

Identifying plant species using architectural features in leaf microscopy images

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Abstract: This work proposes an analytical method to identify plant species based on microscopy images of the midrib cross-section of leaves. Unlike previous shape-based approaches based on the individual shape of external contours and cells, an architectural analysis is proposed, where the midrib is semi-automatically segmented and partitioned into histologically relevant structures composed of layers of cells and vascular structures. Using a sequence of morphological operations, a set of geometrical measures from the cells in each layer is extracted to produce a vector of features for species categorization. The method applied to a database containing 10 species of plants from the Brazilian flora achieved a success rate of 91.7%, outperforming other classical shape-based approaches published in the literature.

Key words: automatic species identification, image analysis, morphological features.

Résumé : Les auteurs de ce travail proposent une méthode analytique permettant d'identifier une espèce végétale à partir d'images en microscopie de coupes transversales de la nervure médiane des feuilles. Contrairement aux approches précédentes basées sur la forme qui reposent sur la forme individuelle des contours externes et sur les cellules, une analyse architecturale est proposée, dans laquelle la nervure médiane est segmentée de manière semi-automatique et divisée en structures histologiquement pertinentes composées de couches cellulaires et de structures vasculaires. En utilisant une séquence d'opérations morphologiques, une série de mesures géométriques des cellules de chaque couche est extraite afin de produire un ensemble de caractéristiques pour catégoriser les espèces. La méthode appliquée à une base de données contenant 10 espèces de plantes issues de la flore du Brésil a obtenu un taux de succès de 91,7 %, surpassant les autres approches classiques basées sur la forme publiées dans la littérature. [Traduit par la Rédaction]

Mots-clés : identification automatisée d'une espèce, analyse d'image, caractéristiques morphologiques.

Introduction

The categorization of plants into species is an important taxonomical task; it provides meaningful information about particular characteristics of a species, which can be used in the context of biodiversity and evolutionary affinities with other species.

Plant identification is an old problem and, classically, it has been based on conventional visual characterisation of plant morphology. It is only recently that computer-based analyses have been used for this purpose (Rossatto et al. 2011; Sá Junior et al. 2011; Florindo et al. 2014; Silva et al.

2014). Computational methods are particularly interesting because they enable a more precise identification of plant leaves, especially when leaves from different species are too similar to each other and the conventional morphological features are not sufficient for their discrimination (Bruno et al. 2008; Backes et al. 2009; Florindo et al. 2014; da Silva et al. 2015). This is especially important in situations where, for example, reproductive structures are not easily obtainable, such as plants in the vegetative phase.

Among the computational methods proposed, those using information from the leaves have become very

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popular, mostly because leaves can be easily found practically all of the time. Image analysis has proven to be a powerful tool in the description of these structures (Florindo et al. 2014). Leaves are diverse and complex structures in terms of anatomy and morphology (Evert 2006), and modern computational approaches can extract large amount of useful information from them.

Most analyses of leaf images use macroscopic features such as leaf contour and venation, among others (Bruno et al. 2008; Backes et al. 2009). More recently, the analysis of microscopic features has been a subject of investigation, using images from midrib cross-sections (Silva et al. 2014). Those studies, however, used approaches to characterise leaf structure, externally or internally, as indivisible elements without taking into consideration the relationship between their parts (for instance, cells and secretory structures observed in cross-sections).

Here we propose a taxonomic analysis of plant leaves, based on quantifying the architecture of the midrib as observed in cross-sections, using microscopy. More specifically, the midrib is characterised using adjacent layers of cells and secretory structures. Visually, it can be seen that cells and secretory structures (represented in analytical terms by pixel regions called virtual cells (v-cells)) are arranged in a seemingly hierarchical order or layers, such that they appear to exhibit characteristic morphological features at various depths from the leaf surface. The analysis, therefore, is aimed at computing various morphological descriptors of this structural organisation (e.g., v-cell area, perimeter, Feret diameter, and others) forming those layers. Those layer descriptors are averaged, and the resulting set of features submitted first to a Karhunen–Loève transform to reduce the data dimensionality and then to a learning machine algorithm to classify the cases into different plant species.

When this novel approach was applied to a database of plants from the Brazilian flora, 91.7% of the samples were successfully identified, suggesting that the proposed method might be useful for taxonomic identification. The results also suggest that the organization of v-cells within a leaf might provide useful descriptors for defining architectural metrics to investigate and compare how species might be morphologically related.

