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NIRS IDENTIFICATION OF *SWIETENIA MACROPHYLLA* IS ROBUST ACROSS SPECIMENS FROM 27 COUNTRIES

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ABSTRACT

Big-leaf mahogany is the world's most valuable widely traded tropical timber species and Near Infrared Spectroscopy (NIRS) has been applied as a tool for discriminating its wood from similar species using multivariate analysis. In this study four look-alike timbers of *Swietenia macrophylla* (mahogany or big-leaf mahogany), C*arapa guianensis* (crabwood), *Cedrela odorata* (cedar or cedro) and *Micropholis melinoniana* (curupixá) have been successfully discriminated using NIRS and Partial Least Squares for Discriminant Analysis using solid block and milled samples. Species identification models identified 155 samples of *S. macrophylla* from 27 countries with a correct classification rate higher than 96.8%. For these specimens, the NIRS spectrum variation was more powerful for species identification than for determining provenance of *S. macrophylla* at the country level.

Keywords: Near infrared spectroscopy, *Carapa guianensis*, *Cedrela odorata*, *Micropholis melinoniana, Swietenia macrophylla,* provenance.

INTRODUCTION

Big-leaf mahogany (*Swietenia macrophylla* King*)* is one of the world's most valuable and widely traded tropical timber species (Grogan & Barreto 2005; Grogan *et al.* 2014). It occurs naturally from North to South America starting in Mexico and ending in Bolivia and south of Brazilian Amazon (Tomaselli & Hirakuri 2008; Pastore *et al.* 2011). Its wood has several desirable features such as beauty, workability and moderate decay resistance (Coradin *et al.* 2009; Pastore *et al.* 2011). In 2003, after decades of extensive selective logging, *S. macrophylla* (along with its congeners *S. humilis* and *S. mahagoni*) was included in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) controlled species, thereby gaining increased regulatory protection (Grogan & Barreto 2005; Tomaselli & Hirakuri 2008). The wood of the big-leaf mahogany is often confused with *Swietenia humilis* and *S. mahagony* in Central American countries where the *Swietenia* species even hybridize (The IUCN Redlist 1998). In Brazil, *S. macrophylla* is the only species of the genus,

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which occurs naturally. Plantations of this and other species of the genus have been established only a few decades ago (Freitas 2015). However, there are many Brazilian native species whose woods are visually similar to *S. macrophylla* (Coradin *et al.* 2009; Pastore *et al.* 2011; Braga *et al.* 2011), and there is evidence that *S. macrophylla* has been smuggled under the guise of other species (Chimelli & Boyd 2010). Thus wood species identification procedures are essential to avoid illegal exploitation and trading and to ensure conservation.

To botanically identify a species it is necessary to look at all its features including leaves, fruits, seeds and flowers. However, in everyday practice of timber identification, the wood has already been logged and typically only boards are available. So, alternative methodologies are needed for correct wood identification. A method currently employed for this purpose is a combination of macroscopic and microscopic identification, where many wood characters such as density, color, smell, brightness, texture, growth rings, vessels, fibers and porosity of an unknown sample are compared with candidate species (Wheeler & Baas 1998; Coradin *et al.* 2009; Pastore *et al.* 2011; Gasson *et al.* 2011). When an expert wood anatomist is available to perform the identification this method can provide forensically highly reliable results. Furthermore, there are identification keys that gather anatomical information about the most relevant species, which represent important databases to support the wood anatomist. Prominent among these tools are the *InsideWood* database (InsideWood 2004-onwards; Wheeler 2011) and the one edited by the Brazilian Forest Service (Coradin *et al.* 2009). Even with access to such tools, forensic identification of wood using wood anatomical information requires an expert wood anatomist. Unfortunately, there are not enough experts to meet the demand (Tsuchikawa *et al.* 2003; Martins-da-Silva *et al.* 2003). Therefore, the development of technological methods for wood identification that does not require specialist knowledge can help to improve field-level inspections and identification as well as forensic wood identification.

Today, there is a global effort to find methods to assist in the correct identification of a forest tree species through its wood (Dormontt *et al.* 2015). New proposals are emerging or are being developed such as machine vision, which is a system that captures an image of a timber under controlled conditions by means of a camera and compares this image with high quality reference images from a database (Hermanson & Wiedenhoeft 2011). Recently, direct analysis in real time (DART) time-of-flight mass spectroscopy (TFMS) has been successfully used in species identifications of American *Dalbergia* species (Espinoza *et al.* 2015).

