LEPY: A Python-Based Pipeline for Automated Morphological Trait Analysis of Lepidoptera Images

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Abstract

1. We present LEPY, a Python-based pipeline for automating the extraction and analysis of morphological traits, including structural and colour properties, from mounted Lepidoptera specimens. It uses a U-Net neural network for image segmentation and a scale bar for precise measurements. LEPY is designed to be easy and reproducible, ensuring efficient and consistent analysis of large Lepidoptera image datasets. It also supports the integration of UV photographs for enhanced colour analysis.

2. LEPY computes structural traits, including body and wing length and area, and colour characteristics such as hue, saturation, and intensity, which are stored in a structured format (CSV) for easy evaluation. It also provides distribution metrics that describe the brightness and dynamic range/contrast, chromaticity, and luminance for four colour channels (R, G, B, and UV). Data from all channels are integrated to calculate colour diversity using the Shannon index. A visual summary of each image pair, including false colour images, is also provided.

3. We validated LEPY using data from Sphingidae and Saturniidae moths, known for their contrasting traits, which were sampled along a complete elevational gradient in the Peruvian Andes. In both families, forewing length increased with elevation. As expected, Sphingidae had smaller wing areas than Saturniidae despite their longer forewings. The brightness of colours decreased with elevation in both families, and Sphingidae were generally darker than Saturniidae. The dynamic range/contrast varied among species but was uncorrelated to elevation.

4. LEPY is a powerful tool for studying key Lepidoptera traits. It integrates advanced computer vision and neural network methods to automated measurements, supporting ecological and

evolutionary research. It also offers new possibilities for analysing Lepidoptera traits along gradients and responses to environmental changes.

KEYWORDS

Computer Vision, Deep Learning, Lepidoptera, Multispectral Colour Analysis, Structural Traits, Python, Photography, UV Photography

1 | INTRODUCTION

Integrating measures of functional traits of organisms and their change with environmental stressors is becoming increasingly important in ecological research (Correa-Carmona et al., 2022; Gámez-Virués et al., 2015; Wellstein et al., 2011). This can be particularly challenging in insects which are the most diverse and abundant organisms on Earth, and it is usually very time-consuming to identify, prepare and measure their morphological traits (van Klink et al., 2022). Lepidoptera – butterflies and moths – is one of the most diverse insect clades, are no exception with this regard (Freitas et al., 2020). They have been used as a model group for many studies in ecology and evolution (Watt & Boggs, 2003; Hill et al., 2021), because at least the largest and most conspicuous groups have traditionally been intensively collected. Lepidopteran taxonomy is on average better studied than in other insect orders, with notable exceptions in which more difficult molecular analysis or other tools for identification are required (Lamarre et al., 2022; Moraes et al., 2021; Murillo-Ramos et al., 2021).

Body size is probably the most important and the most studied functional trait, as it is linked to population vital rates (metabolism, survival, growth, reproduction) and to ecological interactions (Brehm et al., 2019; Woodward et al., 2005). It is also one of the most sensitive traits to environmental change (Chown & Gaston, 2010; Cortés-Gómez et al., 2023; Tammaru & Teder, 2012). Another important morphological trait of insects is their colouration. Colour is particularly important in Lepidoptera, because it is used for a variety of purposes, such as body protection (i.e., camouflage, mimicry), signalling and physiological/thermal adaptation (Heidrich et al., 2018; Koičková et al., 2012). Wing colour patterns are also of key importance for species-level identification (Feng et al., 2015). The UV (ultraviolet) colouration of Lepidoptera is also of vital importance in a wide range of biological and ecological contexts (Brehm et al., 2021; Heidrich et al., 2018; Koičková et al., 2012). UV patterns, invisible to mammals including humans, but perceptible to a wide range of other organisms (e.g., most arthropods and birds), play an important role in communication (Cronin & Bok, 2016; Paul & Gwynn-Jones, 2003). For example, visual signals can attract mates or to distinguish conspecifics from other species (Bálint et al., 2012), they are important for survival (camouflage or mimicry strategies; Lyytinen et al., 2004; Zapletalová et al., 2016) and evolutionary processes (e.g., pollination; Ohashi et al., 2015; Papiorek et al., 2016). However, relatively few studies have been carried out that include UV patterns of insects in general and of Lepidoptera in particular (Stella & Kleisner, 2022), and digitisation programmes in biological collections have usually neglected UV patterns (Brehm, 2025).

Traditional manual methods for measuring morphological traits in entomology are usually time consuming and prone to human error (Fountain-Jones et al., 2015). In the case of Lepidoptera, wing structures are also delicate and can be easily damaged. Recently, several studies investigated ways to automate the detection, classification, and measurement of functional traits such as wing and body size, coloration, and coloration patterns in Lepidoptera by using modern approaches such as computer vision and machine learning, offering ways to save time and improve the accuracy of these measurements (Feng et al., 2015; Høye et al., 2021; Manoukis & Collier, 2019; Palma et al., 2023). Computer vision and deep learning techniques have significantly advanced the field of entomology by reducing reliance on manual measurements, enabling large-scale, high-throughput analyses, and improving the accuracy and reproducibility of trait measurements (Høye et al., 2021; Manoukis & Collier, 2019; Palma et al., 2023). Python is the most used programming language for developing these techniques

(Van Rossum & Drake, 2009), thanks to its advanced image analysis frameworks, comprehensive and extensive scientific libraries/tools, broad user community, and continuous contributions from third-party developers, ensuring its adaptability for new applications. Pipelines already exist in the field of automated analysis of Lepidopteran images, including MothSeg and Mothra. MothSeg (https://github.com/erodner/mothseg) contains Python-based tools for segmenting and analysing images of moths and butterflies in dorsal and ventral position using a scale. Mothseg provides mean, median, and standard deviation of hue, saturation, and intensity of an RGB image, and calculates the width of the moth shape as well as the area of body + wings (Jaimes Nino et al., 2019). However, options for body size measurements are limited, and it takes 3 to 5 minutes to analyse a single image. Another computer vision pipeline is Mothra (Wilson et al., 2023); it automatically detects the specimen and other objects in the image, adjusts the scale, measures wing characteristics (e.g., forewing length), determines the orientation of the image (vertical or horizontal), and identifies sex by assessing patterns of sexual size dimorphism. Mothra is also limited regarding the number of morphological traits and does not analyse colours.

