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Sampling of forest species for genetic studies: vascular cambium storage and efficiency

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Abstract
Genetics studies of tree species using molecular markers usually involve a large number of samples and both the low-cost protocols for DNA extraction and commercial kits were developed for use with leaf tissue samples. However, leaf sampling of tall trees is inherently labour intensive, time demanding, costly and risky. In this context, the objectives of this study were firstly to conduct a comparative analysis of the efficiency (quality, quantity and "shelf-life") between leaf and lignified tissue samples as sources for DNA analyses, the effects of different storing methods and their "shelf-life" on DNA quality and quantity. For the best alternative tissue, were tested variations in the extraction protocol frequently used for isolating genomic DNA from *Eucalyptus* leaves. The results showed that the sample storage method have a significant impact on the quantity of DNA obtained along time, and the reduction of salt quantity during the extraction process makes possible to obtain DNA amounts equivalent to leaves, allowing the use of vascular cambium for genetic studies. Our results also showed that process of vascular cambium sampling was at least two times faster than collecting samples from leaves. Our results demonstrate that vascular cambium sampling is an effective alternative to leaf-based sampling, and additionally to a higher efficiency, vascular cambium sampling also is safer and less expensive than leaf tissue sampling. This way, new genetic studies in forest trees can take advantage of this reliable and cost effective methodology.
Key words: Cambium tissue; DNA isolation; Field work productivity.

Introduction

Sampling of perennial plants from natural and commercial populations for genetic studies, including genetic improvement studies, usually requires a relatively large number of samples (Shepherd et al. 2002). Both the low-cost protocols commonly used for genetic studies of perennial plants and the commercially available kits use leaf tissue as source for DNA isolation (Doyle and Doyle 1990; Ferreira and Grattapaglia 1998; Romano and Brasileiro 1999; Mazzaand Bittencourt 2000; Zucchi 2002). However, collect leaves especially in tropical and subtropical forests is a difficult task, requiring a well-trained climbing team with specialized equipment and a thorough risk assessment. As a result, collecting leaves from tall trees is a time-demanding, costly, and risky activity that has a low rate of productivity.

Therefore, the need for an alternative to leaf tissue sampling for genetics studies is paramount (Arriel et al. 2002; Bittencourt 2007). In this context, alternatives such as vascular cambium and bark sampling are attractive because of the simple procedures involved in sample collection and year-round access to the biological material thus rendering it a much cheaper, safer and faster alternative to leaf sampling.

Along with the logistical difficulties in collecting leaves, some species have inherent characteristics that can impose further difficulties on sample collection. For species that are deciduous or species that occur in hazardous or difficult to access environments (i.e., wetlands, steep slopes), the process of collecting leaves might be temporarily or even permanently prohibited (Raven et al. 2001). As DNA is the basis for genomics research, the procedures for its preparation should produce extracts sufficiently pure in order to minimize the effects of proteins used by molecular techniques (Romano and Brasileiro 1999). The use of commercial kits for DNA isolation is only feasible in cases where a small number of samples is to be analysed or in cases in which DNA extraction is based on tissues collected for different purposes, such as from plant exsiccates (Asif and Cannon 2005) or timber tracking (Deguilloux et al. 2002; Rachmayanti et al. 2006; Tnah et al. 2012). On the other hand, in studies in which a large number of samples are fundamental, the use of low-cost protocols for DNA extraction is necessary (Khan et al. 2004).

Despite the crescent number of studies that recently have used vascular cambium samples as DNA source (e.g., Deguilloux et al. 2002; Colpaert et al. 2005; Rachmayanti et al. 2009; Novaes et al. 2009), no study to date provides a description of the procedures and logistics that allow a simple and efficient method for obtaining samples in the field. In the main studies describing wood collection to DNA isolation purposes (Colpaert et al. 2005; Novaes et al. 2009), the cambium samples are preserved under refrigeration, as usually is made to leaf samples, but kept in a solution with a third part of CTAB 2% (Cetyltrimethylammonium Bromide) and other two parts of ethanol to avoid the freezing of cambium samples. As such, our goal was to compare different sampling methods and to evaluate best practices related to storage and "shelf-life" of samples in order to develop a simplified standard protocol for cambium sampling and the subsequent analysis of tree species genetics. Thus, the main objective of this paperwas develop a standard methodology for sampling, storing and extracting genomic DNA from vascular cambium tissue and bark as low-cost alternatives to leaf sampling of tall trees.

