Article (refereed)

Lemes, Maristerra R.; Dick, Christopher W.; Navarro, Carlos; Lowe, Andrew J.; **Cavers, Stephen**; Gribel, Rogerio. 2010 Chloroplast DNA Microsatellites Reveal Contrasting Phylogeographic Structure in Mahogany (Swietenia macrophylla King, Meliaceae) from Amazonia and Central America. *Tropical Plant Biology*, 3 (1). 40-49. 10.1007/s12042-010-9042-5

Running head: Rangewide phylogeography of *Swietenia macrophylla*

Article type: Research paper

Title:

Chloroplast DNA microsatellites reveal contrasting phylogeographic structure in mahogany (*Swietenia macrophylla* King, Meliaceae) from Amazonia and Central America.

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ABSTRACT

Big-leaf mahogany (*Swietenia macrophylla* King) is one of the most valuable and overharvested timber trees of tropical America. In order to better characterize geographic patterns of genetic variation, we performed a phylogeographic analysis of *S. macrophylla* based on six polymorphic chloroplast genome simple sequence repeat loci (cpSSRs) analyzed in 16 populations (N = 245 individuals) distributed across Central America and the Brazilian Amazon. Of the 31 total cpDNA haplotypes identified, 16 occurred in Central America and 15 in Amazonia with no single haplotype shared between the two regions. Populations from Central America showed moderate differentiation (F_{ST} = 0.36) while within population genetic diversity was generally high (mean Nei's H_E = 0.639). In contrast, the Amazonian populations were strongly differentiated (F_{ST} = 0.91) and contained relatively low genetic diversity (mean H_E = 0.176), except for one highly diverse population (H_E = 0.925) from eastern Amazonia. Spatial analysis of molecular variance (SAMOVA) identified a single Central American phylogroup and four Amazonian phylogroups, indicating stronger phylogeographic structure within Amazonia. The results demonstrate distinctive regional patterns of *S. macrophylla* differentiation, and the first evidence of a strong phylogeographic break between Central American and South American mahogany populations. We suggest that the frequent occurrence of hurricanes in Central America, the differences in the glacial histories and in the duration and intensity of anthropogenic disturbance during the late Holocene may have played important roles in the geographic structuring of cpDNA lineages in the two regions. The high private haplotype diversity in Brazilian populations suggests that cpSSRs can be used as DNA barcodes for regional timber certification.

Key words: Amazon basin, Mesoamerica, tropical trees, big-leaf mahogany, cpSSRs, phylogeography, DNA barcodes, SAMOVA.

INTRODUCTION

Mahogany, *Swietenia macrophylla* King (Meliaceae), is the most valuable hardwood species in Neotropics and is seriously threatened owing to over-exploitation and habitat destruction. S*wietenia macrophylla* has a wide geographic range from Mexico through Central America and across the southern arc of the Amazon basin in Bolivia and Brazil (Lamb, 1966; Pennington, 1981). The species has wide ecological tolerance and occurs in a variety of habitats from wet to seasonally dry, evergreen to deciduous, tropical to subtropical forests, with typically 800 - 2,500 mm of annual rainfall and at altitudes ranging from sea level to 1,400 m (Lamb, 1966; Whitmore, 1983). However, the species reaches its optimum natural development in Holdridge's (1971) tropical dry forest formation (Lamb, 1966). Mahogany tends to occur in widely scattered patches and its density within patches is typically less than one commercial-size tree per hectare (Whitmore, 1983; Verissimo et al., 1995). This patchy distribution is probably related to its mode of regeneration that requires major disturbances such as river course changes, hurricanes, blowdowns, and fire (Snook, 1996). In these situations, stands may be comprised of one or a few cohorts (Grogan et al., 2003).

