

Computer-aided identification of allergenic species of Urticaceae pollen

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Sá-Otero, M. P. de, González, A. P., Rodríguez-Damián, M. & Cernadas, E. 2004. Computer-aided identification of allergenic species of Urticaceae pollen. – Grana 43: 224–230. ISSN 0017-3134.

Plant species of the family Urticaceae have periods of extensive and overlapping flowering. Two genera *Urtica* (*U. urens* L., *U. membranacea* Poir. in Lam) and *Parietaria* (*P. judaica* L.) are abundant throughout the Iberian Peninsula. Both have substantial allergenic potential, but patient sensitivity is specific to each genus. As a result, interest from the medical profession in the identification of the pollen from these genera has arisen. It would be useful to be able to estimate the relative percentages of pollen of these genera in the air, which would allow clinicians to recommend the appropriate safety measures to patients. Unfortunately, palynologists are unable to distinguish between the species of genera (*Parietaria* and *Urtica*). Additionally, traditional methods of pollen counting are laborious and therefore not optimal for routine on-line analysis. Recent biometrical analysis enables us to discriminate between pollen species of the genera *Parietaria* and *Urtica*, but they fail to solve the problem of genera identification.

Alternative methods for counting and distinguishing genera of Urticaceae have long been awaited by medical experts among others. In this paper we present a computational system to discriminate between genera by using image analysis. It is based on the definition and computation of digital shape measures on pollen images taken by an optical microscope. The system correctly classifies more than 86% of all experiments done. A set of shape parameters are tested and the subset with the highest discrimination power includes: area (A), diameter (DM), mean distance to centroid (D_{mean}) and roundness (R) of the pollen grain.

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(Manuscript received 16 December 2002; accepted 10 February 2004)

Urticaceae grows particularly well in soils rich in nitrogen, for example, weeds in cultivated fields or abandoned farmland. Anemophilous monoecious or dioecous or polygamous plants have cymose inflorescences. They have stamens with filaments, inflexed in buds, which are elastically reflecting when pollen is shed. This anatomical characteristic facilitates the propagation of pollen in the air. In the Iberian Peninsula, seven species of Urticaceae grow wild. Some of them are of the genera: *Urtica* (*U. urens* L., *U. membranacea* Poir. in Lam.), and *Parietaria* (*P. judaica* L.). The species of these genera have periods of extensive and overlapping flowering throughout most parts of the year, in which the pollen count in the air is of special importance.

During major flowering months, copious amounts of pollen grain are transported by the wind. When they come into contact with the respiratory tract in humans and in particular on the mucous surface, pollen grains releasing proteins may produce important changes in the organism, provoking “allergic rhinitis” and “asthma type I”. The reaction is caused by the existence of a surface structure named “oncus” on the pollen grains which is a thickening of the inner layer of the pollen wall, referred to as intine, situated under apertures. Apertures are a specialized region of the sporoderm that is thinner than elsewhere on the grain and usually differs in its structure and ornamentation. (Casas et al. 1996, Seoane-Camba & Suarez-Cervera 1986). Although the *Urtica* and *Parietaria* genera are allergenic

(D’Amato & Spiekma 1992, Negrini et al. 1992, Díaz 1995), the pollen of the genus *Parietaria* is one of the most common, and also one of greater allergenic potential (Belmonte & Roure 1991, Ayuso et al. 1995, Liarte & Seras 1993).

Nevertheless, in the medical field it is possible to distinguish the sensitivity IgE-mediated to *Parietaria* or to *Urtica*, and an antigenic cross-reaction between the two genera has not been detected (Corbi et al. 1985 a, b). Since the allergenic potentials are different, the personal sensitivity is specific to each species. Thus, clinical practice has a special interest in the differentiation of the following species: *P. judaica*, *Urtica urens* and *U. membranacea*. In addition, it would be useful for clinical practice to have a count of the relative percentage of pollen of each genus in the air. If the pollen count could be systematically and periodically repeated, it would allow clinicians to recommend patients to take appropriate safety measures.

