Min Yu, Kai Liu, Liang Zhou, Lei Zhao and Shengquan Liu*

**Testing three proposed DNA barcodes for the wood identification of *Dalbergia odorifera* T. Chen and *Dalbergia tonkinensis* Prain**

**Abstract:** *Dalbergia odorifera* T. Chen is a first-grade state protected plant in China. However, it is difficult to distinguish it from the closely related species *Dalbergia tonkinensis* Prain, which is less important in economic value, by wood anatomical features. In this study, three potential DNA barcode sequences, namely rpoC1, *trnH-psbA* and internal transcribed spacer (ITS), were used to differentiate wood of *D. odorifera* from *D. tonkinensis*. The average quantities of DNA extracts from twigs, sapwood and heartwood were 16.3, 11.5 and 6.0 ng mg⁻¹, respectively. The success rates for polymerase chain reaction (PCR) amplification for three loci, namely ITS, *trnH-psbA* and rpoC1, were 62.5, 100 and 81.25%, respectively. The success rate for bidirectional sequencing of amplified products was 100% for all the three loci. The identification power of the three proposed DNA barcodes has been calculated by the BLAST, tree-based method and the TAXONDNA method. The interspecific differences of the *trnH-psbA* region were greater than intraspecific variations. Moreover, the identification power of *trnH-psbA* was higher than that of ITS and rpoC1 regions at the species level. Finally, the *trnH-psbA* region is proposed as a DNA barcode for wood identification between *D. odorifera* and *D. tonkinensis*.

**Keywords:** *Dalbergia odorifera*, *Dalbergia tonkinensis*, DNA barcoding, *trnH-psbA*, wood identification

DOI 10.1515/hf-2014-0234
Received August 27, 2014; accepted February 25, 2015; previously published online March 28, 2015

**Introduction**

Macroscopic and microscopic characters of wood can be very similar among species belonging to the same or similar genera, and thus it is very difficult to identify them accurately at species level by traditional methods (Hanssen et al. 2011). For example, Santos et al. (2013) pointed out this problem in the context of *Myrciaria*, *Neomitrantes*, *Plinia* and *Siphoneugena* species, as closely related genera with very similar wood anatomical features. DNA barcoding is a method of species identification and recognition based on specific regions of short standardized DNA sequences (Hebert et al. 2003a,b; Hajibabaei et al. 2007). The Plant Working Group of the Consortium for the Barcode of Life (PWG-CBOL) has proposed a number of candidate DNA regions for species identification, such as *rbcL*, *matK*, *trnH-psbA*, the nuclear ribosomal DNA internal transcribed spacer (ITS) and ITS2 (CBOL 2009). Several plastid and nuclear ribosomal DNA regions (e.g., *rpoC1*, *matK*, *rbcL*, *trnH-psbA*, *ycf3*, *psbC-trnS* and ITS1) have been successfully introduced for wood identification and recognition (Tang et al. 2011; Tnah et al. 2012; Jiao et al. 2014). The relatively short DNA sequences can be better amplified even in the case of highly degraded DNA templates from wood tissues. Moreover, these barcode markers are well suitable for species differentiation (Gonzalez et al. 2009).

*Dalbergia odorifera* belongs to the family of Leguminosae. It is endemic in Hainan Province, China, mostly distributed in the west and southwest plains or hilly areas with an altitude less than 400 m. *Dalbergia odorifera* was listed in the second-class category of the National List of Local Protected Flora, issued by the Chinese Government in 1999 and regarded the most valuable rosewood in China. Moreover, it was listed in the “Red List of Threatened species” by the International Union for Conservation of Nature (IUCN) in 1998. The heartwood of *D. odorifera* has a typical dark red color, beautiful grain and a favorable odor, which is continuously released for hundreds of years. Furniture made of *D. odorifera* is the most valuable in China. More than 40 individual flavonoids and other...

In recent years, the wood from *D. tonkinensis*, mostly distributed in Vietnam, appeared on the market. Its color, density and odor are very similar to that of *D. odorifera*. The heartwood with red color and grain gives rise to a strong spicy odor. Its wood has anatomical features very similar to *D. odorifera*, except for the number of heterogeneous rays of type III (Luo et al. 2012). However, the two woods have different medicinal uses and economic values, for which *D. odorifera* is much more important. The differentiation of the two wood species is very difficult even for professionals in wood identification (Li et al. 2008).