Near infrared spectroscopy (NIRS) is a powerful technique for wood assessment since it enables rapid and non-destructive analysis, can be applied to solid samples, requires minimal or no sample preparation, most of the times it does not generate chemical waste, commercial portable devices are already available, and operation of the device does not require years of training. Hence, NIRS has been widely applied to study many wood chemical and physical properties and has also been increasingly used for wood identification. Those applications were intensively reviewed by Tsuchikawa (2007), Tsuchikawa and Schwanninger (2013) and Tsuchikawa and Kobori (2015).

Braga *et al*. (2011) and Pastore *et al*. (2011) associated NIRS and Partial Least Squares for Discriminant Analysis (PLS-DA) to successfully discriminate *Swietenia macrophylla* from the three similar species *Carapa guianensis* (crabwood), *Cedrela odorata* (cedar), which is in CITES Appendix III, and *Micropholis melinoniana* (curupixá) using trunk blocks and milled samples, respectively.

Sample surface properties interact with the near infrared radiation so that differences are expected between the way block and powder samples reflect the radiation measured by the device. Nisgoski *et al*. (2015) studied the influence of sample form and granulometry using solid branches and powder samples in the discrimination of *Salix* species using NIRS. They obtained best results with the finest powder, suggesting that for *Salix* samples heterogeneity was relevant at every scale tested.

In this study, NIRS and PLS-DA were used to discriminate *Swietenia macrophylla* from *Carapa guianensis*; *Cedrela odorata* and *Micropholis melinoniana* using both powder and block samples. Model update was used as a strategy to evaluate whether species identification models developed with solid samples could be robust enough to identify a test set of *S. macrophylla* milled samples from 27 countries (Table 1).

Table 1. Distribution of the 155 milled samples from 27 different countries.

Country (Code)*	Number of samples
Barbados (BRB)	2
Belize (BLZ)	4
Bolivia (BOL)	3
Brazil (BRA)	7
China (CHN)	2
Colombia (COL)	2
Costa Rica (CRI)	4
Cuba (CUB)	10
Curacao (CUW)	7
Dominica (DMA)	1
Ecuador (ECU)	1
United States of America (USA)	11
Philippines (PHL)	3
Guatemala (GTM)	4
Honduras (HND)	24
India (IND)	1
Jamaica (JAM)	4
Malaysia (MYS)	1
Mexico (MEX)	18
Nicaragua (NIC)	5
Panama (PAN)	5
Peru (PER)	14
Dominican Republic (DOM)	3
Haiti (HTI)	2
Lanka (LKA)	1
Trinidad and Tobago (TTO)	1
Venezuela (VEN)	15

*Country codes are according to ISO 3166-1.

In addition, we tested whether spectral variations related to species differences were more significant than those due to geographic provenance.

MATERIALS AND METHODS

Species selection

Species were selected based on the studies by Coradin *et al.* (2009), Pastore *et al.* (2011) and Braga *et al.* (2011) where three species, whose woods were the most difficult to distinguish from *S. macrophylla*, were chosen.

Block samples – Each *Carapa guianensis*, *Cedrela odorata* and *Micropholis melinoniana* block sample was obtained from a disk located at the base of an individual tree trunk. These species were collected in authorized forestry exploitation areas in Pará state in Brazil. *Swietenia macrophylla* samples were obtained from tips of apprehended boards coming from the state of Mato Grosso do Sul in Brazil. Altogether, 111 solid samples with dimensions of approximately 2 cm^3 were used: 26 of *Carapa guianensis*, 28 of *Cedrela odorata*, 29 of *Micropholia melinoniana,* and 28 of *Swietenia macrophylla*. Sample surfaces were sanded with 80-grit sandpaper. Besides alleged identity, all samples were identified by a wood anatomist from the Forest Products Laboratory of the Brazilian Forest Service.