Common instruments for pollen identification are optical microscopes or SEM (scanning electron microscopy), the chosen option depends on what palynologists require. At present, palynologists identify pollen in a microscopic preparation by carefully inspecting parameters such as: shape, size, polarity, symmetry, apertures, exine stratification and sculpturing types of them. Analysis results are provided by: genus, species, family or pollen type. The

chosen level depends on how difficult it is to distinguish pollen grain morphology. Most pollen grains are determined only to genus level, and some rarely beyond family level. Determination at finer levels would be possible if sufficient parameters of size, exine structure and sculpture were measured (Stillman & Flenley 1996). Many studies on Urticaceae have been documented in palynological literature. Their main conclusion is the morphology of the pollen is quite similar and the pollen grains are small and of almost equal size (13–17 μm in *Parietaria*, 13–19 μm in *Urtica*). Both genera exhibit similar roundness and do not have excessive ornamentation of exine, which is always micro-echinate. The distribution of small echinae in *U. membranacea* is more regular. There are some apertures of type pore (3–4 porate in *Parietaria* and 3–4 zonoporate or pantoporate in *Urtica*; Sá-Otero et al. 1996). Experts are not able to distinguish these aspects using an optical microscope, however, with the use of SEM it is possible to distinguish the above features. Since SEM is so laborious and relatively costly, it is not practical to use it in systematic routine analyses. Dopazo (Dopazo et al. 2000) tried to discriminate between some species of Urticaceae using biometric studies, however, these studies were only able to discriminate between two pollen types: *Urtica*-type [*U. membranacea* (monospecific)], and *Parietaria*-type in which *U. dioica*, *U. urens* and *P. judaica* were included. Also, as a routine tool for on-line analysis, biometric analysis is also very long, costly, and imprecise and requires expert personal.

The main function of the computer vision system is the manipulation and analysis of images by computer. These systems have been widely used for industrial inspection (Yoshikawa et al. 2000), to aid in the medical diagnosis based on images (Duncan & Ayache 2000), analysis of remote sensing, etc. These systems are an innovation when applied to the automatic classification of pollen grains (Rodríguez-Damián et al. 2002, France et al. 2000) which are respectively based on pollen grain analysis shape and pollen texture analysis. The main aim of the present study will be the design and development of a computational system able to automatically identify species of Urticaceae on the basis of the digital shape of pollen grains. This study provides the basis for the future use of a computer vision system for routine on-line controls of pollen concentration in the air. There are two additional advantages: the system avoids the repetitive counting of each pollen in a microscopic preparation and secondly it discriminates among species of Urticaceae, which is not possible employing current methods. An essential part of the development work is assessing the performance of the system in an objective way. An experimental dataset was created by Duller, which does not incorporate Urticaceae (Duller et al. 1999). As a consequence we created a new experimental dataset with images of this family acquired in our lab.

MATERIAL AND METHODS

Experimental design

Microscopic preparations of every mentioned species were made from herbarium sheet and natural material. Plants were taken from

various villages in Galicia (northwestern Iberian Peninsula) (Table I). In order to preserve shape and size of the pollen, we have used acetolysed pollen (Erdtman 1960).

Optical microscope Nikon SKT connected to a Nikon 35 S photograph camera was used to take analogue photographs. The images under study are the result from 35 mm slides, and containing a vision frame enlarged 400 times, as the connected camera takes the photos bypassing the final lens close to the eye leading to the slides being taken with an amplification of 40 times (this process can be seen graphically in Fig. 1). We have selected well-defined pollens of every species (*P. judaica*, *U. urens* and *U. membranacea*) in the preparation.

Digital image acquisition of pollen grain

The 35 mm analogy slides are rastered by a commercial laser scanner with a resolution of 2048×3072 pixels and 24 color bits, these color images are transformed into grey level images with commercial software. Our computer software system consists of a graphical user interface to show these images to the palynologists, who manually select regions of interest (ROIs), each region containing an isolated pollen grain. First they establish the width and length of the region, and then click in the center of the selected

Table I. Sites where the pollen grain was picked.