In the present paper, the DNA barcoding approach was tested for differentiation of these two similar Dalbergia species. Several plastid and nuclear ribosomal DNA regions, such as *rpoC1* (chloroplast DNA region), *trnH-psbA* (chloroplast intergenic spacer) and the nuclear ribosomal DNA ITS have emerged as good candidates for plant DNA barcoding. These barcodes have high differentiation and identification ability between species, and low variation within species (Chen et al. 2010; Pang et al. 2012). Therefore, the focus will be on the above-mentioned barcodes. Three evaluation methods will be tested, such as BLAST (Altschul et al. 1990), the tree-based method (Kumar et al. 2008) and TAXONDNA (Meier et al. 2006). The goal was to provide a suitable DNA barcode for the identification of *D. odorifera* and *D. tonkinensis*.

## Materials and methods

### Plant materials

A total of 16 plant samples were obtained from China and Vietnam, as indicated in Figure 1. Among them, 12 samples of *D. odorifera* were collected from various sites from southern China, and four samples of *D. tonkinensis* in Vietnam. The fresh leaves and sapwood of *D. odorifera* were collected from Hainan Provincial Forestry Science Institute, Haikou, China. The air-dried heartwood of *D. odorifera* was from Hainan Province, Guangdong Province and Guangxi Province, China, respectively. The fresh leaves of *D. tonkinensis* are from Vietnam Forestry University, Hanoi, Vietnam. The fresh twigs are from Vietnam Forestry University (Hanoi), Hoan Kiem Lake (Hanoi), Vocational College of Electrical Engineering (Qui Nhon) and Cat Tien National Park.

### Table 1: Plant materials investigated.

<table>
<thead>
<tr>
<th>Code</th>
<th>Voucher no.</th>
<th>Collection site</th>
<th>Type of sample</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Dalbergia odorifera</em> A</td>
<td>AHAU001</td>
<td>1, 2, China</td>
<td>sW and leaf</td>
</tr>
<tr>
<td><em>D. odorifera</em> B</td>
<td>AHAU007</td>
<td>1, 2, China</td>
<td>hW</td>
</tr>
<tr>
<td><em>D. odorifera</em> C</td>
<td>AHAU010</td>
<td>1, 2, China</td>
<td>hW</td>
</tr>
<tr>
<td><em>D. odorifera</em> D</td>
<td>AHAU012</td>
<td>2, China</td>
<td>hW</td>
</tr>
<tr>
<td><em>D. odorifera</em> E</td>
<td>AHAU015</td>
<td>1, 2, China</td>
<td>hW</td>
</tr>
<tr>
<td><em>D. odorifera</em> F</td>
<td>AHAU023</td>
<td>3, 4, China</td>
<td>hW</td>
</tr>
<tr>
<td><em>D. odorifera</em> G</td>
<td>AHAU024</td>
<td>5, 7, China</td>
<td>hW</td>
</tr>
<tr>
<td><em>D. odorifera</em> H</td>
<td>AHAU026</td>
<td>6, 7, China</td>
<td>hW</td>
</tr>
<tr>
<td><em>D. odorifera</em> I</td>
<td>AHAU027</td>
<td>5, 7, China</td>
<td>hW</td>
</tr>
<tr>
<td><em>D. odorifera</em> J</td>
<td>AHAU028</td>
<td>5, 7, China</td>
<td>hW</td>
</tr>
<tr>
<td><em>D. odorifera</em> K</td>
<td>AHAU031</td>
<td>5, 7, China</td>
<td>hW</td>
</tr>
<tr>
<td><em>D. odorifera</em> L</td>
<td>AHAU033</td>
<td>5, 7, China</td>
<td>hW</td>
</tr>
<tr>
<td><em>Dalbergia tonkinensis</em> A</td>
<td>AHAU041</td>
<td>8, Vietnam</td>
<td>Twig and leaf</td>
</tr>
<tr>
<td><em>D. tonkinensis</em> B</td>
<td>AHAU042</td>
<td>8, Vietnam</td>
<td>Twig</td>
</tr>
<tr>
<td><em>D. tonkinensis</em> C</td>
<td>AHAU043</td>
<td>9, Vietnam</td>
<td>Twig</td>
</tr>
<tr>
<td><em>D. tonkinensis</em> D</td>
<td>AHAU044</td>
<td>10, Vietnam</td>
<td>Twig</td>
</tr>
</tbody>
</table>

sW, sapwood; hW, heartwood; 1, Haikou; 2, Hainan; 3, Guangzhou; 4, Guangdong; 5, Nanning; 6, Laibin; 7, Guangxi; 8, Hanoi; 9, Qui Nhon; 10, Ho Chi Minh.
In addition, 14 sequences, including the outgroup species *Cinnamomum camphora*, were downloaded from GenBank and tested in terms of species identification ability of the three loci (Table S1).