Milled samples – Milled *Swietenia macrophylla* samples from 27 countries were donated by the Forest Products Laboratory in Madison, Wisconsin, USA. Specimens were chosen from MADw and SJRw registered wood collections. Specimen identifications were based on original collector identifications of standing trees. Small subsamples (approximately 1 by 1 cm across the grain and 2 cm along the grain) were cut from the parent specimen, then a razor blade was used to split the subsamples into approximately 1 by 1 mm slivers which were then Wiley milled to 20 mesh. The mill was brush cleaned between specimens, and water and acetone swabs of the mill blade were made periodically to determine if extractives were building up on the blade. No visible residue was found on the cotton swabs. The milled wood powder was collected directly into glass vials and stored in the dark at room temperature for several years prior to analysis. There were 155 samples from 27 different countries, as shown in Table 1. Country codes are according to ISO 3166-1.

Additionally 31 milled samples from Brazilian *S. macrophylla*'s were obtained from the same set that yielded the Brazilian *S. macrophylla* block samples. Samples were milled to particle sizes between 250 and 420 μm (particles that passed a 40 mesh screen and were retained in a 60 mesh screen).

Near Infrared Spectra acquisition – All spectra were obtained in FT-IR/NIR Tensor 37, Bruker Optics (Germany) with 4 cm^{-1} resolution, 64 scans per second and spectral region from 12,000 cm^{-1} to 3,500 cm^{-1}. Diffuse reflectance measurements were made using an integrating sphere accessory and resultant spectra were in absorbance scale (log (1/R)). The radial face of each block sample was scanned twice, on different spots, resulting in four spectra per sample and totalizing 444 spectra. Milled samples were measured in triplicate totalizing 465 spectra.

Data analysis – Data analysis was carried out in Matlab 7.12.0 (R2011a) software with PLS toolbox 6.5. Spectra were preprocessed with first derivative using Savitzky-

Golay smoothing (13 points and second order polynomial). Data were mean centered and replicate spectra were not averaged. Spectral region was visually selected to minimize spectral noise and spectral differences between milled and block samples. PLS-DA calibration models were built using two thirds randomly selected from the 111 block samples (74 samples) and 10 Brazilian *Swietenia macrophylla* milled samples selected using the Kennard-Stone algorithm. For each species a specific PLS-DA model was developed. To build the PLS-DA models, a class value of 1 was attributed to the calibration samples of the species being discriminated and a class value of 0 was attributed to the calibration samples of other species. Then a PLS regression was performed to build the models where the *y* vector corresponded to the class values. Full cross validation was performed to choose the number of latent variables and exclude outliers. In this process, each spectrum at a time is removed from the calibration set sequentially and the model built with the remaining spectra is used to predict that spectrum. Only block samples were excluded as outliers and they were identified based on the residues of the estimated class values. Models were validated using the remaining third of block samples and 21 Brazilian *S. macrophylla* milled samples. Outliers among block samples of the validation set were excluded. Models were then used to analyze the 155 samples from 27 countries.

RESULTS AND DISCUSSION

NIR spectra of Brazilian *Swietenia macrophylla* block and milled samples before and after preprocessing and the selection of the wavelength range are shown in Figure 1. It is not possible to distinguish the spectra by species through visual inspection since they all appear very similar (Fig. 1A). Figure 1B shows that the application of the first derivative as preprocessing method minimized the additive shifts from baseline as

Table 2. Model parameters and prediction results of the 4 species models for the Brazilian native block and powder samples and 27 countries samples.

Model	Samples	VL*	Calibration outliers	Validation outliers	Discrimination limit	Correct classification rate
Swietenia macrophylla	Block		0	8		100 %
	Powder	8	0	0	0.457	100 %
	Countries		0	0		98.7 %
Carapa guianensis	Block		6	9		100 %
	Powder	8	0	0	0.476	100 %
	Countries		0	0		99.1 %
Cedrela odorata	Block		2	3		100 %
	Powder	8	0	0	0.428	100 %
	Countries		0	0		98.7 %
Micropholis melinoniana	Block		10	14		100 %
	Powder	8	0	0	0.328	100 %
	Countries		0	0		96.8 %

* VL = number of Latent Variables.