Taxa	Sites
<i>Urtica membranacea</i>	Santiago (A Coruña) Monte del Castro, Vigo (Pontevedra) Baiona (Pontevedra) Canido (Pontevedra)
<i>Urtica urens</i>	As Ermitas (Ourense) Sabariz, Lobeira (Ourense)
<i>Parietaria judaica</i>	Vilagarcía (Pontevedra) Parada, Nigrán (Pontevedra) Monte del castro, Vigo (Pontevedra) Canido (Pontevedra) Baiona (Pontevedra)

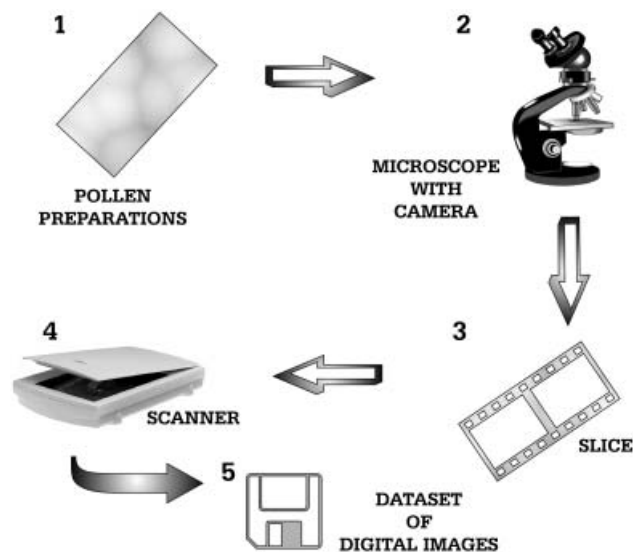


Fig. 1. Step by step the process of image acquisition.

pollen, this pollen is marked by a white box around it (Fig. 2). The computational system contains an image processing module, which processes each ROI and automatically determines the species of pollen grain it belongs to.

Image processing module

The image processing module mentioned above is composed of the following stages:

- identification of the pollen grain boundary,
- computation of shape measures and
- classification.

Firstly, the ROI containing one pollen grain is processed to obtain just the boundary of the pollen grain, this boundary is used to calculate a given shape measure, which will be used to assign the input pollen grain into a determined category (in our case, pollen of *P. judaica*, *U. urens* or *U. membranacea*) in the classification process. In the following subsections, a detailed description of each stage is presented.

Identification of pollen grain boundary

Segmentation is frequently used to separate significant regions from their background. Thresholding is the simplest and most widely used method to carry out this operation (Ritter & Wilson 1996). It is based on the theory that all pixels whose grey levels lie within a certain range, belonging to one class or region. In this case, we are interested in two regions or classes: objects and background, which respectively represents pollen grains and solution. So, one threshold value is necessary to yield these two regions. The main drawback of this type of technique is its sensitivity to speckle noise presented in the original images. So, before applying thresholding, the original image is filtered by a median filter (Petrou & Bosdogianni 2000). In this case the size of the filter used is 5×5 pixels.

The threshold value is automatically determined from the statistical characteristics of the image using the Otsu method (Otsu 1979, Ritter et al. 1996). The statistical information of the image can be graphically illustrated by its histogram (see Fig. 3), which plots the number of pixels in the image of each grey level. The Otsu method determines the optimal threshold value maximizing criterion functions, which depend on the “within-class”, “between-class” and the “total variance” of the grey levels of the image. An example of the resulting binary image after applying a segmentation

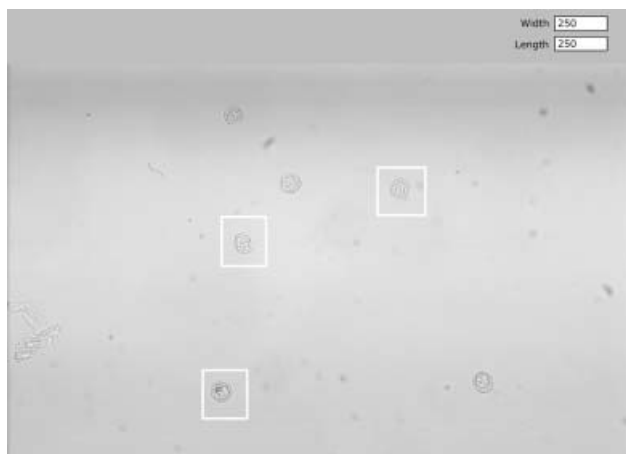


Fig. 2. Selecting ROIs with the graphical user interface.

using the threshold value calculated with this method is shown in Fig. 3.

