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Image-based recognition using advanced neural networks can aid surveillance of *Agrilus* jewel beetles

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Running title: Automatic Image-Based Identification of *Agrilus* Species

ABSTRACT

The genus *Agrilus* includes two species, *Agrilus planipennis* and *A. anxius*, that are of particular phytosanitary concern and that are regulated by the European Union legislation. This implies that phytosanitary agencies of all EU countries are obliged to establish specific surveillance programs to verify the absence of these species from their territory. These activities commonly consist of the use of green-colored traps, which are however attractive not only for *A. planipennis* and *A. anxius*, but also for a wide range of other *Agrilus* species. For this reason, much time and expertise is required to sort and identify specimens to species, impeding an efficient rapid response. In this study, we tested the efficacy of the Entomoscope, a low-cost, open-source photomicroscope that uses high-resolution digital imaging and allows a pre-trained Convolutional Neural Networks (CNN) model to accurately detect, image and classify insect specimens, for automatic identification of 13 *Agrilus* species, including *A. planipennis* and *A. anxius*. We benchmarked models from three different CNN architectures and selected YOLOv8l as the most robust performer; this model achieved a Top-1 accuracy of 90.2% on a "real-world" test set (i.e. a dataset simulating real surveillance conditions). For most species, including *A. planipennis* and *A. anxius*, either no errors or only a few errors were made, whereas for a few native species misidentifications were more common. These results provided proof of concept for an AI-driven surveillance system that can strongly aid in surveillance activities of *Agrilus* species.

Keywords

Agrilus planipennis, *Agrilus anxius*, Bronze birch borer, Deep learning, Early-detection, Emerald ash borer, Entomoscope

INTRODUCTION

The constant increase of global trade over the last hundred years, combined with deliberate plant introductions in the past and ongoing climate changes, has facilitated the movement among continents and the establishment likelihood of an increasing number of insects (Brockerhoff and Liebhold 2017; Pureswaran et al. 2022; Fenn-Moltu et al. 2023; Isitt et al. 2024). The genus *Agrilus* (Coleoptera, Buprestidae) is among the insect taxa that have taken the most advantage of these processes. This genus includes 3341 species and therefore is one of the most species-rich genera of animals in the world (Jendek and Grebennikov 2023). More than 30 species have already been introduced and established outside their native range (Ruzzier et al. 2023), including the emerald ash borer *A. planipennis* Fairmaire, 1888 which has caused massive ecological and economic damage in North America (Kovacs et al. 2010; Klooster et al. 2018), Russia and Ukraine (Orlova-Bienkowskaja et al., 2020). For these reasons, the development of tools and strategies for the early detection of accidentally introduced *Agrilus* species was identified as a research priority to trigger rapid response and reduce potential impacts in the invaded areas.

Among the numerous tools developed for *Agrilus* surveillance programs, baited or unbaited green traps set up at entry points and in their surrounding forests are currently adopted by several phytosanitary agencies worldwide (Evans et al. 2020; Imrei et al. 2020; Silk et al. 2020; Dodds et al. 2024; Duan et al. 2024; Santoiemma et al. 2024a). These traps were primarily developed based on laboratory and field studies targeting *A. planipennis* (Crook et al. 2009; Francese et al. 2010; Poland and McCullough 2014; Poland et al. 2019), but are also attractive for a wide range of other species within the genus *Agrilus* (Rassati et al. 2019; Cavaletto et al. 2020; Khun et al. 2024; Le Souchu et al. 2024; Santoiemma et al. 2024b, 2025). This allows practitioners to survey multiple species simultaneously, but much time and

expertise are required to sort and identify specimens to species due to both the low interspecific morphological differentiation (Kelnarova et al. 2019) and the very high species richness of the genus (Jendek and Grebennikov 2023), impeding an efficient, rapid response (Lyal and Miller 2020). Thus, novel technologies that can help to improve the efficiency of species identification are promptly needed.

Artificial intelligence (AI) systems are being increasingly adopted in entomology for various applications (Teixeira et al. 2023; Hartbauer 2024). Among them, AI is used to enhance the accuracy and speed of insect identification in the laboratory and in the field (De Cesaro Júnior and Rieder 2020; Gao et al. 2024; Hartbauer 2024). Convolutional neural networks (CNNs) trained on image datasets have been shown to reliably classify insects at the family, genus, or even species level (Valan et al. 2019; Ärje et al. 2020; Hansen et al. 2020; Wührl et al. 2022; Tannous et al. 2023; Lertrusdachakul et al. 2025; Marais et al. 2025). Exploiting this technology in surveillance activities for *Agrilus* species would, however, require that laboratories and plant health inspectors are equipped with an affordable device that can capture images of trapped specimens and, subsequently, automatically identify them to species. The Entomoscope, recently developed by the Karlsruhe Institute of Technology (KIT) (Wührl et al. 2024), might satisfy these needs. It is a low-cost, open-source photomicroscope that uses high-resolution digital imaging and allows a pre-trained CNN to accurately detect, image, and classify insect specimens (Wührl et al. 2024). The Entomoscope has been tested so far on parasitoid wasps (Shirali et al. 2024), but its potential application in *Agrilus* surveillance has not yet been investigated.

In this study, we evaluated the Entomoscope's efficacy for *Agrilus* identification, with a specific focus on model robustness for “real-world” deployment, i.e. performance on data that mimic actual surveillance workflows, with new trap collections acquired under realistic

variation in lighting, specimen condition, and operator handling. First, we benchmarked multiple state-of-the-art CNN architectures to select the top performers. Second, we went beyond standard “laboratory” validation, i.e. accuracy measured on randomly shuffled data acquired under controlled and uniform conditions, and tested these models against a held-out dataset designed to simulate a surveillance process based on traps. We compared this “real-world” performance to the often-optimistic results from standard validation on randomly-shuffled data. Finally, we tested the model's ability to reject “unknown” species not included in the training data, with the objective of developing a truly robust, deployable AI tool to be used along with traps for *Agrilus* surveillance.