Swietenia macrophylla has been exploited throughout its natural range since the beginning of the 20th century (Lamb, 1966; Rodan et al., 1992). In recent decades, with the depletion of natural stands in Central America, most of the extraction has come from populations in South America, especially in the Brazilian Amazon. Mahogany extraction is based on selective logging, which usually removes only the tallest trees of good form and with a dbh (diameter at breast height) > 80 cm (Verissimo et al., 1995;

93 Gullison et al., 1996). In addition to removing the most fecund trees, selective logging
94 may have a significant impact on genetic structure and population size, and compromise
95 the evolutionary viability of natural mahogany populations (Cornelius et al., 2005). The
96 inclusion of *S. macrophylla* in CITES Appendix II in 2002 aimed to control
97 international trade by ensuring that logging will not be detrimental to the survival of the
98 species (Grogan and Barreto, 2005).

99 Studies of the organization of genetic diversity of *S. macrophylla* have been
100 carried out in the Brazilian Amazonia (Lemes et. al., 2003) and in Central America
101 (Novick et al. 2003) using nuclear microsatellite DNA markers. The studies sampled
102 populations across a similar spatial scale (ca. 1600 km in Central America; 2103 km in
103 Amazonia) using seven loci in common. Both studies showed significant isolation by
104 distance patterns, and moderate levels of population differentiation (R_{ST}). Furthermore,
105 the Central American populations exhibited significantly lower mean genetic diversity
106 than the Amazonian populations, which Novick et al. (2003) suggested may have
107 resulted from the smaller, more dissected nature of suitable habitat in Central America,
108 combined with more severe vegetation changes during the glacial phases of the
109 Pleistocene.

110 A phylogeographic approach based on chloroplast genome (cpDNA) variation
111 can provide additional insight into the historical patterns of genetic divergence across
112 the range of *S. macrophylla*. Chloroplast DNA is a haploid genome and is maternally
113 inherited in the majority of the angiosperms (Birky, 1995; McCauley 1995; Ennos et al
114 1999). Because of its four-fold smaller effective population size, chloroplast markers
115 can often detect geographic structure that is not apparent in nuclear DNA markers
116 (Cavers et al., 2003; Petit, 2005). Unfortunately the relatively low rates of nucleotide
117 substitution in the chloroplast genome (Wolfe et al., 1987) have often impeded its use in

phylogeographic studies (Schaal, 1998). However, highly variable mononucleotide repeat loci in the chloroplast genome (cpDNA microsatellites or simple sequence repeats [cpSSRs]) have provided a rich source of variation for studies of phylogeography and gene flow (Provan et al., 2001).

In addition to its utility for phylogeographic studies, conservation and management purposes, cpSSRs may be useful as regionally distinct cpDNA barcodes that could permit forensic verification of timber origins (Deguilloux et al., 2002; Dick and Kress, 2009). Here we report on the phylogeographic structure of *S. macrophylla* populations sampled in Central America (Novick et al. 2003) and Amazonia (Lemes et al. 2003) based on cpDNA microsatellites. The main aims of the study were: (1) to evaluate the utility of chloroplast microsatellite loci for assessing intraspecific variation in *S. macrophylla*; (2) to quantify and compare the organization of genetic diversity of *S. macrophylla* populations in Central America and Amazonia ; and (3) to determine if population-specific cpDNA haplotypes are credible as regional DNA barcodes for monitoring timber harvests.

RESULTS

Genetic Variation

Six out of 10 cpSSR loci initially assayed using universal primers (Weising and Gardner, 1999) successfully amplified and were found to be polymorphic. All individuals (n = 245) from eight Central American and eight Amazonian populations were analyzed for these six polymorphic cpSSR loci (ccmp 2, ccmp 3, ccmp 4, ccmp 5, ccmp 7, ccmp 10).