For the purpose of eliminating any false positives presented in the resulting binary image, it is post-processed by morphological operators (Serra 1982). Mathematical morphology refers to a class of spatial filtering operations that are applied to change the shape of a binary-valued region. That is, the morphological operations replace the binary value at a single-pixel location by a value computed from the pixel values within a neighborhood of a chosen shape and size around the location. The most basic operations are erosion and dilation. Erosion is the reduction in the size of the regions of interest, which is most readily accomplished by iterative peeling-off single-pixel layers from the outer boundary of the regions of interest. Dilation is the opposite process, entailing iterative addition of single-pixel layers to the boundary of each region of interest to increase the regions size (Seul et al. 2000). In here, an open operation was applied, this operation implies that first the image was eroded and then dilated; matrix size used is 3×3 pixels. This operation is applied to all pixels in the image. The eroding and dilating operations are also used to attain the boundary of the pollen grain, since the result from the subtraction of the eroded image from the dilated image is the border of the pollen grain. An example of the border obtained with these operations is shown in Fig. 4 in the column labeled “After segmentation”. In here we may observe that the resulting border is thick. A thinner border is achieved by applying the algorithm proposed by Pavlidis (1982). The final border is shown in the third column labeled “After thinning” in Fig. 4. This figure shows graphically the treatment of ROIs from the original image displayed in the column named “Input image” to final image ready to be measured in the next module.

Shape features

Once the external boundary of the pollen grain has been detected, we compute measurements, which describe the digital shape of the pollen grain. With regard to writing done on computer vision, many approaches have been proposed to object classification by shape analysis (Fountoura & Marcondes 2001). Our initial selection of the shape features was done taking into account the morphological characteristics employed by palynologists to distinguish pollen grains. The shape features and their definitions used in this study are explained in Table II. The column named “Centroide” (Fig. 4) graphically illustrates the shape features area and centroid. The area of pollen grain is the black filled ball in the image and the pollen centroid is represented by a small white dot, the centroid corresponding to the mass center of the pollen.

Classification

Once the values of all features have been calculated, the next step is how to assign each image (pollen grain) to a pre-established class or type (*U. membranacea*, *U. urens*, or *P. judaica*). This process was done using a classifier. Minimum distance classifier is the simplest one described in the written studies (Duda et al. 2001). It works as follows: Let M be the number of classes (in this case three classes), and N the number of images (pollen grains). Let $X^i = [x_1^i, \dots, x_p^i]$ be the feature vector (measures of every pollen) of p elements that represents the image or pollen i . We use the Mahalanobis distance D_j to calculate the similarity between an image and the class prototypes denoted as:

