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Evaluation of a hybrid AI model for the automatic identification of pollen morphological features for apitherapy applications

Apiterapi uygulamaları için polen morfolojik özelliklerinin otomatik tanımlanmasına yönelik hibrit bir yapay zekâ modelinin değerlendirilmesi

Uğur Şevik[1,2*]

[1]Department of Computer Science, Faculty of Science, Karadeniz Technical University, Trabzon, Türkiye * usevik@ktu.edu.tr

[2]Retina R&D Software and Engineering Services Ltd, Trabzon Teknokent, Trabzon, Türkiye

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***Corresponding author** /Yazışılan yazar
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Abstract

Melissopalynology is the gold standard for authenticating honey but traditional microscopic analysis is time-consuming and subjective. This study evaluates a hybrid artificial intelligence approach to automate pollen classification using the comprehensive POLLEN73S dataset, which features 73 distinct pollen types from the Brazilian Savanna. To address class imbalance, the dataset was expanded to 7300 images using data augmentation. We extracted morphological features using three pre-trained deep learning models (ResNet50, EfficientNetB0, MobileNetV2) and classified them using 17 traditional machine learning algorithms. The hybrid model combining ResNet50 features with Linear Discriminant Analysis (LDA) achieved the highest accuracy of 97.00%. Error analysis indicated that misclassifications were concentrated among taxonomically similar genera, such as *Serjania*, due to shared exine structures. These results demonstrate that the proposed hybrid model offers a highly accurate and scalable solution for laboratory-based honey authentication, provided it is integrated with debris detection systems to handle real-world samples.

Keywords: Melissopalynology, Pollen Morphology, POLLEN73S, Honey Authentication, Hybrid Classification, ResNet50, Linear Discriminant Analysis

Özet

Melissopalinoloji, balın kimliğini doğrulamada altın standarttır; ancak geleneksel mikroskobik analiz zaman alıcı ve öznel bir süreçtir. Bu çalışma, Brezilya Savanası'na ait 73 farklı polen tipini içeren kapsamlı POLLEN73S veri setini kullanarak, polen sınıflandırmasını otomatikleştirmeye yönelik hibrit bir yapay zeka yaklaşımını değerlendirmektedir. Sınıf dengesizliğini gidermek amacıyla, veri artırma teknikleri kullanılarak veri seti 7300 görüntüye genişletilmiştir. Çalışmada, üç farklı önceden eğitilmiş derin öğrenme modeli (ResNet50, EfficientNetB0, MobileNetV2) kullanılarak morfolojik öznitelikler çıkarılmış ve bu öznitelikler 17 geleneksel makine öğrenmesi algoritması ile sınıflandırılmıştır. ResNet50 özniteliklerini Doğrusal Diskriminant Analizi (LDA) ile birleştiren hibrit model, %97,00 ile en yüksek doğruluk oranına ulaşmıştır. Hata analizi, hatalı sınıflandırmaların, paylaşılan ekzin yapıları nedeniyle *Serjania* gibi taksonomik olarak benzer cinsler arasında yoğunlaştığını göstermiştir. Bu sonuçlar, önerilen hibrit modelin; gerçek dünya örneklerini işleyebilmek adına kalıntı tespit sistemleriyle entegre edilmesi koşuluyla, laboratuvar tabanlı bal kimlik doğrulaması için yüksek doğruluklu ve ölçeklenebilir bir çözüm sunduğunu ortaya koymaktadır.

Anahtar Kelimeler: Melissopalinoloji, Polen Morfolojisi, POLLEN73S, Bal Kimlik Doğrulaması, Hibrit Sınıflandırma, ResNet50, Doğrusal Diskriminant Analizi

Abbreviations: APFA-Net, Attention-Guided Pollen Features Aggregation Network; AUC, Area Under the Curve; CNN, Convolutional Neural Network; CPD-1, Cretan Pollen Dataset v1; DL, Deep Learning; GPU, Graphics Processing Unit; LDA, Linear Discriminant Analysis; LGBM (LightGBM), Light Gradient Boosting Machine; LM, Light Microscopy; ML, Machine Learning; MLP, Multilayer Perceptron; RBF, Radial Basis Function; SEM, Scanning Electron Microscopy; SVC, Support Vector Classifier; YOLOv5, You Only Look Once v5

1. INTRODUCTION

Honey is a high-value natural product known for its nutritional and therapeutic properties (Bicudo de Almeida-Muradian et al., 2020). However, its rising popularity has made it a primary target for fraud, such as mislabeling botanical or geographical origins (Chippa et al., 2025; Pound et al., 2023). Guaranteeing authenticity is critical, and the gold standard method for this is melissopalynology (Von Der Ohe et al., 2004; Bicudo de Almeida-Muradian et al., 2020). This method relies on the analysis of pollen grains, which serve as a natural fingerprint for honey (Pound et al., 2023).

Pollen morphology is defined by characteristics such as size, shape, aperture structures, and exine ornamentation (Hesse et al., 2009; Halbritter et al., 2018). These features hold critical systematic value for distinguishing species and genera (Marques et al., 2021). Even pollen that appears uniform under light microscopy can be distinguished based on detailed exine features (Lu et al., 2022).

Traditional melissopalynology relies on manual identification by expert palynologists using light microscopy (Chippa et al., 2025; Tsiknakis et al., 2022). Although reliable, this method is time-consuming, labor-intensive, and prone to inter-observer variability (Tsiknakis et al., 2022). Moreover, manual analysis cannot meet the high-volume authentication demands of the global market (Chippa et al., 2025). These limitations have made automation necessary. Early attempts using traditional machine learning and handcrafted features were often inadequate, achieving, for example, only 64% accuracy on the POLEN23E dataset (Gonçalves et al., 2016). Consequently, the field has been revolutionized by deep learning, particularly Convolutional Neural Networks (CNNs), which can automatically learn complex features from images (LeCun et al., 2015).

These limitations have made the automation of the pollen classification process a necessity. The first attempts at automatic classification focused on traditional machine learning (ML) methods. These approaches relied on extracting handcrafted features from pollen images, such as Color, Shape, and Texture (CST), or the Bag of Visual Words (BOW), and processing these features using classifiers such as Support Vector Machines (SVM) (Gonçalves et al., 2016). However, these methods are often inadequate for capturing complex variations in pollen morphology. For example, in the POLEN23E dataset (23 classes) presented by Gonçalves et al. (2016) for the flora of the Brazilian Savanna, the highest accuracy achieved using CST+BOW features and a C-SVC classifier remained at 64% (Gonçalves et al., 2016).