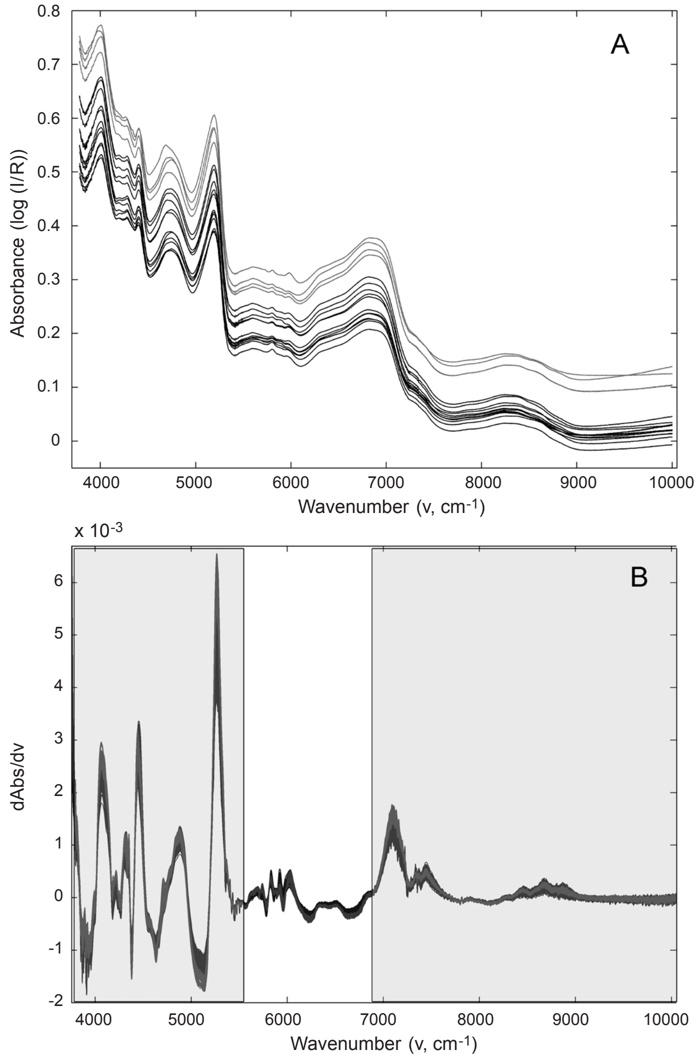
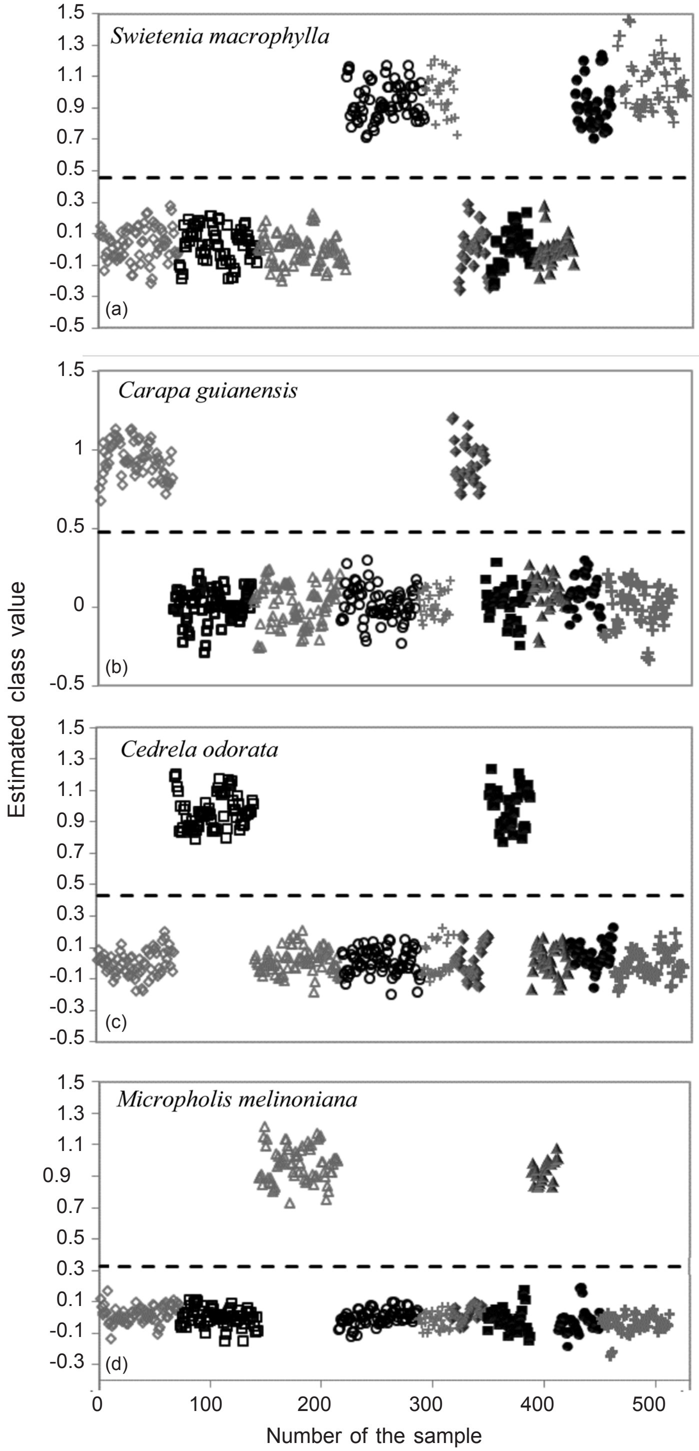


