Primers for Low-Copy Nuclear Genes in the Melastomataceae

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PRIMER NOTE

PRIMERS FOR LOW-COPY NUCLEAR GENES IN THE MELASTOMATACEAE[1]

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- *Premise of the study:* Low-copy nuclear gene primers were developed for phylogenetic studies across the Melastomataceae.
- *Methods and Results:* Total genomic libraries from eight species in the Melastomataceae along with one transcriptome were used for marker identification and primer design. Eight exon-primed intron-crossing markers were amplified with success in taxa of nine tribes in the Melastomataceae. The new markers were directly sequenced for eight samples of closely related species of *Miconia* (Chaenanthera clade) in the tribe Miconieae. The DNA sequences for the eight loci ranged from 660 to 818 aligned base pairs. Compared with four commonly used markers in other studies, the loci developed here had a higher number of variable sites than plastid spacers (7–16 vs. 26–45) and comparable variation to the ribosomal spacers (28–39).
- *Conclusions:* The novel primer pairs should be useful for a broad range of studies of systematics and evolution in the diverse Melastomataceae.

Key words: Melastomataceae; Miconieae; phylogeny; systematics; single copy.

The Melastomataceae Juss. are a diverse group of plants found mainly and throughout the tropical regions (Clausing and Renner, 2001). The family has ca. 5000 species showing great morphological and ecological variability distributed across different habitats (Clausing and Renner, 2001). Recently, several local radiations with great potential to add to our understanding of evolution in tropical regions have been uncovered by molecular studies (Goldenberg et al., 2012; Michelangeli et al., 2013; Michelangeli et al., in prep.). The plastid *rbcL* and *ndhF* genes and the *rpl16* intron have been the popular choice for inferring relationships among major clades in the family (Clausing and Renner, 2001; Goldenberg et al., 2012; Michelangeli et al., 2014), while the plastid spacers *accD-psaI*, *atpF-atpH*, *psbK-psbL*, and *trnS-trnG*, along with the ribosomal spacers (nrETS and nrITS), have commonly being used within lineages of Melastomataceae (Bécquer-Granados et al., 2008; Reginato et al., 2010; Michelangeli et al., 2013; Kriebel et al., 2015). Low-copy nuclear genes have scarcely been explored, being restricted to partial sequences of the genes *GapC* and *waxy* (Stone, 2006; Reginato and Michelangeli, in prep.), very likely due to the lack of specific primers. Low levels of variation in plastid and ribosomal DNA sequences associated with incomplete lineage sorting and/or lateral gene transference have yielded poorly resolved and/or conflicting hypotheses to address evolutionary questions in several lineages within this family (Reginato and Michelangeli, in prep.). Low-copy nuclear markers will likely improve understanding of lineage evolution in Melastomataceae. Here, we developed primer pairs for eight putative single-copy nuclear genes. Amplification was tested in several genera from distinct tribes in the family, while further direct sequencing was performed for eight species of the Chaenanthera clade, a ca. 8-Myr-old lineage within the tribe Miconieae (Michelangeli et al., in prep). Phylogenetic information of the new markers along with previously sequenced markers for the same Chaenanthera samples is presented.

METHODS AND RESULTS

Genomic libraries from eight species in the Melastomataceae (five in the tribe Miconieae and three in the Melastomeae) generated for a phylogenomic study (Michelangeli et al., in prep.) along with the transcriptome of *Tetrazygia biflora* (Cogn.) Urb. (Miconieae) were used for primer design. Total genomic DNA was isolated from silica gel–dried tissue using the QIAGEN DNAeasy Plant Mini Kit (QIAGEN, Valencia, California, USA) following the protocol suggested by Alexander et al. (2007). Total DNA was then quantified using a NanoDrop Spectrophotometer (Thermo Scientific, Waltham, Massachusetts, USA). Total genomic libraries were generated and barcoding was performed at Cold Spring Harbor Laboratory (Cold Spring Harbor, New York, USA) on an Illumina HiSeq 2000 platform (Illumina, San Diego, California, USA) on a single multiplex lane. The number of total reads yielded was on average ca. 20 Gb per sample (SD = 3 Gb). Paired reads were imported into Geneious 7.1 (Biomatters Ltd., Auckland, New Zealand), trimmed by quality (at 0.05 probability), and de novo assembled (Geneious Assembler, "low sensitivity" option, default settings). The assembled *T. biflora* transcripts were downloaded from the oneKP transcriptome database (www.onekp.com). MegaBLAST searches were performed on the *T. biflora* transcripts using the default parameters and mapped against the COSII data set (Wu et al., 2006) along with the *Arabidopsis adh* and *waxy* genes. Mapping was performed in Geneious 7.1, using the medium sensitivity settings, with the "minimum overlap" parameter set to 500. Matches with a single hit and with more than 80% of coverage to the reference were selected for further analyses. Approximately 340 transcripts passed these criteria and were further mapped against the pool of total genomic de novo assembled contigs from all eight libraries. Mapping was performed using the high-sensitivity settings, with the "allowing gap size" parameter set to 1000. Then, we selected eight matches with high coverage, appropriate intronic-sized regions, and a single hit per sample. Primers flanking the target intronic regions were designed