**Light microscopy**

Wood samples were cut into small blocks [10 (L) × 10 (R) × 10 (T) mm³], which were boiled in water for 8 h and then soaked in 10% ethylenediamine (Aladdin, Shanghai, China) for 1 week. Transverse, R and T sections (15 μm thick) were cut by a sliding microtome. Sections were bleached in 3% sodium hypochlorite (Aladdin, Shanghai, China), stained with 1% aqueous safranin (BioDuly, Nanjing, China) and observed under a light microscope (Nikon E100, Japan).

Genomic DNA of leaf samples was extracted with the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer’s instructions. All wood samples, including sapwood, heartwood and twigs, were washed by 75% ethanol several times before removing the surface tissue with a sterile scalpel. After cleaning with distilled water thoroughly, these samples were further cut into small pieces for freeze grinding. About 1 g of the sample was placed in a 50-ml steel jar together with a steel bead and frozen in liquid nitrogen for more than 10 min first, and then milled in a high-throughput ball mill (DHS, Beijing, China) for 5 min. The steel jar and steel bead were washed with distilled water thoroughly after grinding to avoid cross-contamination with other samples.

Genomic DNA was extracted from wood tissue according to Tang et al. (2011), following the protocol for the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) with N-phenacylthiazolium bromide (PTB, Prime Organics, Woburn, MA, USA). Detailed procedure: sample powder was transferred to a tube along with 5 ml of 0.5 M EDTA (Sangon Biotech, Shanghai, China) and soaked for 48 h at room temperature (r.t.) for demineralization of the tissues (Asif and Cannon 2005). Then, 500 μl of proteinase K (Merck, Darmstadt, Germany) and 1 ml of 0.1 M PTB were added. Samples were incubated at 65°C for 12 h in a water bath before adding 0.4 ml of cetyltrimethylammonium bromide (CTAB, Sangon Biotech, Shanghai, China) as a buffer (2%...
CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris–HCl, pH 8.0) for another 15 min. Then the mixtures were centrifuged at 16,000 g for 20 min at 4°C. Clear supernatants were collected and diluted in AP2 buffer in the DNeasy Plant Mini Kit. When the diluted solution was loaded onto a column in the DNeasy Plant Mini Kit, subsequent steps were carried out, strictly following the kit protocol. Concentrations of DNA were estimated with a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA), and confirmed after electrophoresis in 1.0% agarose gels by comparison with DNA Marker (TaKaRa, Dalian, China). Finally, the DNA extracts were purified twice with a High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany) before being used as templates for polymerase chain reactions (PCR).

**PCR amplification and sequencing**

Three barcode sequences were amplified, i.e., the chloroplast DNA region *rpoC1*, the chloroplast *trnH-psbA* intergenic spacer and the nuclear DNA region ITS. We designed two pairs of primers for amplifying the ITS region and *trnH-psbA* intergenic spacer. In addition, the primer for amplifying *rpoC1* region was employed according to Chen et al. (2010). Details are listed in Table S2.

PCR amplification was performed in a 25-μl tube, which contained c. 20 ng of template DNA, 0.5 μl (5 U μl⁻¹) LA Taq DNA polymerase (TaKaRa, Dalian, China), 1 μl dNTP mixture (2.5 mM of each dNTP), 2.5 μl 10× LA PCR Buffer II (TaKaRa, Dalian, China), and 1 μl (20 μM) forward and reverse primer, respectively. The amplification was conducted in a T100 thermal cycler (Bio-Rad, Hercules, CA, USA), for an initial denaturing step of 94°C for 5 min, 30 cycles at 94°C for 1 min, 52–62°C annealing temperature for 1 min and 72°C for 1 min. This was followed by a final extension step at 72°C for 10 min. After amplification, PCR products were subjected to 1.2% agarose gels, stained with SYBR Green I (BioTake, Beijing, China) and photographed with the Gel Doc XR® System (Bio-Rad, Hercules, CA, USA). PCR products were purified with a TIANgel Midi Purification Kit (Tiangen, Beijing, China). The purified bands were cloned with the pEASY-T1 Cloning Kit (TransGen Biotech, Beijing, China). Nucleotide sequencing was carried out by means of an ABI PRISM® 3730xl DNA Analyzer (ABI, Foster City, CA, USA).