In this paper we introduce LEPY, a Python-based tool for the automatic segmentation and analysis of lepidopteran structural and colour traits. Our goal was to integrate key features from existing algorithms with significant new features, especially in quantitative colour analysis, including information on UV reflectance. We aimed to enhance segmentation accuracy through deep learning models and incorporate a configurable calibration step to extract comprehensive measurements more efficiently. We also wanted to develop a structured trait data to store all trait data and a visualization system that highlights key morphological traits alongside density plots of colour channels. To validate LEPY's utility, we applied it to a dataset of two well-studied moth families, viz. Sphingidae and Saturniidae, collected along an elevational gradient in Peru.

2 | MATERIALS AND METHODS

2.1 | Implementation details

LEPY is an open-source pipeline hosted on GitHub (https://github.com/tzlr-de/LEPY), developed and tested under Python 3.9 and 3.12. The recommended installation includes the setup of a virtual python environment like Anaconda (Anaconda, 2023) and the installation of the packages listed in the requirements file. For the detailed installation instructions, we refer to the README.md in the code repository.

LEPY is a command-line program. To run the pipeline, the users must provide the image folder and a configuration file with processing parameters as command-line arguments. In the code repository, we included a configuration file with default values, which worked well on our dataset.

A minimum of one regular (RGB) TIF image is required. For the inclusion of UV information, the corresponding UV image must share the same file name as the RGB image, with 'uv' appended to the UV file name (e.g., Pe-Geo-0016.tif and Pe-Geo-0016uv.tif; see Figure 3). The images should preferably have good lighting conditions, including a neutral grey and homogeneous background, consistent scaling across all images, and the removal of any labels for photography (Brehm, 2025; Figure 3).

Using the UV channel, LEPY generates two new images: *RGB-UV mixed* and *GB-UV*. The first image is a single-channel image, where each pixel is a weighted sum of the R, G, B, and UV channels. It is related to the computation of a grayscale image; in such a normal greyscale image, the grey value is = 0.299 * red + 0.587 * green + 0.114 * blue, corresponding to the sensitivity of a human eye. Since we could not simply extend this weighting to the UV channel, we decided for the most neutral approach in which all four channels where equally weighted (0.25 each). The second image is a false colour image. In this image, the information from the GB-UV channels is shown in the available RGB channels, resulting in the *GB-UV* image.

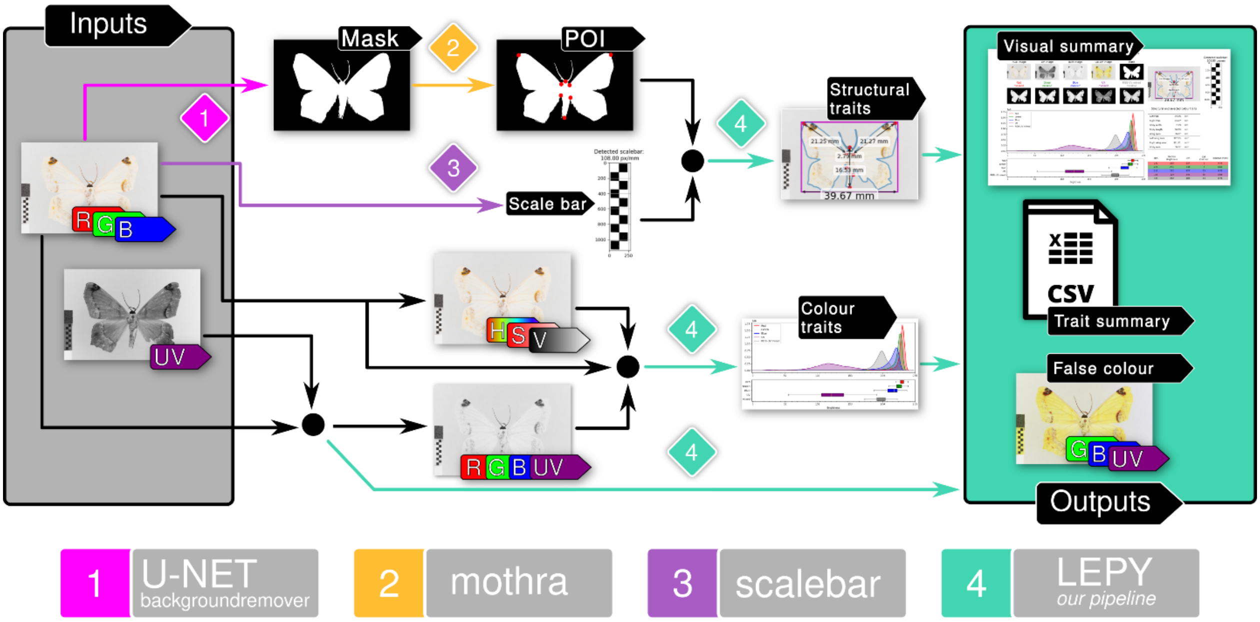

FIGURE 1 LEPY Pipeline - The input images are processed in multiple steps, including a CNN-based pixel-accurate segmentation of the specimen, the localisation of points of interest (POI) using the mothra package, the detection and processing of the scale bar using the scalebar package, and finally the computation of various structural and colouring traits. Besides the main trait summary, which LEPY stores as a CSV table, LEPY stores for each image a visual summary (see Section 3.1), a false colour image (*GB-UV*), all the computed traits in a JSON file, and the contours of the segmentation mask.