We first conducted a comparative analysis of the efficiency (DNA quality, quantity and "shelf-life") between leaf, bark and vascular cambium from *Eucalyptus* samples as sources of DNA analyses. Furthermore, we optimized a DNA extraction protocol for best alternative tissue and report the comparison of their sampling efficiency with traditional leaf sampling.

Material and methods

The wood samples were collected using a circular leather awl and a hammer. Hammering the awl into the tree trunk produces a circular wood sample. This sample can easily be separated in layers, thus enabling the collection of a sterile sample in relation to contact with potential external contaminants. Vascular cambium sampling fromtall *Eucalyptus* trees (DBH - Diameter at Breast Height around 20 cm – 5 years old) were initially carried out following the practices established for leaf sampling, in which samples were stored in paper envelopes and kept frozen (-20ºC) until DNA extraction be performed using a low cost procedure (CTAB 2%).

The CTAB 2% methodology recommends that foliar tissue is frozen using liquid nitrogen and subsequently macerated with a mortar and pestle; however, due to the levels of lignification of the vascular cambium tissue, the suggested freezing process hinders maceration. In order to overcome this difficulty, about 0.5 gof cambium samples were put in a pestle with 600 µL CTAB 2% solution allowing for the start of an early cell lysis and were then macerated; note that the CTAB 2% solution is normally used only after the leaf samples are macerated.

Initially we tested if the ontogenic phase (adults trees - DBH > 40 cm and young trees - DBH < 5 cm) has an impact on the quality and quantity of DNA extracted from leaf, bark and cambium tissue of *Eucalyptus* trees using two different lengths of storage (1 day and 3 weeks). Later we tested different storing methods aiming at defining a procedure that would prevent the process of wood oxidation and consequent DNA degradation. In our subsequent tests we stored vascular cambium in plastic bags, paper envelopes containing silica gel crystals, and plastic centrifuge tubes with CTAB 2% for different periods of time (1 day, 1 week, 1 month) before DNA extraction.

After defining an acceptable longer-term storage method we tested variations of the CTAB DNA extraction protocol described by Ferreira and Grattapaglia (1998), aiming at producing large quantities of genomic DNA. The variations in the extraction protocol startedwith modifications in the componentsof precipitation phaseused after the last cleaning with CIA (Chloroform Isoamyl Alcohol), which began replacing the isopropanol for absolute ethanol in the first phase of precipitation with alcohol, and the inclusion of an additional step using a NaCl (1M) cleaning solution after the first cleaning phase with alcohol 70%. Later were tested alterations in the concentrations components of CTAB 2% buffer [CTAB 2% (p/v); NaCl 1.4 M; Tris-HCl 100 mM, pH 8.0; EDTA 20 mM; β-mercaptoethanol 0.2% (v/v)], that isused in the initial steps of the extraction process. These variations involved the addition of antioxidant component polyvinylpyrrolidone (PVP 1%) to buffer, and a fivefold increase in the β-mercaptoethanol component (from 2 µL/mL to 10 µL/mL).

The DNA concentration in the samples was estimated using agarose gel by comparing 5 µL of the DNA from each sample with 5 µL of Phage Lambda DNA size marker in concentrations of 20, 50 and 100 ng. Thirty minutes after the gel (agarose gel 1%) electrophoresis began, the gel was dyed with ethidium bromide for visualisation inthe Electrophoresis Documentation and Analysis System – EDAS 290 (Kodak 2000). The results from the software estimates were divided by 5 in order to obtain a DNA concentration in ng/µL and the total DNA amount obtained was estimated by multiplying the DNA results from EDAS software (given in ng/µL) by 50 (see optimized protocol in the Results section).

