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Automatic pollen recognition using convolutional neural networks: The case of the main pollens present in Spanish citrus and rosemary honey

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ABSTRACT

The automation of honey pollen visual sorting overcomes the limitations of the conventional procedure helping the specialist in this time-consuming task. In this work, a novel and comprehensive Ground Truth of almost 19,000 images (from optical microscopy) of the 16 most abundant types of grains/pollen particles present in citrus and rosemary honey from Spain was constructed. This task was assisted by a HoneyApp (also developed herein) for the labelling and annotation process. Subsequently, the effectiveness of different pre-existing automatic pollen recognizers based on convolutional neural networks (CNN) (VGG16, VGG19, InceptionV3, Xception, ResNet50, DenseNet201, MobileNetV2 and EfficientNetV2M) was tested together with a new network proposed in this paper (PolleNetV1). The extreme complexity of those pre-existing CNN and extensive use of millions of parameters makes this new proposal especially promising. Although with a slightly lower accuracy (average 96%) in determining the relative frequencies of different types of pollen grains/particles, it has considerable advantages such as simplicity and ability to be included in the future functionality to automate pollen recognition in honey. This is the first step to finally achieving an objective tool that allows the correct labelling of any types of pollen in honey, thus contributing to its transparency in the market.

1. Introduction

In beekeeping, monofloral honey is especially valuable due to its commercial importance. Several analytical methods have been used to verify the monoflorality, such as GC-MS or HPLC-MS, both of which applicable in the identification of specific compounds that can be attributed to certain botanical species (Escriche et al., 2023a; Matkovits et al., 2023). However, the most traditional technique used in this sector to classify a honey as monofloral is through the optical microscopy analysis of the pollen grains present in a sample, since bees impregnate themselves with pollen when they collect nectar from the flowers. This melissopalynological method is based on identifying and quantifying the

pollen grains morphology of the different botanical species present in a honey sample. To this end, highly skilled analysts are required, although there is an evident lack of experimented professionals in this field (Escriche et al., 2023b). The first step in this laborious and time-consuming process by using a microscope, find at least 500 grains of pollen in the dried honey sediment. Subsequently, the morphology of each grain is identified and given to a certain botanical species. Finally, if the percentage (which differs depending on the species) of the most abundant type of pollen is enough compared to the total pollen present in the slide, the analyst can assign a specific monoflorality to the sample (Louveaux et al., 1978). However, this type of analysis proves difficult in certain monofloral honeys since its pollen is underrepresented due to the

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low quantity of pollen present in their flowers (Juan-Borrás et al., 2015). This conventional method, based on the visual inspection and pollen counting, is slow, complex and forces the analyst (despite being an expert in this field) to give real-time results of what was observed in the microscope. Consequently, these results, in part influenced by personal pressure, are subject to the intrinsic variability of the manual method currently being applied (Escriche et al., 2023b). Therefore, if an objective technical information is made available, this problem could be minimized, preventing an incorrect labelling of a monofloral honey (Thrasyvoulou et al., 2018). In this regard, the Directive 2014/63/EU related to honey outlines the need to establish appropriate objective analytical methods for ensuring the veracity of the information provided to the consumer.

A possible option to overcome the limitations of the manual procedure could be to have an automated tool based on a computer image analysis facilitating the identification and classification of the pollen grains present in a honey, and subsequently its attribution as monofloral. Over the last years, the automated pollen grain analysis and classification in different areas such as palynology, aerobiology and melissopalynology, has evolved supported by the significant increase in computational capacities and the development of Deep Learning techniques (Daood et al., 2016; del Pozo-Banos et al., 2015; Holt and Bennett, 2014; Sevillano and Aznarte, 2018). The initial feature-based approach for grain classification related to the pollen morphology has been eclipsed by a new tool based on a deep sequence of convolutional layers, which after training determines and extracts features by itself, rather than being defined by human specialists. These kinds of models, named Convolutional Neural Network (CNN) or ConvNets, were firstly introduced in the context of the ImageNet Large Scale Visual Recognition Challenge (ILSVRC) (Russakovsky et al., 2015). Different types of CNN networks have been developed in recent years related to the advanced classification of pollen grains as as a result are being applied in different fields of knowledge (ImageNet.org, 2021); for instance, in aerobiological/allergenic pollens (Battiato et al., 2021; Khanzhina et al., 2018), or for the botanical origin classification with hyperspectral imaging of New Zealand honey (Zhang and Abdulla, 2022).

In any case, for any cataloguing approaches for the pollen grains and for the different areas of interest (palynology, aerobiology and melissopalynology), there is a requirement for the construction of the corresponding pollen images datasets obtained using light microscope and properly categorized by experts. In palynology, two different pollen datasets of flowers from Central America stand out. The first, created by Travieso et al. (2011) contained 564 colour images from 47 botanical species. These authors considered morphological details of the pollen grains contour as discriminative features and applied classical computer vision techniques (Hidden Markov Models and a Support Vector Machine classifier) obtaining a mean accuracy of 93.8%. The second was built by García et al. (2012), with 426 images of 17 pollen grain types, relied on contour analysis of pollen grains achieved a higher average precision of 98.8%. Most recently, Astolfi et al. (2020) developed the POLEN73S dataset (2523 images) taking pollens directly from the flowers collected in the Brazilian Savannah. With an approach based on eight pre-trained ImageNet CNNs, these authors classified up to 73 different types of pollens, achieving the best accuracy with the Dense-Net201 (95.7%) and ResNet50 (94.0%) networks. In the aerobiology area (especially for allergy prevention), it is noteworthy the recent dataset POLLEN13K with 13,000 images in combination to the Pollen Grain Classification Challenge 2020, reached an accuracy of 97% (Battiato et al., 2021). With respect to the field of melissopalynology, the datasets of pollens grains from honey samples, are very scarce, despite being of paramount importance as a prerequisite for developing automated pollen classification. The first dataset of this type (POLEN23E) was obtained using honeys from Mato Grosso state in Brazil (Goncalves et al. 2016). It was built with 805 images from 23 different pollen types annotated by an expert botanist using a light microscope and Corel-Draw® software. These authors with classical methods: CST (Color -