MATERIALS AND METHODS

Agrilus specimens

The dataset for this study comprised specimens from 13 *Agrilus* species, including 11 native species to Europe and two non-native species of high phytosanitary concern: *A. planipennis* (native to Asia) and *A. anxius* (native to North America) (Table S1, Fig. 1). The native species were selected as they are commonly collected in trapping studies carried out in Europe targeting *Agrilus* spp. (Le Souchu et al. 2024; Santoiemma et al. 2024b, 2025), whereas the non-natives were selected because they are regulated by the European Union legislation and surveillance is mandatory in EU countries under Regulation (EU) 2019/2072 (EFSA et al. 2020a,b). Specimens were collected in Canada, France, Poland, and Slovenia (Table S1) using green multi-funnel traps. The preservation methods in the trap-collecting cups varied by location and included: a 2:1 mixture of propylene glycol and water (Slovenia); a 1:1 mixture of ethylene glycol and water (Poland); a 2:1 mixture of monopropylene glycol and water with a drop of liquid dish detergent, or kept dry but integrated with a section of mesh impregnated with α-cypermethrin insecticide (Storanet®, BASF Pflanzenschutz Deutschland, Germany) (France); and a saturated salt water solution plus a drop of liquid dish detergent to reduce surface tension (Canada). Specimens were preserved in ethanol until identification. Species-level taxonomic identifications were performed by initially Eva Groznik, Maarten de Groot, Jerzy M. Gutowski, Alain Roques, Aurélien Sallé and Kate Van Rooyen, based on morphological traits, keys, and other reference materials (Schaefer 1950; Farrugia 2007; Paiero et al. 2012), and then confirmed by Gianfranco Curletti.

Image acquisition

A standardized imaging protocol was established using the "plug-in" version of the Entomoscope (Wührl et al. 2024). Two Entomoscope (Fig. S1) units were utilized: one at the University of Padova's DAFNAE department and another at the Entomology Museum of La Sapienza University of Rome. To simulate realistic conditions faced by phytosanitary inspectors, specimens were not manipulated or positioned (or at least to a minimal extent for the identification process). Instead, they were photographed in their natural preservation state (i.e., "death position") and without distinguishing between sexes. Each specimen was photographed from two to five standard points of view to ensure comprehensive morphological coverage: dorsal, laterodorsal, lateral, lateroventral, and ventral (Fig. S2). For image capture, specimens were submerged in 70% ethanol to minimize distortion effects. All images were acquired using the Entomoscope fixed, standard-intensity lighting. To create a single, fully-focused image for each angle, a focus stacking technique using Helicon Focus (v8.2.2) was employed.

Dataset preparation and evaluation strategy

Our evaluation strategy was designed to assess model robustness against the challenges of surveillance. The dataset consisted of 14 classes: 13 *Agrilus* species and one “Background” class. The “Background” class consisted of 89 images of the empty Entomoscope setup (empty Petri dish, with and without ethanol) taken under various lighting conditions to simulate non-target images. The entire dataset was first partitioned at the specimen level (to prevent data leakage). This partitioning was stratified by class (to maintain the distribution of all 14 classes) but ordered by acquisition time (to create a realistic temporal split). Two set of images where created: i) the development set, including the first 85% of images from the ordered, stratified dataset. This set represents the data available for all model training and validation; ii) the held-

out “real-world” set, including the final 15% of images from the ordered, stratified dataset. Because these images were collected later, they may contain subtle variations in lighting, operator setup, or new specimen batches. This set served as our challenging OOD test set, simulating the data drift common in surveillance (often termed an Out-Of-Distribution, or OOD, dataset).

Our evaluation followed a two-stage process. Stage 1 consisted in the model benchmarking. In particular, we trained 11 model variants (from You Only Look Once [YOLOv8, YOLO11], and EfficientNet families) using an ordered 70/15 (a total of 85% of the whole set) split of the Development Set. This means the first 70% of the Development Set was used for training, and the 15% was used as a validation set to select the champion models. The models from this stage were also evaluated on the OOD Set to establish a baseline performance from a simple, ordered training strategy. Stage 2 consisted in the robustness evaluation. The top-performing models were advanced for further evaluation. We performed a randomly-shuffled, stratified 5-fold cross-validation (CV) only on the 85% Development Set. The folds were partitioned at the specimen ID level to prevent data leakage. For each of the 5 trained folds, we performed two distinct evaluations: i) Standard Validation (IID): the performance was measured on the held-out validation fold from the cross-validation. This represents a standard test on randomly-shuffled data (Independent and Identically Distributed, or IID); ii) Real-World Validation (OOD). The exact same model was then tested against the 15% (remaining 15% not used in training and validation process) OOD Set to measure robustness to data drift. The final reported metrics for our recommended methodology were the average and standard deviation across these 5 folds. This process validated the training recipe. To test the model's rejection capabilities, one additional class was created and used only for testing the

"unknown" species class (see below). This class included two species not in the training set, *A. biguttatus* and *A. convexicollis*.