Material and methods

The material analysed is composed of 10 species from the Cerrado vegetation in Brazil. Table 1 shows the names of the collected species (six samples per species). More details about the database can be found in da Silva et al. (2015). The central part of the leaf (including the midrib) was fixed in formalin – acetic acid – 70% alcohol (FAA) for 48 h (Johansen, 1940), then dehydrated in an ethanol series and embedded in paraffin. Afterwards, cross-sections 8 µm-thick were cut, rehydrated, and stained with Astra Blue and basic fuchsin, dehydrated, and finally mounted with Entellan. The images of mid-

Table 1. Plant species in the database analysed here (six samples per species).

Family	Species
Anacardiaceae	<i>Anacardium humile</i> A. St.-Hil.
Annonaceae	<i>Annona crassiflora</i> Mart.
Aristolochiaceae	<i>Aristolochia galeata</i> Mart. & Zucc.
Bignoniaceae	<i>Arrabidaea brachypoda</i> (DC) Bur.
Apocynaceae	<i>Aspidosperma subincanum</i> Mart.
Asteraceae	<i>Baccharis salzmännii</i> DC.
Fabaceae	<i>Bauhinia pulchella</i> Benth.
Fabaceae	<i>Bauhinia unguolata</i> L.
Malpighiaceae	<i>Banisteriopsis stellaris</i> (Griseb.) B. Gates
Malpighiaceae	<i>Byrsonima laxiflora</i> Griseb.

ribs were captured with a 10× objective, in a trinocular Axio Lab A1 microscope that was coupled to a digital AxioCam ICc 1 camera (Zeiss). After images were captured, the region containing the midrib was manually separated from the background.

Method

The method proposed here consists of 4 steps: pre-processing of the cross-section, segmentation of v-cells, labelling of layers of v-cells, and extraction of features from each layer.

Pre-processing

First, the midrib area in the section is manually selected (by the operator) and separated from the mesophyll region. The midrib image (Fig. 1a) is then pre-processed for noise suppression and background illumination by applying Gaussian Blur, where the original colour image is converted to grey-levels, inverted, and convolved with a Gaussian filter with a radius of 5 around each pixel to attenuate some noise and outliers by smoothing (Fig. 1b).

These steps were carried out in ImageJ (version 1.48). Figure 1 shows the result for an image after processing.

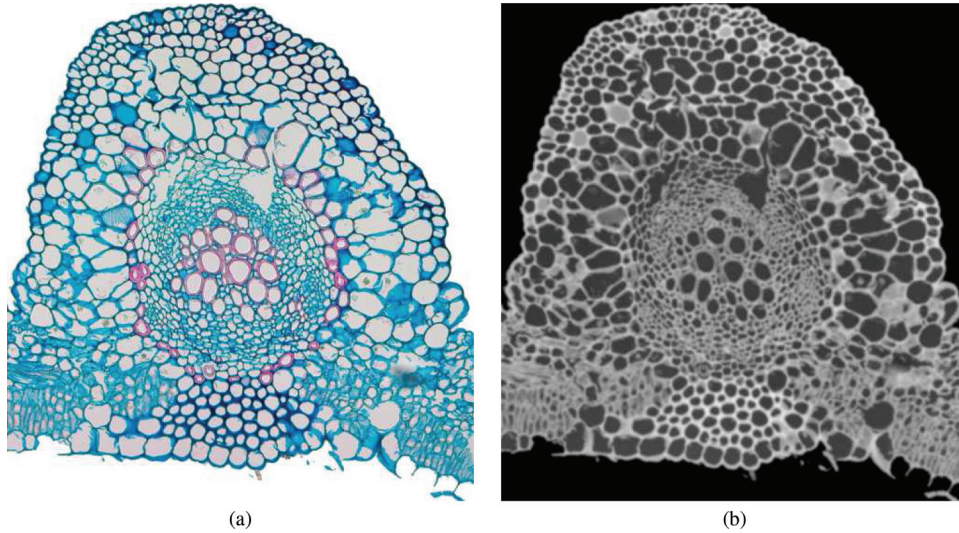
Morphological segmentation

The morphological segmentation of grey-level images used here and also presented in Vincent and Dougherty (1994), is based on the watershed transform, which relies on detecting catchment basins on the grey-level image, taking into consideration that pixel greyscale values represent the height of the greyscale function. The catchment basins are those regions where imaginary drops of water falling over the image would tend to accumulate. The borders where the different catchment basins meet are called watershed lines.

