	KX767917, KX767919	1330–1570			X		X		X	X		X					
AT3G10530 ^{c,d}	F: GGTCCTCTTGCAATAATGAGCTGTT R: TCAAAATTCGCAAGTCCCAAATC			700–1960			X	X	X	X	X	X	X	X					
AT3G61620 ^c	F: ATTTCCCATTCAAATTCCACTCG R: ACCTCATGCGATCTCCAGTACTAA			1330					X										
AT4G21770 ^d	F: TGGAGCTGTTTATATGCCCCTGT R: TAGTCCTAGCTAAACACACACAGC	KX767992	KX767993	730–1130			X	X	X	X	X		X						
AT3G22990 ^d	F: TTCTAGGTCCATCTCTTCAAGTGC R: TTATGATATTTGAGGCAGCAACGG			900–1130			X	X	X	X	X	X	X		X				
AT4G18810B	F: CCTAAAAGGTGATGGTCAAGGTA R: TCTCCCTTCAACTGAATGTGAGA	KX767988	KX767989	540–640			X	X	X	X	X	X	X	X					
AT1G77550B ^d	F: ACAGTTCAGAAAGCACATGGATC R: CTGGTGTGTTTCATGTGATTGAGTC	KX767922	KX767923	760–820			X	X	X	X	X			X					
AT5G16690 ^d	F: TGCTTCCTCTAGACAAATTGTTCACT R: TCCAAAAGAAAGCGCTATGAATTG			760–820			X	X	X			X							
AT4G00560	F: CTGCTATGCTCTATAACGTTCTCTCC R: GTCCACCAACATCAACAGTAACT	KX767984 (5' only)	KX767985 (5' only)	900–1180			X		X	X	X			X	X				

TABLE 1. Continued.

Locus ID ^a	Primer sequences (5'–3') ^a	GenBank accession no. ^b		Amplicon length range among all taxa											
		<i>B. simaruba</i>	<i>C. grandifolia</i>		<i>Arabidopsis thaliana</i>	<i>Aucoumea klaineana</i>	<i>Beiselia mexicana</i>	<i>Boswellia neglecta</i>	<i>Bursera simaruba</i>	<i>Bursera tonkinensis</i>	<i>Canarium pilosum</i>	<i>Commiphora grandifolia</i>	<i>Phellodendron amurense</i>	<i>Protium guianense</i>	<i>Schinus fasciculatus</i>
AT3G17170 ^d	F: GATGATGAAGTATTTTCCCTGAGGC R: TCTTGACTTCTCATTCACACTGC			630–900				X	X	X	X	X		X	34
AT3G14910 ^e	F: GGAGCTATTTTATCAAAAGTTGTGCC R: AAAGCAATATACGACCAAGAGAAATCTG	KX767968	KX767969	360–840	X		X	X	X	X	X	X		X	54
Total no. of primers amplified/taxon					2	16	47	68	90	53	71	72	26		

Note: INT = reverse primer is an internal primer for the locus.
^aPrimer originally developed by Gostel et al. (2015).
^bGenBank accession numbers from loci used in 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.
^cUniversal Burseraceae primer (excluding *Aucoumea*).
^dPrimer for which high-fidelity TAQ increased amplification success for *Commiphora grandifolia*.
^ePrimer for which high-fidelity TAQ increased amplification success for *Bursera simaruba*.
^fPrimer for which high-fidelity TAQ increased amplification success for *Bursera simaruba* and *Commiphora grandifolia*.
^gUniversal Sapindales primer (excluding *Aucoumea*).
^hFaint 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 (%)
<i>Arabidopsis thaliana</i> (Brassicales; Brassicaceae)	2/91 (0.02)
<i>Aucoumea klaineana</i> (Sapindales; Burseraceae)	16/91 (17)
<i>Beiselia mexicana</i> (Sapindales; Burseraceae)	47/91 (52)
<i>Boswellia neglecta</i> (Sapindales; Burseraceae)	68/91 (75)
<i>Bursera simaruba</i> (Sapindales; Burseraceae)	90/91 (99)
<i>Bursera tonkinensis</i> (Sapindales; Burseraceae)	53/91 (58)
<i>Canarium pilosum</i> (Sapindales; Burseraceae)	71/91 (78)
<i>Commiphora grandifolia</i> (Sapindales; Burseraceae)	72/91 (79)
<i>Phellodendron amurense</i> (Sapindales; Rutaceae)	26/91 (28)
<i>Protium guianense</i> (Sapindales; Burseraceae)	54/91 (59)
<i>Schinus fasciculatus</i> (Sapindales; Anacardiaceae)	34/91 (37)

GOSTEL, M. R., K. A. COY, AND A. WEEKS. 2015. Microfluidic PCR-based target enrichment: A case study in two rapid radiations of *Commiphora* (Burseraceae) from Madagascar. *Journal of Systematics and Evolution* 53: 411–431.

GRUDINSKI, M., C. M. PANNELL, M. W. CHASE, J. A. AHMAD, AND A. N. MUELLNER-RIEHL. 2014. An evaluation of taxonomic concepts of the widespread plant genus *Aglaia* and its allies across Wallace's Line (tribe Aglaieae, Meliaceae). *Molecular Phylogenetics and Evolution* 73: 65–76.