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TABLE 1. Primer sequences for the eight putative single-copy nuclear markers developed to amplify across Melastomataceae.

Locus	Primer sequences (5′–3′)	Putative *Arabidopsis* homolog	Putative product	T_a (°C)
ADH	F: TGGCAAGCACYGCTGGTCAGG R: GAGATGCCGCAGCTSAGGA	At1g77120	Alcohol dehydrogenase	56
UNC50	F: CGGGAGGAGGCACCAAATAG R: AGARGCGGCCACCATGAAGA	At2g15240	UNC-50 family protein	53
PCRF1	F: RCTCAAGTTCGAGAGTGGAGT R: YAGCTTTGACCGATCCRAGT	At2g47020	Peptide chain release factor 1	50
UFP	F: AAGTGGAGCGGGAAGGAGTA R: RGGAGMCTCCACTTGGTCCA	At4g06599	Ubiquitin family protein	53
At5g10780	F: AGYGCCTTGCAGGGTGTTGG R: RAGTAGGCCCAATGTGTTTA	At5g10780	Unknown protein	50
SOS4	F: GCAACAAGTCGGCTGTCTTT R: CGGAGCTTCTTGACAACTTCC	At5g37850	Pyridoxal kinase	51
ZBADH	F: TCCACGAGCTYGGGGGTRCC R: GGCCGGAAGAATCTGCTCTT	At5g61510	Zinc-binding alcohol dehydrogenase family	53
waxy	F1: GRGGTCTTGGGGACGTGCTC F2: ACACTTGCGTGGTCGTYCAG R: AGCAGTGTGCCARTCGTTGG	At1g32900	Granule-bound starch synthase 1	54

Note: T_a = annealing temperature used in the PCR.

with Primer3, using the default settings (Rozen and Skaletsky, 1999). Primer sequences and their putative *Arabidopsis* homolog are presented in Table 1, and the gene models showing primer locations are shown in Fig. 1.

The primers were tested on total genomic DNA of eight species from different tribes across the family (PCR and electrophoresis only, voucher information in Appendix 1), while PCR and direct sequencing was performed for eight closely related species of *Miconia* Ruiz & Pav. within the Chaenanthera clade in the tribe Miconieae (voucher information and GenBank accessions in Appendix 2). PCR amplification was performed using the following mix: 7.5 µL of EconoTaq Plus Green Master Mix (Lucigen Corporation, Middleton, Wisconsin, USA), 2 µL of $MgCl_2$ (25 mM), 2 µL of bovine serum albumin (BSA) (10 mg/mL), 1.2 µL of each primer (3 µM), and 0.7 µL of DNA template. A general amplification program was used, varying the annealing temperature for each marker (see Table 1): 2 min at 94°C; 36 cycles of 45 s at 94°C, 45 s at 50–56°C, 1 min and 15 s at 72°C; and a final extension of 10 min at 72°C. Direct sequencing was performed with the same forward and reverse primers used for amplification at the High-Throughput Genomics Unit sequencing service at the University of Washington (Seattle, Washington, USA). Contigs were assembled with Sequencher 4.9 (GeneCodes Corporation, Ann Arbor, Michigan, USA), and sequence alignment was performed with MAFFT version 7 using the strategy E-INS-i (Katoh, 2013).