Depending on the application, the watershed lines are computed from the image itself, or from its gradient (so the watershed lines tend to be located at the edges of image objects). Here we exploit a mathematical morphology approach and use the Beucher's gradient, defined for image I as

$$(1) \quad \text{grad}(I) = (I \oplus B) - (I \ominus B)$$

Fig. 1. [This figure is in colour on the journal Web site only: <http://www.nrcresearchpress.com/doi/suppl/10.1139/cjb-2015-0075>] Pre-processing operation. (a) Original image. (b) Grey-level inversion and Gaussian blurring.



where B is a unitary ball and $\oplus$ and $\ominus$ represent morphological dilation and erosion, respectively.

The extraction of watershed lines from the greyscale gradient sometimes tends to over-segment the image (i.e., produces too many basins). The method described in Vincent and Dougherty (1994) seeks to avoid this by re-defining the gradient through a morphological grey-level reconstruction driven by a marker image. Thus given the gradient image $J = \text{grad}(I)$ and the marker image M , a new reconstructed gradient J' can be computed.

First, for every pixel p , J and M are transformed into J^* and M^* by

$$(2) \quad J^*(p) = \begin{cases} h_{\min} & \text{if } M(p) = 1 \\ J(p) & \text{otherwise,} \end{cases}$$

and

$$(3) \quad M^*(p) = \begin{cases} h_{\min} & \text{if } M(p) = 1 \\ h_{\max} & \text{otherwise,} \end{cases}$$

where $h_{\min}$ and $h_{\max}$ are chosen such that $\forall p, h_{\min} < J(p), h_{\max} > J(p)$.

At this point, it is important to define the procedure called dual grey-scale reconstruction. This procedure is not exactly straightforward and so it is explained next using simpler concepts. The first concept is the n^{th} geodesic dilation $\delta_X^n(Y)$ of a set $Y \subseteq X$. In terms of morphological operations, for $n = 1$:

$$(4) \quad \delta_X^1(Y) = (Y \oplus B) \cap X$$

and $\delta_X^n(Y)$ is defined recursively:

$$(5) \quad \delta_X^n(Y) = \delta_X^1(\delta_X^{n-1}(Y))$$

Based on that, the reconstruction $\rho_X(Y)$ is given by

$$(6) \quad \rho_X(Y) = \lim_{n \rightarrow \infty} \delta_X^n(Y)$$

In practice, only a few steps are necessary to achieve the expected reconstruction.

Given two grey-scale images I and J defined over the same domain, assuming discrete grey-levels $\{0, 1, \dots, N\}$, and satisfying $\forall p, J(p) \geq I(p)$, the reconstructed image of I from J is given by

$$(7) \quad \rho_I^*(J)(p) = N - \rho_{N-I}(I - J)$$

where ρ is the reconstruction defined by

$$(8) \quad \rho_I(J)(p) = \max\{k \in [0, N] | p \in \rho_{T_k(I)}(T_k(j))\}$$

and $T_k(I)$ is the set of pixels p such that $I(p) \geq k$.

Finally, after the above definition, J' is provided by a dual grey-level reconstruction:

$$(9) \quad J'(p) = \rho_{J'}^*(M^*)$$

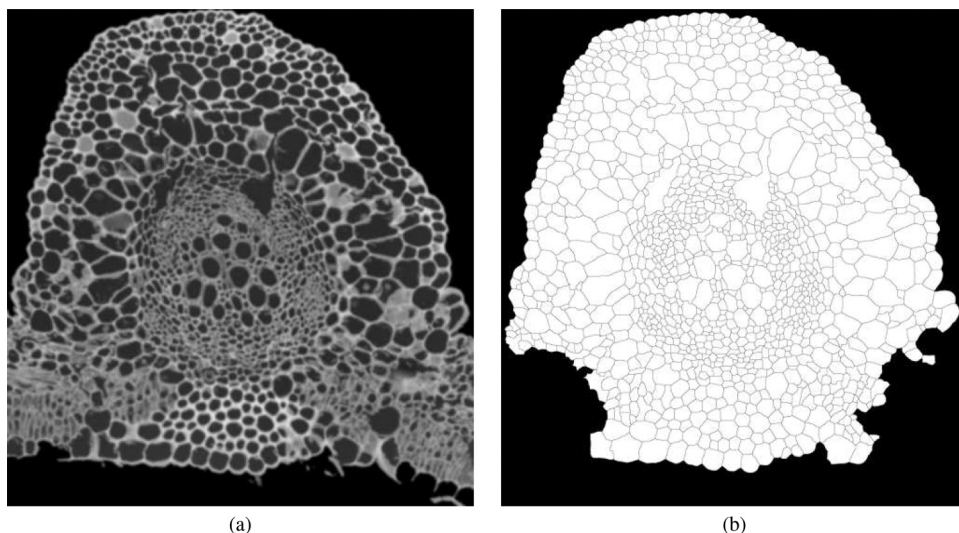
The final step of the segmentation is to extract the watershed lines of J' .