$$D_j = (X^i - m_j)^T \Sigma^{-1} (X^i - m_j) \text{ for } j = 1, \dots, M$$

Where Σ is the covariance matrix of the training set and m_j is the

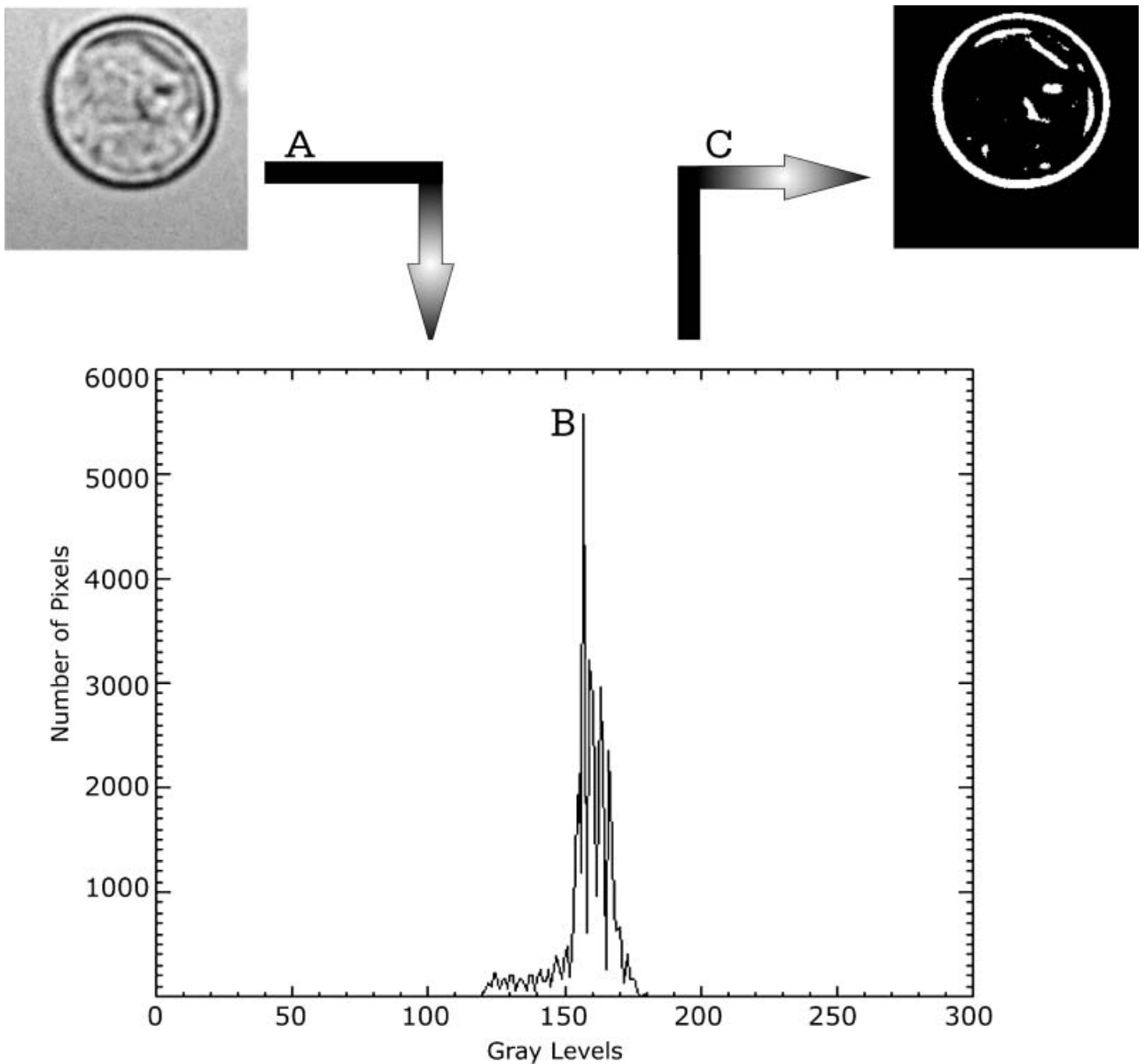


Fig. 3. ROI contained a pollen grain (A). Histogram of a (B). Binary image resulting after thresholding of a (C).

mean class prototype of class j . The mean class prototype is calculated taking the mean vector of each class in the training set.

We assume the same covariance matrix for all classes. The training set is constructed using $N-1$ (i.e. leave-one-image-out approach) images and the test is carried out using the excluded image. If this image is correctly classified, a hit is counted. The process is repeated N times, each time excluding a different image in the dataset (Duda et al. 2001).

RESULTS AND DISCUSSION

Computational System

To evaluate our computational system we tested the performance of the system under optimal conditions. As test cases, we used a set of 30 images containing ten pollen grains of each type (*P. judaica*, *U. membranacea* and *U.*

urens). Using all the shape features described in Table II the percentage pollen grains correctly classified is 73.33%. Although system performance is higher than random classification (33.33%), it is rather low for an acceptable pollen discrimination system. The result may be due to the fact that some of the shape features could have meaningless classification capabilities or do not improve overall system performance due to correlation among them.