The real revolution in pollen analysis began with the rise of deep learning (DL) techniques, particularly Convolutional Neural Networks (CNNs) (LeCun et al., 2015). CNNs can automatically learn hierarchical and complex morphological features directly from raw image pixels without manual feature engineering. In recent years, DL-based approaches have demonstrated remarkable success in pollen classification, surpassing the accuracy of manual analysis.

State-of-the-art results obtained on various publicly available pollen datasets in the literature confirm this trend.

POLLEN23E Dataset (23 classes): This dataset, presented by Gonçalves et al. (2016), was reanalyzed using DL approaches by Sevillano and Aznarte (2018), achieving a classification accuracy of over 97%.

Cretan Pollen Dataset (CPD-1) (20 classes): Compiled from plant species native to Crete by Tsiknakis et al. (2021), this dataset achieved 97.5% accuracy using transfer learning and ensemble models (Tsiknakis et al., 2022).

POLLEN73S Dataset (73 Classes): Introduced by Astolfi et al. (2020), this challenging Brazilian Savanna dataset contains 73 classes and is one of the most comprehensive datasets available. Studies on this dataset have reported the following: Astolfi et al. (2020) achieved 95.7% precision using DenseNet-201; Garga et al. (2024) reported 97.2% accuracy with optimized DenseNet201; and Mahmood et al. (2023) obtained an F1-score of 97.37% using a custom model with an attention mechanism called APFA-Net.

These studies demonstrate that more advanced DL techniques, such as sophisticated attention mechanisms (Mahmood et al., 2023; Soares et al., 2025), joint optimization with morphological features (Zhang & Mao, 2026), and even the integration of phylogenetic data (Adaïmé et al., 2024), have the potential to further improve accuracy. However, these high-performance end-to-end DL models face two main challenges: high computational costs due to the need for training from scratch and low interpretability due to their black-box nature. An alternative and more efficient approach is the hybrid DL-ML architecture, which combines the strong feature representation capabilities of DL models (Sevillano & Aznarte, 2018) with the speed, efficiency, and interpretability of traditional ML classifiers. In this approach, a CNN pre-trained on large datasets, such as ImageNet, is used as a feature extractor, and these deep features are classified by a simpler ML model, such as SVM or Linear Discriminant Analysis (LDA) (Boldeanu, 2022; Li et al., 2023).

Although the effectiveness of this hybrid approach has been mentioned in the literature (Sevillano & Aznarte, 2018), a systematic and comprehensive comparison between various ML classifiers and different DL extractors, whether lightweight, balanced, or powerful, was lacking, especially on a morphologically complex dataset with a high number of classes, such as POLLEN73S. This study aims to fill this research gap. In this study, using the POLLEN73S dataset, the performance of 51 unique hybrid model combinations was experimentally analyzed; these combinations paired different pre-trained DL feature extractors—MobileNetV2, EfficientNetB0, and ResNet50—with 17 different ML classifiers to distinguish the morphological characteristics of 73 different pollen types. The main purpose of our study was to determine the model that provides the highest accuracy for melissopalynology applications and the most optimized hybrid model in terms of computational efficiency, scalability, and practical applicability.

2. MATERIALS AND METHODS

This section details the dataset used in the study, the data preprocessing and augmentation steps, the deep learning models selected for the automatic extraction of pollen morphological features, the machine learning algorithms used for classification, and the experimental evaluation metrics used.

2.1. Polen Data Set: POLLEN73S

This study was based on the POLLEN73S dataset developed by Astolfi et al. (2020) and made publicly available. This dataset consists of 2,523 microscope images representing 73 different pollen types (classes) belonging to the rich flora of the Brazilian Savanna (Cerrado) biome (Astolfi et al., 2020; Garga et al., 2024). Images were obtained using a Carl Zeiss Microimaging microscope with a 40x objective lens after staining the pollen grains collected from the anthers of flowers with fuchsin (Astolfi et al., 2020). Each image was manually segmented to ensure that only a single pollen grain remained in each image (Astolfi et al., 2020).

The dataset contains 35 images for the vast majority of the 73 classes, whereas some classes have fewer samples, such as *Gomphrena* sp. with 10 images, *Trema micrantha* with 34 images, and *Zea mays* with 29 images (Astolfi et al., 2020; Garga et al., 2024). This reflects a natural class imbalance, which is commonly encountered in real-world scenarios but poses challenges for deep learning models (Tsiknakis et al., 2022). The dataset also presents classification challenges because it includes multiple morphologically similar species within the same genus, such as *Serjania* (four species) and *Mimosa* (two species) (Astolfi et al., 2020).