More details, rationale, and illustrated examples can be found in Vincent and Dougherty (1994). In terms of computational implementation, an ImageJ plug-in was used (Arganda-Carreras and Legland 2014) with a tolerance threshold of 10. Figure 2 illustrates how the v-cells are identified by the method.

Extraction of layers

The tissue structure in the cross-sections has a relatively symmetrical arrangement, resembling layers of

Fig. 2. Morphological segmentation of the cells in cross-section. (a) Pre-processed image (resulting from Fig. 1). (b) Morphological segmentation.



cells and secretory structures with similar properties (size, shape) across the species studied.

The v-cell layers are sets of v-cells that fulfill specific adjacency relationships and these can be computed using the algorithm described by Landini and Othman (2003).

The first step in this procedure is to identify the outermost external layer. This is achieved by identifying those v-cells adjacent to the background region. The v-cells in the segmented cross-section C are merged into C' , by applying a closing operation:

$$(10) \quad C' = (C \oplus S) \ominus S$$

where S is a 3×3 structuring element.

The background is then dilated, which gives rise to a region R of intersection with the v-cells, computed using

$$(11) \quad R = C \wedge \bar{C}' \oplus S$$

where $\bar{C}'$ is the binary inversion of C' .

The first layer is obtained by the binary reconstruction of C from R and is labelled as 1. Once the first layer has been identified, the remaining layers are computed by considering the previous layer as the background and applying two consecutive dilations in eq. 11 (the first one fills the gap between the v-cells, the second provides the overlap with not-yet-labelled v-cells and which is used by the reconstruction operation to identify the next adjacent layer). The layers are increasingly labelled 2, 3, 4, and so on. Here, such labelling procedure was applied in two directions (from the dorsal to the ventral surface and vice-versa) and the minimum of these labels defines the layers from the closest free surface of the leaf.

More details and a pseudocode can be found in Landini and Othman (2003). Figure 3 shows the layers identified in the segmented image.

Extraction of layer features

The final step in the proposed method was to compute the features of the v-cells, according to the layer where they were located.

A straightforward and efficient way of representing layer features is to calculate the mean $\mu_L^M(x)$ of the measure M over the cells x in the layer L . A potential problem of using the mean is that segmentation inaccuracies can result in very large or small v-cells that are not strictly cellular or vascular structures, e.g., empty spaces and (or) over- or under-segmented regions. To minimise this, outliers with areas 95% larger or smaller than the mean were removed, and $\mu_L^M(x)$ was computed in two steps. In the first, an auxiliary mean $m_L^M(x)$ is computed by

$$(12) \quad m_L^M(x) = \frac{1}{N} \sum_{x \in L} M(x)$$

and from that $\mu_L^M(x)$ is obtained through

$$(13) \quad \mu_L^M(x) = \frac{1}{N} \sum_{x \in L'} M(x)$$

where $L' = \{x \mid \|M(x) - m_L^M(x)\| < 0.95 \sup(\|M(x) - m_L^M(x)\|)\}$.

For M , 25 different measures were employed. They were inspired by the reports of Landini (2006 and 2008) and are listed in Table 2. A complete description of each measure can be found in Landini (2006 and 2008).

Finally, all the values of μ_L^M for each layer L and each measure M , were concatenated to create a vector of features. This results in a large set of features, depending on

Fig. 3. [This figure is in colour on the journal Web site only: <http://www.nrcresearchpress.com/doi/suppl/10.1139/cjb-2015-0075>] External layers highlighted in different colours over the segmented image. (a) Segmented image (resulting from Fig. 2). (b) Extracted layers.