Model architecture

We evaluated and compared three distinct state-of-the-art Convolutional Neural Network (CNN) architectures for our fine-grained classification task, i.e., YOLOv8, YOLO11, and EfficientNet. All models were initialized with weights pre-trained on the ImageNet dataset (Deng et al. 2009) and subsequently fine-tuned on our *Agrilus* dataset. The Ultralytics YOLO (Limberg et al. 2022; Jocher et al. 2023; Khanam and Hussain 2024) framework provides a suite of models optimized for various computer vision tasks. Each model version is available in five sizes (nano, small, medium, large, and extra-large), which offer a trade-off between speed and accuracy. For this study, we selected their dedicated classification models. These models utilize the powerful and highly efficient YOLO backbone for feature extraction but employ a standard classification head, making them distinct from the object detection variants and ideally suited for image classification. Their inclusion allows us to evaluate architectures that provide a state-of-the-art balance between inference speed and accuracy, a critical consideration for future deployment in high-throughput surveillance systems. To benchmark the YOLO models against a well-established, pure classification architecture, we included EfficientNet (Tan and Le 2019). This model family is renowned for achieving high accuracy through a principled compound scaling method and serves as a strong baseline for classification performance. We employed the EfficientNetV2 large variant (Tan and Le 2021). This comparative approach allows for a robust evaluation, pitting models optimized for speed and efficiency (YOLO series) against an architecture designed to maximize classification accuracy (EfficientNet).

233

234 ***Model training and implementation***

235 All models were trained on the HAICORE high-performance computing cluster at the
236 Karlsruhe Institute of Technology (KIT), utilizing nodes equipped with NVIDIA A100-SXM4-
237 40GB GPUs. The software environment consisted of Python (v 3.10) and PyTorch (v 2.0.1)
238 with the Ultralytics framework for the YOLO models, and TensorFlow (v 2.10) with Keras for
239 the EfficientNetV2 models. The training process was conducted in the two stages described in
240 Section 2.3. We employed transfer learning for all models, initializing them with weights pre-
241 trained on the ImageNet dataset (Deng et al. 2009) to accelerate training and improve
242 generalization. Input images were resized to the input dimensions required by each architecture
243 (e.g., 640x640 for YOLOv8, 480x480 for EfficientNetV2L) and standard data augmentation
244 techniques (e.g., random flips, rotations, color adjustments, erasing) were applied. To ensure
245 robust generalization and prevent overfitting, we employed a consistent optimization strategy.
246 All models were fine-tuned using the AdamW optimizer (Loshchilov and Hutter 2019) and
247 included dropout regularization (rate = 0.3). An early stopping mechanism was used, halting
248 training if the validation loss did not improve for 15 consecutive epochs, with a maximum limit
249 of 150 epochs. To address the inherent class imbalance in the dataset, we applied class weights
250 to the loss function. For the EfficientNetV2 models, a categorical focal cross-entropy loss was
251 used to further prioritize difficult-to-classify examples, while the YOLO models used their
252 framework's standard weighted loss function. The training and validation curves, as well as
253 accuracy curves are shown in Fig. 2. The learning dynamics showed similar patterns across
254 folds, with consistent loss decay and accuracy stabilization; therefore, one representative
255 example (fold 4) is shown here. All the images used for training and testing, as well as the

scripts employed in the classification pipeline, are available in the Zenodo (www.zenodo.org) repository.

Performance evaluation metrics

Model performance was assessed using a comprehensive set of metrics, evaluated at two distinct stages. In stage 1 (benchmarking) models were ranked based on Top-1 validation accuracy. Their performance on the OOD Set was also recorded. In stage 2 (robustness) the 5-fold CV models were evaluated on both the IID validation fold and the OOD test set, using Top-1 Accuracy and Weighted F1-Score. The final performance of the methodology was reported as the average of the 5-fold CV results on the OOD set. A full classification report and an aggregated confusion matrix, summing the predictions from all 5 folds, were generated. To test the model's ability to handle species not included in the training process, we added an additional class named "unknown". In fact, our model does not include an "out-of-class" mechanism to exclude images that do not belong to any trained class, so it still tries to associate the image with the most similar species. When the image is very different from those used for training, the confidence level (max_conf) of the prediction is typically low. In our case, we set this confidence level to 0.5, meaning that the model will ignore all predictions with less than 50% confidence. This threshold was adopted following the standard convention, as it represents the neutral decision boundary in probability-based classification tasks. To test this mechanism, we used 19 images belonging to two native species that were not used for the training procedure (i.e., 8 images belonging to 2 specimens of *A. biguttatus* and 11 images belonging to 3 specimens of *A. convexicollis*).

Model explainability

To gain insight into the models' decision-making process, we employed the Eigen-CAM explainability technique (Muhammad and Yeasin 2020). Eigen-CAM produces saliency maps that highlight the image regions most influential for a given classification, allowing for a qualitative assessment of whether the models focus on relevant morphological features.

RESULTS

Stage 1: model benchmarking

The 11 model variants were trained on an ordered 70% training set and ranked by their Top-1 accuracy on the ordered 15% validation set (Table 1). The EfficientNetV2L and YOLOv8l emerged as the top performers, tying with an identical validation accuracy of 88.9% (0.8885). These two models were selected for the comprehensive Stage 2 evaluation. When tested on the OOD Set, the models from this simple, ordered training strategy performed modestly. The top-performing YOLOv8l achieved a Top-1 accuracy of 85.0% (Table 1), establishing a baseline for our subsequent robustness tests.

Stage 2: Robustness evaluation of standard vs. real-world testing

The top two models, YOLOv8l and EfficientNetV2L, underwent a 5-fold CV. Both models achieved near-perfect accuracy on the IID validation folds, with YOLOv8l averaging 97.9% ± 1.4% and EfficientNetV2L averaging 97.2% ± 2.4% (Table 2 and Fig. 3). However, when these same models were tested against our 15% OOD Set, two key findings emerged. First, a performance gap was observed. The YOLOv8l model's accuracy, for example, was 97.9% on the IID fold but 90.2% on the OOD set, a difference of 7.7 percentage points. Second, the models trained with the robust, randomly-shuffled CV methodology (Stage 2) achieved higher accuracy on the OOD set than the models from the simple, ordered training (Stage 1). The CV-trained YOLOv8l achieved an average Top-1 accuracy of 90.2% ± 1.9%, compared to the

85.0% from the Stage 1 ordered-split model. On this OOD test, the YOLOv8l methodology had a higher average Top-1 accuracy, with 90.2% ± 1.9% and a weighted F1-score of 88.2% ± 2.4%. This was consistently higher than the EfficientNetV2L (89.8% ± 1.7% Top-1, 88.0% ± 1.6% F1).