Shape - Texture), BoW (Bag of Words) and a combination CST+BOW, achieved a low performance 68% in pollen grains classification. Sevillano and Aznarte (2018) improved the pollen classification accuracy, reporting much better results than the previous non-CNN based approaches. More recently, Tsiknakis et al. (2022) reported the Cretan pollen dataset, containing 4034 images from 20 different pollen species commonly found in Cretan honeys (Pefkothymaromelo Kritis PDO). They obtained the best accuracy result (97%) using a CNN suite with Xception, Inception and ResNet50. In the present year Mahmood et al. (2023) proposed a new CNN, called APFA-Net, with good results, but using the datasets aforementioned: POLLEN73S, POLLEN23E, and Cretan Pollen datasets.

The scarcity of information about datasets of pollen images obtained from honey samples and the automation for identifying pollen grains in honey is a fact. Hence, the aim of this work is twofold: firstly, to build a dataset of pollen images obtained (following the melissopalynological conventional procedure) using a labelling and annotation tool developed in this work; and secondly, to test the effectiveness of different preexisting and a new CNN-based automatic pollen recognizers in determining the relative frequencies of different types of pollen grains. This work focuses solely on the most abundant types of pollen present in Spanish citrus and rosemary honeys as a starting point for further developments of this recognizer in other types of pollen and honey.

2. Material and methods

2.1. Honey samples and classical pollen analysis

Forty monofloral honey samples (20 of each citrus and rosemary), collected in 2021, were used in the present study, which were provided by: Different beekeepers from *Melazahar Cooperativa Apícola* (Valencian Region); experimental beehives located in citrus fields property of *Sant Vicent Ferrer de Benaguasil Cooperativa* in Pedralba (Valencian Region) and Ministerio de Agricultura, Pesca y Alimentación (MAPA) from Valencian and Andalusia regions. Only those honeys previously analysed under the microscope and that met the monofloral requirements (minimums of 10% *Citrus* sp. pollen grains for citrus honey and 10% *Rosmarinus officinalis* for rosemary honey, in comparison with the total observed) were considered valid honey samples and subsequently images of their pollens were considered for this study (Saenz-Laín and Gómez-Ferreras, 2000; DOGV, 2002).

The palynologic analysis of each honey sample was performed in the Laboratory of honey at Universitat Politècnica de València (LABMIEL). This laboratory is accredited in this methodology as per ISO 17025 (ISO/IEC 17025:2017, 2017). In addition, LABMIEL annually takes part in all available Proficiency Tests Schemes specifically designed for pollen and other quality parameters analysis in honey such as BIPEA (Bureau Interprofessionel d'Etudes Analytiques, Gennevilliers, France-Proficiency Testing Scheme; http://www.bipea.org).

The pollen analysis was carried out as described by Escriche et al., (2023b). The slides were imaged at \times 40 magnification by means of a light optical microscope (Zeiss Axiolab, Göttingen, Germany) coupled with a digital camera Axiocam 305 color, Zeiss of 2454×2056×24 bits resolution (true color) and dark field LED. To allow repeatability of image acquisition, each slide was placed in a microscope X-Y positioner. Through the associated capture software (ZEN Module Tiles and Positions), a mosaic of positions (tile regions) was defined to which the positioner will move to acquire each image, thus sweeping the main field of view of the sample holder. All the captured information was saved in a configuration file (xml) that allows repeating the same acquisition if necessary. Initially, a 10 \times 10 image mosaic was used for each slide, yielding a total of 100 images of each slide. If required, images of multiple sample holders could be created. An expert technician in pollen cataloguing according to its morphology and visual appearance inspected each honey image (PalDat, 2022; Saenz-Laín and Gómez-Ferreras, 2000).

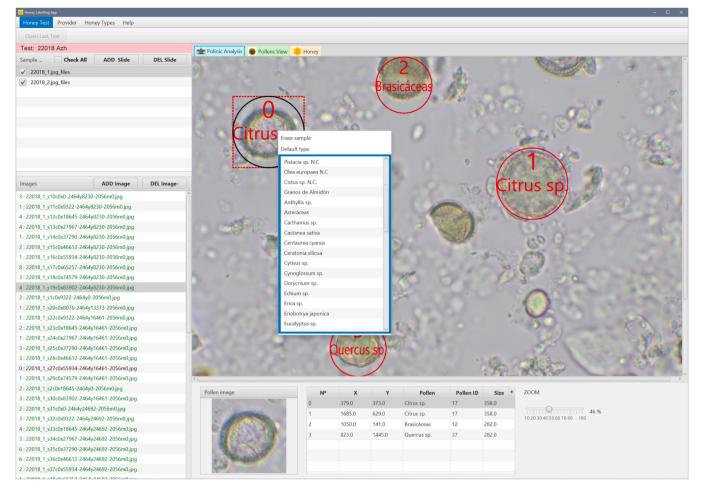


Fig. 1. Analysis-View: main window of the HoneyApp application, where a typical image of honey acquired with the optical microscope is shown. The red circles display the pollen markers entered by the user. The drop-down menu shows the list of pollen/particle types that the user can choose.

