# Intra and interspecific spectral variability of *Campo Rupestre* plant species

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**Abstract***:* We assessed variability in leaf reflectance for individuals of eight sympatric *campo rupestre* species having a high degree of phenotypic plasticity. From each selected species, three individuals were sampled from different microhabitats, to account for intra-specific variability. Spectroscopy measurements were acquired between 350-2500nm, using an ASD FieldSpec Standard spectroradiometer, for all leaves of three sampled branches from each individual. We show that spectral reflectance is able to differentiate among *campo rupestre* species; among individuals within the same genus; and among species of the same family. We did not find, however, that spectral similarity was associated with phylogenetic similarity among the studied species. Our results highlight the potential of leaf spectroscopy to identify species from the megadiverse and endangered *campo rupestre* vegetation.

**Palavras-chave:** *spectroscopy, leaf reflectance, individual variability*

## 1. Introduction

Leaves are the fundamental energetic unit of biology (Blonder et al. 2011). Leaf structural and biochemical components represent trade-offs between leaf construction costs and photosynthetic carbon uptake, and are known to be a central part to the understanding of plant ecological strategies (Shipley et al. 2006; Wright et al. 2004). Leaf spectroscopy expresses fundamental chemical interactions of canopies with solar radiation, and spatial and temporal variations in resource utilization by plants result in chemical, metabolic, structural, and phenological differences, that ultimately influence the optical properties of leaves and canopies (Ustin and Gamon 2010). These variations lead to unique optical patterns that are detectable by remote sensing, allowing for the separation of multiple levels of diversity (e.g. taxonomic, phylogenetic, and functional) based on optical properties alone (Asner and Martin 2011; Asner and Martin 2009; Asner et al. 2014; Serbin et al. 2014).

At the leaf level, studies have successfully identified tree species with hyperspectral data (Price 1994; Cochrane 2000; Serbin et al. 2014; Ferreira et al. 2013; Ferreira et al. 2016), but understanding the spectral variance in different levels of biological organizational, taking plasticity into account, is still a challenge (Cavender-Bares et al. 2016). Generally, variation among individuals within populations or species is smaller than variation among species, so it is expected that inter-specific spectral variability should exceed intra-specific spectral variability (Ferreira et al. 2013; Price 1994). Despite the generality of this pattern, however, convergent and divergent evolutionary processes can lead distantly related species to have more similar phenotypes, and close relatives to be more distinct, than expected under a Brownian motion model of evolution (Cavender-Bares et al. 2016).

The *campo rupestre* is a vegetation type associated with the Cerrado biome, having its core distribution area in the highlands of the Espinhaço Mountain Range, in the States of Bahia and Minas Gerais (Brazil). These areas are known for its megadiverse vegetation (Silveira et al. 2015), where high floristic richness is favoured by the influence of three adjacent biomes (Cerrado, Atlantic Forest, and Caatinga), high soil diversity, high habitat heterogeneity, and a large degree of isolation among vegetation “islands” (Fernandes 2016; Giulietti and Pirani 1988). The large environmental heterogeneity supporting *campo rupestre* vegetation results in a large variety of plant life forms and phenotypes, and several endemic species can be usually found within relatively small areas. Although knowledge on plant diversity and biogeography of the area has increased, it relies on locally-restricted surveys and large areas are still virtually uncovered (Silveira et al. 2015).

State of the art remote sensing methods can be a powerful tool to overcome this limitation, and in this sense, assessing the extent to which spectral reflectance can be associated with evolutionary changes at both intra- and interspecific levels is essential to fully understand how we can use hyperspectral methods to effectively monitor biodiversity, across multiple spatial, temporal and biological scales. In the present work, we answered the following questions: (i) do closely related species have more similar spectral profiles than unrelated species? (ii) can leaf spectra detect variation among a) different *campo rupestre* species; b) different genus within the same family; and c) species of the same genus?