In Figure 1, we show the major processing steps of LEPY: (1) the pixel-accurate segmentation of the specimen, (2) the localisation of the points of interest (POI), (3) the detection and analysis of the scale bar, and (4) the computation of the structural and colouring traits based on the scale bar information and the POI.

For the first step, we used the backgroundremover (https://github.com/nadermx/backgroundremover) package. It utilises a U-NET-based (Ronneberger et al., 2015) neural network which is trained for general background removal. This solution works well with the homogeneous light grey background we used (see Brehm, 2025), as the specimen can be usually easily distinguished from the background. We also evaluated other methods for background separation, e.g., GrabCut (Rother et al., 2004), Otsu's thresholding (Otsu, 1979), etc.), but the backgroundremover package delivered the most stable results, especially in the cases where the specimen had a very bright colouring. After postprocess the binary mask returned by the backgroundremover by filling minor holes, we identify contours in the binary mask and select only the largest contour under the assumption that this contour encloses the specimen.

Based on the extracted binary mask, we estimated eight points of interest (POI) in each specimen (Figure 2A), which helps to separate the body from the wings and calculate the sizes of different specimen parts. We used and extended the Mothra package (Wilson et al., 2023), which estimates four of the POI we are interested in, i.e., the wing tips and base of both forewings. LEPY also estimates also the two lower points which connect the wings with the body, and the upper and lower tips of the body (Figure 1).

Next, for the extraction of the scaling information from the scale bar present in the image, we used scalebar (https://github.com/DiKorsch/scalebar), a reworked and improved version of the MothSeg pipeline (https://github.com/erodner/mothseg). To locate the scale bar, it uses a simple pattern matching method (https://docs.opencv.org/4.x/d4/dc6/tutorial_py_template_matching .html) by matching a template image at different sizes (ranging from 2.5% to 100% of the original template size in steps of 2.5%) across the left 15% area of the image. As a template, we used a single image of a chessboard reference (see Figure 3) with a pre-computed ratio of 290 pixels per mm (a scale bar template is supplied in SI4; if printing, ensure it's at 100% for accurate measurements; scale purchased from Sphere Optics, https://sphereoptics.de/uebersicht-produkte/). Using this pre-computed scale and the resize factor matched best during pattern matching, the final scale is computed and returned. If the scale of the template image is unknown, then another algorithm identifies up to 50 corners of the chessboard and estimates the scale based on the distances between these corners. Both the location of the template image and the pre-computed scale can be modified in the configuration file if our provided scale does not accurately represent the scale bar of the analysed images.

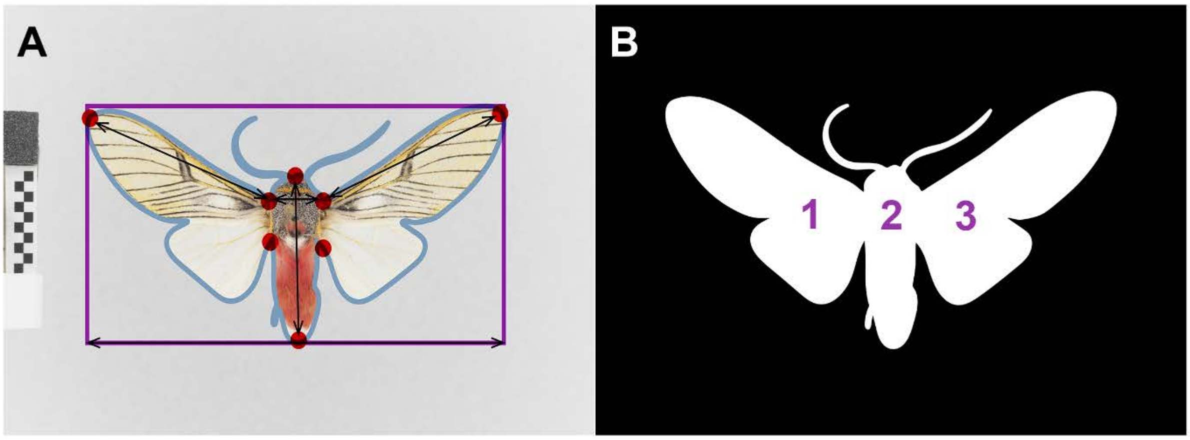

FIGURE 2 A: Points of interest (POI, shown as red dots) detected on a binarized moth image, highlighting the body and length of both forewings, and the thorax width generated by the pipeline. B: The binarized moth image is segmented into three sections: 1. the left forewing and hindwing, 2. the body, and 3. the right forewing and hindwing.

Finally, after we estimated the eight POI and the scale of the image, LEPY quantifies various structural and coloration traits. For structural measurements, it assesses thorax width, body length, and forewing length (Figure 2A). By segmenting the mask into distinct body and wing regions, LEPY also calculates their respective areas (Figure 2B). These measurements are recorded in the visual summary generated for each image and the corresponding .csv file (see 2.2). For the colouring traits, LEPY analyses each channel of the RGB (red, green, and blue) image separately. LEPY transforms the RGB image into HSV (hue, saturation, and value) colour space. A distinguishing feature of LEPY is its capability to compute statistical metrics for the ultraviolet (UV) channel. Finally, using the segmentation mask estimated in previous image processing step, we calculate mean, median, minimum, and maximum pixel values for the RGB-UV and HSV. For the all four channels (RGB-UV), LEPY calculates the 25% quartile, 75% quartile, the interquartile range (IQR) which represents dynamic range / contrast, Shannon index, luminance, and chromaticity (formulas detailed in SI1). In the visual summary, we also add density and box plots for each channel and the *RGB-UV mixed* image (Figure 4).