**DNA barcode analysis**

Five independent colonies were selected from each sample for sequencing. Bidirectional sequences were assembled by the EditSeq program in the Lasergene Suite 7 (DNASTAR, Madison, USA) (Burland, 1999) and aligned by Clustal X 1.83 (UCD Conway Institute, Dublin, Ireland) (Gu et al. 2011). The alignments were adjusted manually by means of the BioEdit software (North Carolina State University, Raleigh, USA) (Hall 1999). Intraspecific and interspecific genetic distance were calculated by MEGA 5.0 (The Biodesign Institute, Tempe, USA) (Kumar et al. 2008) with a Kimura two-parameter (K2P) model for the three DNA regions. Three evaluation methods of species identification, including the BLAST method (Altschul et al. 1990), tree-based method (Kumar et al. 2008) and TAXONDNA analysis (Meier et al. 2006), were applied to evaluate the discrimination power of the three DNA barcodes (Ross et al. 2008; Pang et al. 2011; Tamura et al. 2011).

In the BLAST method, the query was searched against the NCBI (National Center for Biotechnology Information) nucleotide database. The origin of the sequence that showed the highest similarity was compared with the sample. The barcoding sequences were phylogenetically analyzed by MEGA v. 5.0 with the K2P model for

![Figure 3: Agarose gel electrophoresis pattern of polymerase chain reaction (PCR) products of amplified nuclear DNA internal transcribed spacer (ITS) (a), chloroplast intergenic spacer *trnH-psbA* (b) and chloroplast DNA *rpoC1* (c) regions, the arrow indicated target band. (1–12) Code of *Dalbergia odorifera* A–L; (13–16) code of *Dalbergia tonkinensis* A–D; (17–18) leaves of *D. odorifera* and *D. tonkinensis*; (CK) control check: sterile water is employed for DNA template to amplified three DNA barcode regions. *D. Dalbergia.*](image-url)
Table 2: Evaluation of the three DNA loci.

<table>
<thead>
<tr>
<th></th>
<th>ITS</th>
<th>trnH-psbA</th>
<th>rpoC1</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR success rate (%)</td>
<td>62.5</td>
<td>100</td>
<td>81.25</td>
</tr>
<tr>
<td>Sequencing success rate (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Aligned sequence length (bp)</td>
<td>627</td>
<td>320</td>
<td>529</td>
</tr>
<tr>
<td>Indel length (bp)</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Informative sites</td>
<td>8</td>
<td>8</td>
<td>51</td>
</tr>
<tr>
<td>Variable sites</td>
<td>24</td>
<td>17</td>
<td>55</td>
</tr>
<tr>
<td>Mean intraspecific distance (range)</td>
<td>0.0059 (0–0.0262)</td>
<td>0.0040 (0–0.0164)</td>
<td>0.0422 (0–0.1168)</td>
</tr>
<tr>
<td>Mean interspecific distance (range)</td>
<td>0.0061 (0–0.0162)</td>
<td>0.0263 (0.0229–0.0329)</td>
<td>0.0864 (0–0.1193)</td>
</tr>
</tbody>
</table>

ITS, internal transcribed spacer; PCR, polymerase chain reaction.

neighbor-joining (NJ) trees. The bootstrap supporting option was set to 1000 random addition replicates to determine statistical support for the clusters and clades (Koski and Golding 2001).

The software applied was TAXONDNA 1.77 (National University of Singapore, Singapore), which is a Java program for exploring intra- and interspecific genetic distances, matching sequences and clustering sequences based on pairwise distances. For analyzing identification rates of DNA barcodes, the criteria, “best match”, “best close match” and “all species barcodes” were employed (Meier et al. 2006).

Results and discussion

Wood anatomy

Light microscopic observations in Figure 2 can be summarized to show that both species are: diffuse-porous with semi-ring porous tendency; vessel outlines mostly circular to oval; solitary pores and few radial multiple pores (two or three) are visible; axial parenchyma in abundance, as aliform, aliform-confuent and banded paratracheal parenchyma type; rays are storied, with uniseriate and multiseriate rays (two or three cells wide), and heterogeneous rays of type III are present. Li et al. (2008) and Luo et al. (2012) described these features in more detail and confirmed that *D. odorifera* and *D. tonkinensis* cannot be differentiated based on wood anatomy.

