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TECHNICAL NOTE

Development of nuclear SNP markers for Mahogany (*Swietenia* spp.)

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Abstract

Swietenia species are the most valuable American tropical timbers and have been heavily overexploited for decades. The three species are listed as either vulnerable or endangered by IUCN and are included on Appendix II of CITES, yet illegal exploitation continues. Here, we used restriction associated DNA sequencing to develop a new set of 120 SNP markers for *Swietenia* sp., suitable for MassARRAY®iPLEX™ genotyping. These markers can be used for population genetic studies and timber tracking purposes.

Keywords SNPs · Mahogany · *Swietenia* spp. · MassARRAY®iPLEX™

The genus *Swietenia* includes the species: *Swietenia mahagoni* (L.) Jacq. (Small-leaved mahogany, native to Florida and the Caribbean islands), *Swietenia macrophylla* King. (Big-leaved mahogany, native to Central and South America) and *Swietenia humilis* Zucc. (Pacific Coast mahogany, native to the relatively dry Central American Pacific coast) (Schütt et al. 2014). While *S. mahagoni* is no longer traded commercially because of past overexploitation, *S. macrophylla* is now the most valuable and economically important American tropical timber (Louppe et al. 2008). *Swietenia* wood is used for high-class furniture, boat building, musical instruments etc. All three mahogany species are listed on CITES (Convention on International Trade in Endangered Species, Appendix II) and on the IUCN red list, where *S. macrophylla* and *S. humilis* are listed as vulnerable and *S. mahagoni* is classified as endangered.

Illegal logging is a major threat to the sustainable use of natural forests. Laws like the European Union Timber Regulation and an amendment of the Lacey Act in the US prohibit the trade of illegally sourced timber. However, existing timber-tracking/chain-of-custody systems are based mainly on electronic tags or paper-based documentation, and are vulnerable to falsification. Therefore, efforts to establish genetic methods for timber tracking are being undertaken. Several academic studies documenting population genetic structure in *Swietenia* species have been completed, based on the analysis of nuclear and chloroplast microsatellite (SSR) markers (e.g. Degen et al. 2013; Lemes et al. 2010; Lemes et al. 2003; Novick et al. 2003). However, genotyping of SSR loci from timber material can be difficult as DNA extracts are often low quality, which impedes the amplification of relatively long SSR-fragments (typically 100–300 bp). Consequently, single nucleotide polymorphism (SNP) markers have become the marker of choice for timber forensics, as they can be characterized in much shorter fragments, are

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simple to standardize among laboratories, and are fast and low-cost to develop in large numbers (Blanc-Jolivet et al. 2017). SNP sets have been developed for timber tracking purposes in other commercially important tropical tree species, e.g., *Hymenaea* spp. (Chaves et al. 2019), *Dipteryx* spp. (Honorio Coronado et al. 2019), *Cedrela* spp. (Paredes-Villanueva et al. 2019), *Jacaranda copaia* (Sebbenn et al. 2019), *Carapa* spp. (Tysklind et al. 2019), *Entandrophragma cylindricum* (Blanc-Jolivet et al. 2018), *Handroanthus* spp. (Meyer-Sand et al. 2018) and *Milicia* spp. (Blanc-Jolivet et al. 2017).

Here, we describe a new set of 120 SNPs for *Swietenia* spp., which can be used for population genetic studies and timber tracking purposes.

Tissues (predominantly leaves) from *S. macrophylla*, *S. humilis* and *S. mahagoni* were harvested in Central and South American countries (Brazil, Belize, Bolivia, Costa Rica, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, and Panama). Samples were dried with silica gel. DNA isolation from dried cambium and leaf material was carried out according to Dumolin et al. (1995). DNA isolation from wood material was carried out according to Lowe et al. (2015). SNP discovery was based on restriction-associated DNA sequencing (RADseq, Miller et al. 2007). RADseq was carried out by Floragenex (Portland, USA) using genomic DNA from five individuals of *S. macrophylla* originating from Bolivia, Brazil, Costa Rica, Honduras and Mexico. Libraries were prepared with *Sbf*I and sequenced using 2×100 bp paired-end Illumina HiSeq. The Brazilian sample was used for reference assembly. A total of 2450 SNPs (variant call format 4.1) were identified on 1011 reference contigs (stringent mapping conditions). An initial set of 480 SNP markers was compiled based on the results of the RADseq approach. Selection criteria were: strictly no other SNP present within at least 50 bp on both sides of a selected SNP, location on different scaffolds, sufficient sequence coverage and different patterns of allele distribution among the different individuals used for sequencing. The 480 selected SNPs were used for MassARRAY®iPLEX™ (Agena Biosciences, Hamburg, Germany) genotyping. Alleles were called using Typer Viewer v.4.0.24.71 (Agena Biosciences). The screening included 94 samples (Suppl. 1), representing all three species (*S. macrophylla:* 69 individuals, *S. humilis*: 11 individuals, *S. mahagoni*: 10 individuals), two wood samples and two individuals of the related species *Carapa guianensis* and *Carapa procera*. The individuals originated from at least 13 different countries (Brazil, Belize, Bolivia, Costa Rica, Dominican Republic, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Panama, USA and Venezuela). Some samples—especially *S. mahagoni*—originated from botanical gardens. The two DNA samples extracted from wood were included to test the performance of the markers for timber genotyping. Failed markers with no amplification or contaminated negative control were removed from the dataset, leaving a total of 384 loci for further analysis. Individuals were then grouped according to: (1) species (all individuals) and (2) country of origin (individuals of each species separately). For each grouping we calculated: the percentage of analyzable individuals per marker, the fixation index (F_{IS}) as an indicator of potential null alleles or allele fixation within a group (Nei and Chesser 1983), the effective number of alleles (A_e), the genetic differentiation (δ) among groups (Gregorius 1987), the correlation between genetic and geographic distance (only when grouped according to country of origin), and population fixation index F_{ST} (Nei and Chesser 1983), using GDANT (Degen, unpublished, Suppl. 2). A final marker set of 120 SNPs was determined, prioritizing markers with high rates of amplification, high differentiation power among groups, and—if available—high correlation between genetic and geographic distance. The list of the 120 SNP loci included in the final marker set is in Suppl. 3.

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