## 2. Methods

The study was carried at the Serra do Cipó region, corresponding to the southern portion of the Espinhaço Mountain Range, in Minas Gerais, Brazil (Figure 1). The regional climate is mesothermal and sub-humid, with dry winters and warm summers, and temperatures averaging between 18ºC and 20ºC during summer, reaching averages of 13ºC on winter. The region has a marked seasonality, with two distinguishable seasons: a warm rainy season from November to April and a cool dry season from May to October. During the rainy season, precipitation can reach 300 mm/month, while average precipitation is about 27mm during the dry season (Sobreiro et al. 2015). Natural fires occur at the end of the dry season, whereas man-made fires are frequent in the mid dry season (Silveira et al. 2015).

The vegetation structure of Serra do Cipó changes in response to the elevational gradient. Our study focuses on the natural areas of *campos rupestres sensu lato*, which can be found on shallow soils above 800m (Fernandes et al. 2014, Le Stradic et al. 2015, Silveira et al. 2015). This vegetation is characterized as a montane, grassy-shrubby, fire-prone vegetation mosaic, with rocky outcrops of quartzite, sandstone or ironstone and grasslands growing on sandy, stony or waterlogged soils, interspersed with patches of transitional vegetation such as Cerrado, gallery forests, and relictual hilltop forests occurring along the drainage network (Alves and Kolbek 2010, Silveira et al. 2015).

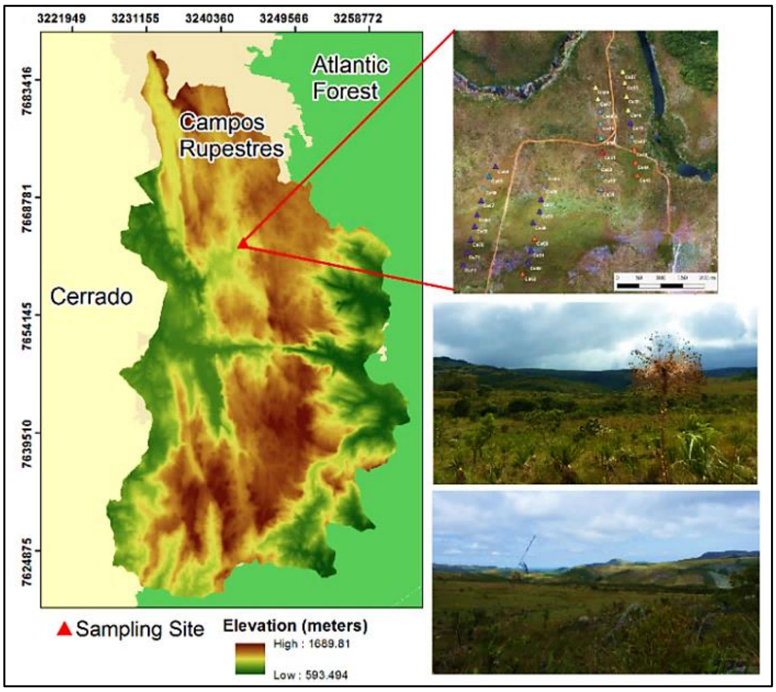


**Figure 1**: Left: overview of the topography and extent of the Serra do Cipó in the state of Minas Gerais, Brazil, and its unique location as an ecotone of vegetation types: Cerrado to the west; Atlantic Forest to the east, and *campos rupestres* (*sensu lato*) formations along the highlands. Upper right: aerial image of the sampling area showing the transects on which species were sampled. The photographs illustrate the vegetation types found within the sampling site, along a mosaic of rocky outcrops, sandy and rocky grasslands and peat bogs.

## 2.1 Species selection

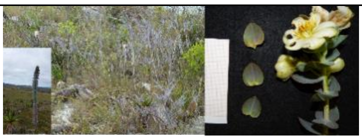
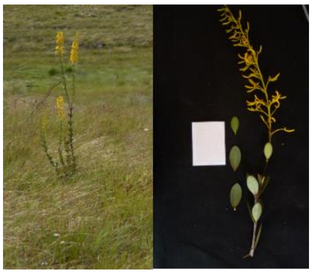
Species were selected based on their phenotypes, abundance and distribution along the sampling site. We chose four related species from the Melastomataceae family, two species from the Vochysiacea family, one micro-endemic species from the Polygonaceae family, and a common tree species of the Cerrado, from the Callophylaceae family (Table 1). Sclerophylly, thick cuticles, and sunken stomata are common characteristics of leaves of all selected species (Hoffmann et al. 2005). We sampled three individuals from each selected species, in different rock outcrops, sandy and rocky grasslands, ranging from small shrubs to tall trees.