DNA extraction and PCR amplification

DNA extraction with PTB has proved an effective process for DNA isolation. This approach is common for DNA extraction from ancient bones in paleontological studies, which cleave glucose-derived protein cross-links and help release the DNA that might have been trapped within sugar-derived condensation products (Kelmann and Kelman 1999; Gugerli et al. 2005). It was demonstrated

Figure 4: Nucleotide variability distribution of chloroplast intergenic spacer *trnH-psbA* (a); seven diagnostic sites between *Dalbergia odorifera* and *Dalbergia tonkinensis* (b).
that the PTB method was suitable for DNA extraction from the heartwood of *D. odorifera* (Yu et al. 2013, 2014). In the present work, DNA was successfully extracted from the sapwood, heartwood and twigs of both the species in focus (Figure S1).

The average quantities of DNA extracts from twigs, sapwood and heartwood were 16.3, 11.5 and 6.0 ng mg⁻¹, respectively. The DNA extracts from heartwood were invisible in 1% agarose gels after electrophoresis (due to their low yield below the threshold of fluorescence; Ohyama et al. 2001), whereas its counterparts from sapwood and twig were well observable (Figure S1). Overall, the efficacy of DNA extraction from twigs and sapwood was higher than that of heartwood, as was already reported by Rachmayanti et al. (2009), Tnah et al. (2012) and Jiao et al. (2014). This might be because of the large number of living parenchyma cells in the sapwood. On the other hand, the dead parenchyma in the heartwood contained mainly degenerated DNA (Bamber 1976; Panshin and Zeeuw 1980), whereas the degradation occurred during heartwood formation via the programmed cell death (Fukuda 2000; Rachmayanti et al. 2006). Thus, most DNA fragments in heartwood were shorter (Cano 1996).

The nuclear DNA region ITS, the chloroplast *trnH-psbA* intergenic spacer and the chloroplast DNA region *rpoC1* were successfully amplified (Figure 3a–c). The success rates for PCR amplification for these loci were 62.5%, 100% and 81.2%, respectively. The success rate for bidirectional sequencing of PCR products was 100% for all three loci (Table 2). The PCR success rate for long fragments was lower than for the short fragments. It was more difficult to amplify DNA from the heartwood than sapwood, as the relative short DNA fragments were probably washed out in the course of DNA purification. Moreover, tyloses and polyphenols in heartwood were potentially inhibitors of DNA isolation (Rachmayanti et al. 2009).

### Alignment and variability

For the ITS region, the length of the aligned sequences was 627 bp, with 24 variable sites and eight informative sites dispersing across the alignment. In the *rpoC1* matrix, the aligned sequences were 529 bp in length, with 55 variable sites and 51 informative sites (Table 2). Unfortunately, no diagnostic sites were found between the two species in these two DNA barcoding regions. Seven diagnostic sites, suited for distinguishing the two species by character-based methods (Gu et al. 2011), were detected in the *trnH-psbA* region (Figure 4a and b). The aligned *trnH-psbA* matrix was 320 bp long, with 17 variable sites and 8 informative sites (Table 2). The mean interspecific distances were greater than the intraspecific distances in the present study for the tests of three loci (Table 2). The frequency distribution of inter- and intra-specific K2P

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**Table 3:** Applicability of species identification based on BLAST and neighbor-joining (NJ) tree methods.

<table>
<thead>
<tr>
<th>Ability to discriminate</th>
<th>ITS</th>
<th><em>trnH-psbA</em></th>
<th><em>rpoC1</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>BLAST (genus level)</td>
<td>100% (10/10)</td>
<td>100% (16/16)</td>
<td>100% (13/13)</td>
</tr>
<tr>
<td>NJ tree (species level)</td>
<td>66.7% (10/15)</td>
<td>100% (21/21)</td>
<td>78.6% (11/14)</td>
</tr>
</tbody>
</table>
Figure 6: A taxon identification tree constructed using neighbor-joining analysis of Kimura two-parameter (K2P) distances showing patterns of internal transcribed spacer (ITS) sequence divergence (a), trnH-psbA (b) and rpoC1 (c) for Dalbergia odorifera and Dalbergia tonkinensis. Bootstrap values (>50%) are shown above the relevant branches. D, Dalbergia; C, Cinnamomum.
distances for the three proposed DNA regions is presented in Figure 5. The results demonstrated that the interspecific differences of the chloroplast trnH-psbA intergenic spacer were greater than intraspecific variations (Figure 5b).