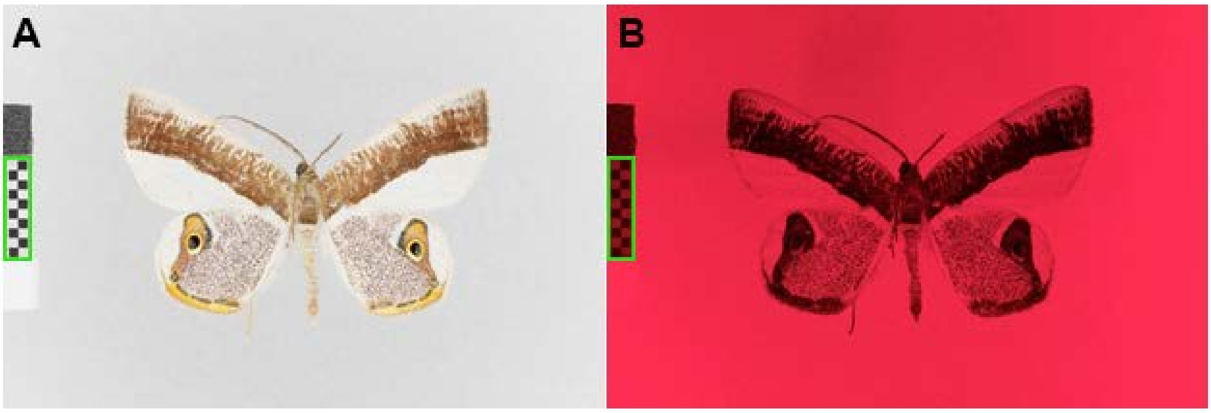

FIGURE 3 Example of a pair of original images of a moth (Geometridae: *Opisthoxia* sp.), taken with a modified camera, UV lens and respective lighting and filtering (Brehm 2025). A: normal RGB photograph. B: UV photograph in which the red channel is the most sensitive to UV. A 10 mm scale bar with chessboard pattern is placed along the left edge for accurate and reliable size reference (green box).

2.2 | Pipeline outputs

The LEPY pipeline generates a new folder to store all result files. These files are saved in a directory specified by the command-line argument (--output). If this argument is omitted, the results are saved by default in a new created folder within the same directory of the input folder. This folder is named after the input folder, with "_results" appended to the name.

The primary result file is a trait summary document in CSV format, containing all traits calculated by LEPY. Each row represents a single input image, with structural and coloration traits stored in separate columns (for detailed descriptions, see SI1).

Besides the trait summary file, the current version of LEPY creates the following outputs: (1) a contour of the segmentation mask, (2) a false colour image (if the UV-channel was detected; GB-UV), (3) a trait file in JSON format containing the same structuring and colouring traits as the corresponding row in the CSV summary file, and (4) a visual summary of the main structural and colouring traits, and of the different colour channels in form of density and box plots. The visual summary is explained in more detail in Section 3.1. Each of these outputs is stored in a separate subfolder: “contours”, “gbuv”, “json”, and “visualisations”, respectively.

2.3 | Validation of structural trait measurements and performance in an example dataset

We performed a validation of size measurements derived from LEPY against a traditional manual method, using ImageJ, a widely used software for morphometric analysis (Rasband, 2015). For this, we used a dataset of 100 moth images of moths of the families Sphingidae (54 images) and Saturniidae (46 images; See SI2). Moths were sampled in Peru in 2022 and 2023 as part of the ANDIV project (Holzmann et al., 2025). Moths were collected at 26 sampling sites between 250 and 3650 m a.s.l. in habitats ranging from Amazon lowland rainforest up to the treeline in the Andes. Size measurements included forewing length (both sides), body width (BW), body length (BL), and wingspan (WS). We applied paired Welch's t test when normality assumptions were met, and a Wilcoxon rank sum test when deviations were detected. We checked the normality of model residuals using the Shapiro-Wilk test. Statistical significance was determined at a threshold of p<0.05.

Furthermore, we explored selected morphological traits (size and colour) using data derived from LEPY. This also included moths from the same taxa collected in the same region as above. A total of 224 photographed moth specimens with 109 image pairs of Saturniidae and 115 image pairs of Sphingidae were analysed (See SI3). Photographs were taken at the Phyletisches Museum Jena (PMJ), Germany, according to the methods of photography and lighting described in detail by Brehm (2025). LEPY was run on a computer with an Intel(R) Core (TM) i5-13500 2.50GHz processor and 16.0 GB RAM (15.7 GB usable). As an example of the software's ecological application, we investigated the relationship between elevation and several functional traits, including forewing length, wing area and colour properties such as brightness (median of the four colour channels) and dynamic range / contrast (expressed as interquartile range of four colour channels).

Statistical analyses for the validation and exploration of performance were conducted in R v.4.4.0 (R Core Team, 2024). To evaluate whether elevation had a linear or non-linear effect on the traits, we constructed generalized additive models (GAMs) separately for each taxonomic family and trait. In GAMs, non-parametric smoothers are used to define the relationship between a response and a predictor variable, allowing flexible, semi-automatic estimations of both linear and non-linear relationships. To avoid overparameterization, we set the basis dimension of the smoothing term (k) to five for all GAMs. The models were implemented using the ‘mgcv’ package (Wood, 2023).

The number of images LEPY can process in one run depends on the computer’s memory capacity. To address this, we developed a script called LepyLoop, for analysing large datasets (available at https://github.com/DesBoe/LepyLoop). This script must be run in the same environment as LEPY. It will ask users for an input directory containing images or subfolders. All images are stored in a new folder and checked for unmatched RGB and UV image pairs. Small packages are created that contain a smaller number of images to be analysed consecutively by LEPY. Users can specify the individual count for each package (we recommend 100 from computational capacity). After all packages are analysed, input images are stored in their original directory, results are merged, and a .xlsx file containing all statistics is generated.