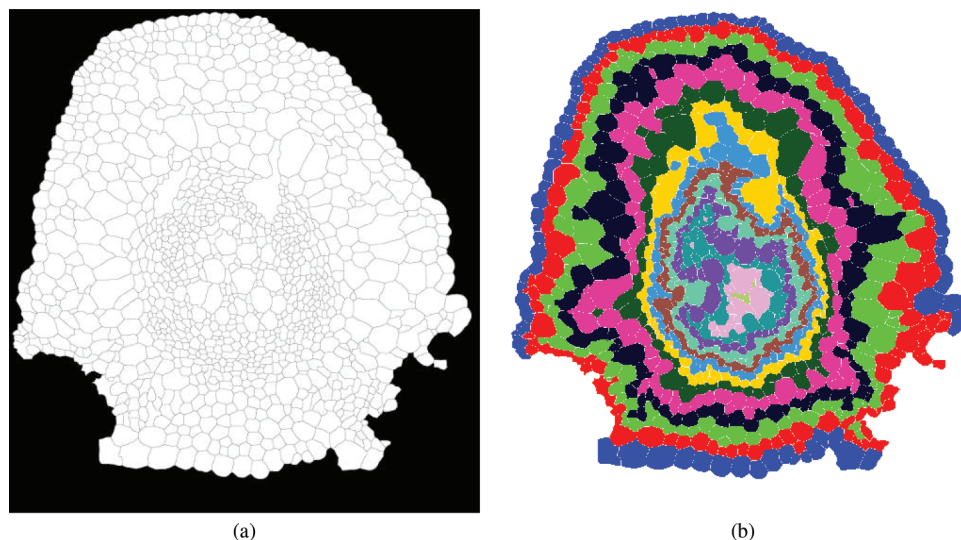


Table 2. Measure of marker image (M) computed for each virtual cell.

Perimeter	PerimEquivD
Area	EquivEllipseAr
MinR	Compactness
MaxR	Solidity
Feret	Concavity
Breadth	Convexity
CHull	Shape
CArea	RFactor
MBCRadius	ModRatio
AspRatio	Spericity
Circ	ArBBox
Roundness	Rectang
AreaEquivD	

Note: for a complete description of the measures in this table see Landini (2006, 2008).

the number of layers and the number of measures considered in each layer. To address this and produce results comparable with other classification methods, the concatenated features were submitted to a Karhunen–Loève transform (also called principal component analysis or PCA) to reduce the data dimensionality. The most significant scores were used in the next machine learning step. Figure 4 shows a simple diagram of the method described for the analysis of a leaf cross-section.

Assessment

The categorization of species was carried out by means of a learning machine method using the PCA scores as input. A cross-validation strategy was employed, where the samples were split into training and testing sets, following a 10-fold procedure. This consisted of dividing the samples into 10 subsets with similar sizes, and in

each round nine subsets were used for training and the remaining one for testing (as described in Duda and Hart 1973). The categorization was finally accomplished with linear discriminant analysis (LDA) (Duda and Hart 1973), following similar approaches used in other reported plant image analyses (Backes et al. 2009; Florindo et al. 2014).

We compared our results with those from shape-based methods previously reported in the literature, namely: multiscale fractals (Bruno et al. 2008) (using the entire multiscale curve); invariant moments (Hu, 1962) (7 moments); as well as a classification based on the measures computed over the v-cells (irrespective of the layers where they were located). Those methods were implemented following the published descriptions to analyse the contour of the v-cells. The multiscale fractal descriptors were computed on the shapes of v-cells as the object of interest, whereas the moment descriptors were averaged over all the v-cells. To make results comparable across methods in terms of descriptor numbers, the extracted feature vectors were submitted to the Karhunen–Loève transform and the first n scores (up to a maximum of 100) were used in the assessment.

Results and discussion

Table 3 shows the success rates obtained with the proposed method and other published approaches. Our method outperforms the direct use of v-cells and all the others by at least 5%, showing how the architectural analysis provides additional information on the structural morphology of leaf cross-section. We also investigated whether unbalanced sized groups of species in the database made significant differences to the results. We repeated the analysis using all the samples in the database (total $n = 96$, instead of six samples per species, $n = 60$) and found that the correct rate of identification for the

Fig. 4. [This figure is in colour on the journal Web site only: <http://www.nrcresearchpress.com/doi/suppl/10.1139/cjb-2015-0075>] A basic diagram of the proposed method. From left to right, the layered cross-section, the average measures estimated over two layers, and the resulting concatenation after Karhunen–Loève reduction.