The classification of quality of the DNA obtained (high, medium and low) is related to purity patterns obtained from absorbance wave length of 260 nm and 280 nm (Sambrook and Russell 2001). The ratio among these two wave length (A260/A280) reflect the purity of sample. Samples are considered of high quality if values are higher than 1.80, medium quality for values between 1.79 and 1.50, and low quality corresponds to values of less than 1.50 (Sambrook and Russell 2001).

Finally, in order to compare the efficiency (in terms of time) between leaf sample collection and vascular cambium collection we recorded the amount of time a team of three people spent to collect samples, from the moment of arrival at a target tree (which includes unpacking, preparing the collection equipment, collecting and storing a sample) until the team is ready to leave (including re-packing). We recorded the time spent to collect samples for three species in which this methodology was used by authors (*Araucaria angustifolia*, *Cedrela fissilis* and *Eucalyptus* in planted and natural forests) during one week of field work per species. The significance of all comparisons tested (p-value) was carried out using F-test for multiple factors followed by a pairwise comparison of means (Tukey-Kramer test).

Results

Initially we tested quantity and quality of the DNA extractedfromvascular cambium and bark (adults and young trees) as an alternative to leaf tissue extraction. The results showed that the DNA extracted from leaf tissue allowed for significantly higher levels of DNA than vascular cambium and bark for both storage periods, independently of the ontogenic stage ($p<0.01$), DNA extracted from leaves reached a quantity of 100 ng with very low levels of impurity (Table 1).

Table 1. Quantification of DNA extracted from *Eucalyptus* trees for two storage periods considering the tissue sample type and ontogenic stage (samples kept in silica gel).

Factors (A, B, C, D)	Tissue type (A)	Ontogenic stage (B)		DNA quantity (ng) Storage time		Interaction factors	
				1 day (C)	3 weeks (D)	p-values	
p-values	$p<0.001^{*}$	$p>0.05^{ns}$	AB ×	$p<0.001^{*}$	$p<0.001^{*}$	(A) × (B)	(AB) × (CD)
	foliar	young		120^{a}	90^{a}	$p>0.05^{ns}$	$p<0.05^{*}$
		adult		120^{a}	90^{a}		
	cambium	young		45^{b}	20^{b}	$p>0.05^{ns}$	$p<0.01^{*}$
		adult		60^{b}	20^{b}		
	bark	young		30^{c}	5^{c}	$p<0.05^{*}$	$p<0.001^{*}$
		adult		0^{d}	0^{c}		

*significantat $\alpha = 0.05$; ^{ns} not significant; ^{a,b,c,d} – columns with identical letters represent means that did not differ statistically based on pairwise comparison (Tukey-Kramer Test; $p<0.05$).

Additionally, except for bark tissue, the ontogenic stage showed no significant differences between tissue types for the storage periods considered ($p>0.05$). The extractions based on vascular cambium of young trees conducted one day after sampling were a slightly lower than 50 ng, while for adult trees the extractions were above 50 ng with no significant difference between the results ($p>0.05$); in both cases the DNA extracted contained some levels of impurity and drag (Figure 1).

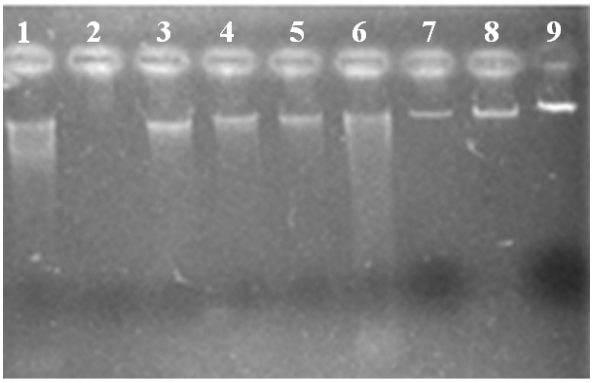

Figure 1. Results of DNA isolation obtained from different tissue types analyzed one day after collection (slot 1, 2 and 3: DNA from leaf, bark and vascular cambium of an adult tree; slots 4, 5 and 6: DNA from leaf, bark and vascular cambium from a young tree; slot 7, 8 and 9: DNA Lambda phage (λ) standards of 20 ng, 50 ng e 100 ng).