3 | RESULTS

3.1 | Visual summary

As described in Section 2.2, LEPY creates a variety of output files after processing the images. The most condensed summary is the visual summary as explained in Figure 4. It visualises the most important structural and colouring traits, different colour channels (including the UV channel), as well as statistics of each channel in the form of density and box plots. The visual summary is both useful and practical, as it displays the main traits generated by LEPY and provides a quick check to ensure that the measurements and all LEPY steps were executed correctly for each image.

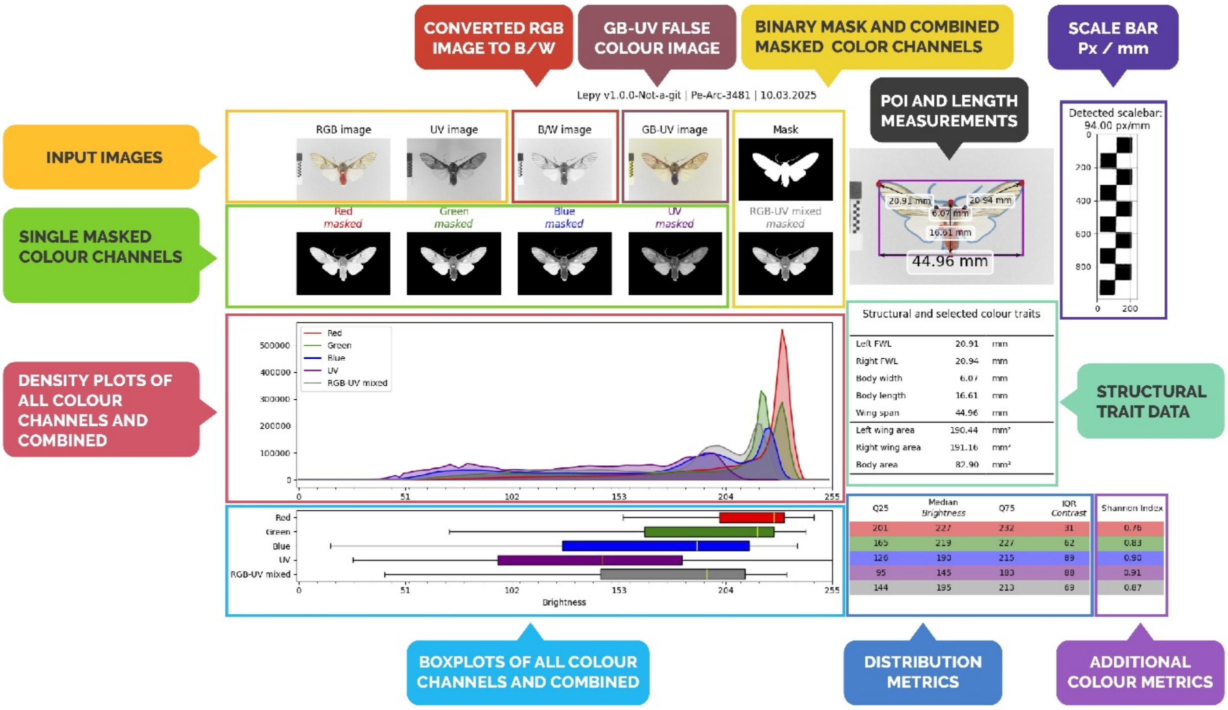

FIGURE 4 Example of a visual summary of the results of LEPY for a moth specimen (Arctiinae: *Idalus* sp.) with explanations (coloured boxes). The visualisation shows the input images (orange), the binary mask (yellow), the POI, and the scale bar (purple). It also shows structural trait data (blue green), density plots (pink), and boxplots (light blue) of four colour channels with colour metrics for each (blue), and the Shannon index (violet). The density plots indicates that the moth has relatively high brightness values in all channels (in the order red, green, blue, and ultraviolet). The boxplots visualise the interquartile range (IQR) of the dynamic range / contrast. The bars of the green, blue and UV channel are relatively broad, i.e., they have a wide dynamic range / contrast. The red bar is rather narrow, indicating lesser dynamic range / contrast in the red channel (See SI1 for further explanation of colour metrics).

In the upper left part, it displays a series of images. From left to right in the first row those are the original input images, the UV (ultraviolet) channel, classic grayscale image (B/W; black and white), the false colour image (*GB-UV*) as described in Section 2.1, and finally the binary mask estimated with the backgroundremover package. In the second row the images from left to right are four channels masked according to the binary mask and the weighted average of those channels, the *RGB-UV mixed* image. In the upper right section, the estimated points of interest (POI), the length measurements of the specimen, and the detected scale bar along with the estimated pixel scale in pixel per millimetre are visualised.

The lower part of the visual summary displays the density and box plots of the four colour channels and the *RGB-UV mixed* image on the left. The most important structural and colour traits of the specimen are displayed in two tables on the right. Based on the eight POI, LEPY calculates eight structural traits: five lengths and three areas as shown in the visual summary (Figure 4). The colour traits include the median, Q25, Q75, IQR (interquartile range), and the Shannon index of the four channels and of the *RGB-UV mixed* image. The median pixel value is a measure for the brightness of each channel. The dynamic range / contrast of an image is usually defined by the difference between maximum and minimum pixel value. We use the IQR of the dynamic range / contrast because it is less sensitive to statistical outliers and therefore probably provides more stable results. As mentioned in Section 2.2, additional colouring traits are stored in the main trait summary file. Those are explained in more detail in supplementary information (SI).

3.2 | Validation of structural trait measurements

The comparison between LEPY and ImageJ measurements (n = 100) revealed no statistically significant differences for any of the measured parameters. For normally distributed data (both forewing lengths), Welch's t-tests produced p-values of 0.80 and 0.96, respectively. For non-normally distributed data (BW, BL, and WS), Wilcoxon rank-sum tests resulted in p-values of 0.50, 0.85, and 0.49, respectively. Visual representation of the data, presented in boxplots, further supported the similarity between the two measurement methods (Figure 5).