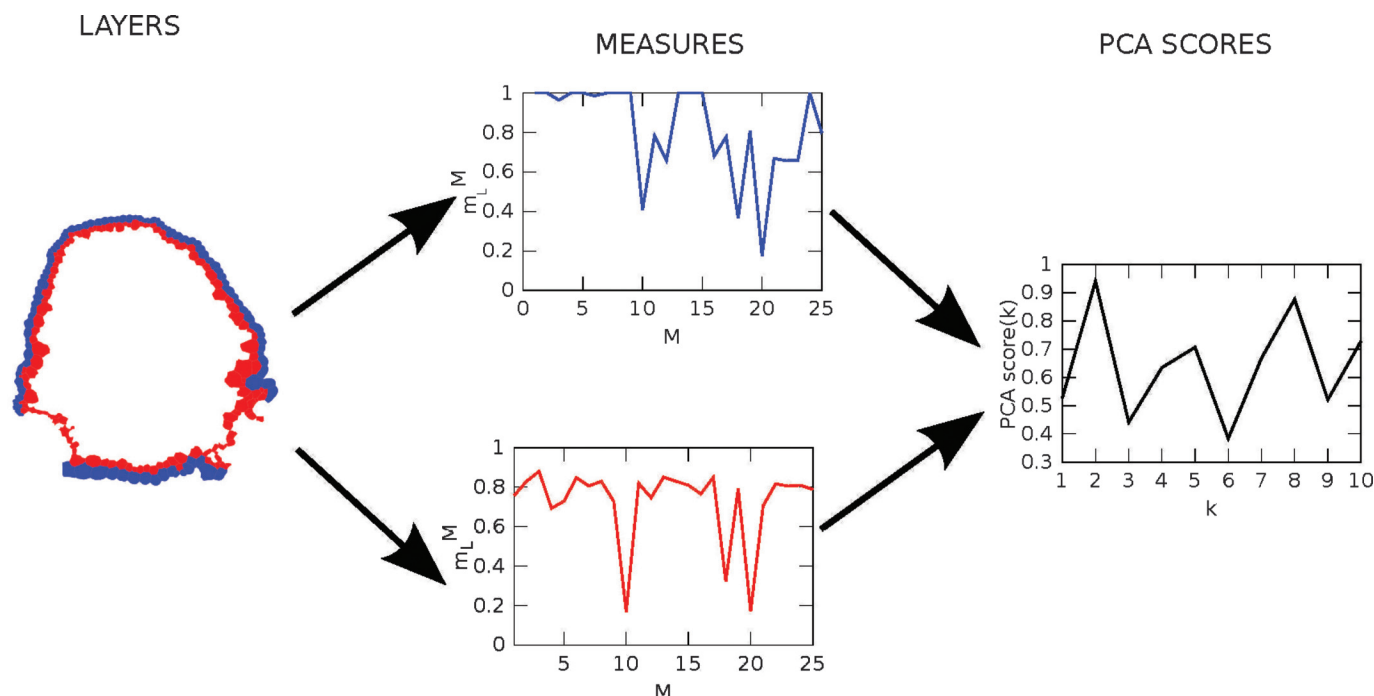


Table 3. Correctness rate obtained by the proposed method, in comparison with other shape-based approaches in the literature.

Method	Correctness rate (%)	Number of features
Invariant moments	46.7±0.2	4
Multiscale fractal	68.3±0.2	3
Cells	86.7±0.1	19
Proposed method	91.7±0.1	12

proposed method was practically the same (91.8%), and this result was also higher than for the “Cells” approach (87.3%).

The results show that the analysis of midrib architecture is useful to describe its complex structure in cross-section, and highlights the importance of microanatomy in plant taxonomy, in particular how the cellular structures are assembled following a characteristic order and pattern, which appears to be characteristic and conserved in samples of the same species, but statistically different across species.

There are still some challenges that need to be resolved to fully automate this type of analysis. Two of these are the spatial orientation of the specimen within the image frame and the identification of the midrib section (the region of interest), which currently require operator input.

Conclusions

This work presents a new type of analysis for the identification of plant species based on microscopical images

of midrib cross-sections. Instead of the classical shape-based analysis focused on leaf contours or individual cells, we have the partitioning of the midrib cross-sections into histologically relevant structures (cell and secretory structures) and their spatial organization (layers). This provided a level of description that machine learning procedures were able to exploit for the identification of plant species.

The performance of the method was tested using a database of plants from the Brazilian flora (da Silva et al. 2015) and compared with previous approaches published in the literature. Our method achieved a success rate of 91.7% over a set of 10 plant species. This is an encouraging result, which is higher than the performance of other computational approaches to this problem. Furthermore, this also gives some idea of how a layer-wise analysis can improve a shape-based analysis of this type of material, suggesting a more in-depth investigation in the future.

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