After 3 weeks of storage, vascular cambium samples kept in paper wrappers with silica gel resulted in significantly lower quantities of DNA (from 50 ng to 20 ng; $p<0.05$) and higher levels of impurity, a decrease apparently related to length of storage. According the comparisons performed the bark samples only resulted in usable quantities of DNA from young trees after one day and three weeks of storage the quantity and quality of DNA obtained with low-cost protocol were inadequate for genomic studies, independent of ontogenic stage (Table 1).

In relation to the influence of different storing techniques on DNA quality and quantity obtained from vascular cambium, the results showed a significant difference for DNA in terms of storage type and also a significant difference for storage time (Table 2). Specifically, DNA extracted one day after collection shows results statistically similar regardless of the storage method (50 ng, minimal drag). The plastic bag technique showed significantly lower DNA levels when compared with plastic storage tubes but statistically similar results to paper envelopes (Table 2).

Finally, DNA extraction carried out one month after sampling showed more varied results between storage methods: plastic tubes with CTAB 2% maintained the DNA quantity levels (no significant difference across storage length) with negligible difference in drag, whereas the other two methods showed a proportional deterioration of quality related to length of time in storage and a significant reduction in DNA quantity (70% reduction in comparison to one day of storage) (Table 3).

The protocol optimization using vascular cambium as a DNA source for genomic analysis began replacing the isopropanol for absolute ethanol in the first phase of precipitation with alcohol, but this change did not influence the quantity or quality of the DNA extracted (Table 3). The second variation included an additional step using a cleaning solution (NaCl 1M) after the first phase of (alcohol) precipitation; such a change was designed to reduce the impurities in the isolated DNA. The results of the second protocol variation showed a sharp reduction in the DNA quantity while not improving the overall quality of the products obtained when compared to the use of the original protocol.

As the changes in the precipitation phase were not successful, we tested variations in the components of the CTAB 2% buffer used in the initial phase of DNA extraction as a means to inhibit the effects of secondary metabolites that can degrade or immobilize the DNA, hindering the isolation. Initially we tested the addition of PVP 1%, and later a fivefold increase in the β-mercaptoethanol concentration (from 2 µL/mL to 10 µL/mL) in the CTAB 2% buffer solution. However, those changes did not result in improvements in terms of quality or quantity of the DNA extracted. Because the only variation in the DNA quantity during the initial tests was related to the salt concentration found in the solution used for the DNA extraction, we tested the removal of the CTAB 10% buffer from the second DNA cleaning. With the removal of CTAB 10% the results showed a significantly higher DNA yield, from 50 ng to near the 100 ng (Figure 2), results that are comparable to the results commonly obtained from leaf tissue in laboratory (Table 3).

Optimized protocol for DNA extraction based on vascular cambium tissue.