2.2. Pre-existing convolutional neural networks used

Eight pre-existing CNN networks, most of them from the ImageNet Challenge, were selected in this work as adequate representatives of the networks developed in recent years related to the advanced classification of pollen grains (Battiato et al. 2021). Six of them (VGG16, VGG19, InceptionV3, Xception, ResNet50, and DenseNet201), are among the most successful reported networks, and the others (MobileNetV2 and EfficientNetV2M), are the result of optimization processes in training speed, memory usage or CPU power. Table S1 shows important information about the details of architecture levels and complexity of these networks. In addition, this table includes references where the data can be supplemented. Furthermore, to these pre-existing CNN networks, in this work a new one (PolleNetV1) was developed whose description and functionality is shown in Section 3.2.

2.3. Evaluation metrics

Accuracy (also called Correct Classification Rate-CCR) was used as the metric for evaluating classification models, since it is the most common related classification problems. However, for imbalanced classification, with different number of samples per class, as is the case in this study, other metrics have been reported in the literature, such as those proposed by Battiato et al. (2021) in the Pollen Grain Classification Challenge 2020: Precision, Recall, and F1-Score. The latter (used in the present study) is the harmonic mean of Precision and Recall; however, the classic F1-Score was modified to account for label imbalance and calculate the weighted F1 score that averages the F1 with the number of instances of that class in the dataset. In these cases, the closer the value is to 1, the better the classifier.

The Matthews Correlation Coefficient (MCC) has also been computed; a metric widely used in bioinformatics and machine learning, which is in essence a correlation coefficient between observed and predicted binary classification. This coefficient is useful even if the classes are imbalanced. MCC takes possible values between +1 and -1: a coefficient of +1 represents a perfect prediction, 0 no better than random prediction and -1 indicates total disagreement between prediction and observation.

What is interesting about this coefficient is that it has a generalization to the multi-class case. This MCC value is called the RK statistic and is defined using a K x K confusion matrix C, where K represents the number of classes, and can be computed as showed in Eq. 1:

$$MCC = \frac{c \bullet s - \vec{t} \bullet \vec{p}}{\sqrt{s^2 - \vec{p} \bullet \vec{p}} \bullet \sqrt{s^2 - \vec{t} \bullet \vec{t}}}$$
(1)

where:

 \vec{t} : Row vector of matrix C. t_k is the number of times class k occurred. \vec{p} : Column vector of matrix C. p_k is the number of times class k was predicted.

c: Sum of the diagonal of matrix C. Total number of samples correctly predicted.

s: Sum of the entire matrix C. Total number of samples.

The MCC coefficient is more informative in imbalanced multiclass cases because it considers the size of each class in the confusion matrix in their final score computation, whereas the accuracy is Accuracy = c/s.

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Fig. 2. Pollens-View of the HoneyApp: A mosaic appears with the pollens of the selected type. In this visualization, the errors can be detected and corrected. This is the case for the pollen indicated by the arrow, classified as Brassicaceae rather than Asteraceae.

Table 1

Different classes considered in the dataset (type of pollen and particles), as well as the number of samples labelled in each case. The two main pollen types (from citrus and rosemary honeys) appear in bold letters.

Class	Name	Number	Class	Name	Number
Type_0	background	870	Type_30	Leguminoseae and others	174
Type_3	bubble	137	Type_33	Onobrychis sp.	1431
Type_7	Olea europaea	1579	Type_36	Prunus dulcis	254
Type_8	Cistus sp.	962	Type_37	Quercus sp.	1225
Type_9	starch	1014	Type_40	Rosmarinus officinalis	1400
Type_12	Brassicaceae	4919	Type_43	Thymus sp.	897
Type_17	Citrus sp.	1093	Type_47	Taraxacum type	352
Type_21	Echium sp.	1580	Type_50	Umbelliferae	977

3. Results and discussion

3.1. Honey image labelling and annotation using our own tool HoneyApp

A variety of free image annotation and labelling tools are widely available on the market; however, a tool that considers specific characteristics more adapted to the field of melliferous pollen analysis has been developed by the authors, at the Universitat Politecnica de Valencia, referred to as the HoneyApp. In addition to labelling and annotating the images, it was also developed with the aim of forming the core of the newly introduced automated pollen recognizer.

HoneyApp is a desktop application developed in Java, with JavaFx, JavaXB plugins and OpenCV library. It consists of a root folder where the files of the honey images captured in the microscope are contained. An XML file is also created with complete information of labels and annotations and the images of the target pollens and particles.