## 2.2 Spectroscopy measurements

We selected three branches of each species and measured all attached leaves. For *L. cordata*, *V. pygmaea* and C. *cereifera*, we selected 10 leaves from each individual to acquire the measurements. Leaf spectral measurements followed the protocols and standards by Asner et al. (2014) and Asner and Martin (2011), available from the Carnegie Spectranomics website (http:// spectranomics.ciw.edu). All measurements were acquired in the same date (October 30th, 2016), so no phenological differences between individuals were expected.

Spectral measurements were taken using an ASD FieldSpec 4 Standard portable spectroradiometer (Analytical Spectral Devices, Boulder, Colorado, USA), with a spectral range of 350nm to 2500nm, spectral resolution of 3nm in the VNIR and 10nm in the SWIR, and wavelength accuracy of 0.5nm. To acquire high-resolution full-range spectra, we use the ASD probe optic accessory, which measures the spectral reflectance by direct contact with the leaf, avoiding variations in sun geometry during the measuring. The probe contains a light source perpendicular to the contact surface, and the end of a bare fiber-optic cable bundle, mounted at 42° to perpendicular. This configuration minimizes unwanted specular reflections (Serbin et al. 2014).

Table 1. Description of the species sampled along the *campos rupestres*, at Serra do Cipó, Minas Gerais.

| Family | Photo | Species |
|---|---|---|
| Melastomataceae |  | *Lavoisiera cordata* is a shrub associated to rock outcrops that can be found in large populations or isolated. Presents sessile, purple leaves. |
| |  | *Trembleya laniflora* is an endemic shrub from rock outcrops of Minas Gerais state. This shrub is characterized for having soft and white colour leaves. |
| |  | *Miconia albicans* is an aluminium accumulator shrub or tree, sensitive to fire, and is very commonly found across the cerrado. |
| |  | *Miconia ferruginata* is a small tree or shrub, with evergreen sclerophylls leaves known for itss reddish coloration. |
| Polygonaceae |  | *Coccoloba cereifera* is a narrow endemic highly sclerophyllous shrubby plant, conspicuous against the open grassland, associated with sandy soils and rock outcrops (Ribeiro e Fernandes, 1999). |
| Vochysiaceae |  | *Vochysia pygmaea* is a small shrub with sclerophyllous and persistent leaves, found mainly in rocky and sandy grasslands at Serra do Cipó. |
| |  | *Vochysia tyrsoidea* can be found as a tree or shrub and have a wide distribution along the cerrado. Presents highly sclerophyllous and deciduous leaves. |
| Calophyllaceae |  | *Kielmeyera coriacea* is a tree usually found in open areas, but has been seen in cerrado forests. Leaves are deciduous and are positioned in the top part of the branches. |

## 3. Results and discussions

Overlap in vegetation spectral reflectance is usually very common, due the relatively small number of factors controlling plant spectral responses (Cochrane 2000). Despite this being true, spectral behaviour is a phenotypic expression of the aggregated signals of leaf chemical and structural components that have evolved through time, and reflect adaptations to certain ecological conditions. Our results suggest that leaf spectroscopy is able to discriminate *campo rupestre* species reasonably well (Figure 2). The wide variety of life forms and functions associated with *campos rupestres* (Silveira et al. 2015) may be the responsible for allowing species spectral discrimination. In the visible region of the spectrum, where pigment response is more prominent, the diversity of leaf colours allowed the separation of *T. laniflora* (green-to-white leaves, with a higher spectral response from 400-700nm with no distinguishable green peak). *L. cordata* and *C. cereifera* had a spectral peak at 450-500 nm, expected for its purple leaves. Melastomataceae species had higher NIR-SWIR reflectance, which suggest more resource allocation to leaf construction within species of this family than on the remaining studied species.