1. Place 0.5 g of shredded vascular cambium tissue into a mortar and add 600 µL of pre-heated CTAB 2% buffer [CTAB 2% (p/v); NaCl 1.4 M; Tris-HCl 100 mM, pH 8.0; EDTA 20 mM; β-mercaptoethanol 0.2% (v/v)]. Macerate the tissue into a homogeneous "doughy" consistency.
2. Fill the microtube up to its conic level and water bath at 65ºC for 30 minutes; shaking every 10 minutes.
3. Let microtube cool down in room temperature and add 600 µL of CIA (24:1 chloroform isoamyl alcohol).
4. Stir samples in vortex for 5 minutes and, then centrifuge microtubes for 5 minutes at 13,000 rpm (at least 2,500 RCF or G-force).
5. Transfer supernatant (about 600 µL) to new microtubes and add 600 µL of CIA for another 5 minutes of vortex stiring.
6. Centrifuge samples for 5 minutes at 13,000 rpm. Transfer supernatant (about 400 µL) to new microtubes.
7. Add 500 µL of refrigerated isopropanol (4ºC) and keep microtube in freezer (-20°C) for at least 2 hours (ideally overnight).
8. Centrifuge samples kept in freezer for 3 minutes at 7,000 rpm (at least 700 RCF or G-force).
9. Discard alcohol carefully so pellet is not lost and add 1 mL of alcohol 70%. Keepsamples in freezer for atleast 1 hour.
10. Discard alcohol 70% (again being careful with pellet) and add 500 µL of absolute alcohol.
11. Homogenize by inversion (without breaking pellet) and centrifuge for 3 minutes at 5,000 rpm (at least 350 RCF or G-force). Discard absolute alcohol.
12. Place samples to dry at room temperature for abour 1 hour and, then dissolve DNA pellets in 100 µL of TE buffer (10 mM Tris; 0.1 mM EDTA; pH 8.0) with RNAse (1 µg/ml), in the proportion of 2 µL of RNAse for each 1 mL of TE. After the DNA isolation procedure, the work solution must be prepared diluting an amount of DNA with ultrapure water, to avoid the interference of contaminants in the activity of DNA polymerase enzyme.

Table 2. Quantification of DNA extracted from vascular cambium tissue considering length of storage and storage type.

Factors (A, B)	Storage time (A)	Storagetype (B)	DNA quantity (ng)	Interaction (A) × (B)
p-values	p<0.001*	p<0.001*		p<0.001*
		Paper envelope	50[a]	
	1 day	Plastic bag	50[a]	
		Tube (CTAB2%)	50[a]	
		Paper envelope	40[ab]	
	1 week	Plastic bag	37[b]	
		Tube (CTAB2%)	50[a]	
		Paper envelope	15[c]	
	1 month	Plastic bag	15[c]	
		Tube (CTAB2%)	50[a]	

* significant at α=0.05. [a,b,c] – columns with identical letters represent means that did not differ statistically based on pairwise comparison (Tukey-Kramer Test; p<0.05).

Table 3. Results of the changes in the CTAB 2% DNA extraction protocol in terms of quality and quantity for vascular cambium samples (DNA quality evaluated in terms of levels of drag and impurity: low, medium and high).

Protocolphase	Treatment	DNA quantity (ng)	DNA quality
Precipitation phase	Isopropanol	50	medium
	Absolute ethanol	50	medium
	Cleaning with NaCl 1M	< 20	high
CTAB2% buffer component	Addition PVP on buffer	50	medium
	5× β-mercaptoethanol increase	50	medium
DNA second cleaning with CIA	no CTAB 10%	> 100	medium

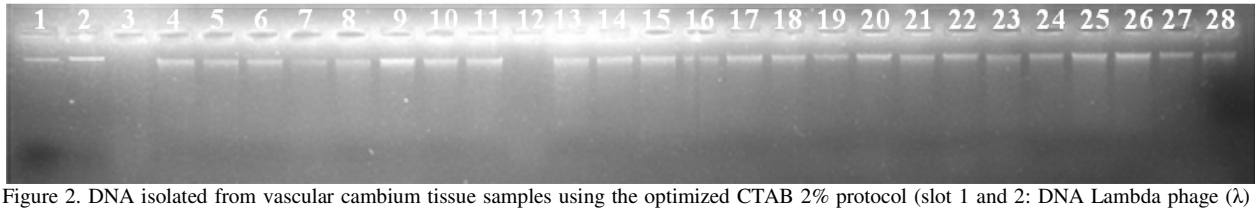

Figure 2. DNA isolated from vascular cambium tissue samples using the optimized CTAB 2% protocol (slot 1 and 2: DNA Lambda phage (λ) standards of 50 ng and 100 ng; slot 3 and 12: empty; slot 4 to 11 and 13 to 28: DNA obtained from vascular cambium of *Eucalyptus* sp. adult trees).