Final YOLOv8l model performance (5-Fold Average)

Based on its higher robustness and performance, YOLOv8l was selected as our final recommended methodology. The trained CNN correctly assigned the highest percentage of correspondence to the correct species 100% of times for 7 species, i.e., the non-native *A. anxius* and *A. planipennis*, and the native *A. angustulus*, *A. betuleti*, *A. cuprescens*, *A. graminis*, and *A. olivicolor* (Fig. 4). For four of the other species, the trained CNN assigned the highest percentage of correspondence to the correct species between 86% and 98% of times (Fig. 4). In particular, the native *A. laticornis* was identified with a 92% accuracy (respectively 4% and 5% of the pictures with *A. laticornis* have been misidentified with *A. angustulus* and *A. graminis*), the native *A. obscuricollis* with a 95% accuracy (3.5% misidentified as *A. olivicolor* and 1.5% as *A. hastulifer*), the native *A. sulcicollis* with 86.0% accuracy (9% misidentified as *A. laticornis*, 1% as *A. planipennis* and 3% as *A. angustulus*) and the native *A. viridis* with a 98% accuracy (2% misidentified as *A. graminis*). The *Agrilus* species for which a misidentification was common were *A. hastulifer* and *A. pratensis*. For *A. hastulifer*, the trained CNN assigned the highest percentage of correspondence to *A. graminis* 65% of the times and to *A. viridis* 21% of the times (Fig. 4), making *A. hastulifer* the class with the lowest percentage of correct classification (9%). For *A. pratensis*, the trained CNN assigned the highest percentage of correspondence to the wrong species 58% of the times to *A. obscuricollis* and

2% times to *A. hastulifer* (Fig. 4). Full per-class metrics (Precision, Recall, F1, Support; mean ± SD over five folds) are provided in Supplementary Table S2.

Identification of out-of-distribution species

The model's rejection capability was tested on the OOD set. Averaged across the 5 folds, the YOLOv8l methodology correctly classified 100% of the 19 images from the “unknown” class (*A. biguttatus* and *A. convexicollis*) as unknown species using a 0.5 confidence threshold.

Model explainability

Eigen-CAM analysis of the trained YOLOv8l models revealed that the classification decisions relied on specific morphological regions. For example, the model consistently focused on the distinctive shape and texture of the elytra in most specimens in dorsal and laterodorsal views (*A. angustulus*, Fig. 5A; *A. olivicolor*, Fig. 5C), as well as on head morphology—particularly the pronotal margins, eyes, and clypeus (*A. olivicolor*, Fig. 5C). In lateral and ventral images, key features such as the shape of the abdominal sternites (*A. angustulus*, Fig. 5B; *A. planipennis*, Fig. 5D), the structure of the eyes (*A. planipennis*, Fig. 5D), and, in many cases, the elytra, were especially influential.

DISCUSSION

The genus *Agrilus* includes two species, *A. planipennis* and *A. anxius*, that are of particular phytosanitary concern due to the economic and ecological impacts they can have in the invaded areas (Baranchikov et al. 2008, 2014; Kovacs et al. 2010; Klooster et al. 2018; Evans et al. 2020) and as such, they are regulated by the European Union legislation (EFSA et al. 2020a, 2020b). This implies that phytosanitary agencies of all EU countries are obliged to establish specific surveillance programs to verify the absence of these species from their territory (Evans et al. 2020). These activities commonly consist of the use of green-colored traps, which are however attractive not only for *A. planipennis* and *A. anxius* but also for a wide range of other *Agrilus* species (Rassati et al. 2019; Cavaletto et al. 2020; Le Souchu et al. 2024; Santoiemma et al. 2024a, 2024b, 2025). Here we showed that the Entomoscope, in combination with our validated deep learning methodology, can strongly aid in discriminating *A. planipennis* and *A. anxius* from other commonly collected native *Agrilus* species, a process that otherwise demands much time and taxonomic expertise.

Our validated YOLOv8l methodology, which averaged 90.2% Top-1 accuracy on the real-world set, serves as a powerful proof-of-concept for a practical triage tool. Its perfect identification (100% F1-score) of the key pests *A. planipennis* and *A. anxius*, as well as its perfect identification of the "Background" class, demonstrates its immediate value for phytosanitary agencies. This system could be used to autonomously sort through thousands of specimens, filtering out empty images and flagging only those identified as high-priority pests for expert review. However, the model is not an infallible taxonomist. The aggregated confusion matrix shows that misclassifications still occur among a few native species, namely *A. hastulifer* and *A. pratensis*. This is quite surprising, as these species are easily separated using classic taxonomic approaches (Schaefer 1950; Farrugia 2007). This might be due to the

characters used by the models. As for many other CNNs, the models are only partially based on the same morphological characteristics (such as genitalia, setae and hairs distribution, size, etc.) (e.g., Volkovitsh et al. 2020) used by taxonomists but instead on the arrangement and intensity of pixel clusters which are identified as discriminating features by the algorithm (Goodfellow et al. 2016; Redmon et al. 2016). Our Eigen-CAM analysis supports this, showing that, depending on the position of the specimen, the general shape of the body and the margins of the head, elytra, and pronotum or the shape of the abdominal sternites, the forehead and the clypeus were used as main characters to discriminate among the tested *Agrilus* species. This can result in accurate classification of species that are difficult to distinguish (i.e., *A. obscuricollis*), but also failures when attempting to discriminate between species that are easily separated using classic taxonomic approaches (e.g., *A. hastulifer* and *A. pratensis*). Overall, these results indicate that the Entomoscope and the current methodology are highly useful for surveillance of *A. planipennis* and *A. anxius*, but are not yet ready to identify all native species collected in traps to a species level.