A total of 31 different haplotypes and 30 cpSSR alleles were found. The composition of the haplotypes and their distribution in the populations are given in

143 Table 1. The number of size variants (alleles) per locus varied from three to six. Gene
144 diversity indices (H_E) showed a high range of variation across populations (0.000 to
145 0.925) (Table 2).
146
147 **Structuring and geographical distribution of haplotypes**
148 The hierarchical analysis of genetic variation within and among populations performed
149 for each geographical region (Amazonia and Central America) showed contrasting
150 patterns. Most of the variation found in Amazonia was partitioned among populations
151 (91%), while in Central America most variation was partitioned within populations
152 (64%, Table 3).
153 The pattern of cpSSR haplotype organization provides evidence of a strong
154 phylogeographic break between *S. macrophylla* populations in Central America and
155 Amazonia. Of the 31 haplotypes detected, 15 occurred exclusively in Amazonian
156 populations and the remaining 16 in Central America (Table 2), with no single
157 haplotype shared between the two geographical regions. A maximum parsimony
158 median-joining network (Figure 1), based on the 31 cpDNA haplotypes, exhibited a sole
159 median vector and a total of 69 mutations, 38 of which occurred along the long branch
160 separating the Central American and Amazonian haplotype clusters. The median vector
161 connected the single Boca do Acre haplotype 17 with the other Amazonian haplotypes.
162 Haplotype 17 was separated by 19 mutations from the closest Central American
163 haplotype from Panama (haplotype 7). The long branch between the Central American
164 and Amazonian clusters is further evidence of a deep phylogeographic break.

165 The organization of haplotype diversity within the two geographical regions also
166 differed. The populations from Central America exhibited a relatively low level of
167 differentiation ($F_{ST} = 0.36$) compared to Amazonian populations ($F_{ST} = 0.91$) but

genetic diversity of populations was generally high (H_E ranging from 0.233 to 0.857). Some common haplotypes were shared among distantly separated Central American populations. For example, haplotypes 4 and 5 were sampled in all eight Central American populations. Haplotype 3, exhibited by 21 individuals and closely related to haplotype 4, was also widespread, occurring in four populations. These three widely distributed haplotypes represented 60% of the individuals sampled in Central America. Despite the generally weak genetic structure observed in Central American populations, haplotypes 9-16 formed a cluster comprised of individuals from the Pacific region of Panama, Costa Rica, and Guatemala, and a few individuals from a north-central Costa Rican population (El Parque).

Consistent with its higher level of population differentiation ($F_{ST} = 0.91$), there were few widespread haplotypes in Amazonia. The most common and widely distributed haplotype 28 was sampled in three adjacent populations (Pimenta Bueno, Cahoeira Parecis E, Resex Chico Mendes). Four Amazonian populations were fixed for one haplotype and three other populations exhibited only two haplotypes. Haplotype diversity within populations was relatively low (mean $H_E = 0.176$), with the exception of Marajoara, which contained nine haplotypes among the sixteen individuals sampled ($H_E = 0.925$). One of haplotypes found in Marajoara was shared with neighboring Agua Azul, located 107 Km to the north. Populations from the western Amazon tended to cluster genetically, except for Boca do Acre, which was relatively isolated in the network and clustered with eastern rather than western populations. Haplotypes from southernmost Amazonian population (Pontes e Lacerda) tended to occupy network tips.

Spatial analysis of molecular variance (SAMOVA) indicated the most likely presence of five genetic groups ($F_{CT} = 0.24$, $P<0.05$). Under all values of K, Central American populations grouped together. With K = 5, the populations grouped as

follows: 1 – Central American populations, 2 - Boca do Acre, 3 - Marajoara, Agua Azul, 4 - Pontes e Lacerda, 5 - Cachoeira Parecis A, Cachoeira Parecis E, Resex Chico Mendes, Pimenta Bueno (Figures 1 and 2). These haplotype-defined genetic groups tended to cluster the most geographically proximate populations with the exception of Resex Chico Mendes, which grouped with the populations at Cachoeira Parecis and Pimenta Bueno rather than the closer Boca do Acre.

DISCUSSION

The cpSSR haplotype data revealed a strong phylogeographic break between *S. macrophylla* in Central America and Amazonia. Similarly large phylogeographic breaks between cis- and trans-Andean populations have been reported for other rain forest tree species (e.g. Dick et al. 2003; Dick and Heuertz 2008; Hardesty et al this issue). There were also notable differences in the distribution of cpDNA variation within Central America and Amazonia. Central American populations harboured widespread haplotypes that occurred from Mexico to Panama. The Amazonian haplotypes, on the other hand, were more localized and most cpDNA variation was partitioned among populations. This pattern is not likely to be explained by sampling effects, since the sample sizes in Brazil were consistently high (>16 individuals per population) where differentiation was also the highest.