MATASCI, N., L.-H. HUNG, Z. YAN, E. J. CARPENTER, N. J. WICKETT, S. MIRARAB, N. NGUYEN, ET AL. 2014. Data access for the 1,000 plants (1KP) project. *GigaScience* 3: 17.

MATHIEU-DAUDE, F., J. WELSH, T. VOGT, AND M. MCCLELLAND. 1996. DNA rehybridization during PCR: The 'C₀t effect' and its consequences. *Nucleic Acids Research* 24: 2080–2086.

MUELLNER-RIEHL, A. N., A. WEEKS, J. W. CLAYTON, S. BUEKI, L. NAUHEIMER, Y.-C. CHIANG, S. CODY, AND S. K. PELL. 2016. Molecular phylogenetics and molecular clock dating of Sapindales based on plastid *rbcl*, *atpB* and *trnL-trnF* DNA sequences. *Taxon* 65: 1019–1036.

URIBE-CONVERS, S., M. L. SETTLES, AND D. C. TANK. 2016. A phylogenomic approach based on PCR target enrichment and high throughput sequencing: Resolving the diversity within the South American species of *Bartisia* L. (Orobanchaceae). *PLoS ONE* 11: e0148203.

WANG, H., M. J. MOORE, P. S. SOLTIS, C. D. BELL, S. F. BROCKINGTON, R. ALEXANDRE, C. C. DAVIS, ET AL. 2009. Rosid radiation and the rapid rise of angiosperm-dominated forests. *Proceedings of the National Academies of Science, USA* 106: 3853–3858.

WEEKS, A., D. C. DALY, AND B. B. SIMPSON. 2005. The phylogenetic history and biogeography of the frankincense and myrrh family (Burseraceae) based on nuclear and chloroplast sequence data. *Molecular Phylogenetics and Evolution* 35: 85–101.

WEEKS, A., AND B. B. SIMPSON. 2007. Molecular phylogenetic analysis of *Commiphora* (Burseraceae) yields insight on the evolution and historical biogeography of an "impossible" genus. *Molecular Phylogenetics and Evolution* 42: 62–79.

WEEKS, A., F. ZAPATA, S. K. PELL, D. C. DALY, J. D. MITCHELL, AND P. V. A. FINE. 2014. To move or to evolve: Contrasting patterns of intercontinental connectivity and climatic niche evolution in "Terebinthaceae" (Anacardiaceae and Burseraceae). *Frontiers in Genetics* 5: 409.

ZIMMER, E. A., AND J. WEN. 2012. Using nuclear gene data for plant phylogenetics: Progress and prospects. *Molecular Phylogenetics and Evolution* 65: 774–785.

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^a.

Species	Voucher (Herbarium)	Country of origin	Geographic coordinates	DNA extraction method ^a
Sapindales				
Burseraceae				
<i>Aucoumea klaineana</i> Pierre	Walters <i>et al.</i> 466 (MO)	Gabon	00°07'12"S, 11°42'57"E	1
	McPherson 16293 (MO)	Gabon	00°27'S, 11°45'E	1
<i>Beiselia mexicana</i> Forman	Pell <i>s.n.</i> (TEX)	Mexico	NA	1, 2
<i>Boswellia neglecta</i> S. Moore	Weeks 00-VII-29-1 (TEX)	Ethiopia	NA	2
<i>Bursera simaruba</i> (L.) Sarg.	Weeks 16-VI-16-01 (GMUF)	USA	NA	1
	Goldman <i>s.n.</i> (BH)	USA	NA	2
<i>Bursera tonkinensis</i> Guillamin	Daly <i>et al.</i> 13929 (NY)	Vietnam	20°15'12.6"N, 105°43'2.5"E	1
<i>Canarium pilosum</i> A. W. Benn.	Bogler <i>s.n.</i> (TEX)	Malaysia	NA	2
<i>Commiphora grandifolia</i> Engl.	Gostel 121 (GMUF)	Madagascar	23°39'19.64"S, 44°37'44.36"E	1
	Weeks 10-I-09-10 (GMUF)	Madagascar	12°14'16.14"S, 49°22'12.906"E	1
<i>Protium guianense</i> (Aubl.) Marchand	Miller and Hauk 9391 (MO)	Suriname	04°45'22"N, 056°52'30"W	1
Anacardiaceae				
<i>Schinus fasciculatus</i> (Griseb.) I. M. Johnst.	Silva-Luz 287 (NY)	Argentina	24°52'05.4"S, 65°32'41.4"W	1
Rutaceae				
<i>Phellodendron amurense</i> Rupr.	Weeks 15-VII-13-01 (GMUF)	USA	38°49'53.76"N, 77°18'32.04"W	1
Brassicales				
Brassicaceae				
<i>Arabidopsis thaliana</i> (L.) Heynh.	Gostel <i>s.n.</i> (GMUF)	USA	NA	1

Note: NA = not available.

^a 1 = FastDNA, 2 = CTAB.