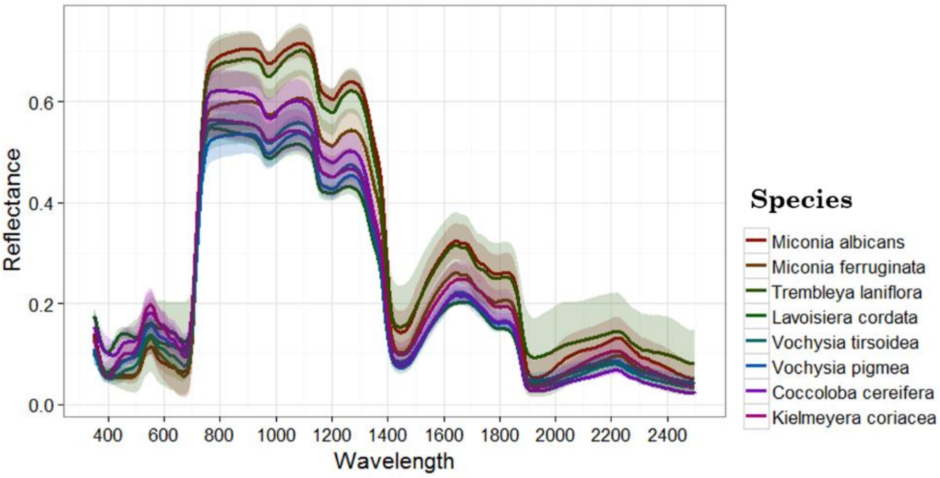


Figure 2: Average spectral response and spectral variability of leaf reflectance from the selected species of *campos rupestres* vegetation, Serra do Cipó, MG, Brazil.

We found an extensive amount of variability in leaf reflectance within Melastomatacea species, but still, using the full spectral average response, we could observe spectral features that were able to distinguish individuals that are phylogenetically related (Figure 3). We did not find any evidence that more phylogenetically related species, such as species from the *Miconia* genus (or between *T. laniflora* and *L. cordata*, which belong to the tribe Microliceae), were more spectrally similar. This could indicate a phylogenetic overdispersion of *campo rupestre* species, caused by habitat heterogeneity and strong environmental filtering, or by competition processes (Cavender-Bares et al. 2009). Spectral variation among taxa is generated by phenotypic variation, and does not directly measure the underlying genetic or phylogenetic relationships among species (Cavender-Bares et al. 2016).