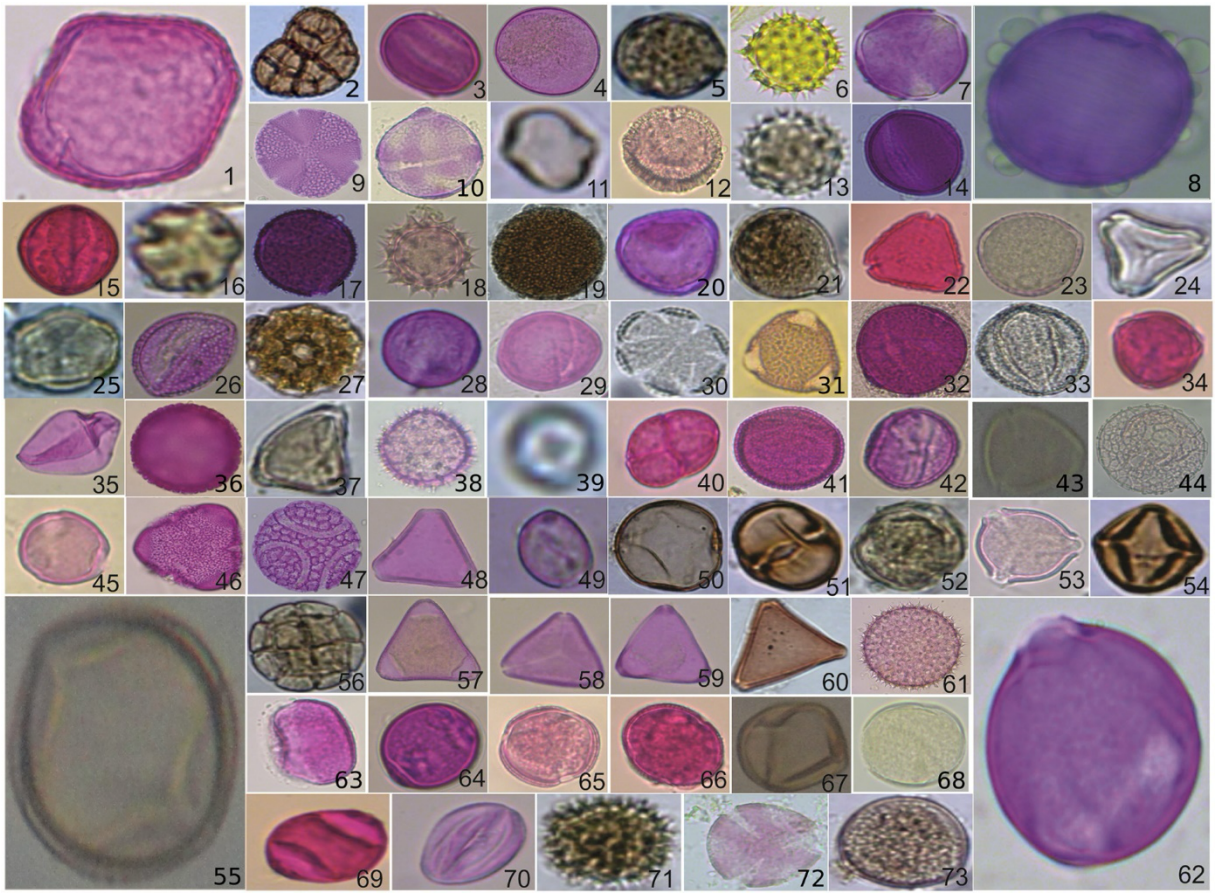


Figure 1. Samples of the POLLEN73S dataset. 1 - Acrocomia aculeata, 2 - Anadenanthera colubrina, 3 - Arachis sp., 4 - Archontophoenix cunninghamiana, 5 - Arrabidaea florida, 6 - Aspilia grazielae, 7 - Bacopa australis, 8 - Brugmansia suaveolens, 9 - Caesalpinia peltophoroides, 10 - Caryocar brasiliensis, 11 - Cecropia pachystachya, 12 - Ceiba speciosa, 13 - Chromolaena laevigata, 14 - Cissus campestris, 15 - Cissus spinosa, 16 - Combretum discolor, 17 - Cordia trichotoma, 18 - Cosmos caudatus, 19 - Croton urucurana, 20 - Dianella tasmanica, 21 - Dipteryx alata, 22 - Doliocarpus dentatus, 23 - Erythrina mulungu, 24 - Eucalyptus sp., 25 - Faramea sp., 26 - Genipa americana, 27 - Gomphrena sp., 28 - Guazuma ulmifolia, 29 - Hortia oreadica, 30 - Hyptis sp., 31 - Ligustrum lucidum, 32 - Luehea divaricata, 33 - Mabea fistulifera, 34 - Machaerium aculeatum, 35 - Magnolia champaca, 36 - Manihot esculenta, 37 - Matayba guianensis, 38 - Mauritia flexuosa, 39 - Mimosa ditans, 40 - Mimosa pigra, 41 - Mitostemma brevifilis, 42 - Myracrodruon urundeuva, 43 - Myrcia guianensis, 44 - Ochroma pyramidale, 45 - Ouratea hexasperma, 46 - Pachira aquatica, 47 - Passiflora gibertii, 48 - Paullinia spicata, 49 - Piper aduncum, 50 - Poaceae sp., 51 - Protium heptaphyllum, 52 - Qualea multiflora, 53 - Ricinus communis, 54 - Schinus terebinthifolius, 55 - Schizolobium parahyba, 56 - Senegalia plumosa, 57 - Serjania erecta, 58 - Serjania hebecarpa, 59 - Serjania laruotteana, 60 - Serjania sp., 61 - Sida cerradoensis, 62 - Solanum sisymbriifolium, 63 - Syagrus romanzoffiana, 64 - Symplocos nitens, 65 - Tabebuia chrysotricha, 66 - Tabebuia roseoalba, 67 - Tapirira guianensis, 68 - Tradescantia pallida, 69 - Trema micrantha, 70 - Trembleya phlogiformis, 71 - Tridax procumbens, 72 - Vochysia divergens, 73 - Zea mays. (Astolfi et al., 2020)

2.2. Data Preprocessing and Augmentation

Data scarcity and class imbalance are among the most common challenges in pollen datasets used to train deep learning models (Tsiknakis et al., 2022; Astolfi et al., 2020). To enable the models to learn rare pollen classes and enhance their generalization capability, a comprehensive data augmentation strategy was implemented in the first stage of this study. All 73 classes, including those with insufficient samples, were completed with 100 images each using standard

data augmentation techniques. The data augmentation pipeline was implemented using the Keras Sequential API with the following specific hyperparameters: RandomFlip (horizontal and vertical), RandomRotation (factor: 0.4), RandomZoom (height and width factor: 0.2), RandomTranslation (height and width factor: 0.1), and RandomContrast (factor: 0.2). These parameters were selected to generate robust morphological variations while preserving the diagnostic features of the pollen grains. These processes aim to prevent overfitting and increase the model robustness by generating morphological variations that the model has not previously encountered. As a result of this augmentation process, a balanced and enriched training dataset consisting of 7,300 images, with 100 samples in each of the 73 classes, was created. All subsequent stages of the study were conducted using this dataset of 7,300 images.