Our results showed some inconsistency with the nuclear SSR (nSSR) analyses of Lemes et al. (2003) and Novick et al. (2003). The nSSR data from Central America showed phylogeographic structure in the form of high levels of differentiation (R_{ST}) across geographic barriers (Novick et al., 2003). Central American populations also had relatively low allelic richness per locus (mean 13 alleles/locus) compared to the

Amazonian populations (mean of 18 alleles/locus). In contrast, there was no discernible phylogeographic structure in the Central American cpSSR data and the haplotype diversity (16 haplotypes) was similar to levels found in the Brazilian Amazonia (15 haplotypes). Some of the discrepancy between these results may be explained by differences between the nuclear and chloroplast genomes. First, genetic drift is expected to act more strongly on the chloroplast because of its fourfold lower effective population size. Furthermore, the cpDNA results reflect the sorting of a single genetic locus, whereas the nSSR results were summed over seven nSSR loci and thus provide several independent estimates of population genetic structure.

Geographic structuring of the cpSSR haplotypes does not appear to correspond with contemporary climatic or altitudinal barriers in Central America. The occurrence of widespread cpDNA haplotypes across Central America strongly implies a role of long distance dispersal and suggests that mountains have not been effective barriers to mahogany seed dispersal here. Hurricanes, which are frequent in Central America, can carry the winged seeds of mahogany over long distances and the accompanying wind throws are thought to play an important role in mahogany dispersal and establishment in this region (Snook 1996), which would lead to the present-day haplotype distribution. In the Amazon basin, on the other hand, hurricanes are absent or very rare.

On the other hand, topography may provide physical and climatic barriers for pollinator movements, as suggested by the significant divergence among Central American populations across geographical barriers found by Novick et al. (2003) using nSSR markers. Similarly, the divergences among three close populations from different valleys of the Parecis mountains in west-central Brazil, studied by Lemes et al. (2003) using nSSRs, were also highly significant, although these populations belong to the same cpSSR haplotype genetic group (group 5) in the present study.

A non-exclusive alternative explanation for to the observed phylogeographic structure in Central America is the severity of the impact of Pleistocene glaciations (Whitmore and Prance, 1987) coupled with the relatively small areas of suitable habitat for mahogany establishment. The reconstructed vegetation of lowland Central America between 20,000 and 10,500 B.P. (Piperno and Pearsall, 1998) showed restricted areas with moist and dry forests and widespread thorn woodlands, low scrub, and wooded savanna vegetation in the region. These factors are expected to have caused local extinctions and much more dramatic reduction in effective population size for Central American than for Amazonian mahogany populations (Novick et al. 2003). Under this scenario, any ancient signal of structuring and diversification would have been modified by Pleistocene vegetation changes. Thus, the current pattern of genetic variation may reflect only the most recent geographic expansion of a few founder haplotypes from a limited refugial source and the subsequent formation of newly derived haplotypes. It is worth noting the occurrence of a few rare and highly differentiated lineages (e.g. haplotypes 6 and 16) in Central America that may be a relict of the ancestral polymorphism.

The significantly lower number of nuclear microsatellite alleles and lower heterozygosity in Central American (Novick et al., 2003) than Amazonian populations (Lemes et al., 2003) suggests either a more recent geographic expansion, or lower effective population sizes in the more topographically dissected Central American region. Mating system analysis has shown that mahogany is somewhat tolerant of selfing (Lemes et al., 2007) and the lower nuclear microsatellite diversity in Central America may be influenced by ecological pressures favoring inbreeding in individuals colonizing new areas in this region.