Our results also demonstrated the ability of the trained CNN to reject non-target images. Our methodology correctly assigned 100% of correspondence to the class “unknown” when identifying specimens belonging to species not used in the training process (*A. biguttatus* and *A. convexicollis*). Several trapping studies showed that many *Agrilus* species can be collected when using green-colored traps (e.g., Santoiemma et al. 2024a, 2024b). These species might include native species present at low density or non-native species not initially targeted. In this scenario, the capacity of the trained methodology to discriminate between known and unknown species can alert the phytosanitary personnel of the presence of a potential non-native species that should be further scrutinized via classic morphological or molecular approaches.

Our results also indicate that the method of validation is critical for determining real-world-viability. Our study demonstrates a significant "validation gap" between IID tests and our more realistic, OOD dataset. While our top models achieved near-perfect accuracy (97-98%) in the “laboratory” setting, this performance dropped by over 7 percentage points when faced with data simulating new collection events. This finding strongly suggests that studies reporting accuracy from simple, random splits may be dangerously optimistic, and that testing against OOD data is essential for validating deployable biosecurity tools. Furthermore, our study provides a key insight into how to train a robust model. The model from our initial benchmark, trained on a simple ordered split, achieved a respectable 85.0% on the OOD set. However, the models trained using a randomly-shuffled, 5-fold CV (on the exact same development data) were demonstrably superior, achieving an average of 90.2%. This proves that a robust training strategy, which exposes the model to more diverse and randomized data combinations, is just as important as the model architecture itself for building a tool that can generalize to new data. This performance gap was not uniform. Interestingly, while the EfficientNetV2L tied for the highest score in our initial benchmark, the YOLOv8l model proved to be more robust, consistently performing better on the challenging OOD set. This suggests that the architectural properties of YOLOv8l may offer better generalization to the subtle data drift (e.g., in lighting, pose, or operator handling) inherent in real-world surveillance.

Conclusions

This study provides a robust proof-of-concept for an AI-driven system, combining the Entomoscope with a validated deep learning methodology, for the surveillance of *Agrilus* jewel beetles. We have demonstrated that our final YOLOv8l methodology is a reliable triage tool,

capable of perfectly identifying the high-priority pest *A. planipennis* and *A. anxius*, correctly filtering background noise, and effectively rejecting unknown species. In addition, we have presented a validation framework that moves beyond standard "laboratory" accuracy to test for robustness against real-world data drift. This methodology of evaluating on a OOD dataset is critical for bridging the gap between a promising model and a trustworthy, deployable tool for phytosanitary surveillance. In fact, the open-source nature of this technology, combined with the relatively low cost of setting up and operating an Entomoscope, makes AI-based identification systems highly suitable for technology transfer initiatives. These systems could be integrated into training projects for phytosanitary personnel, foresters and environmental agencies, increasing knowledge on AI-based identification technologies, and encouraging their active participation in large-scale monitoring efforts. Future work will focus on expanding the species library and integrating this model into a field-ready, rapid-alert system without the need for extensive laboratory infrastructure (Brydegaard et al. 2024; Chiavassa et al. 2024), as well as hybrid approaches that integrate CNN-based image classification with other diagnostic methods, such as molecular tools. A cloud-based or mobile application linked to the Entomoscope could allow users to upload images and receive automated identifications in real time, greatly increasing the accessibility and practical applicability of the system. Such an innovation would not only facilitate rapid species identification in phytosanitary surveillance but also contribute to broader biodiversity assessment initiatives.

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Data Availability

All image data used and the code developed for this study are available on Zenodo repository at https://doi.org/10.5281/zenodo.14998760.

CRediT authorship contribution statement

Valerio Caruso: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Visualization; **Hossein Shirali:** Writing – original draft, Methodology, Investigation, Formal analysis, Visualization; **Christophe Bouget:** Writing – review & editing; **Pierfilippo Cerretti:** Writing – original draft, Conceptualization, Supervision, Funding acquisition; **Gianfranco Curletti:** Writing – review & editing, Data curation; **Maarten de Groot:** Writing – review & editing, Resources; **Eva Groznik**: Writing – review & editing, Resources; **Jerzy M. Gutowski:** Writing – review & editing, Resources; **Pyliatuk:** Writing – review & editing, Resources, Methodology; **Radosław Plewa**: Writing – review & editing, Resources; **Alain Roques:** Writing – review & editing, Resources; **Aurelién Sallé:** Writing – review & editing, Resources; **Jon Sweeney:** Writing – review & editing, Resources; **Kate Van Rooyen:** Writing – review & editing, Resources; **Lorenz Wührl:** Writing – review & editing, Methodology; **Davide Rassati:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Supervision, Visualization, Funding acquisition, Project administration

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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.

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FIGURES

Fig 1. Examples of pictures taken using the Entomoscope to some of the *Agrilus* specimens used during the training process. (A) *A. angustulus*, (B) *A. anxius*, (C) *A. cuprescens*, (D) *A. graminis*, (E) *A. hastulifer*, (F) *A. laticornis*, (G) *A. obscuricollis*, (H) *A. olivicolor*, (I) *A. planipennis*, (J) *A. pratensis*, (K) *A. sulcicollis*, (L) *A. viridis*. Note: the pictures are not to scale with each other.