Table 1. Features of the POLLEN73S Dataset and the Applied Data Augmentation Process

Feature	Original Data Set (Astolfi vd., 2020)	Data Set Used in the Study
Number of Classes	73	73
Total Number of Images	2523	7300
Image per Class	Number of Variables (between 10 and 35)	Balanced (Each class 100)
Data Partitioning	K-fold	80% Training (5840 images) / 20% Test (1460 images)

2.3. Deep Learning-Based Morphological Feature Extraction

In this study, we adopted a hybrid approach to capture the complex morphological features of pollen grains. In this approach, instead of training an end-to-end DL model from scratch, powerful CNN architectures that have been pretrained on massive datasets, such as ImageNet (Russakovsky et al., 2015), are used as feature extractors through transfer learning (Sevillano & Aznarte, 2018; Tsiknakis et al., 2022). For this purpose, three popular and proven models that differ in terms of architectural complexity, computational efficiency, and representational power were selected:

ResNet50: This architecture, presented by He et al. (2016), solves the vanishing gradient problem using deep residual connections (Astolfi et al., 2020; Szegedy et al., 2017). It has demonstrated high performance in the literature for tasks where fine-textured patterns, such as pollen classification, are important (Astolfi et al. 2020; Garga et al. 2024; Li et al. 2023). Li et al. (2023) reported that ResNet50 achieved an outstanding success rate of 99.4% in

distinguishing between Urticaceae pollens (Parietaria and Urtica), which are morphologically very similar.

EfficientNetB0: This model, developed by Tan and Le (2019), offers a good balance between high accuracy and computational efficiency by optimizing the depth, width, and resolution of the network using a compound scaling method.

MobileNetV2: This lightweight architecture, designed by Sandler et al. (2018), significantly reduces the number of parameters and computational cost by using depth-wise separable convolutions (Khalane et al., 2025). It is especially ideal for use in systems such as mobile or resource-constrained automatic pollen-counting devices (Boldeanu, 2022).

The final fully connected classification layer of each pretrained model was removed from the architecture. After each image in the dataset of 7,300 images was resized to 224 × 224 pixels, it was passed separately through each of these three models (Garga et al., 2024). As the output of this process, a feature vector representing what the model has learned was obtained for each image: a 2048-dimensional feature vector for ResNet50, a 1280-dimensional feature vector for EfficientNetB0, and a 1280-dimensional feature vector for the MobileNetV2.

2.4. Machine Learning Classifiers

The deep feature vector sets of ResNet50, EfficientNetB0, and MobileNetV2, which were obtained separately in the previous step, were used to train and test 17 different traditional machine learning (ML) classifiers.

The selection of these 17 classifiers aimed to systematically compare different algorithmic approaches, such as linear, tree-based, kernel-based, and ensemble learning. This comprehensive analysis was designed to determine which type of ML classifier performs best in the feature space generated by the DL. To ensure reproducibility and establish a fair baseline comparison, standard hyperparameter settings were employed for the majority of the machine learning classifiers, primarily relying on the default configurations provided by the Scikit-learn library, unless otherwise noted. Deep learning feature extractors utilized pre-trained ImageNet weights to leverage transfer learning capabilities. For ensemble and boosting algorithms such as XGBoost, LightGBM, and CatBoost, GPU acceleration was enabled to optimize training efficiency. A comprehensive summary of the specific hyperparameter configurations and experimental settings for both feature extraction and classification models is provided in Table 3. The feature extractors and classifiers used in this study are summarized in Table 2.

Table 2. Feature Extractors and Machine Learning Classifiers Used in the Experimental Setup

Feature Extractors (DL)	Classifiers (ML)
ResNet50	Logistic Regression (Ridge, Lasso, ElasticNet)
EfficientNetB0	K-Nearest Neighbors (KNeighbors, K=5)
MobileNetV2	Decision Tree (DecisionTree)
	Random Forest (RandomForest)
	Linear Support Vector Classifier (LinearSVC)
	Linear Discriminant Analysis (LDA)
	Stochastic Gradient Descent (SGDClassifier)
	Ridge Classifier (RidgeClassifier)
	XGBoost
	LightGBM
	CatBoost
	Support Vector Classifier (SVC, RBF Kernel)
	Multi-layer Perceptron (MLPClassifier)
	Extra Trees (ExtraTrees)
	Bagging Classifier (BaggingClassifier)

Table 3. Hyperparameter settings for feature extractors and classifiers.

Model Category	Algorithm / Model	Key Hyperparameters & Settings
Feature Extraction	MobileNetV2, EfficientNetB0, ResNet50	Weights: ImageNet; Pooling: Average; Input Shape: (224, 224, 3); Batch Size: 64; Include Top: False.
Linear Models	Logistic Regression (Ridge)	Penalty: L2; Solver: lbfgs; Max Iter: 1000.
	Logistic Regression (Lasso)	Penalty: L1; Solver: liblinear; Max Iter: 1000.
	Logistic Regression (ElasticNet)	Penalty: ElasticNet; Solver: saga; L1 Ratio: 0.5.
	Ridge Classifier	Alpha: 1.0 (Default); Solver: auto.
	SGD Classifier	Loss: log_loss; Penalty: L2; Max Iter: 1000.

Model Category	Algorithm / Model	Key Hyperparameters & Settings
	Linear Discriminant Analysis (LDA)	Solver: svd (Default).
Support Vector Machines	Linear SVC	Kernel: Linear; C: 0.1; Probability: True.
	SVC (RBF)	Kernel: RBF; C: 1.0; Gamma: scale; Probability: True.
Tree-Based & Ensemble	Decision Tree	Criterion: Gini; Splitter: Best.
	Random Forest	N_estimators: 100; Criterion: Gini; Bootstrap: True.
	Extra Trees	N_estimators: 100; Criterion: Gini.
	Bagging Classifier	Base Estimator: Decision Tree; N_estimators: 10.
	AdaBoost	N_estimators: 100; Learning Rate: 1.0.
	Gradient Boosting	N_estimators: 100; Learning Rate: 0.1; Max Depth: 3.
	XGBoost	Objective: binary/multi:softprob; Tree Method: gpu_hist; Eval Metric: mlogloss.
	LightGBM	Device: GPU; Boosting Type: gbdt; Objective: multiclass.
	CatBoost	Task Type: GPU; Loss Function: MultiClass; Verbose: 0.
Neighbors & Neural Nets	K-Neighbors (KNN)	N_neighbors: 5; Metric: minkowski (p=2); Algorithm: auto.
	MLP Classifier	Hidden Layers: (100, 50); Activation: relu; Solver: adam; Max Iter: 500; Early Stopping: True.
General Settings	All Models	Random State: 42 (for reproducibility); Test Size: 20% (Check text); Cross-Validation: None (Hold-out).

2.5. Experimental Setup and Evaluation Metrics

Figure 2 summarizes the general workflow of the proposed hybrid approach. The experimental process consists of three main stages: First, data augmentation techniques were applied to address the class imbalance in the POLLEN73S dataset. In the second stage, high-level morphological features were extracted from the augmented images using pre-trained deep

learning models (ResNet50, EfficientNetB0, MobileNetV2). Finally, these feature vectors were classified using various machine learning algorithms, such as Linear Discriminant Analysis (LDA), to evaluate model performance.