The eight markers were successfully amplified in the majority of the nine genera tested (Appendix 1). The only three failures were registered for the genus *Mouriri* Aubl., a member of the small subfamily Olisbeoideae, which is sister to the remainder of the family (Clausing and Renner, 2001). Direct sequencing was successful for all samples of *Miconia* included in this study. However, sequencing was problematic for some other samples of the *At5g10780* marker (also in the Chaenanthera clade, not included in this study), due to a repetitive sequence of ca. 15 Ts. Sequences of the eight amplified loci for the samples of the Chaenanthera clade of *Miconia* ranged from 660 to 818 aligned base pairs, while 20 to 45 variable and one to six parsimony informative sites were observed across the individual alignments. The level of polymorphism of most of the new markers is comparable to the ribosomal spacers nrETS and nrITS, and higher than the chloroplast spacers that are widely used in phylogenetic studies in the Melastomataceae (Table 2).

CONCLUSIONS

Amplification of eight novel primer pairs was successful in samples from nine tribes across Melastomataceae for a majority

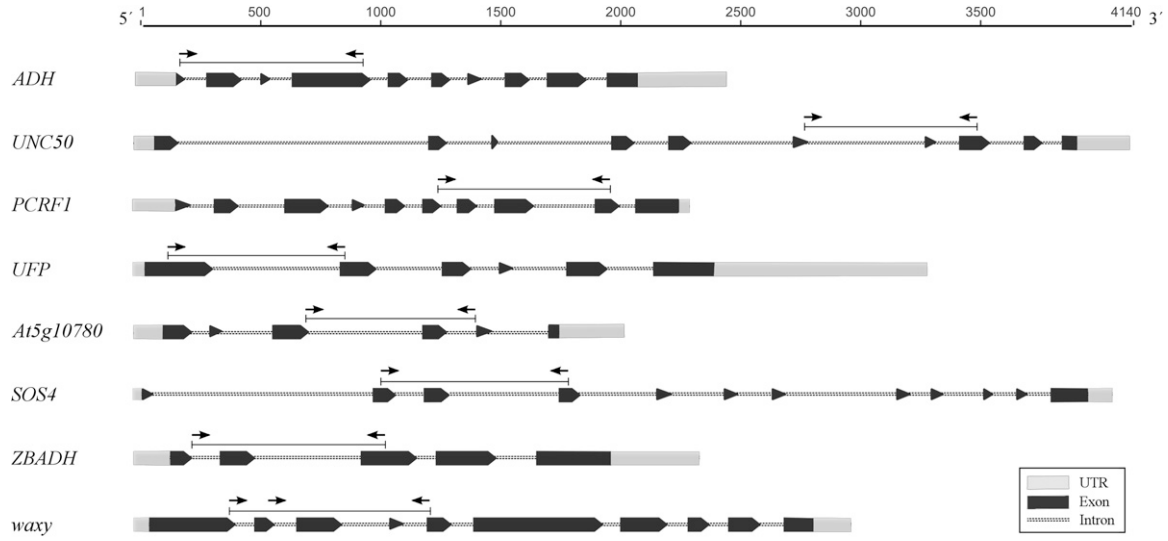

Fig. 1. Gene models for the eight putative single-copy nuclear markers developed to amplify across Melastomataceae. Primer locations are indicated with arrows; intron/exon boundaries are based on the *Tetrazygia biflora* transcripts.

TABLE 2. Summary statistics of the eight putative single-copy nuclear markers developed to amplify across Melastomataceae. Metrics are given for alignments including the same eight samples of the Chaenanthera clade and are compared with markers previously sequenced for these samples (indicated by an asterisk, *accD-psaI* and *psbK-L* are chloroplast spacers, and rETS and rITS are ribosomal spacers).

Locus	Mean no. of bases (range)	Aligned bases	Variable sites (%)	PIS (%)
ADH	768 (745–808)	809	32 (4)	3 (0.4)
At5g10780	799 (740–815)	818	27 (3.3)	1 (0.1)
PCRF1	671 (667–691)	692	20 (2.9)	5 (0.7)
SOS4	730 (728–732)	732	26 (3.6)	2 (0.3)
UFP	654 (645–658)	660	35 (5.3)	5 (0.8)
UNC50	669 (664–670)	670	26 (3.9)	1 (0.1)
waxy	767 (767–768)	770	38 (4.9)	6 (0.8)
ZBADH	707 (697–764)	776	45 (5.8)	5 (0.6)
*accD-psaI**	675 (671–697)	699	16 (2.3)	3 (0.4)
*psbK-L**	350 (349–352)	352	7 (2)	2 (0.6)
rETS*	586 (585–587)	590	39 (6.6)	6 (1)
rITS*	802 (801–803)	807	28 (3.5)	8 (1)

Note: PIS = parsimony informative sites.

of markers. Although the usefulness of the markers at a broader family scale still needs to be evaluated, the markers should be useful to increase resolution of phylogenetic relationships within lineages in the family.