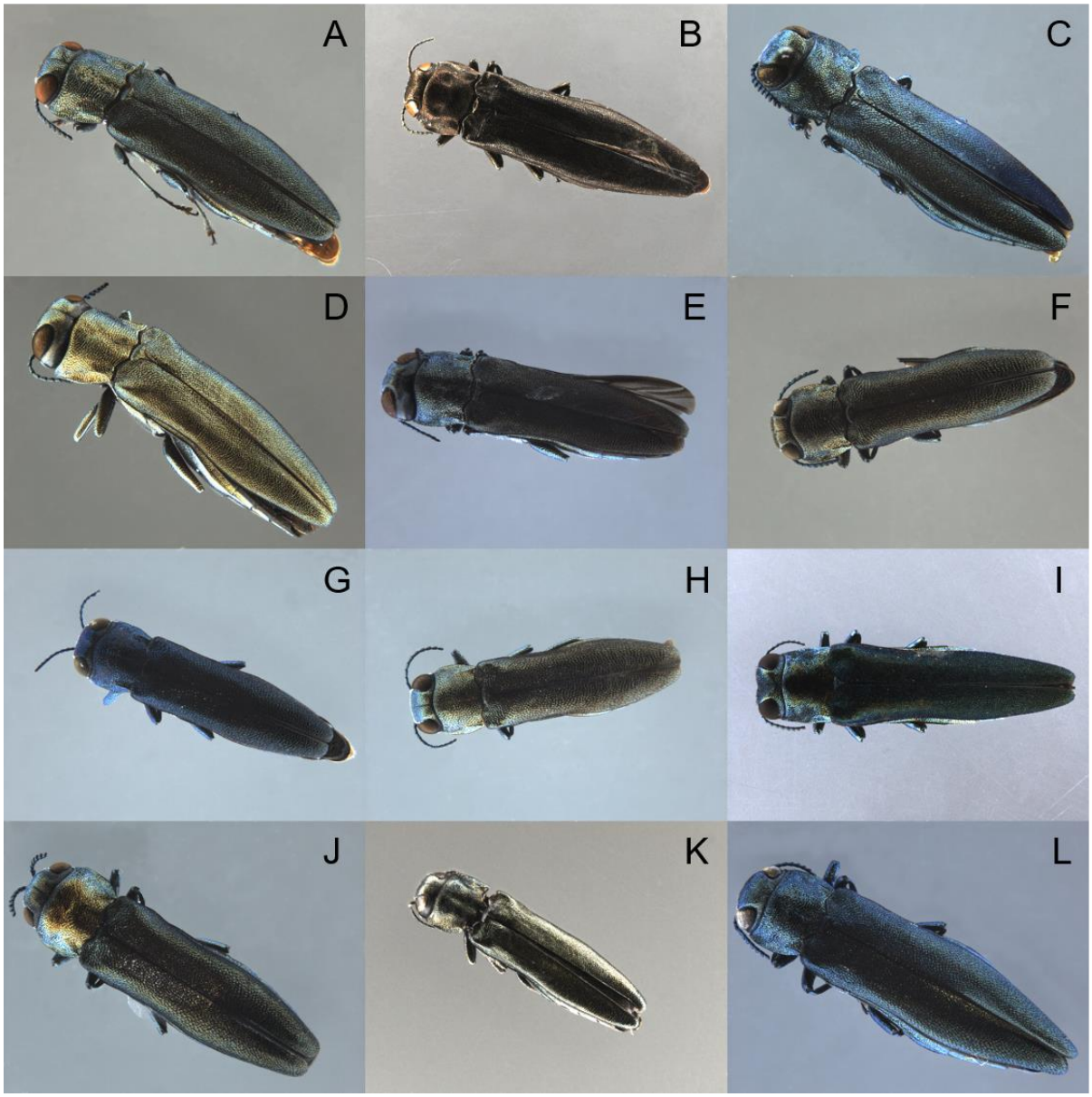


Fig 2. Representative training and validation curves for the YOLOv8l model during one of the cross-validation folds. The plots show (top left) training loss and (top right) validation loss decreasing over epochs, indicating effective learning; (bottom left) Top-1 accuracy and (bottom right) Top-5 accuracy on the validation set increasing and stabilizing, confirming robust convergence without overfitting. Blue full line = results; orange dashed line = smooth.

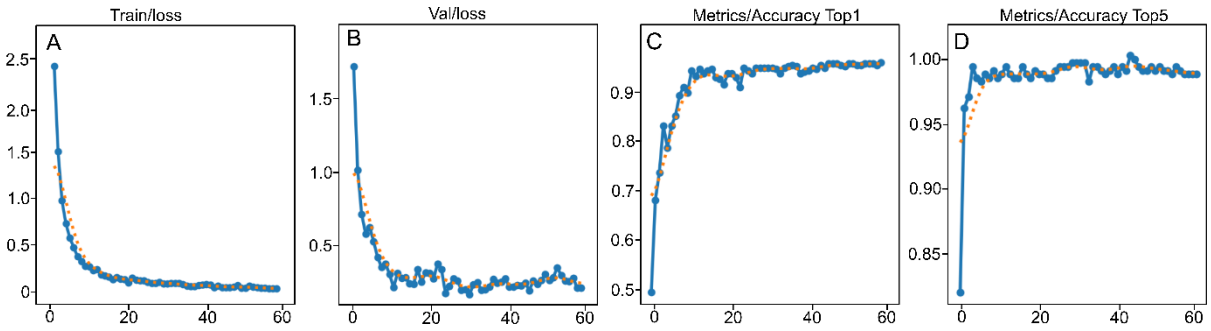


Fig 3 Comparison of Top-1 Accuracy between Standard Validation (IID) and "Real-World" Validation (OOD) for YOLOv8l and EfficientNetV2L models. Bars show mean accuracy across five folds, illustrating the performance gap between IID and OOD evaluations.