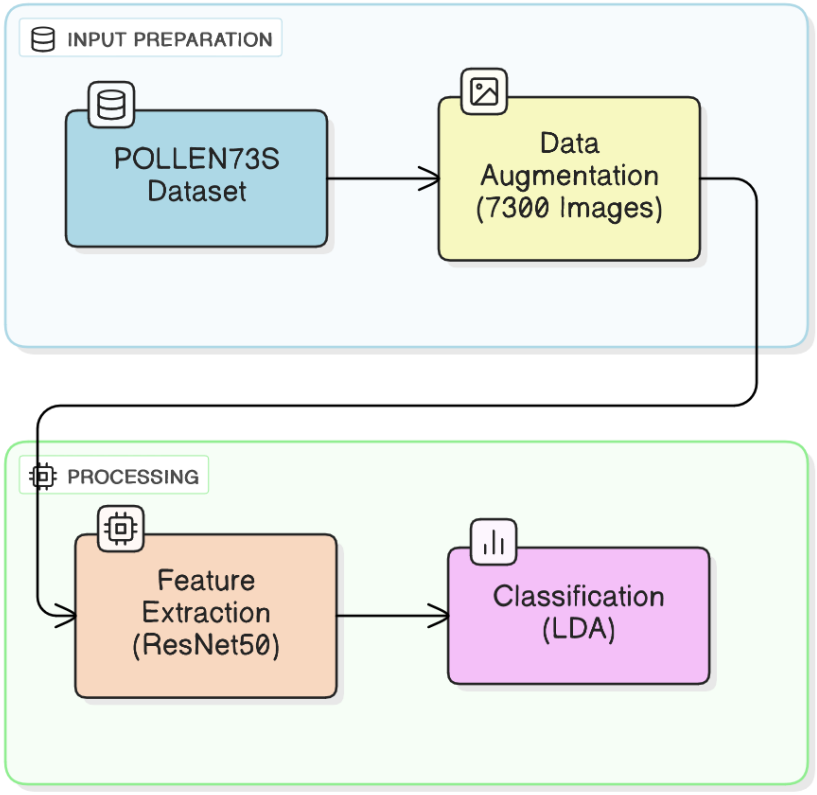


Figure 2. The proposed workflow of the hybrid classification system.

The dataset, consisting of 7,300 augmented and balanced images, was split into two parts: 80% training (5,840 images) and 20% testing (1,460 images), ensuring the preservation of class distribution. All ML classifiers were trained on the training set, and their performance was evaluated on the previously unseen test set. The performance of the models in this challenging 73-class task was measured using standard classification metrics, such as Accuracy, Precision, Recall, and F1-Score (Garga et al., 2024; Tsiknakis et al., 2022). Owing to class imbalance (before augmentation) and the high number of classes (73), both macro (giving equal weight to each class) and weighted (weighted by the number of samples in each class) averages were considered for Precision, Recall, and F1-Score (Tsiknakis et al., 2022).

3. RESULTS AND DISCUSSION

In this section, the classification performances of 51 different hybrid model combinations, conducted using three different DL feature extractors and 17 different ML classifiers on the POLLEN73S test dataset, are presented. The findings are discussed in the context of pollen morphology and the current state-of-the-art results in the literature.

3.1. Comprehensive Analysis of Classification Performance

The features extracted from MobileNetV2, the lightest architecture, were tested using 17 different ML classifiers. The results are summarized in Table 3. When MobileNetV2 features were used, LinearSVC achieved the highest test accuracy of 92.33%.

Table 3. Test Accuracy of ML Classifiers with MobileNetV2 Feature Extractor

Classifiers	Test Performance(%) *Accuracy*	Classifiers	Test Performance (%) *Accuracy*
LinearSVC	**92.33**	LogReg (ElasticNet)	90.14
LogReg (Ridge)	91.42	LogReg (Lasso)	89.41
SVC (RBF Kernel)	89.50	RidgeClassifier	88.95
Linear Discriminant Analysis (LDA)	89.50	ExtraTrees	79.36
CatBoost (GPU)	86.53	KNeighbors (K=5)	79.63
MLPClassifier	86.16	RandomForest	78.49
SGDClassifier	85.84	LightGBM (GPU)	77.85
BaggingClassifier	56.94	DecisionTree	35.71

Second, features obtained from the EfficientNetB0 architecture, which has moderate complexity and high efficiency, were tested. This architecture provided a significant performance improvement across all classifiers compared with MobileNetV2 (Table 4). In this group, the highest test accuracy was again achieved by LinearSVC, with 95.39%. Linear Discriminant Analysis (LDA) (95.02%) also performed above 95%.

Table 4. Test Accuracy of ML Classifiers with EfficientNetB0 Feature Extractor

Classifiers	Test Performance (%) *Accuracy*	Classifiers	Test Performance (%) *Accuracy*
LinearSVC	**95.39**	LogReg (ElasticNet)	93.06
Linear Discriminant Analysis (LDA)	95.02	LogReg (Lasso)	93.01
LogReg (Ridge)	94.57	CatBoost (GPU)	89.82
RidgeClassifier	93.61	SGDClassifier	89.13
MLPClassifier	91.69	RandomForest	82.92
SVC (RBF Kernel)	91.69	ExtraTrees	82.65
KNeighbors (K=5)	80.91	LightGBM (GPU)	80.00
BaggingClassifier	63.97	DecisionTree	40.82

Finally, the features extracted from the ResNet50 architecture, which is known to extract the deepest and morphologically richest features, produced the best results among the 51 combinations (Table 5). In this group, Linear Discriminant Analysis (LDA) achieved the highest test accuracy of 97.00%. This result was the best overall score obtained in the study. LinearSVC (96.30%) also demonstrated extremely high success in the feature space.