Discussion

The low-cost protocols for DNA extraction based on CTAB recommend that the leaf tissue is frozen in liquid nitrogen for later maceration. However, because of lignification such a recommendation was not feasible for vascular cambium and bark tissues without the use of the appropriate equipment to macerate/grind those tissues. In order to overcome this difficulty, vascular cambium tissues were macerated (skipping the freezing process) with the addition of 600 µL CTAB solution allowing for the initiation of early cell lysis.

Genomic studies can be based on either foliar or vascular cambium tissues although relative differences in DNA quantity and quality are to be expected. Although initial results showed vascular cambium as a viable alternative to foliar tissue as a source of DNA, the storage time before extraction significantly affected the quantity of DNA available. The quality of DNA – expressed in terms of level of impurity and the existence of drag – showed that foliar tissue yields DNA with negligible levels of impurity and drag after one day of storage while cambium and bark produced higher (but still acceptable) levels of impurity for the same period. After three weeks all types of tissue produced higher levels of impurity and drag, but the foliar and cambium tissue samples were still considered satisfactory for genomic research.

The development of a DNA extraction protocol based on bark samples showed results that varied depending on the tree's ontogenic stage. Specifically, DNA extraction from the bark of adult trees did not yield detectable quantities of DNA whereas extraction from the bark of young trees yielded usable quantities of DNA (Table 1). One possible explanation for such a difference is related to inherent characteristics of cells at various life stages. In adult trees, samples contain a reduced amount (or an absence) of living cells that are fully differentiated (not meristematic). In the case of young trees we suspect that there is a considerable presence of living cells and undifferentiated cells (meristematic) which are a viable source for DNA extraction.

In relation to the techniques for storing tissue samples, vascular cambium samples kept in CTAB 2% solution was the most efficient storage method among the techniques tested as it reduces tissue oxidation and subsequent DNA degradation. For the extractions performed one week after sampling, the results showed that only storage in plastic centrifuge tubes with CTAB 2% and paper envelopes with silica gel were able to maintain a quantity of DNA similar to that after one day of storage. This is a consequence of the natural process of tissue oxidation and its subsequent degradation that is unavoidable when paper envelopes or plastic bags are used to store dry samples without freezing. Although we tested DNA storage for up to three weeks, we expect that vascular cambium samples stored in CTAB should be viable for much longer periods (possibly years) without degradation or visible signs of oxidation; such a method also allows for longer storage periods under refrigeration, eliminating the need for freezing.

The results showed that storing vascular cambium in tubes with CTAB 2% is a more efficient method for DNA extraction and it allows for the DNA analysis to take place after much longer periods in storage (e.g., more than three

years based on authors tests running in the laboratory, unpublished data).Other advantages of using vascular cambium includes removing the need to refrigerate samples in the field as well as reducing contact with external contaminants which is a concern with foliar tissue as leaves are in constant contact to potential contaminants.

Although the results show that vascular cambium is a viable source of tissue sampling for DNA extraction, the quantity of DNA obtained might be lower than desired in some situations. By eliminating CTAB 10% from the second cleaning stage (with CIA) we managed to improve the amount of DNA as the component has a higher concentration of salt which in turn reduces the quantity of DNA at the end of the extraction process. The CTAB 10% solution is an important component in the process of obtaining DNA from leaves when there are high levels of secondary metabolites in the tissue (Rogstad 1992).

The CTAB 10% solution is composed mainly of salt, CTAB and water, and this component is not listed in the original protocol described by Doyle and Doyle (1991), being introduced later during the adaptation of the protocol for genomic DNA isolation based on *Eucalyptus* leaf samples by Ferreira and Grattapaglia (1998). Although eliminating this step resulted in an increase in the DNA extracted with a slight reduction in quality, the resulting DNA extract proved to be appropriate for molecular studies both to planted or natural forests.