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APPENDIX 1. List of genera across the Melastomataceae in which amplification of the eight putative single-copy nuclear markers was successful.

Clade	Species	*ADH*	*At5g10780*	*PCRF1*	*SOS4*	*UFP*	*UNC50*	*waxy*	*ZBADH*	Voucher (Herbarium)[a]
Bertolonieae	*Bertolonia mosenii* Cogn.	+	+	+	+	+	+	+	+	*Goldenberg, R. 804* (NY, UPCB)
Blakeeae	*Blakea bracteata* Gleason	+	+	+	+	+	+	+	+	*Goldenberg, R. 964* (NY, UPCB)
Melastomeae	*Brachyotum quinquenerve* (Ruiz & Pav.) Triana	+	+	+	+	+	+	+	+	*Michelangeli, F. A. 1979* (NY)
Marcetia alliance	*Comolia microphylla* Benth.	+	+	+	+	+	+	+	+	*Michelangeli, F. A. 2499* (NY)
Henrietteeae	*Henriettea trachyphylla* (Triana) Penneys, Michelang., Judd & Almeda	+	+	+	+	+	+	+	+	*Almeda, F. 10165* (CAS)
Cambessedesia alliance	*Huberia consimilis* Baumgratz	+	+	+	+	+	+	+	+	*Michelangeli, F. A. 1618* (NY)
Merianieae	*Meriania nobilis* Triana	+	+	+	+	+	+	+	+	*Clark, J. L. 13051* (UNA)
Olisbeoideae	*Mouriri chamissoana* Cogn.	—	—	+	+	+	+	+	—	*Reginato, M. 1025* (UPCB)

Note: + = successful amplification; — = no amplification.

[a]Vouchers are deposited at the herbaria of the California Academy of Sciences (CAS), San Francisco, California, USA; New York Botanical Garden (NY), Bronx, New York, USA; University of Alabama (UNA), Tuscaloosa, Alabama, USA; or Universidade Federal do Paraná (UPCB), Curitiba, Paraná, Brazil.

Appendix 2. Voucher information and GenBank accessions of the species in the Chaenanthera clade sequenced for the eight putative single-copy nuclear markers.

Species	*ADH*	*At5g10780*	*PCRF1*	*SOS4*	*UFP*	*UNC50*	*waxy*	*ZBADH*	Voucher (Herbarium)[a]
Miconia caudigera DC.	KT377070	KT377078	KT377086	KT377094	KT377102	KT377110	KT377118	KT377126	*Lima, J. 729* (NY)
Miconia dorsaliporosa R. Goldenb. & Reginato	KT377071	KT377079	KT377087	KT377095	KT377103	KT377111	KT377119	KT377127	*Kollmann, L. 8572* (UPCB)
Miconia inaequidens (DC.) Naudin	KT377072	KT377080	KT377088	KT377096	KT377104	KT377112	KT377120	KT377128	*Goldenberg, R. 732* (NY, UPCB)
Miconia longicuspis Cogn.	KT377073	KT377081	KT377089	KT377097	KT377105	KT377113	KT377121	KT377129	*Kollmann, L. 8562* (UPCB)
Miconia paucidens DC.	KT377074	KT377082	KT377090	KT377098	KT377106	KT377114	KT377122	KT377130	UPCB 59855
Miconia ramboi Brade	KT377075	KT377083	KT377091	KT377099	KT377107	KT377115	KT377123	KT377131	*Goldenberg, R. 793* (NY, UPCB)
Miconia staminea (Desr.) DC.	KT377076	KT377084	KT377092	KT377100	KT377108	KT377116	KT377124	KT377132	*Goldenberg, R. 784* (UPCB)
Miconia tristis Spring	KT377077	KT377085	KT377093	KT377101	KT377109	KT377117	KT377125	KT377133	*Goldenberg, R. 812* (NY, UPCB)

[a]Vouchers are deposited at the herbaria of the New York Botanical Garden (NY), Bronx, New York, USA, and/or Universidade Federal do Paraná (UPCB), Curitiba, Paraná, Brazil.