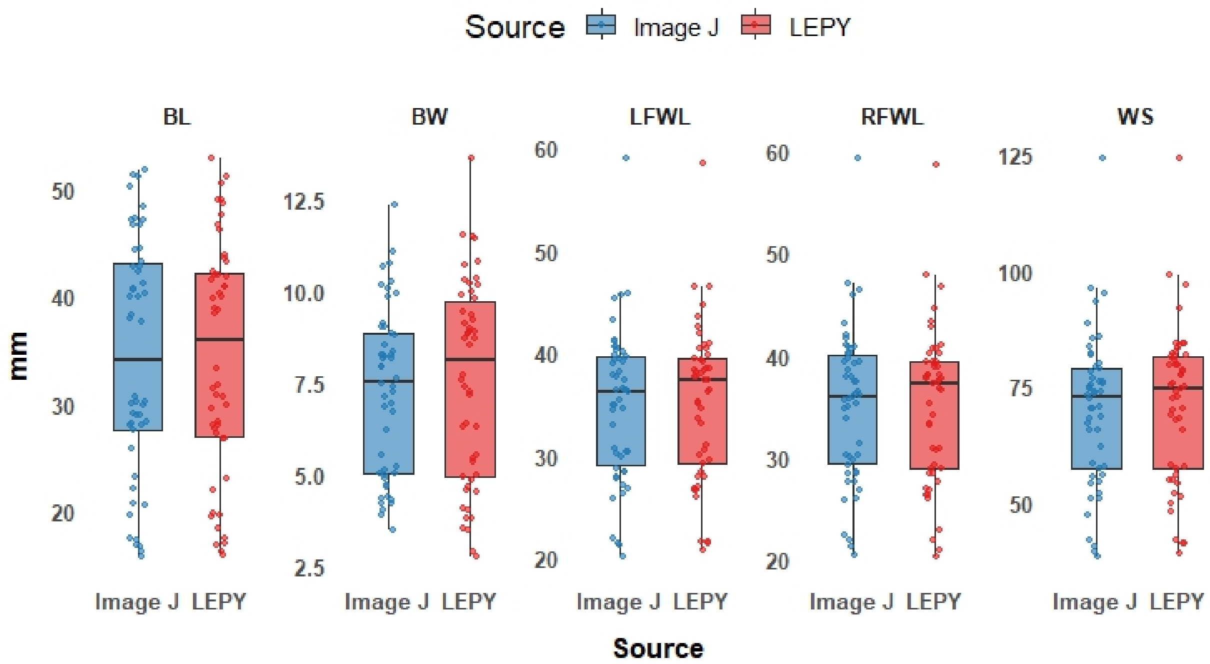

FIGURE 5 Comparison of structural measurements obtained using ImageJ and LEPY. Boxplots with jittered points represent measurements for body length (BL), body width (BW), left forewing length (LFWL), right forewing length (RFWL), and wingspan (WS) across the two methods.

3.3 | Performance of LEPY in the example data set

General performance. The processing of 224 image pairs (RGB image, UV image) took between 15 and 50 seconds per pair. The performance of LEPY is influenced by specific image and specimen characteristics. For example, visible antennae or legs in the images occasionally led to errors in POI identification, resulting in inaccuracies in morphological measurements. Specimen preparation and mounting also played a role: errors in wing positioning, such as misalignment of the wings at a 90° angle relative to the body or asymmetry between the two sides of the body, led to POI detection errors.

Forewing length and wing area. The effect of elevation on forewing length and wing area varied between Sphingidae and Saturniidae, as indicated by the Generalised Additive Models (GAM). For forewing length, elevation had a significant effect in both families, but the trend of this relationship differed. In Sphingidae, the effect was linear (edf = 1, $F = 5.227$, $p = 0.0241$), though the model explained only 4.42% of the deviance. In Saturniidae, the effect was non-linear (edf = 3.281, F = 2.267, p = 0.0487), and the model explained a slightly higher proportion of variance (10% deviance explained). These results suggest that elevation influences forewing length in both families, but with a non-linear effect in Saturniidae (Figure 6A). For wing area, elevation had no significant effect in either family. In Sphingidae, the relationship was non-significant (edf = 1.58, F = 1.562, p = 0.267 deviance explained = 2.91%). In Saturniidae, wing area changed in a complex manner with elevation, but the effect was only marginally significant (edf = 3.183, F = 2.205, p = 0.0579, explained deviance = 9.4%). These results suggest that elevation has a limited effect on wing area variation in both families, though Saturniidae shows a slightly stronger non-linear response (Figure 6B).