Table 5. Test Performance of ML Classifiers with ResNet50 Feature Extractor

Classifiers	Test Performance (%) *Accuracy*	Classifiers	Test Performance (%) *Accuracy*
Linear Discriminant	**97.00**	SGDClassifier	92.19
LinearSVC	96.30	MLPClassifier	92.10
LogReg (ElasticNet)	95.48	RandomForest	88.26
LogReg (Ridge)	95.48	ExtraTrees	87.76
RidgeClassifier	95.30	LightGBM (GPU)	86.99
LogReg (Lasso)	94.75	KNeighbors (K=5)	80.46

Classifiers	Test Performance (%) *Accuracy*	Classifiers	Test Performance (%) *Accuracy*
CatBoost (GPU)	94.66	BaggingClassifier	72.79
SVC (RBF Kernel)	93.61	DecisionTree	53.11

When all three experimental groups in Table 3, 4, and 5 are examined, simple linear classifiers (LDA, LinearSVC, Logistic Regression) have achieved higher accuracy in almost every scenario compared to complex, non-linear models such as Decision Trees, Random Forest, Boosted Models (LGBM, CatBoost), and Multilayer Perceptron (MLP).

This striking finding strongly demonstrates that pre-trained DL models (particularly ResNet50) successfully transform the complex morphological structure of pollen images into a high-dimensional, but largely linearly separable, feature space. This indicates that the model has learned fundamental morphological features, such as exine pattern, aperture shape, and pollen size, which are palynologically meaningful, and has created a representation in which these features can be separated by a linear boundary.

3.2. In-depth Analysis of the Best Model (ResNet50 + LDA) and Its Morphological Interpretation

The performance of the ResNet50 (feature extractor) + LDA (classifier) model, which showed the best performance among all combinations with 97.00% accuracy, was analyzed in more detail to understand the morphological basis of the classification errors. This analysis revealed the strengths and weaknesses of the model for practical melissopalynology.

To visualize the performance of the best model across 73 classes and analyze the error patterns, the confusion matrix shown in Figure 3 was created.

The confusion matrix in Figure 1 shows that the model distinguished the vast majority of the 73 classes with high accuracy. The concentration along the main diagonal of the matrix confirmed the overall success of the model.

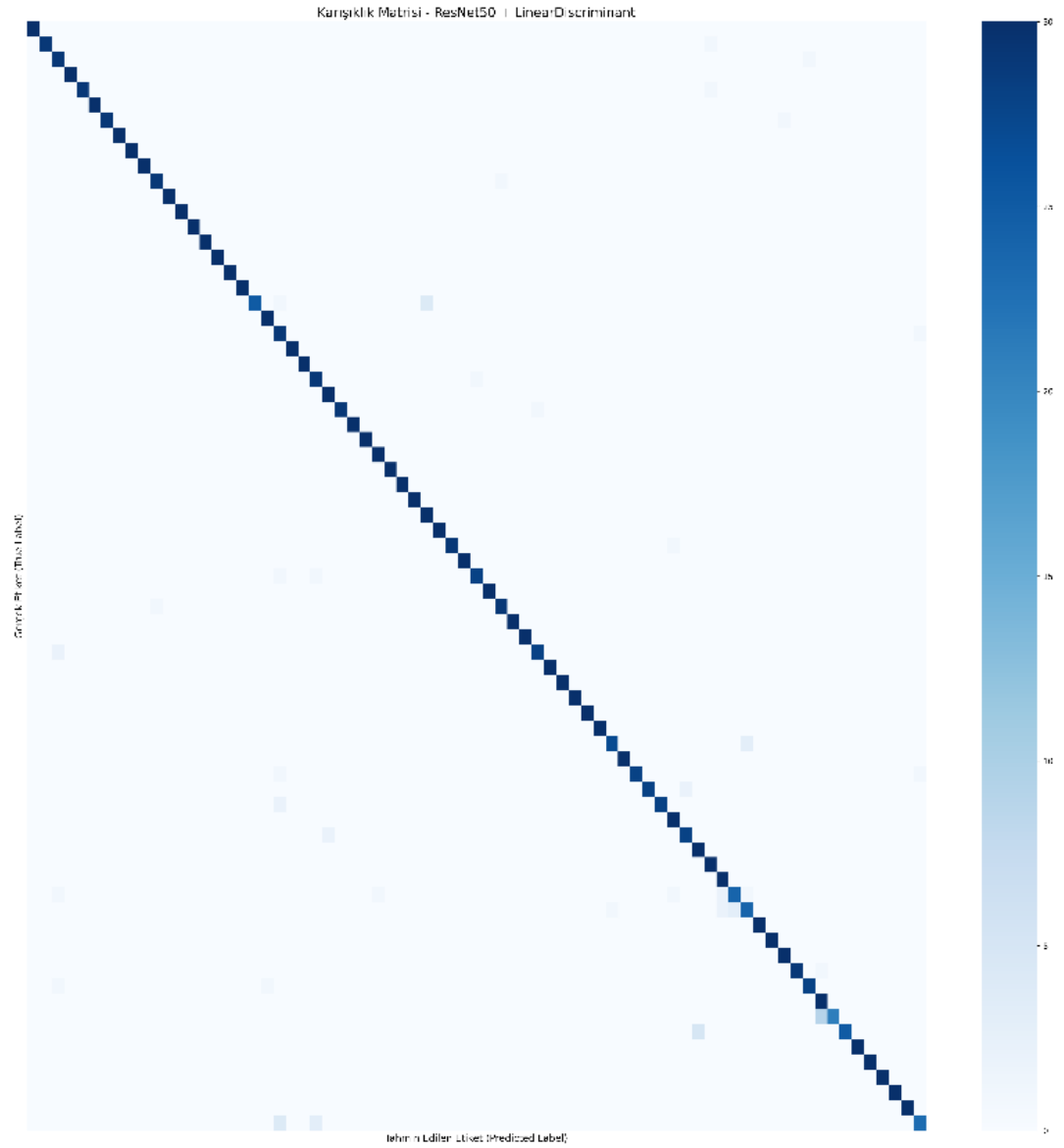


Figure 3. Normalized Confusion Matrix of the ResNet50 + LDA Model on the Test Dataset with 73 classes.