In addition to the impacts of the Pleistocene glaciations, Holocene events may have contributed to the current phylogeographic pattern observed for mahogany in Central America. For at least 1500 years before European conquest, tens of millions of Pre-Columbian agriculturalists practiced shifting agriculture, cultivating maize and other light-demanding crops in this region (Denevan 1992). Notably, four of our sample sites (sites A-D) are in what was the most highly populated core Mayan zone of influence where several loosely associated city-states coexisted and rapid forest clearance began about 2800 B.P. (Hodell et al.2000, Islebe et al. 1996). As a result of the intensive land use by these dense sedentary agrarian communities, Central America was probably covered, at the time of the European first arrival, by a mosaic of crop lands and abandoned fields with secondary vegetation at different successional stages. Despite the death of adult trees caused by forest clearances, this type of anthropogenic landscape will likely have enhanced dispersal of mahogany, a long-lived pioneer tree (Grogan et al. 2003), over the region. With the population decline accompanying the collapse of the Classic Mayan society between A.D. 800 and 900 (Hodell et al. 1995) and the demographic collapse experienced by Amerindian populations after European contact (Denevan 1992), human pressures were strongly curtailed and most of the fragmented landscape in Central America was abandoned . The subsequent large-scale forest regeneration (Nevle and Bird 2008) would also have accelerated the expansion of the remnant mahogany lineages in this region.

CpSSR variation in the peripheral Amazon basin exhibited a comparatively stronger phylogeographic structure than in Central America. Most of the Amazonian populations were fixed for one haplotype, or exhibited only a few related haplotypes. Spatial analysis indicates that there is significant within-region structuring in South America, primarily reflecting geographic proximity. The exception to this general

292 pattern is the clustering of ‘Resex Chico Mendes’ with Rondonian populations rather
293 than the more proximate ‘Boca do Acre’. These two populations, 200 km apart and
294 having the Juruá River as the sole geographical barrier between them, were probably
295 derived from separate lineages. Resex Chico Mendes, located in the transitional zone
296 between the Brazilian Shield and the Tertiary deposits of the Amazon basin was likely
297 formed by lineages coming from the relict populations in the Serra dos Parecis
298 mountains. Boca do Acre, located at the north-western limit of the species distribution
299 in Brazil, seems to be more genetically related to populations from eastern Amazonia.
300 It seems most likely that cycles of demographic expansion followed by
301 population bottlenecks and isolation have shaped the phylogeographic pattern in the
302 region. Clearly, there has been a distinctly different demographic history in Amazonia
303 compared with Central America, with an older colonization suggested by longer branch
304 lengths and a higher level of geographic isolation for the Amazonian populations as
305 indicated by higher F_{ST}.
306 The current distribution of mahogany in the Amazonia is characterized by
307 aggregations of trees in deciduous and semi-deciduous forests along an arc following
308 the southern boundary of the basin (Grogan, 2001; Grogan et al., 2002). These
309 seasonally dry forests are areas of “ecological tension” between the Amazonian and
310 Cerrado biomes and are bounded by the evergreen rain forests to the north and by
311 savannas to the south. Seasonally dry forests, which provide optimum habitat for
312 mahogany, probably expanded during the cool and dry glacial intervals (Pennington et
313 al. 2000; Bush and Silman, 2004; Bush and Oliveira, 2006; Colinvaux et al. 1996,
314 2001) and mahogany population sizes would have been correspondingly larger and
315 possibly more continuous in some areas. During the wetter and warmer Holocene,
316 rainforests expanded south and eastwards replacing the deciduous forests and savannas

317 in the Amazonian lowlands and in the foothills of the Brazilian Shield. Thus, it is
318 possible that the retraction and isolation of mahogany populations since the end of the
319 Last Glacial Maximum and the lack of long distance seed dispersal among the
320 remaining mahogany aggregations have influenced phylogeographic structure in the
321 Brazilian Amazon.