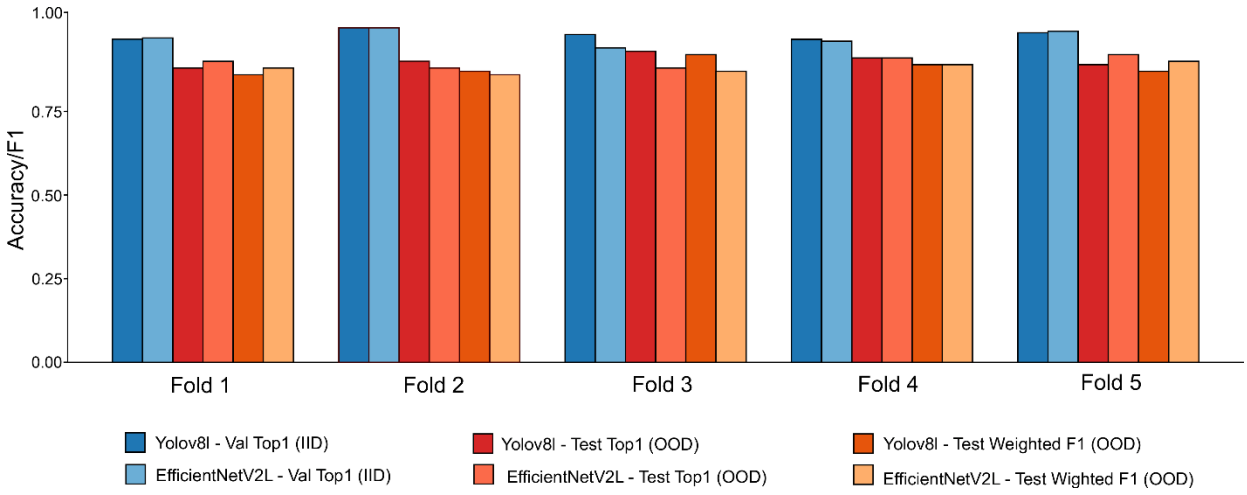


Fig 4 Aggregated confusion matrix for YOLOv8l on the held-out real-world (OOD) test set. The matrix represents the sum of all predictions from the five cross-validation folds across 14 classes, showing classification accuracy per class and highlighting the main sources of misclassification. True classes (Y axis) indicate the true species identity. Predicted classes (X axis) indicate the species to which the model assigned analyzed specimens. The degree of accuracy is reported both in the form of numerical value, showing the percentage of specimens that were assigned to a given species, and color-coded with blue for correct predictions and red for incorrect ones (light blue and red are associated with low degree of prediction, both correct and incorrect).