In the main window there are, for now, two views: (1) Analysis-View and (2) Pollens-View. The Analysis-View is where the user observes all the honey images captured (an example is shown in Fig. 1). The user can select (with the mouse) a pollen or particle, adjusting the size with a circle marker. Then, from a context menu, the label that corresponds to the pollen being observed is selected, leaving this information stored in the XML file that defines the test. The second window is the Pollens-View, which is shown in Fig. 2. In this view, a mosaic is displayed with the images of all the pollens labelled up to that moment and it is possible to select only those of a specific type of pollen. This allows the technician to check for possible errors, since having a global view helps to detect a previous incorrect labelling and therefore immediate

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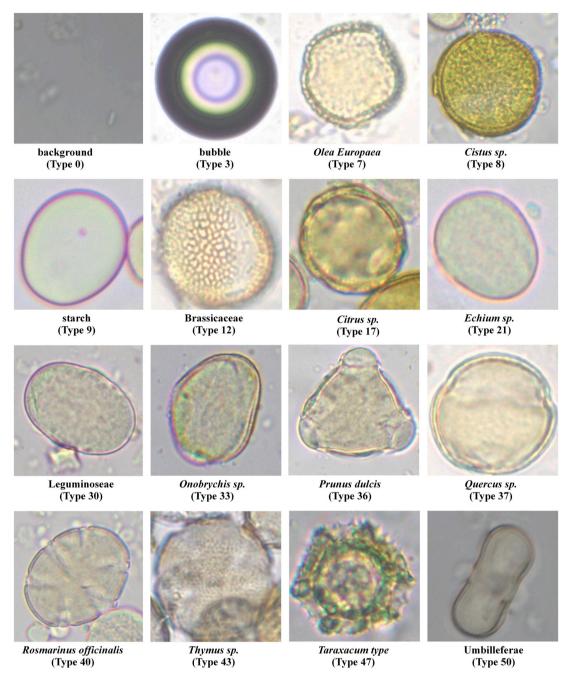


Fig. 3. Image examples of the 16 pollen and element types identified in citrus and rosemary honeys: Type_0 (background), Type_3 (bubble), Type_7 (*Olea europaea*), Type_8 (*Cistus* sp.), Type_9 (starch), Type_12 (Brassicaceae), Type_17 (*Citrus* sp.), Type_21 (*Echium* sp.), Type_30 (Leguminoseae), Type_33 (*Onobrychis* sp.), Type_36 (*Prunus dulcis*), Type_37 (*Quercus* sp.), Type_40 (*Rosmarinus officinalis*), Type_43 (*Thymus* sp.); Type_47 (*Taraxacum* type), Type_50 (Umbelliferae).

correction is possible. In the near future, there will be some other views, such a Classification-View that will show the result of applying the best CNN networks developed here; and a Statistics-View that will summarize the statistics/frequency of the labelled pollens and will assign, according to previously defined rules, the possible type of monoflorality of the analysed honey. This will only be possible when our tool learned to recognize all the pollen present in those honeys.

3.2. Pollen dataset creation

From each of the 40 honey samples (20 of each citrus and rosemary) used in this study, 100 images of the field observed through the optical microscope were obtained, following the procedure outlined in section

2.1. The total number of images was approximately 4000 since the invalid ones were discarded. Using the HoneyApp, the LABMIEL expert technician labelled and annotated the different pollen grains and elements present in each image. In this way, the Ground Truth dataset used in this work was created. In this dataset each botanical species of pollen, each element observed and even the background of these images was classified as a "Type". Table 1 lists the 16 "Types" used in this study, since these are the most abundant pollens present in the honeys here considered (citrus and rosemary honeys). Among the related pollens appear both the target main pollens (*Citrus* sp. and *Rosmarinus officinalis*, highlighted in bold in Table 1), since they define the monoflorality of the honeys considered herein, and other 11 accompanying pollens that come from other plants also visited by the bees in the area where these

Table 2

Mean accuracy and variance of the used Networks, from 5 training prediction experiments, with and without data augmentation.

Networks	No Data Augme	ntation	Data Augmentation		
	Mean Accuracy (%)	Variance (%)	Mean Accuracy (%)	Variance (%)	
VGG16	96.30	0.381	97.10	0.232	
VGG19	96.30	0.870	97.11	0.132	
InceptionV3	97.06	0.160	97.99	0.037	
Xception	96.34	0.033	97.52	0.052	
ResNet50	96.69	0.062	97.70	0.283	
DenseNet201	97.69	0.067	97.78	0.256	
MobileNetV2	96.93	0.058	96.13	0.368	
EfficientNetV2M	96.96	0.047	97.59	0.105	
PolleNetV1	83.09	1.033	95.66	0.130	

honeys are harvested. This table shows the different classes considered (type of pollen or element), as well as the number of samples labelled in each case, that constitute the Ground Truth dataset. As examples, Type_17 represents Citrus sp. pollen, appearing 1093 times, and Type_40 Rosmarinus officinalis, appearing 1400 times into the microscope images. All of them were labelled by lab experts using the aforementioned HoneyApp. Likewise, Type_0 for the "background" with 870 samples and Type_3 for air "bubbles" with 137 samples have been included. Both items (Type_0 and Type_3) are not pollen grains, and therefore not be marked by a lab technician as they would not make any contribution to the pollen analysis. Nevertheless, they have also been considered following the recommendations of the Pollen Grain Classification Challenge 2020 (Battiato et al., 2021), since their identification can avoid a misclassification of pollens. Also, the "starch" class (Type 9), with 1014 samples, have also been included since this type can allow for the detection of honey contamination. Finally, the Type_30 has been labelled as "Leguminosae and others", resembling the Unknown Class, as it includes a variety of pollens scarcely present in the honey content. For this reason, only 174 samples of this type have been identified.

In total, the Ground Truth dataset was composed of 18,864 images of elements, corresponding to 16 different classes. Fig. 3 shows an example of each pollen type and other particles considered.