322 The high level of private haplotype diversity found in the Marajoara population,
323 in contrast to other Amazonian populations, suggests additional intricacy to the pattern.
324 One possible explanation concerns the stability of a dry and seasonal climate in this
325 region, even during interglacial periods. Marajoara is located on the SW border of the
326 dry transverse corridor that crosses central Amazonia in a NW-SE direction, separating
327 humid upper and lower Amazonia (Haffer, 2008). The private haplotype diversity of the
328 Marajoara population may be explained by the greater size and stability of the dry
329 forests in this area, which could have led to the accumulation and maintenance of
330 cpSSR diversity.

331 The Pre-Columbian cultivation practices in Amazonia also changed the
332 environment in different ways especially along of the headwater basins of the main
333 rivers coming from the northern flanks of the Brazilian Shield (Heckenberger et al.
334 2007). This region is dominated by semi-deciduous forests and represents the natural
335 area of distribution of *S. macrophylla* in the Brazilian and Bolivian Amazon. Several
336 small to medium-sized complex societies flourished in this broad region with
337 agricultural and parkland landscapes occurring around villages (Heckenberger et al.
338 2003, Erickson 2006). However, the lower Amerindian density, the large distances
339 between the main settlements, the prevalence of a hunter-gatherer subsistence strategy
340 in many groups, and the management of the landscape using agricultural systems that
341 did not require intensive forest clearance (Denevan, 2001, Balée 2006) suggest that the

342 impact of anthropogenic disturbance in the southern Amazon during the Late Holocene
343 was likely smaller than in Central America. One would expect that there were
344 proportionally fewer anthropogenically-altered habitats available for mahogany
345 colonisation in the Amazon basin than in Central America during Pre-Columbian times.
346 Thus the present-day haplotype distribution in Amazonian mahogany appears to have
347 been most likely shaped by Pleistocene events.

348 In addition to providing genetic evidence of regional demographic history, our
349 study should also be useful for genetic conservation and management. In mahogany,
350 important traits such as resistance to shoot borers, growth rate and degree of branching
351 show heritable variation (Newton et al., 1999). The deep phylogeographic break
352 between Central and South American mahogany populations suggests that there may be
353 major genomic differences between these sources of mahogany. While the major
354 provenance trials for mahogany (CATIE, Turrialba, Costa Rica) contain only Central
355 American samples, our study strongly suggests that Central and South American
356 provenances should be jointly studied for silviculture programs.

357 The findings also have relevance for the conservation of natural mahogany
358 populations. Recent advances in DNA extraction technology permit genotyping of DNA
359 from dried timber samples (Deguilloux et al., 2002). Using a DNA barcoding approach,
360 it is possible to determine the species origin of tropical timbers. However, standard
361 plant DNA barcodes often display little variation among closely related species, let
362 alone between populations within a species (Dick and Kress, 2009), making it difficult
363 to determine the provenance of timber, which is essential in order to monitor illegal
364 logging activities. Our study demonstrates that combinations of cpSSR loci can provide
365 distinct regional cpDNA haplotypes for mahogany. With the six cpSSR loci in this
366 population, it was possible to definitively assign samples to either Central American or

South American provenances. With more loci, it should be possible to provide distinct genotypes at finer geographic scales. These DNA barcode approaches should be especially useful in the Amazon basin, which displays the highest level of cpDNA phylogeographic structure, and which contains the largest commercial and protected tracts of mahogany.

In summary, our data have highlighted a strong phylogeographic break and an intriguing contrast between Amazonian and Central American mahogany populations in terms of phylogeographic structure. In order to clarify the occurrence of points of historical dispersal between the two geographical regions, more extensive sampling in South America is needed, particularly from the Peruvian, Ecuadorian, Colombian and Venezuelan Amazon. Based on the data available so far we suggest that differences in glacial history for Central America and Amazonia may have been a key factor in determining these very divergent patterns. Differences in terms of duration and intensity of anthropogenic disturbance between the two regions during the late Holocene may have also affected the vegetation history and played an important role in structuring cpDNA lineages. In addition, these findings indicate that any *in situ* conservation program or germplasm collection initiatives for this valuable and endangered tree should take into consideration the distinct genetic structures shaped by the contrasting history of the species in these regions.