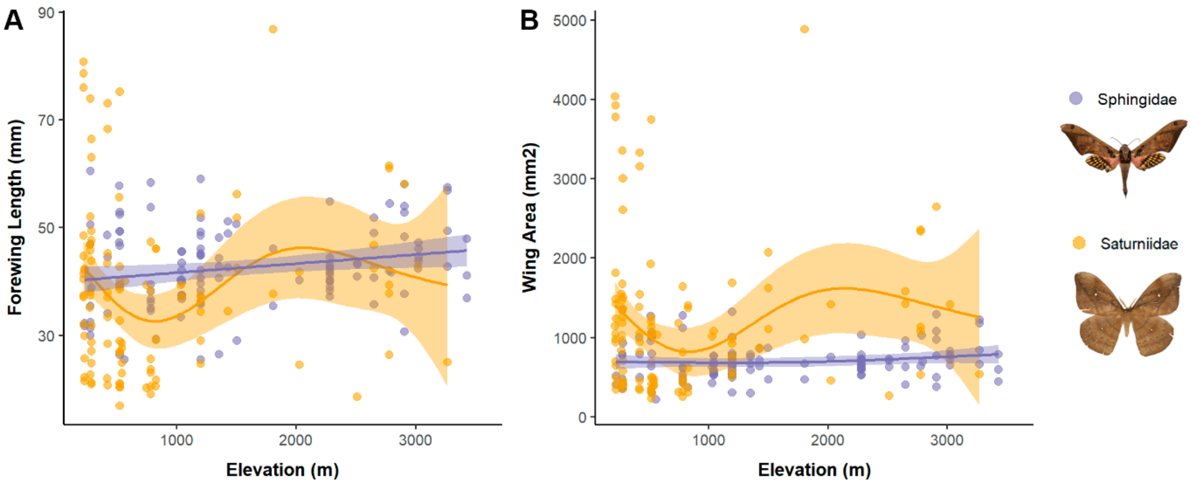

FIGURE 6 A. Relationship between elevation and forewing length of Sphingidae (purple) and Saturniidae (orange). B. Relationship between elevation and wing area of Sphingidae (purple) and Saturniidae (orange). Each point represents an individual moth, and the shaded regions indicate 95% confidence intervals around the fitted GAM predictions (lines).

Brightness and dynamic range / contrast. The effect of elevation on brightness and dynamic range / contrast was weak in both Sphingidae and Saturniidae. For brightness, elevation had a significant effect in Sphingidae (edf = 1.083, F = 12.05, p = 0.000384), suggesting a nearly linear relationship. The model explained 11.5% of the deviance, indicating that elevation played a moderate role in shaping this trait in Sphingidae. In Saturniidae, trends in brightness were similar but the effect was not significant (edf = 3.216, F = 1.62, p = 0.129). The model explained only 7.53% of the deviance, suggesting a minimal impact of elevation on brightness in this family (Figure 7A). For dynamic range / contrast, elevation had a non-significant effect in both families. In Sphingidae, the model showed a weak relationship with elevation (edf = 1, F = 2.106, p = 0.149), explaining only 1.83% of the deviance. Similarly, for Saturniidae, the effect was weak and non-significant (edf = 1.917, F = 1.675, p = 0.194), with 4.56% of the deviance explained. These results suggest that elevation had little influence on contrast variation in both families (Figure 7B).

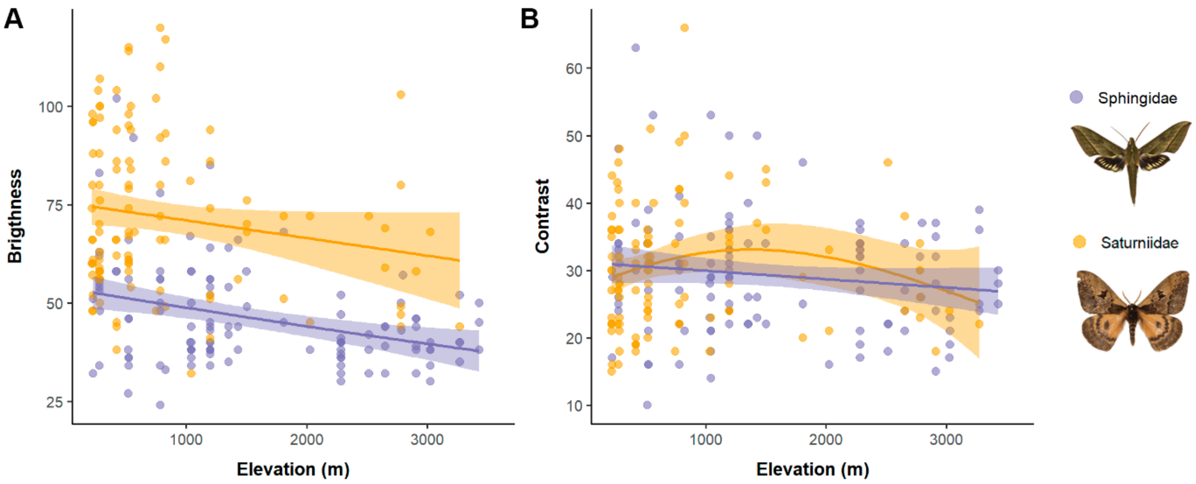

FIGURE 7 A. Relationship between elevation and brightness (median of four colour channels) in Sphingidae (purple) and Saturniidae (orange). B. Relationship between elevation and dynamic range / contrast (IQR of four colour channels) in Sphingidae (purple) and Saturniidae (orange). Points represent individual data, and the shaded regions indicate confidence intervals around the fitted GAM predictions (lines).

4 | DISCUSSION

4.1 | Capabilities of LEPY

LEPY demonstrated significantly enhanced capabilities compared to previous algorithms such as Mothseg and Mothra. LEPY can easily analyse more than thousand image pairs overnight with a normal computer and provides a wealth of data for analysing traits of body size and colouration of moths and butterflies at once. Results automatically derived by LEPY were highly like those measured manually with ImageJ. Our examples showed only a selection of the possibilities and demonstrate that different size measurements can lead to different results. For example, a longer wing length does not automatically mean a larger wing area, as this ratio varies between different taxa. In many studies, only a single measure, such as wing length, was used, but this is only possible if the groups are relatively homogeneous (e.g., Brehm et al., 2019).

With the automated methods used in LEPY, it is now easy to record different measures simultaneously and compare them. The comparison of colouration parameters such as brightness only give a foretaste of future possibilities. In our tested dataset, members of both families become darker with elevation whereas we found no significant changes in the dynamic range /contrast. These results highlight how morphological traits can vary in response to environmental gradients. Stronger patterns are expected in groups with aposematic or chemically defended species, such as butterflies and Arctiinae and other moths, which use coloration to signal toxicity or unpalatability (Prudic et al., 2007).