Figure 1. – A: Raw NIR spectra of the block (black) and powder (grey) samples of Brazilian *Swietenia macrophylla*. – B: The derivate NIR spectra with selected spectral range used in the discrimination models (between 5,547 and 6,897 cm^{-1}) highlighted.

well as the differences between the spectra of the block and powder samples. In addition, the spectral region between 5,547 and 6,897 cm^{-1} was selected for the model development once no visible difference between the block and milled samples were observed in this region.

Results and parameters of the models applied for the discrimination of the block test samples for the four Brazilian native species, the Brazilian *S. macrophylla* milled test samples and the 155 milled samples from 27 countries are presented in Table 2. The estimated class values for calibration and validation/test sets are shown in Figure 2.


Swietenia macrophylla
(a)
Carapa guianensis
(b)
Cedrela odorata
(c)
Micropholis melinoniana
(d)
Estimated class value
Number of the sample
1.5
1.3
1.1
0.9
0.7
0.5
0.3
0.1
-0.1
-0.3
-0.5
1
0
0
100
200
300
400
500

All four models correctly classified 100% of block samples and *S. macrophylla* milled samples, as can be seen in Table 2 and Figure 2. Models built using only block samples also gave 100% of correct classifications for both block and powder samples, except for the *Carapa guianensis* model, which misclassified more than 45% of Brazilian *S. macrophylla* powder samples (results not shown). The updating of the calibration set of the models by the inclusion of 10 *S. macrophylla* milled samples resulted in robust models that successfully classified all the test/validation samples, showing that these models were robust regarding differences in sample forms.

Figure 3 (*see next page*) shows the estimated class values for the 27 countries' samples. As can be seen in Table 2, the rates of correct classifications were > 96% for those samples. These results indicate that the method succeeded to discriminate *S. macrophylla* samples between similar species and that the spectral differences between the species were more relevant than differences due to the provenance of the samples.

The misclassified samples in the *S. macrophylla* model (Fig. 3a) were one from Colombia, which was also misclassified as *Carapa guianensis* (Fig. 3b) and one from Cuba, which was also the only sample misclassified as *Cedrela odorata* (Fig. 3c). The *Micropholis melinoniana* model had the higher number of misclassified samples as can be seen in Figure 3d and Table 2. Those samples were one sample from Ecuador, one sample from the USA, and two samples from Mexico.

Due to the relatively easy handling and portability of equipment, added to high speed to obtain the results, the NIRS technology was included in the list of new methodologies for illegal timber identification (Dormont *et al.* 2015). However, the NIRS technology for wood identification is still in an early stage, requiring further study of the variables that influence the spectrum and the building spectra banks of endangered forest species. Promising results as obtained in this study stimulates our team to continue further research.

CONCLUSIONS

Swietenia macrophylla milled samples from 27 countries could be discriminated from three macroscopically confusable Brazilian species using spectral range selection, spectral preprocessing and PLS-DA models. The updated strategy of mixing block samples from the four species and a few Brazilian *S. macrophylla* milled samples resulted in > 96% of correct classifications. The results show that spectral variations due to species differences were more significant than the variations related to provenance countries and corroborate the possibility of application of NIRS and PLS-DA as a tool for timber trade control and species conservation.