Although rpoC1 regions had the most variable information after multiple sequence alignment, these sites were mostly detected from the samples of D. odorifera, which were collected from various sites in China. Meanwhile, the results showed that the distribution ranges of intraspecific genetic distances were greater than the interspecific genetic distances (Figure 5c). Thus, the rpoC1 is not suitable as a barcode to identify the wood of D. odorifera and D. tonkinensis (Gu et al. 2011).

Applicability of species identification

Three published methods, including BLAST, tree-based method and the TAXONDNA method, were used to test the feasibility in different regions to identifying unique species (Ross et al. 2008). BLAST searches with the ITS, trnH-psbA and rpoC1 sequences of D. odorifera and D. tonkinensis matched all the sequences with the Dalbergia genus in GenBank (Table 3). It was reported that the best hit of the query sequence is often not the expected one or the closest phylogenetic relative (Koski and Golding, 2001; Gu et al. 2011). We propose that the BLAST method is a good candidate for evaluating the identification power of the three proposed DNA barcodes at the genus level.

A phylogenetics tree was obtained by the NJ method based on the ITS, trnH-psbA and rpoC1 sequences, respectively (Figure 6a–c). Accordingly, D. odorifera and D. tonkinensis can be distinguished accurately at the species level by the NJ tree method based on trnH-psbA sequences, which has reached a 100% (21/21) success rate with respect to species identification. The rpoC1 sequences correctly identified 78.6% (11/14) of the species, followed by ITS 66.7% (10/15) (Table 3).

Based on the TAXONDNA method, the criteria “best match” and “best close match” were applied to analyze the identification rates. The success rates for all of three DNA barcode regions were ≥50%, and the trnH-psbA region was most successful with 100% identification rate. Based on the “all species barcodes” evaluation criteria, the trnH-psbA region had a 100% success rate, whereas, in contrast, the ITS and rpoC1 regions failed to identify the species (Table 4).

How powerful trnH-psbA is as DNA barcode for species identification has already been demonstrated (Kress and Erickson 2007; Lahaye et al. 2008; Newmaster et al. 2008; Nitta 2008). The results of the present study confirm this finding.

Conclusions

In the present study, DNA was successfully extracted from several kinds of wood samples, including sapwood, heartwood and twigs, of D. odorifera and D. tonkinensis. Sapwood or twigs were more suitable for extracting DNA than heartwood. Furthermore, the identification power of the chloroplast trnH-psbA intergenic spacer was higher than the ITS and rpoC1 regions at the species level according to BLAST, the tree-based method and the TAXONDNA method. The trnH-psbA was easy to amplify by PCR with one pair of primers and showed the highest interspecific divergence and a relatively low level of intraspecific divergence. The trnH-psbA region provided the highest identification efficiency (100%) of the three regions tested and the trnH-psbA region also satisfied all of the rigorous standards. Thus, the chloroplast trnH-psbA intergenic spacer is a suitable DNA barcode for wood identification between D. odorifera and D. tonkinensis, which are difficult to differentiate by traditional methods.

Acknowledgments: This work is supported by a grant from the National Natural Science Foundation of China (No. 31070636) and a project of the Chinese State Forestry Administration (No. 201304508). We are grateful to vice director Dazhou Li (Hainan Provincial Forestry Science Institute, Hainan, P.R. China) and Professor Feng Xu (Guangxi University, Guangxi, P.R. China) for providing the heartwood samples of D. odorifera. Our special thanks also go to Professor Vu Huy Dai (Vietnam Forestry University, Hanoi, Vietnam) for courtesy in helping collect D. tonkinensis samples in Vietnam.

Table 4: Species identification success rate based on TAXONDNA analysis.

<table>
<thead>
<tr>
<th>Evaluation criteria</th>
<th>ITS</th>
<th>trnH-psbA</th>
<th>rpoC1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Best match</td>
<td>11 (78.57%)</td>
<td>21 (100%)</td>
<td>7 (50%)</td>
</tr>
<tr>
<td>Best close match</td>
<td>11 (78.57%)</td>
<td>21 (100%)</td>
<td>7 (50%)</td>
</tr>
<tr>
<td>All species barcodes</td>
<td>0 (0.0%)</td>
<td>21 (100%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

References


Ogata, T., Yahara, S., Hisatsune, R., Konishi, R., Nohara, T. (1990) DNA barcoding markers for the identification of *Cyclobalanopsis* species (Fagaceae) based on DNA polymorphism of the intergenic spacer between trn T and trn L.


Supplemental Material: The online version of this article (DOI: 10.1515/hf-2014-0234) offers supplementary material, available to authorized users.