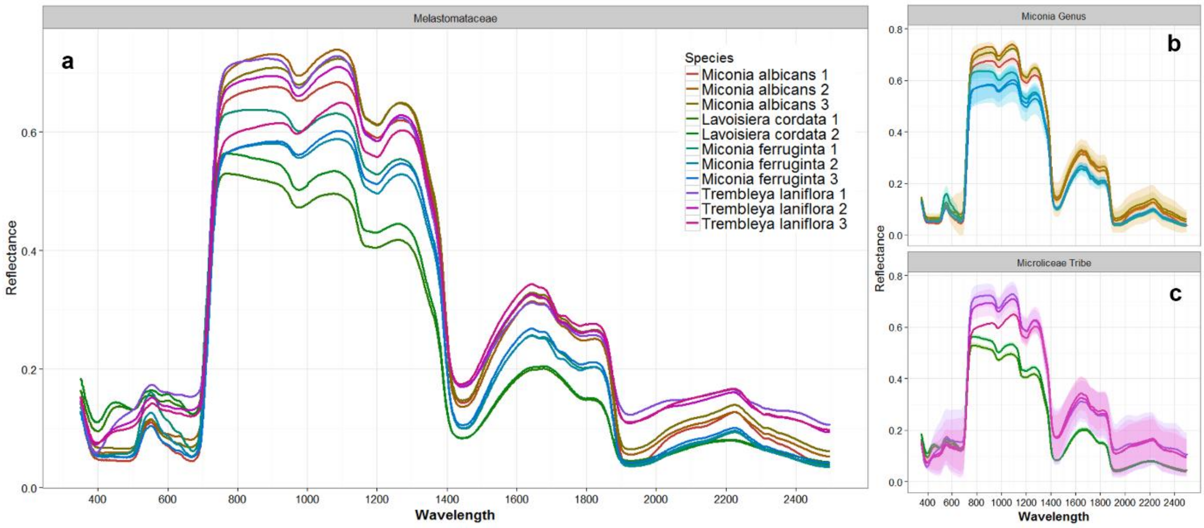


Figure 3: Average leaf reflectance spectral response and variability within individuals of different species of the Melastomatacea family (a), located in different *campos rupestres* microhabitats. Average spectral variation among Miconia genus individuals (b), and between the individuals of different genus within Microliceae tribe (c). All measurements were acquired in the same date.

Within the genus taxonomic level, a lower but still considerable amount of variability was observed (Figure 4). *Lavoisiera* had the lowest variability between individuals, and also the lowest spectral response in the SWIR region, indicating low investment in cellulose and lignin content. The micro-endemic *Coccoloba* also had low variability between individuals, with only one of the three sampled individuals being visually distinguishable. The different species of *Miconia* could be differentiated in the NIR region, among individuals of the same species, and in the SWIR region, between the two species sampled. *Trembleya* had the highest variability between individuals, with the NIR region being more spectrally dissimilar.

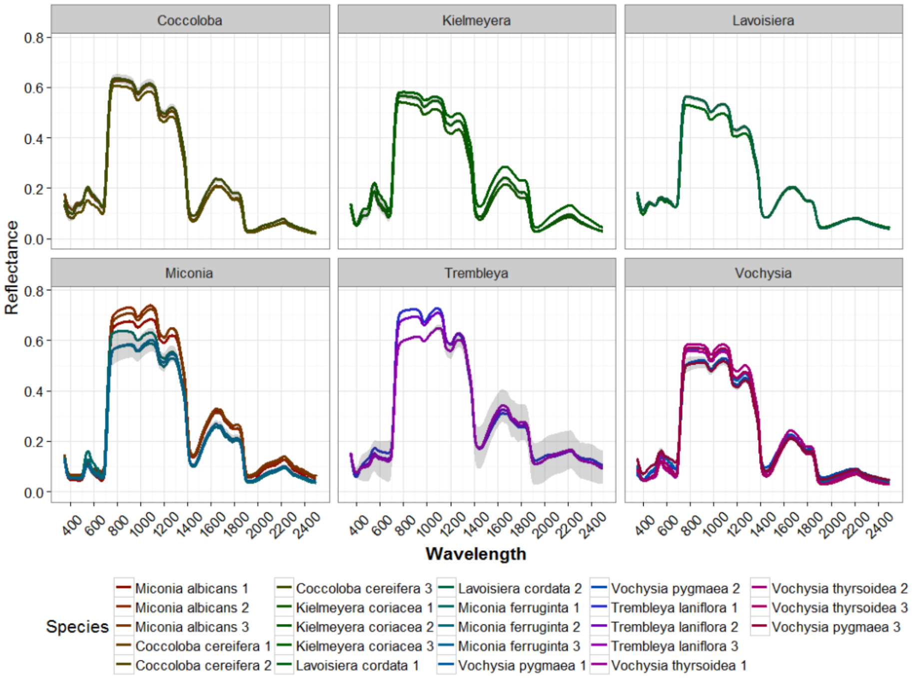


Figure 4: Leaf reflectance average variability between individuals within the same genus taxonomic level. All measurements were acquired in the same date.

## 4. Conclusion

Our results highlight the potential use of spectroscopy to identify *campo rupestre* species, across different biological levels. We were able to discriminate species and individuals within and among clades, despite their genetic relatedness. Although we did not find evidence of a phylogenetic signal on the average spectral response, more investigations are needed to disentangle phenotypic, spectral and phylogenetic variations and similarities, to better elucidate the underlying processes of optical differentiation among plants.

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