←

Figure 2. Dispersion graphs of the estimated class values of the Brazilian native woods (a) *Swietenia macrophylla* (○), (b) *Carapa guianensis* (◇), (c) *Cedrela odorata* (□) and (d) *Micropholis melinoniana* (Δ) models for mahogany block and powder samples (+) and discrimination limits (---). Empty markers stand for calibration samples and full markers for validation.

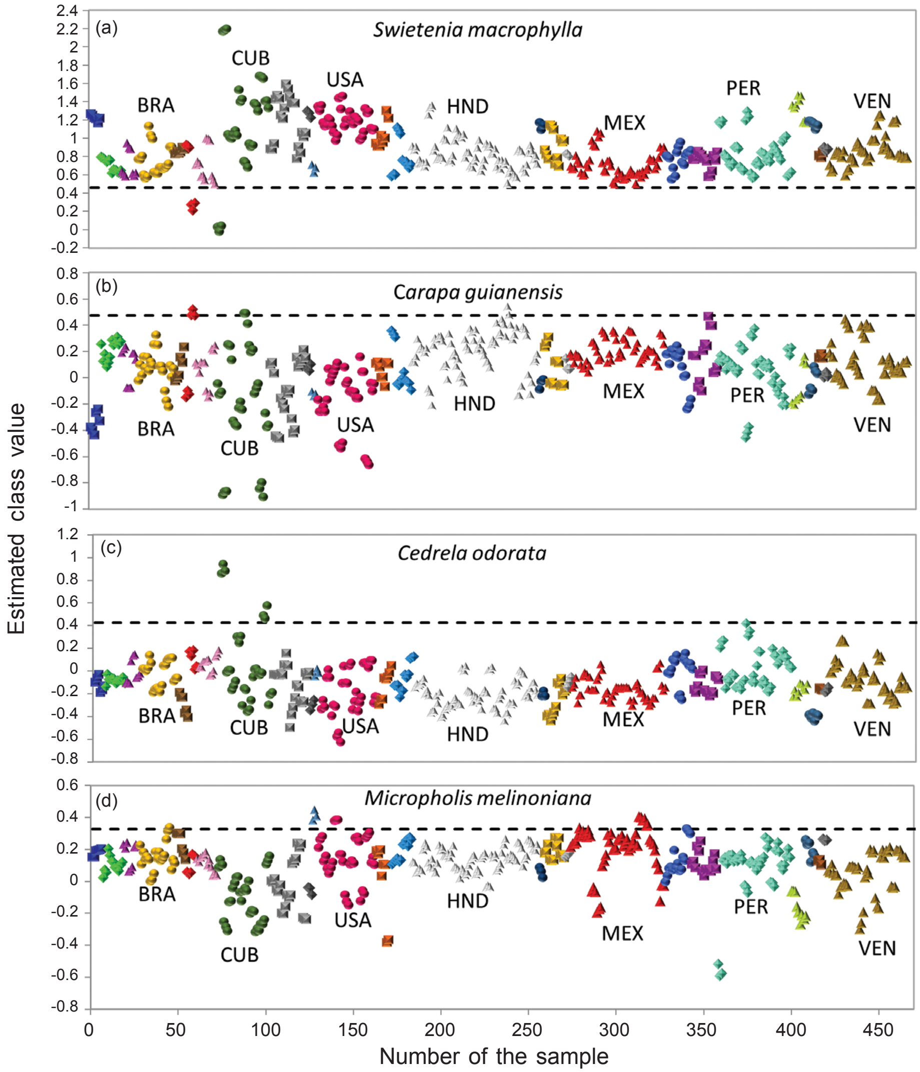


Figure 3. Dispersion graphs of the class values estimated for the 155 powder samples of *Swietenia macrophylla* from the 27 countries analyzed in the discrimination models for (a) *Swietenia macrophylla*, (b) *Carapa guianensis*, (c) *Cedrela odorata*, and (d) *Micropholis melinoniana*. Discrimination limit (---). For the country codes, see Table 1.

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