More importantly, the points at which the model makes errors (off-diagonal) are not randomly distributed. Errors tend to cluster among species that are taxonomically close to each other or display similar morphological characteristics. This suggests that the challenging cases for the model stem from subtle distinctions that are also difficult for expert palynologists to distinguish under a light microscope, representing palynologically challenging fine differences (Li et al., 2023). Some morphologically challenging groups in the POLLEN73S dataset are as follows:

***Serjania Genus (Sapindaceae)*:** The dataset includes four different *Serjania taxa: Serjania erecta, Serjania hebecarpa, Serjania laruotteana, and Serjania sp*. These pollens constitute a group that is difficult to distinguish even through manual microscopy because of

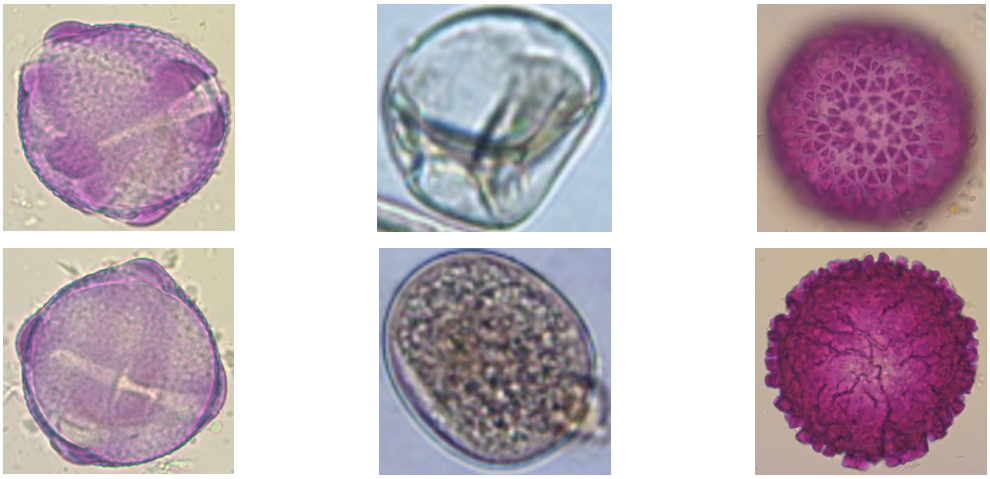
their similar size, shape, and exine structures. The confusion matrix shows that the model experiences significant confusion among the four classes. Morphologically, these species share a very similar heteropolar, tricolporate structure with a triangular ambitus in polar view. The subtle differences in the reticulate exine ornamentation are often indistinguishable even to expert palynologists, leading to the observed model confusion.

Mimosa Genus (Fabaceae): Similarly, because of the morphological similarity between *Mimosa ditans* and *Mimosa pigra*, some degree of confusion has been observed, albeit at a low level.

Appearance Differences (Equatorial and Polar): The POLLEN73S dataset contains images of pollen grains captured from different angles. In palynology, the equatorial view of a pollen grain (from the side) and the polar view (from the poles) can appear morphologically different (Soares et al., 2025; Lu et al., 2022). The fact that the model must learn these two different views belonging to the same class as a single category can be a factor that increases the difficulty of classification and may lead to errors.

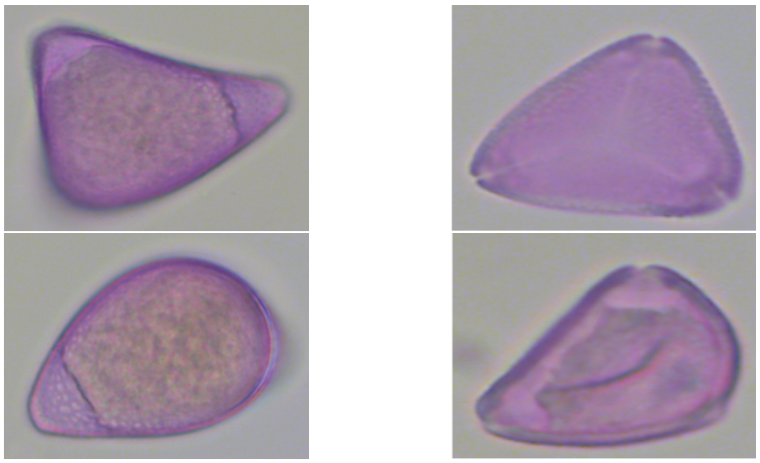
3.3. Examples of Classification Success and Failure

To better understand the model's performance, examples of both correctly classified (High Reliability) and misclassified (Failure Cases) pollen types are shown in Figure 4 and 5.



Caryocar brasiliensis *Zea mays* *Manihot esculenta*

Figure 4. Pollen Samples Correctly Classified with High Reliability. These pollen samples have distinct and unique morphological features.



(a) *Serjania erecta* (b) *Serjania hebecarpa*

Figure 5. Pollen types for which the model failed owing to morphological similarity. The most common type of error was when the model misclassified a pollen, such as (a) *Serjania* erecta as (b) *Serjania* hebecarpa, which is morphologically very similar.

3.4. Literature Comparison and Advantages of the Hybrid Model

The 97.00% test accuracy achieved with ResNet50 + LDA, which is the main finding of our study, is compared in Table 6 with State-of-the-Art DL models that are generally more complex and trained end-to-end, and also work on the same POLLEN73S dataset.

Table 6. Comparative Performance of Different Models on the POLLEN73S Dataset

Study	Model	Approach	Performance Metric	Result (%)
Astolfi et.al. (2020)	DenseNet-201	End-to-End DL	Precision	95.7
Garga et.al. (2024)	DenseNet-201	End-to-End DL	Accuracy	97.2
Mahmood et.al. (2023)	APFA-Net	End-to-End DL (Attention)	F1-Score	97.4
Recommended Study	**ResNet50 + LDA**	**Hybrid(DL Features+ ML)**	**Accuracy**	97.0

Table 6 clearly shows that our hybrid "ResNet50+LDA" model offers almost equivalent accuracy (97.0%) compared to State-of-the-Art DL models with specialized attention mechanisms or complex end-to-end architectures, such as APFA-Net (Mahmood et al., 2023) or the optimized DenseNet-201 (Garga et al., 2024), which achieve 97.2–97.4% accuracy.

This finding demonstrates that to achieve 97% accuracy in pollen classification, complex and custom-designed networks that can take days to train from scratch are not required. The deep morphological feature representation power of a strong, pretrained backbone, such as ResNet50, is sufficient for this complex classification task.

3.5. Implications and Challenges for Melissopalynology and Apitherapy

The findings of this study have important practical implications for apitherapy and honey authentication. A model capable of distinguishing between 73 different pollen classes with 97% accuracy can form the basis of a scalable and reliable system for the automatic verification of the botanical and geographical origins of honey.