It is noteworthy that the scattered numbering of the 16 "Types" listed in Table 1 were extracted from a larger dataset being developed (as part of a more extensive research) with more than 50 different types of pollens present in other honeys. The original numbering was kept so as not to cause confusion with other works. Another detail to highlight in this table is the non-uniformity in the number of samples for each class (imbalanced class distortion). This is because they are exactly the types of pollen that appeared in the honey samples considered; just as any expert who carried out the pollen analysis would obtain them.

3.3. Performance of pre-existing used networks

The eight "pre-existing networks" were used with five different image datasets built randomly from the Ground Truth pollen dataset created, in a 5-fold cross-validation method (Hastie et al., 2009). Each dataset contained 80% of the images for training, 10% for validation and 10% for testing. The use of training and validation sets (for the training stage) made it possible to avoid overfitting, reducing the difference between the training error and the validation error. In the prediction stage, the test data set containing new or unseen data was submitted to the trained model to carry out the predictions. If the prediction error is low, the model positively generalizes new data. Thus, a total of 5 training-prediction experiments per network were carried out with the averaged accuracy, as a measure of observational error, and the variance as a measure of model precision (Table 2).

The standard use of these networks is through the 'Transfer Learning' technique. It consists in re-using these deep networks by training them with a new dataset, modifying the input and output stages. All these networks are overparametrized, containing millions of coefficients in their convolutional stage filters, and all of them have already been learned in ImageNet Challenge through millions of real-life images. Transfer learning implies that many of these coefficients are frozen in the early stages, most related with low level image details, like edges, circles, corners, or blob colours. The rest of stages are zeroed or initialized randomly, and then learned with the high-level details of the new dataset (Fine-Tuning). Nevertheless, in this study transfer learning was not used in its strictest sense. Some initial experiments were performed to determine the appropriate number of stages needed to freeze the coefficients, but the best results were obtained without freezing. This was probably because the pollen images have quite different pixel details than the images used in the ImageNet Challenge (dogs, cats, cars and houses). Thus, a Fine-Tuning was performed on all the layers, without freezing coefficients of any stage. The network was trained using as initial coefficients those obtained for pre-existing networks in ImageNet (Chollet, 2018).

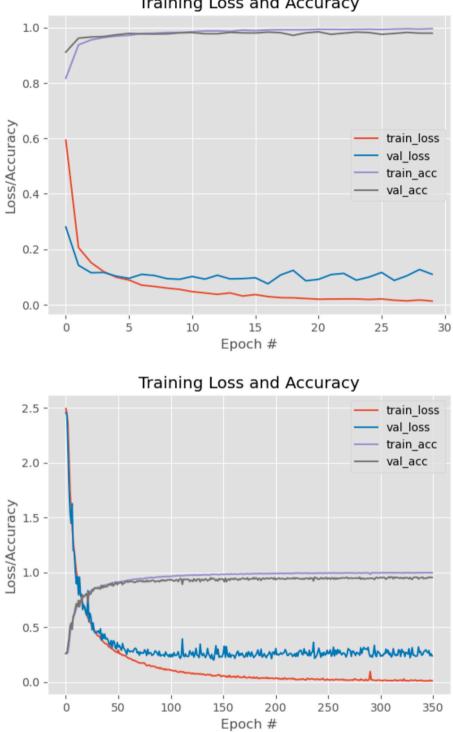
Regarding the hyperparameters of the CNN models, in addition to those defined by the respective authors, in the present work the following have been selected: 30 epochs (during the training stage was sufficient, since from pre-trained initial coefficients the network converges more quickly); learning rate of 0.005; Stochastic Gradient Descent optimizer; batch size of 32; loss function Categorical Cross-Entropy (measured how well the network modelled the training data).

In the training experiments a *Data Augmentation* technique was used. This technique consisted in applying some affine transformations (rotation, translation and reflection) to original images to increase their number during the training. The results shown in Table 2 include the mean and variance of the accuracy obtained in the test stage of each network, trained with and without data augmentation. Results obtained by data magnification include 18° rotation range, 10% X-Y translation range, 10% reflection, and 10% zoom. It is evident that this technique allowed a notable increase in the rate of correct classification, therefore the results are considered below.

A total of 80 experiments were carried out, five without and five with data augmentation, for each one of eight CNNs. From bias-variance trade-off in machine learning, the results shown in Table 2 indicate that most of the considered pretrained CNNs have achieved low bias error (high precision) and low variance. This means that they have all succeeded in capturing the essential features of the 16 pollen types/ particles in the proposed pollen dataset. The small errors detected was mainly due to the similarity between certain types of pollen. In addition, it must be considered that the low variance of the error in the 5 data sets implies that the model is capable of adapting to new data. In any case, the best results were achieved by the InceptionV3 network, with an accuracy mean of 97.99% and variance of 0.037%. Similar behaviour of all the networks was relevant, with results in general over 97%, for the five versions of the dataset and the use of data augmentation. However, it is noteworthy that some of them increased their accuracy and their variance, such as ResNet50, Xception, DenseNet201, or EfficientNetV2M, what was probably indicative about the noise introduced by data augmentation. Instead, other networks increased the accuracy but also decreased their variance, like VGG16, VGG19 and InceptionV3. A separate case is that of the MobileNetV2 network, which obtained the worst results, both with and without data augmentation, and therefore it was not considered adequate for the application.