An important issue for a better pollen classification is how to select the best features to choose between the different species of pollen. If an optimal subset of s features were searched, all possible combination of s features out of the p available would be formed. This is a large number even for quite small number of s and p . In practice, other suboptimal searching techniques are suggested like the FSM (Floating Search Method; Pudil & Kittler 1994). We consider for the

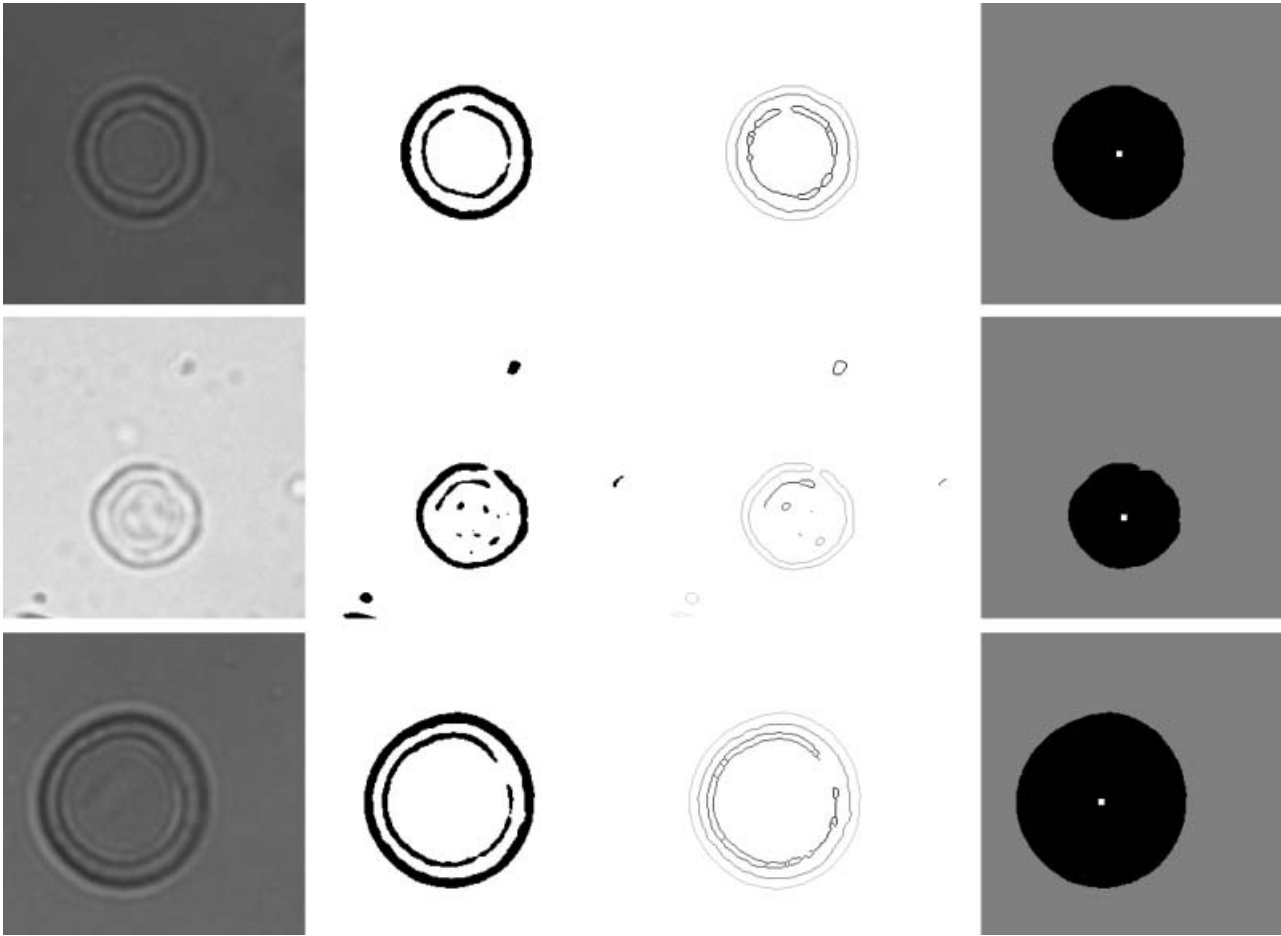


Fig. 4. The processing applied to original image until is ready to compute shape features. (*Urtica membranacea* – upper row; *U. urens* – middle row; *Parietaria judaica* – lower row) .