The inclusion of UV information represents a significant further development. UV patterns can be important for communication, camouflage, and mating signals (Pinna et al., 2021; Prabhulinga et al., 2022), but the systematic consideration of UV information has received little attention to date, e.g., in digitisation programmes (Brehm, 2025). To better understand this potential, we encourage studies to compare the contribution that this information can make. LEPY can also be used without UV photographs and represents the traditional parameters such as HSV (hue, saturation, and intensity). Our four-channel approach enables the full integration and visualization of UV information using false-colour images. We also encourage to work on further models for this colour information and possibly derive further meaningful parameters. LEPY provides scalable analyses of large Lepidoptera datasets, allowing the accurate extraction of morphological traits relevant to studies of phenotypic diversity, ecological adaptations, and trait-environment relationships. The comparison between LEPY and ImageJ measurements demonstrated strong consistency across all measured parameters, with no statistically significant differences observed. This suggests that LEPY provides an accurate and automated alternative to ImageJ for morphological measurements in Lepidoptera. Future studies could leverage LEPY to streamline data collection processes while maintaining measurement accuracy. Data generated by LEPY can easily be registered in publicly accessible repositories.

4.2 | Current limitations of LEPY

Despite its strengths, LEPY has some limitations that may require further development for other types of input data, for example regarding the type of scale bar used. LEPY currently requires a specific chessboard pattern. Adapting to different scales or formats would require additional programming, which in most cases appears possible, given a standardization (e.g., a uniform 10 mm black scale bar). Furthermore, extending compatibility to a broader range of file formats could enhance a broader accessibility and usability. Accuracy and efficiency of the pipeline are dependent on the quality of specimen preparation. Errors in segmentation and POI detection can arise from poorly prepared specimens, visible legs, or pale or translucent wings. Such problems can never be completely avoided but the visual summary of results make it possible to identify such errors relatively quickly and replace them with manual measurements.

A broader question is whether LEPY could be adapted in such a way that it will be possible to investigate also other insect groups. Although this is certainly desirable, we expect that insects characterized by pronounced three-dimensional body shapes will be more difficult to analyse, and additional technologies (such as image stacking) are likely to be required. However, LEPY could serve as a good starting point, as its modularity and extensibility are likely to allow such adaptations.

4.3 | Ecological relevance and potential applications of LEPY

LEPY enables the study of Lepidoptera adaptation to environmental changes, such as climate change or habitat loss (Clusella-Trullas & Nielsen, 2020; Duarte et al., 2017; Henriques et al., 2022). For example, body size is related to individual mobility, whereas individuals with reduced mobility tend to be more sensitive to habitat loss (Mattila et al., 2008). The tool enables the monitoring of changes in Lepidoptera populations and communities over time. By facilitating the study of how environmental changes and habitat degradation influence their morphology, coloration, and survival, it provides critical insights into species responses. Such information is essential for predicting whether species will adapt or face decline, as well as for informing the development of effective conservation strategies (Koneru & Caro, 2022; Mikitová et al., 2022).

In our example dataset, morphological traits varied with elevation, likely due to associated environmental changes. However, the magnitude and direction of these effects differed by trait. Notably, forewing length increased significantly with elevation, particularly in Sphingidae. In contrast, Saturniidae species had significantly larger wing areas, but this trait did not follow a clear elevational pattern. These findings align with those of Brehm et al. (2019), who found a significant increase in body size (measured as forewing length) along an elevational gradient of nearly 2,900 meters in Costa Rica, with temperature as the main predictive factor. However, Brehm & Fiedler (2004) reported a negative relationship between body size and elevation of Geometridae moths in Ecuador along a gradient of more than 1,600 m, suggesting that geographical differences and gradient length may influence these patterns.

The increase in forewing length at higher elevations may reflect adaptations to flight constraints in montane environments. Reduced atmospheric pressure may require larger wings to maintain efficient flight (Brehm et al., 2019). The relatively low R^2 for forewing length suggests that while elevation has an effect, other factors – such as taxon-specific adaptations or phylogenetic relationships – are likely to contribute to variation in this trait. In contrast, traits with higher R^2 values (brightness and wing area), indicate that moth taxon and, to some extent, elevation explain most of the variation. These results support previous findings that colour-related traits change with elevation (Fiedler & Brehm, 2021).

Previous studies found that larger and darker insects are favoured in colder environments and that body size and coloration might play an important role for thermoregulation, even in nocturnal species. For instance, Heidrich et al. (2018) found that noctuid moths were larger and darker at high elevations in Europe, whereas geometrids showed an opposite trend in brightness and no clear trend in body size.

For future research, integrating morphological patterns with phylogenetic analyses would help to clarify whether the observed changes are primarily driven by elevation or result from species-specific evolutionary adaptations. Combining trait-based approaches with phylogenetic frameworks will be essential for understanding how moth communities respond to environmental gradients over evolutionary timescales (Shrestha et al., 2014).

5 | CONCLUSION

LEPY is a tool that addresses key challenges in Lepidoptera trait research by automating the analysis of structural traits and colour properties, including UV information, across large datasets of specimens and/or images. Our results highlight the effectiveness of LEPY in quantifying morphological trait variation and detecting the influence of elevational gradients on Lepidoptera communities. By incorporating both morphological and ecological data, this approach can provide valuable insights into how environmental factors shape trait distributions in insect communities. We encourage users to test LEPY and expand its possibilities through further adaptation steps.

AUTHOR CONTRIBUTIONS

Yenny Correa-Carmona, Dennis Böttger, Dimitri Korsch, and Gunnar Brehm conceptualised and developed the LEPY pipeline, contributing to both its design and the manuscript's content and revisions. Dimitri Korsch implemented the pipeline code. Yenny Correa-Carmona drafted the initial manuscript and conducted the validation data analysis with advice from Marcell Peters. Gunnar Brehm organized the example dataset and took all photographs. All authors reviewed and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest.

DATA AVAILABILITY STATEMENT

Code used for this study are available from the GitHub repository at https://github.com/tzlr-de/LEPY.

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SUPPORT INFORMATION

SI1. Explanation of variables generated by LEPY

SI2. LEPY vs. ImageJ Measurement Comparison Dataset

SI3. LEPY Morphological Traits Dataset

SI4. Scale bar template