With regards the overfitting, the upper part of Fig. 4 shows one plot of 'Accuracy vs. Loss' during training and validation for one of the 80 experiments performed. Experiments with the other networks obtained similar results. It can be observed that there is no overfitting, that is, the difference between training and validation errors is not greater than 15%.

In view of these results, it can be considered that the proposed methodology is appropriate for automating the identification of pollens in honey. This is concluded after verifying that the accuracy shown in Table 2 is of the same order of magnitude and even better than those



Training Loss and Accuracy

Fig. 4. Training Loss and Accuracy plots for InceptionV3 (up) and PoleNetV1 (down) networks.

reported by other authors who used CNN-based pollen classifiers in aerobiology and botany. For example, Sevillano and Aznarte, (2018) reported 97.28% of accuracy with the POLEN23S dataset of 23 types of pollens of plants using an AlexNet CNN. The best result of the 2020 Pollen Grain Classification Challenge was reported by Battiato et al. (2021), with an accuracy score of 97.53% with the challenging POL-LEN13K dataset and 5 classes. Astolfi et al. (2020) used 8 pre-trained ImageNet CNNs to classify 73 pollen types with the POLEN73S dataset, obtaining the best results with DenseNet 201 (95.7%) and ResNet50 (94.0%). This shows that increasing the number of classes does not lead

to a substantial increase in mean precision. Finally, recent results by Mahmood et al. (2023), using their proposed APFA-Net, obtained 97.21% on POLLEN73S, 97.39% on POLLEN23E, and 98.33% on the Cretan Pollen dataset. It can be seen that these results are very similar to those reported in this study.

Another aspect to point out from the results in Table 2 is that some networks, initially optimized to improve its performance, such as Xception, DenseNet201 or EfficientNetV2M in this work showed worse behaviour than the InceptionV3 network. This raises the question of whether for those tenths of difference it is better to use one network or

Table 3

Time-Memory usage of each CNN network. The values are calculated on own dataset using an AMD Rizen9 PC with standard Nvidia RTX 3090 GPU board.

Networks	Training time (minutes)	Inference time (milliseconds)	Number of parameters (Millions)	Depth (Conv. layers)	Memory usage (MB)
VGG16	51	2.84	138	16	160.1
VGG19	51	3.18	144	19	181.4
InceptionV3	91	11.15	23.9	48	613.2
Xception	96	4.03	22.9	71	903.9
ResNet50	50	2.45	25.6	50	497.1
DenseNet201	56	10.84	20.2	201	452.4
MobileNetV2	51	2.10	3.4	53	261.1
EfficientNetV2M	57	5.30	55	43	466.3
PolleNetV1	67	2.11	15	6	147.6

Table 4

PollenetV1 architecture.

Layer (type)	Filter size	Filter #	Output Shape	Params #	Connected to
Input shape			(256, 256, 3)	0	RGB Image
Conv2D1 (Convolution2D)	3×3	64	(256, 256, 64)	1792	Input_shape
MaxPool2D1 (MaxPooling2D)	Max		(128, 128, 64)	0	Conv2D1
Conv2D2 (Convolution2D)	3×3	128	(128, 128, 128)	73856	MaxPool2D1
MaxPool2D2 (MaxPooling2D)	Max		(64, 64, 128)	0	Conv2D2
Conv2D3 (Convolution2D)	3×3	256	(64, 64, 256)	295168	MaxPool2D2
MaxPool2D3 (MaxPooling2D)	Max		(32, 32, 256)	0	Conv2D3
Conv2D4 (Convolution2D)	3×3	512	(32, 32, 512)	1180160	MaxPool2D3
MaxPool2D4(MaxPooling2D)	Max		(16, 16, 512)	0	Conv2D4
Conv2D5 (Convolution2D)	3×3	512	(16, 16, 512)	2359808	MaxPool2D4
MaxPool2D5 (MaxPooling2D)	Max		(8, 8, 512)	0	Conv2D5
Conv2D6 (Convolution2D)	3×3	512	(8, 8, 512)	2359808	MaxPool2D4
MaxPool2D6 (MaxPooling2D)	Max		(4, 4, 512)	0	Conv2D5
Flatten_1 (Flatten)			(8192)	0	MaxPool2D5
Dense1 (Dense)			(1000)	81921000	Flatten_1
Dense2 (Dense)			(200)	200200	Dense1
FinalStage (Dense + Softmax)			(16)	3216	Dense2
			TOTAL	14666998	

another. Therefore, these aspects of complexity and speed must be considered.

In this regard, Table 3 shows a summary of Time-Memory for each network. Timing was obtained using a computer with AMD Ryzen 5 processor and standard RTX 3090 GPU. In the software section, the programming language Python and library Keras for Deep Learning with Tensorflow were used. It took about an hour to train most of the models, and mere milliseconds to predict each new particle image. The memory value indicated the size of the ".h5" format file used to store the architecture and resulting network parameters. This was also the memory usage on the CPU to manage de models. Both were the time and memory requirements that any application, including HoneyApp, need to use each CNN. The most successful networks, InceptionV3 and Dense-Net201, required between 500 and 600 MB of memory to be used, which is very difficult for any computer. The best time-memory trade-off were due to VGG16/VGG19 networks. The VGG16 network also achieved very good accuracy result (97.11%) and low variance (0.232) with an accuracy difference from the InceptionV3 network of only 0.89%. However, the former had the advantage of being simpler (16 vs.48 deep) and low memory usage (160 vs 613 MB). For this reason, the VGG16 was used as a reference to build our own network.