Table II. Definition and description of digital shape features.

Shape features	Description
Area (A)	Number of pixels covered by the pollen
Perimeter (P)	Number of pixels on the pollen boundary
Roundness (R)	Relation between the squared perimeter and area: $4\pi A/ P^2$.
Centroide (CT)	Center of gravity
Mean Distance to centroide (D_{mean})	Average distance between CT and pollen boundary
Maximum Distance to centroide (D_{max})	Largest distance between CT and pollen boundary
Minimum Distance to centroide (D_{min})	Smallest distance between CT and pollen boundary
Rate of change (R1)	Maximum Distance to centroide divided by Minimum Distance to centroide: $(D_{max})/(D_{min})$
Rate of change (R2)	Maximum Distance to centroide divided by Mean Distance to centroide: $(D_{max})/(D_{mean})$
Rate of change (R3)	Minimum Distance to centroide divided by Mean Distance to centroide: $(D_{min})/(D_{mean})$
Diameter (DM)	Largest distance between two points on the pollen boundary
Holes (H)	Sum of differences between D_{max} and the distance between CT and pollen boundary
Boundary roughness (BR)	Border points are adjusted to a normal distribution: height, center, and width. The rate height /width is BR.
Radius Dispersion(SD)	Standard deviation of distances between CT and pollen boundary

system’s sensitivity that the rate of correct classification is based on the Mahalanobis distance with a leaving-one-image-out approach. Because normal FSM can be trapped in cycles, we additionally stop the search after three times the number of features. The percentage of correct pollen classification using this searching approach is 86.66%. The

best subset includes the following pollen shape features: area (A), diameter (DM), mean Distance to centroide (D_{mean}) and roundness (R).

A detailed performance of the system is shown in Table III, which contains information about actual and predicted class done by means of our system for every class. The column labeled success signifies the percentage of correctly classified grain of pollens, whereas the column labeled error signifies the percentage of misclassifications. The system behavior is quite uniform for every class. It correctly classifies the 100% of *U. membranacea*, and only misclassifies the 20% of the other two pollen types.

Feasibility of pollen-on-line-analysis

The results presented in the above subsection show the efficiency of a computer vision approach (86.66% of correct pollen classification). However, we believe that further evaluations of the method should be carried out with a wider and more realistic number of pollens in order to demonstrate its feasibility to work on-line in routine analysis. So a new experiment was carried out taking an equal number of photographs from each species (*U. membranacea*, *U. urens*, and *P. judaica*). Nevertheless, the concentration of pollen grains by photographs was different among the species under study. The experts have marked over the digital photographs 100 pollens in the next proportions: 69 *U. membranacea*, 9 *U. urens* and 13 *P. judaica*. Some of this pollen is not in optimal conditions for identification as it can be seen by comparing Fig. 3 (optimal pollens) and Fig. 4. The percentage of correct classification using the subset of features chosen previously (Area (A), Diameter (DM), Mean Distance to centroide (D_{mean}) and Roundness (R)) is 90%. This score (90%) is even higher than the one obtained using optimal pollens (86.6%). We believe that this score is produced because of the fact that the pollen categories are equal in number in the first experiment, whereas the number of pollen per category in the second experiment is asymmetric. A detailed performance of the

system using realistic dataset is shown in Table IV. In the species *U. membranacea* the percentage of correct classification is 98.5%, but in the other two *U. urens* and *P. judaica* the percentages are lower: 55.5% and 77.2% respectively.

Comparing Tables III and IV some conclusions can be derived: our digital approach discriminates the species *U. membranacea* from *P. judaica* and *U. urens* satisfactorily. This result ties with biometrical analysis done by Dopazo (Dopazo et al. 2000). *U. urens* and *P. judaica* pollens were correctly classified at nearly 80%. We believe that lower percentage in Table IV for *U. urens* species (55.5%) may be due to the limited number of *U. urens*, only nine pollens in relation with the number of pollens of the other species in the dataset.