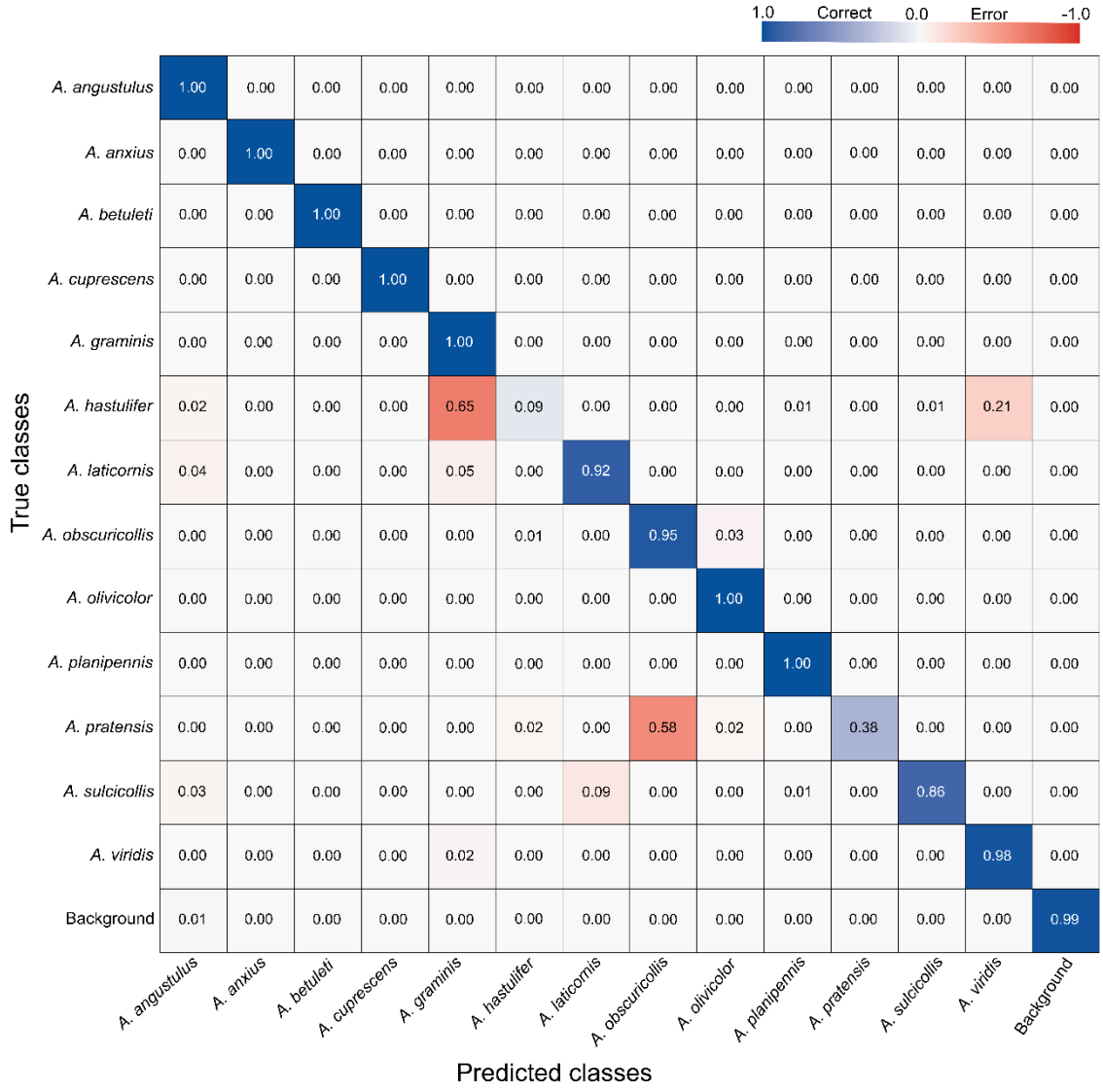
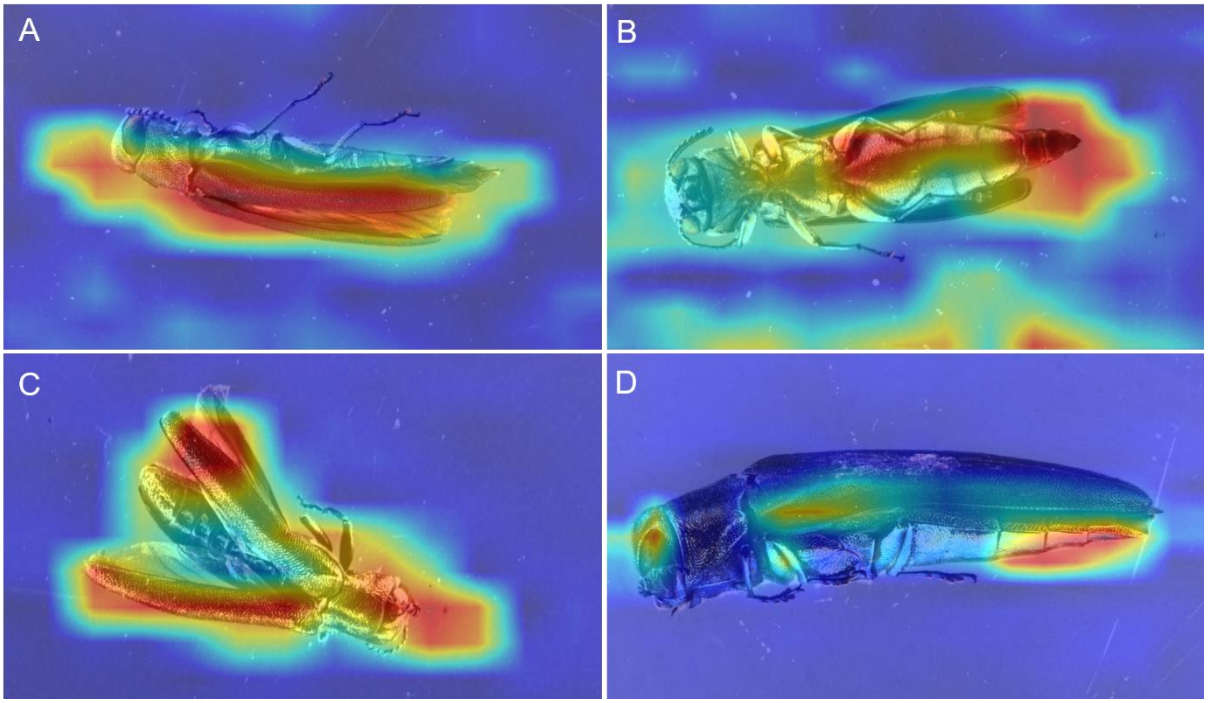


Figure 5. Example of class activation heatmaps obtained for pictures of A) *Agrilus angustulus* (laterodorsal view), B) *A. angustulus* (ventral view), C) *A. olivicolor* (dorsal view), D) *A. planipennis* (lateral view). The heatmaps indicate the image regions most influential for the model's prediction (from red = high influence, to blue = low influence).



TABLES

Table 1 Benchmarking of 11 model variants (YOLOv8l and EfficientNetV2L). Reports Validation Top-1 (ordered split) and, on the held-out real-world OOD test set, Test Top-1 and Test Weighted F1.

	Validation Top1 accuracy	Test Top1 accuracy	Test Weighted F1 score
Yolov8n	0.8500	0.8100	0.7900
Yolov8s	0.8852	0.8100	0.7800
Yolov8m	0.8787	0.8200	0.8000
Yolov8l	0.8885	0.8500	0.8300
Yolov8x	0.8852	0.8300	0.8100
Yolo11n	0.8623	0.8100	0.7800
Yolo11s	0.8525	0.7800	0.7600
Yolo11m	0.8787	0.8000	0.7800
Yolo11l	0.8400	0.7600	0.7400
Yolo11x	0.8426	0.7700	0.7500
EfficientNetV2L	0.8885	0.8500	0.8200

770 **Table 2** Results (%) of the 5-fold cross-validation for YOLOv8l and EfficientNetV2L models

Model	Metric	Fold 1	Fold 2	Fold 3	Fold 4	Fold 5	Mean ± SD
EfficientNetV2L	Validation Top1 Accuracy	97.0	100.0	94.0	96.0	99.0	97.2 ± 2.4
EfficientNetV2L	Test Top1 Accuracy	90.0	88.0	88.0	91.0	92.0	89.8 ± 1.7
EfficientNetV2L	Test Weighted F1	88.0	86.0	87.0	89.0	90.0	88.0 ± 1.6
YOLOv8l	Validation Top1 Accuracy	96.6	100.0	98.0	96.6	98.5	97.9 ± 1.4
YOLOv8l	Test Top1 Accuracy	88.0	90.0	93.0	91.0	89.0	90.2 ± 1.9
YOLOv8l	Test Weighted F1	86.0	87.0	92.0	89.0	87.0	88.2 ± 2.4

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