3.4. PolleNetV1 Network

An initial result of this research was to propose a new network with good precision but with less computational and memory effort, despite the known advantages of the very deep and overparameterized networks (Dar et al., 2021; Tran et al., 2022). For this reason, the PolleNetV1 network was based on the VGG16 model although the distribution of the convolutional layers was changed, and their number reduced to 6.

The PolleNetV1 architecture contains the following elements: firstly, an input image of $256 \times 256 \times 3$ target size, next a sequence of six

convolution layers (size: 3×3 , stride 2) and increasing output size (64, 128, 256, 512, 512 and 512), each one followed by a MaxPool layer of 2×2 filter with stride of size 2. Subsequently, a Flatten layer and 3 Dense layers (units: 1000, 200 and 16 respectively) ends the net. Table 4 shows the specific architecture of the network provided in Python with Keras software. The simplicity of this network should be noted: with depth of 9, all filters of fixed size (3×3) and less than 15 M parameters are far from the millions of parameters of the other networks.

PolleNetV1 was trained from scratch in the five experiments, using random coefficients in filters and applying more epochs (350) to reach a stable behaviour. Other hyperparameters were learning rate 0.005;

Table 5

Results per class using InceptionV3 in one of the 5-fold experiments. Global average accuracy and multiclass metrics of this experiment are also included.

Name	Туре	Precision	Recall	F1-score	Support
background	type_0	1.00	1.00	1.00	87
bubble	type_3	1.00	1.00	1.00	15
Olea	type_7	0.96	0.98	0.97	159
Cistus sp.	type_8	0.99	0.98	0.98	97
starch	type_9	1.00	0.99	1.00	102
Brasicaceae	type_12	0.99	0.99	0.99	493
Citrus sp.	type_17	0.95	0.99	0.97	110
Echium sp.	type_21	0.99	0.99	0.99	158
Leguminosae	type_30	0.65	0.61	0.63	18
Onobrychis sp.	type_33	0.98	0.99	0.99	144
Prunus	type_36	0.96	1.00	0.98	26
Quercus sp.	type_37	0.99	0.96	0.98	123
Rosmarinus	type_40	0.99	0.99	0.99	140
Thymus sp.	type_43	0.99	0.98	0.98	91
Taraxacum	type_47	1.00	1.00	1.00	36
Umbelliferae	type_50	1.00	1.00	1.00	99
Weighted avg		0.98	0.98	0.98	1898
Accuracy	0.98314				
MCC	0.9810				

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Class	Name	VGG16	VGG19	InceptionV3	Xception	ResNet50	DenseNet201	MobileNetV2	EfficientNetV2M	PolleNetV1
Гуре_0	background	98.62	98.85	98.85	99.54	99.08	99.08	99.08	98.62	98.62
Туре_З	bubble	100.00	98.67	99.02	97.33	100.00	98.67	100.00	100.00	98.67
Type_7	Olea	97.36	96.10	89.33	97.36	97.11	96.60	90.69	97.10	92.83
Гуре_8	Cistus sp.	99.38	99.18	98.11	98.56	99.59	99.59	99.79	99.38	98.14
Гуре_9	starch	99.80	99.61	99.45	99.80	100.00	100.00	99.80	100.00	98.82
Type_12	Brasicaceae	98.26	98.50	98.91	98.66	98.86	98.74	98.86	98.74	97.81
Type_17	Citrus sp.	93.46	93.45	95.51	94.55	96.00	97.09	96.72	97.27	88.73
Type_21	Echium sp.	98.48	98.10	98.83	99.11	98.86	99.11	95.70	98.48	97.85
Type_30	Leguminosae	58.89	48.89	72.22	57.78	57.78	60.00	64.45	66.67	54.44
Type_33	Onobrychis sp.	96.25	98.19	98.89	97.50	97.92	98.89	96.23	98.33	95.41
Type_36	Prunus	88.46	89.23	98.46	93.85	93.08	93.08	92.31	92.31	89.23
Type_37	Quercus sp.	95.28	95.12	97.07	97.07	96.58	95.77	93.00	97.23	93.01
Type_40	Rosmarinus	98.43	98.71	99.19	98.86	99.15	99.29	98.57	99.14	98.00
Type_43	Thymus sp.	94.93	93.85	94.95	91.85	91.65	92.09	84.83	86.82	91.67
Type_47	Taraxacum	100.00	98.89	100.00	100.00	100.00	100.00	99.44	100.00	97.22
Type_50	Umbelliferae	97.98	99.80	99.07	99.39	99.39	79.80	98.18	99.19	97.58
Accuracy		0.9710	0.9711	0.9799	0.9752	0.9770	0.9778	0.9613	0.9759	0.9566
F1-score		0.9720	0.9700	0.9800	0.9760	0.9760	0.9780	0.9620	0.9760	0.9580
MCC		0.9674	0.9675	0.9773	0.9721	0.9741	0.9751	0.9565	0.9728	0.9511

Stochastic Gradient Descent optimizer; batch size of 32; loss function Categorical Cross-Entropy. Once training has been finalised, after 350 epochs, it is considered that the network is already trained and the tests can be analysed. Again, there is no overfitting, as is shown in Fig. 4 (lower part). The average result achieved by PolleNetV1, after analysis of the 5 test data sets, was approximately 95.66% accuracy (see Table 2). Another observation was the low variance of this network, indicating its adaptability to changing data sets and unknown honey pollen samples. The achievement of almost 96% was a promising result, plus this network was simpler than the other networks, resulting in lower memory usage and faster computations. These factors were essential for the adequate introduction of this network into standard applications, like HoneyApp.