In conclusion, the potential for the use of computer vision for discriminating species (*U. membranacea*, *U. urens*, and *P. judaica*) of Urticaceae was demonstrated. These results may be seen as encouraging for the use of this methodology for automatic discrimination of pollen grains.

CONCLUSIONS AND FUTURE WORK

An innovative methodology to discriminate Urticaceae species (*U. membranacea*, *U. urens* and *P. judaica*) using computer vision has been put forward. This approach is based on the definition of digital shape parameters to represent a pollen grain. The system performance has more than an 86% of success rate for all experiments done. The set of pollen measures which offer the best discrimination results includes the following: Area (A), Diameter (DM), Mean Distance to centroide (D_{mean}) and Roundness (R).

These initial results are very encouraging and suggest further evaluation (other species, a wider number of pollen by species, other recollection places, etc.), although the detection of isolated pollen still needs to be automated in our prototype. This approach provides the basis for the future development of systems to be used in routine on-line pollen counting, with important repercussions in allergenic treatment in medicine.

Table III. Percentage of correct classification within every class in the well-defined dataset of pollens (30 pollens).

Taxa	Predicted pollen (%)			Success (%)	Error (%)
	<i>Urtica membranacea</i>	<i>Urtica urens</i>	<i>Parietaria judaica</i>		
Observed Pollen (%)					
<i>Urtica membranacea</i>	100	0.0	0.0	100	0.0
<i>Urtica urens</i>	10	80	10	80	20.0
<i>Parietaria judaica</i>	10	10	80	80	20.0

Table IV. Percentage of correct classification within every class in the extended dataset of pollens (100 pollens).

Taxa	Predicted pollen (%)			Success (%)	Error (%)
	<i>Urtica membranacea</i>	<i>Urtica urens</i>	<i>Parietaria judaica</i>		
Observed pollen (%)					
<i>Urtica membranacea</i>	98.5	0.0	1.4	98.5	1.4
<i>Urtica urens</i>	11.1	55.5	33.3	55.5	44.4
<i>Parietaria judaica</i>	4.5	18.1	77.2	77.2	22.7

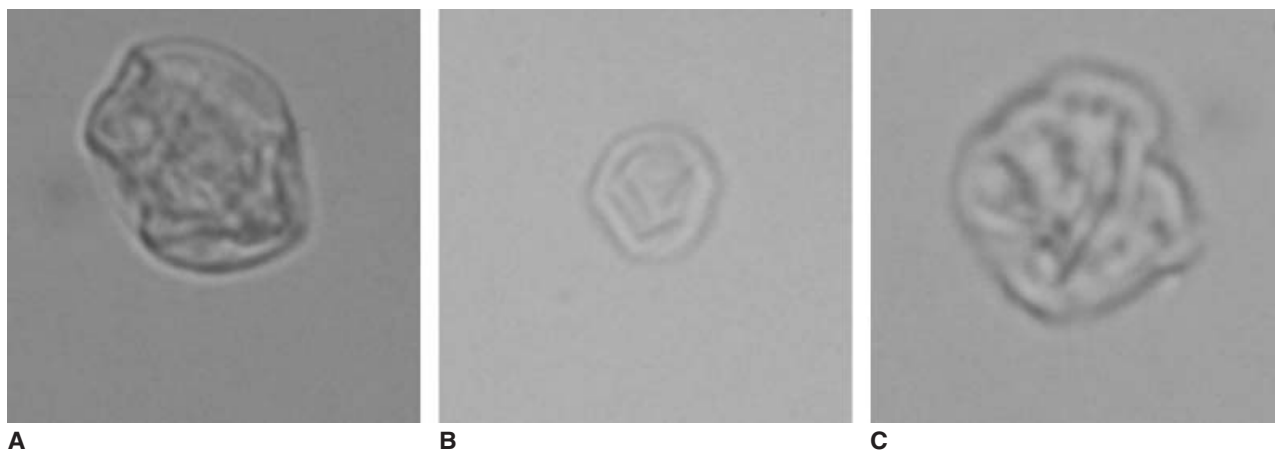


Fig. 5. Examples of pollen in the complete dataset: A. *Parietaria judaica*; B. *Urtica membranacea*; C. *U. urens*.

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