However, there are some significant challenges that these models, which are trained in laboratory settings, face in practical applications. The most important limitation of our study is the issue known in the literature as the domain gap (Tsiknakis et al., 2022). Our model was trained on a dataset consisting of images from the POLLEN73S dataset under ideal conditions (images taken directly from plants that were well-lit, clean, and perfectly segmented). In contrast, real melissopalynology applications work with environmental samples obtained from honey sediments (Gimenez et al., 2024). These samples inevitably contain debris, air bubbles (Battiato et al., 2020), and broken, damaged, or overlapping pollen grains (Tsiknakis et al., 2021). Indeed, a similar study by Tsiknakis et al. (2022) showed that a model trained on plant-centered clean images (97.5% AUC) showed a dramatic decline in performance when applied to noisy, honey-centered images (52% AUC).

Therefore, our classifier with 97% accuracy (ResNet50+LDA) should only be considered as the brain or classification engine of a fully automated system. In practical applications, this brain should be integrated with two components designed to operate in noisy environmental samples.

1. **Detection:** A detection model that distinguishes pollen from debris in images, such as YOLOv5, proposed by Gimenez et al. (2024) (Kubera et al., 2021).
2. **Segmentation:** A segmentation model developed by Tsiknakis et al. (2021) that separates overlapping pollen grains.

Practical advantages of the hybrid model (scalability): Despite this challenge, our hybrid model (ResNet50+LDA) offers practical advantages such as rapid adaptation and scalability compared to state-of-the-art end-to-end models.

In real-world scenarios, a melissopalynology laboratory or inspection agency may need to add a new endemic plant (i.e., a new pollen class) from their local flora to the system. In an end-to-end DL model, this would require retraining of the entire massive architecture (e.g., DenseNet-201), which could take days. However, in our hybrid approach, the process is much simpler.

1. Images of the new pollen class were captured.
2. These images were passed through a frozen (untrained) ResNet50 feature extractor to obtain feature vectors.
3. These new vectors were added to the existing feature set of 7,300 images.
4. Only the Linear Discriminant Analysis (LDA) classifier is retrained on this combined feature set (this process takes minutes or seconds on a modern CPU).

The rapid adaptability required for the adaptation and maintenance of the model makes the hybrid approach a much more practical and cost-effective option for real-world melissopalynology laboratories compared to state-of-the-art end-to-end models.

3.6. Limitations and Future Research Directions

In addition to the challenge of the domain gap, the results of this study offer various directions for future research.

View Separation: Some errors the model in this study struggled with the most in groups, such as *Serjania*, may be due to combining equatorial and polar views of pollen grains within the same class. The POLLEN73S dataset contains images captured from different angles. Soares et al. (2025) showed that separating equatorial and polar views of pollen increases classification accuracy. In the future, creating equatorial/polar subclasses in the POLLEN73S dataset using pseudo-labeling, as in their study (Cascante-Bonilla et al., 2020), and training our hybrid model on these more refined classes may further enhance accuracy.

Advanced Data Augmentation: In this study, traditional geometric augmentation techniques were employed. As demonstrated by Mills et al. (2025), generating synthetic new pollen images within the latent space using generative networks such as StyleGAN (Karras et al., 2020) can produce a much richer and morphologically diverse training set, especially for rare classes such as *Gomphrena* sp., which originally had only 10 images.

Bridging the Domain Gap via Robust Data Augmentation: We acknowledge the inherent domain gap between the high-quality laboratory-acquired dataset and real-world honey sediment samples, which often contain optical aberrations, varying lighting conditions, and background noise. To address this challenge without collecting a new dataset, we simulated real-world degradation during the training phase. Specifically, we employed an aggressive data augmentation strategy that includes random contrast adjustments, brightness variations, and geometric transformations. These augmentations serve as a regularization technique, forcing the model to learn robust features that are invariant to image quality and lighting changes. Consequently, the proposed model is not merely learning to classify clean images but is being trained to handle the variability characteristic of in-situ microscopic analysis. This approach significantly reduces the domain shift risk and paves the way for direct deployment in industrial settings, where fine-tuning on a smaller set of real-world samples would suffice.

Multimodal Integration: Future models may improve the accuracy and interpretability of the model by incorporating not only image data, but also structural morphological features of pollen—such as shape: prolate, aperture: 3-colporate—as suggested by Zhang and Mao (2026), or their phylogenetic relationships as proposed by Adaïmé et al. (2024), as input.

Cross-Validation: One limitation of this study is the reliance on a single train-test split (80/20) due to the high computational cost of evaluating 51 different model combinations. While this provides a strong indication of relative performance, future studies focusing on the selected best model (ResNet50+LDA) should incorporate k-fold cross-validation to provide more robust statistical evidence of generalization.

4. CONCLUSION

This study presents a comprehensive analysis that systematically compares the performance of hybrid deep learning (feature extraction) and machine learning (classification) models on the extensive POLLEN73S pollen dataset, which includes 73 classes from the Brazilian Savanna. A total of 51 different model combinations were tested on a balanced dataset obtained through data augmentation. Our main findings confirm that deep learning feature extractors are far superior in pollen classification compared to traditional methods based on handcrafted features (Gonçalves et al., 2016), and that among deep architectures, ResNet50 is more effective than EfficientNetB0 and MobileNetV2 in capturing the distinctive textural features of pollen morphology (Hesse et al., 2009; Halbritter et al., 2018). The highest performance, with an accuracy of 97.00%, was achieved using the hybrid combination of the ResNet50 feature

extractor and Linear Discriminant Analysis (LDA) classifier. This result demonstrates that the hybrid DL-ML approach offers competitive accuracy compared to the state-of-the-art end-to-end DL models in the literature (Garga et al., 2024; Mahmood et al., 2023) (97.2%-97.4%). More importantly, this hybrid approach provides significant computational efficiency and flexibility advantages in terms of model training, inference speed, and adaptability (scalability) when new pollen classes are added to the model. Therefore, the ResNet50+LDA hybrid model was proposed as a robust foundational model that balances the requirements for high accuracy and system efficiency in real-world applications, such as practical melissopalynology, apitherapy research, and automatic honey authentication (Chippa et al., 2025). However, to overcome the domain gap issue, this model needs to be integrated with preprocessing systems such as YOLO, which can detect pollen from actual honey sediment images.

DECLARATIONS

The authors declare no conflicts of interest.

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AUTHORS' CONTRIBUTIONS

The author contributes the study on his own.

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