3.5. Accuracy per class and metrics results

This section outlines the results of accuracy per class for all networks and the multi-class metrics indicated in Section 2.3. In this context class means "type of pollen/particle." These metrics have been computed for the 5-fold scheme followed in previous experiments, that is, the results obtained for dataset_1 to dataset_5. Table 5 shows the specific metrics of each class (Precision, Recall and F1-Score) and the global values in one of these five experiments, obtained using InceptionV3 network. It should be noted that, in measuring the precision per class, only the positive values are considered, that is, the rows of confusion matrix. Then, the precision per class is also accuracy per class. Recall, on the other hand, considers the columns of the confusion matrix, that is, the false negatives. Therefore, recall per class is also True Positive Rate. In contrast, global measures consider all values, so that the global precision, F1-Score, or MCC can be used to compare different networks.

The good metrics of Precision and Recall per class showed in Table 5 indicates that, between particles of a given type, the network will give low false positives (high Precision) and low false negatives (high Recall); in addition to both measures being balanced. Consequently, the F1-Score maintains similar values to the others. In the rest of the experiments, very similar values were obtained.

Regarding the other networks, the measurements by class and the global multiclass measurements are shown in Table 6. It can be observed that the resulting F1-Score and MCC multi-class metrics are correlated with the global accuracy obtained in each network. F1-Score is near 1 in all the networks which accounts no imbalance in classification. Regarding de MCC metric, once again, the value is near 1 in all the networks, resulting in a good correlation between observed and predicted classification.

The InceptionV3 was generally the most appropriate with regards to

the results of accuracy per class or type. For some types, the accuracy was quite good on all networks (e.g., Type_47: Taraxacum; Type_9: starch) while for others it was not. This is the case of Type_30: Leguminosae, for which the accuracy ranged between only 48.89% (VGG19) to 72.22% (InceptionV3), being PolleNetV1 with low score of 54.44%. This behaviour is not random and is repeated in the five experiments. Type_30 is the Leguminosae family and other that includes many botanical species, which obviously hinders its classification. For this reason, this Type is also especially difficult to be defined under the microscopy for the analyst expert due to the great variety of the different morphologies that make up the pollens. To improve this accuracy, future research should not only increase the number of samples of this Type_30, but also divided into different subtypes, including perhaps the previously mentioned Unknown class.

In general, PolleNetV1 has similar behaviour to other networks. Analysing the accuracy per class in Table 6 for all networks, in any one of the experiments, the most common mistake is the confusion of Type_30: Leguminosae and Others with Type_21: *Echium* sp. Observing Fig. 3, it is clear that for a non-expert in pollens, it proves difficult to tell the difference between them. Probably, with a larger number of samples in Leguminosae, the errors will be significantly reduced. However, Type_3 (air bubbles) with just a few samples obtained good precision in all cases, since it is obviously distinguishable from the others and several networks score 100% accuracy.

It is interesting to point out that the simplest networks, such as MobileNetV2 and PolleNetV1, achieve a result of average around 96% in accuracy and MCC, with a minimal difference between them. Therefore, it seems evident that PolleNetV1 network (despite needing improvements and optimization) is a valid tool for the field of honey pollen analysis.

4. Conclusion

This work offers a step forward for the automated process of pollen grain analysis with the aim of facilitating the monofloral honeys cataloguing. There is a lack of reliable databases on honey pollens, especially from the Mediterranean area, making it necessary as a first step to create and develop a tool that produces more accurate results for this analysis. Hence, in this work, an extensive dataset, with approximately 19,000 microscope images from 16 types of pollen/ particles (present in citrus and rosemary honeys) was created. The new and specific application HoneyApp has been developed to better label and annotate these images. This software proves crucial in supporting the creation of the dataset and to form the core of the newly introduced automated recognizers. Different CNN-based automatic pollen recognizers have shown to be effective in counting pollen grains in honey images, allowing their use in melissopalynology. Positive results were obtained using several pre-existing CNN networks, especially with the InceptionV3 in terms of accuracy. However, its great complexity and the extensive use of millions of parameters complicate the future development of the HoneyApp as a tool for automatic pollen recognition. Therefore, the new proprietary network conceived in this study (PolleNetV1), although with a slightly lower accuracy (around 96%), has the great advantage of a simpler and low memory. With some improvements, PollenNetV1 could be a robust network, to be included in this future App.

The results of this research have demonstrated the efficacy and efficiency of the automatic pollen recognition developed in this work. Although proven to be successful only for the most abundant pollens present in Spanish citrus and rosemary honeys, it shows promise in its application to other pollens with the final aim of determining the monoflorality of the honeys. This will create an opportunity to provide an objective tool that guarantees the correct labelling of monofloral honeys and contribute to their transparency in its commercialization.

CRediT authorship contribution statement

José Miguel Valiente: Funding acquisition, Project administration, Conceptualization, Supervision, Writing – review & editing. Isabel Escriche: Funding acquisition, Project administration, Conceptualization, Supervision, Writing – review & editing. Marisol Juan-Borrás: Formal analysis, Methodology, Data curation, Original Draft, Fernando López-García: Formal analysis, Methodology, Software, Data curation

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2023.105605.

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