According the author's experience that worked with different forest species, the use of RNAse or proteinase for RNA and protein elimination must be assessed in accordance with the quality of the DNA obtained, as each species has its own particularities that might influence the process of DNA extraction (Porebski et al. 1997). However, the application of laboratorial concepts during the sampling stage is extremely relevant to maintain the sample asepsis independent of the studied species, avoiding contamination either by another external DNA source, as selective microorganisms.

In relation to logistics, leaf sampling requires a team with two specialized climbers involving specific training and equipment and a third supporting team member whose activities might include clearing trails, collecting samples, identifying trees and measuring additional variables (coordinates, DBH and height of trees, etc). For vascular cambium sampling, we also used a team of three people but with a more homogenous distribution of responsabilities: one person was responsible for tree identification and opening access to trees; the second member was responsible for cambium data collection and decortication; the third person was responsible for handling the samples (collecting, tagging and transporting). The difference between the two methods is not restricted to efficiency but also involves very different levels of risk, cost and physical effort. While leaf sampling requires two members to be exposed to significant risk while climbing, teams involved in sampling vascular cambium can have a dedicated person to collect samples (which tends to reduce errors) while others conduct much less physically demanding and risky activities. This way, vascular cambium sampling is more efficient, safer and cheaper to collect from a wide range of forest species.

The DNA extraction protocol and sampling method system established in this paper was tested on samples obtained from the vascular cambium of natural and planted forests: 1,500 samples of the native conifer *Araucaria angustifolia* (Bittencourt 2007; Medina-Macedo 2014); 200 samples of native broadleaf *Cedrela fissilis* (Medina-Macedo, unpublished data), and finally 1,500 samples of exotic *Eucalyptus* spp. (Carvalho 2009; Medina-Macedo 2009; Sena 2009). The results of our tests showed that vascular cambium is a viable alternative to leaf tissue especially when the research is focused on tree species whose leaves are mostly found in the canopy of tropical and sub-tropical forests; bark, on the other hand, is not a viable alternative. Moreover, collecting vascular cambium dramatically reduces the time required for collecting samples and it is significantly lessrisky when compared to climbing trees to collect leaf samples. Along with the difficulties related to the logistics of collecting leaves, some species have inherent characteristics that prevent or prohibit the process of leaf collection, including their occurrence in high-risk environments or on steep-slopes.

Although our evaluation of the efficiency of sampling methods was designed to include a variety of forest conditions and species in order to ensure its applicability in broader conditions, we must emphasize that differences are to be expected depending on specific conditions (i.e., type of forest and species studied). It is important to note that studies on genetic diversity usually evaluate relatively small sections of the genome, which require isolated quantities of DNA samples that can be stored in small tubes (1.5 mL). On the other hand, genome mapping uses a much larger section of the genome that requires larger quantities of DNA; in this case we recommend that larger amounts of vascular cambium should be collected and stored in larger plastic tubes, such as the 15 or 5 mL tubes.

The most commonly used method for DNA extraction from leaf tissue is a difficult task especially in tropical and subtropical forests that requires well-trained climbing teams with specialized equipment in a time demanding, costly and risky field operation. In order to find an alternative to foliar sampling, we compared different sampling methods and developed a method for DNA extraction based on vascular cambium that we expect will allow researchers to use a much faster and safer method of sampling while guaranteeing the quality and quantity of DNA required for molecular studies. Some caution should be advised in using our results for planning field-work logistics and applying the protocol developed for DNA extraction as testing with other species may be an important first step in implementing this protocol. Because of the variety of tree species considered in this study, we expect that the protocol has the potential to reduce significantly the costs and risks involved in collecting and analysing samples for molecular studies of trees in a large varying of forest conditions.

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