METHODS

Study sites and collection

Leaves of 245 plants were collected from eight populations from six countries in Central America and from eight populations spread across 2,100 km in the southern arc of the Amazon drainage basin in Brazil (Figure 2). The samples were used in previous

nuclear microsatellite analyses of Lemes et al. (2003) and Novick et al. (2003). In addition, new leaf material from adult trees was sampled from Boca do Acre in Brazil (N = 28). The leaf material came from adult trees from natural populations in Amazonia except for Cach E and Agua Azul or from progeny arrays for the Central American populations and Cach E and Agua Azul, in which a single progeny was used as a proxy for an adult tree. Living material from the Central American populations is maintained by CATIE in Turrialba, Costa Rica. The leaves were dried in silica gel and stored at -20^{o}C until DNA extraction.

Microsatellite analysis

Total genomic DNA was extracted using a Fast Prep (Bio101 Corporation) following standard CTAB procedure (Doyle and Doyle, 1987) or alternatively using Plant DNeasy kits (Qiagen Corporation, Valencia, CA). DNA quantification was performed by comparison with known concentrations of a DNA standard (Lambda DNA) in ethidium bromide-stained 1% agarose gels.

PCR was initially performed using 10 universal angiosperm primers developed by Weising & Gardner (1999) for cpSSR analysis in tobacco. Reactions were carried out in a total volume of 10 µl containing 1X PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$), 200 µM dNTPs, BSA (2.5 mg ml^{-1}), 1.25 µM of each forward and reverse primers, 1 U Taq DNA polymerase and 5.0 ng of genomic DNA using a MJ Research Incorporated PTC 200 thermal cycler under the following parameters: (1) initial denaturation at 94^{0}C for 4 min; (2) 30 cycles of denaturation at 94^{0}C for 1 min, annealing at primer-specific T_m for 1 min and extension at 72^{0}C for 1 min; (3) final extension at 72^{0}C for 10 min. PCR products were eletrophoresed on 5% polyacrylamide gels in an Applied Biosystem Incorporated (ABI) Prism 377 sequencer

417 and analysed with Genescan and Genotyper softwares (ABI). The cpSSR allele sizes
418 were binned and normalized using *AlleloBin* software (Idury and Cardon, 1997).
419
420 **Data analysis**
421 Unique multi-locus combinations of cpSSR alleles (size variants) were
422 considered as distinct haplotypes. Genetic diversity was estimated for each population
423 based on the number of alleles (A), the number of haplotypes (N_H), and gene diversity
424 index (H_E, Nei, 1987). Partitioning of genetic variation within and among populations
425 was tested for each geographical region (Amazonia and Central America) separately by
426 analysis of molecular variation (AMOVA; Excoffier et al., 1992) using *Arlequin 2.001*
427 (Schneider et al., 2001). The significance of the fixation index was tested with 1000
428 permutations. Relationships among the haplotypes were inferred using median-joining
429 network analysis (Bandelt et al., 1999) implemented by *Network* software (Forster et al.,
430 2000). Spatial structuring of variation at chloroplast loci was examined using
431 SAMOVA (Dupanloup et al, 2002), considering values of K (phylogroup number)
432 between 2 and 10, using 100 initial conditions for each run and the sum of squared size
433 differences as a measure of molecular distance.
434
435 **ACKNOWLEDGMENTS**
436
437 The study was funded by the Brazilian Ministry of Science and Technology (CNPq/CT-
438 Amazonia grant no. 554017/2006-7 to M.R.L), and the European Commission,
439 (SEEDSOURCE project - contract number 003708). We are grateful to Jimmy Grogan
440 and José Ribeiro for field assistance and Alessandra E. Pinto for help in laboratory
441 analyses. We also acknowledge logistical support provided by CATIE (Costa Rica) and
442 the timber companies Serraria Marajoara, Madeireira Juary and Cacique Madeireira in

443 Brazil. We thank Rachel Novick for providing DNA from Central American
444 populations.

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