LEPY: A Python pipeline for automated trait extraction from standardised Lepidoptera images

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ABSTRACT

We present LEPY, a free and openly available Python-based pipeline for the automated extraction and analysis of morphological and colour traits, from mounted specimens of Lepidoptera (butterflies and moths). The pipeline uses an automatically detected scale bar for accurate morphological measurements, together with image segmentation that separates the specimen from the background, with users able to pre-select from a set of segmentation models. We designed LEPY to be user-friendly and reproducible, ensuring efficient and consistent analysis of large image datasets. The pipeline also supports the integration of ultraviolet (UV) photographs for improved colour analysis, an innovative feature rarely available in existing trait-analysis tools.

LEPY computes morphological traits such as body length, forewing length, and specimen area. It also extracts colour traits including hue, saturation, intensity from the red, green, and blue (RGB) channels, as well as brightness, contrast, chromaticity, and luminance from both RBG and UV channels. The pipeline uses the data to calculate colour diversity with the Shannon index, exports results in a structured, machine-readable format, and it also generates visual summaries of each image pair.

We tested LEPY on different moth groups spanning a wide range of body sizes and colouration patterns. As an ecological case study, we applied the pipeline to complete datasets of Sphingidae and Saturniidae collected along an elevational gradient in the Peruvian Andes. The resulting trait data revealed taxon-dependent morphological and colour responses to elevation, thereby demonstrating LEPY's utility for analysing large-scale trait datasets.

LEPY provides a robust and fully automated approach for the analysis of morphological and colour traits in Lepidoptera, supporting ecological and evolutionary research. Its scalability and ability to generate standardised, high-resolution trait datasets make it a valuable tool for biodiversity monitoring, macroecological research, and the development of global trait databases.

1. Introduction

Measuring functional traits and understanding how they vary across taxa, habitats, and ecological contexts has become increasingly important in ecological research (Correa-Carmona et al., 2022; Gámez-Virués et al., 2015; Wellstein et al., 2011). This is especially challenging for insects, the most diverse and abundant animal group on Earth, as identifying, preparing, and measuring their morphological traits is often time-consuming (van Klink et al., 2022). Lepidoptera, comprising butterflies and moths, represent one of the most diverse insect clades and

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are no exception in this regard (Freitas et al., 2020). They serve as a model group in ecological and evolutionary studies (Boggs et al., 2003; Hill et al., 2021), partly because Lepidoptera have been extensively collected and curated worldwide. Compared with many other insect orders, lepidopteran taxonomy is relatively well established, although some challenging taxa still require molecular analyses or other tools for accurate identification (Lamarre et al., 2022; Moraes et al., 2021; Murillo-Ramos et al., 2021).

Body size of animals is a widely studied functional trait, as it relates to key physiological and life-history processes, such as survival, growth, and reproduction, as well as ecological interactions (Brehm et al., 2019; Woodward et al., 2005). Body size can be influenced by environmental conditions and can vary substantially across environmental gradients (Chown and Gaston, 2010; Cortés-Gómez et al., 2023; Tammaru and Teder, 2012). Another key trait in insects is colour, particularly in Lepidoptera, where it serves various functions including protection (e.g., camouflage, mimicry), communication, and thermal regulation (Heidrich et al., 2018; Kočíková et al., 2012). Wing colour patterns are also crucial for species identification (Feng et al., 2015). Ultraviolet (UV) colour also plays an important role in many biological and ecological processes (Brehm et al., 2021; Heidrich et al., 2018; Kočíková et al., 2012). While invisible to mammals, UV patterns are visible to most arthropods and birds, aiding in communication, mate attraction, species recognition (Bálint et al., 2012), survival strategies as mimicry (Lyytinen et al., 2004; Zapletalová et al., 2016), and pollination (Ohashi et al., 2015; Papiorek et al., 2016). However, studies on UV colour patterns remain limited, especially in Lepidoptera, and digitisation efforts in biological collections have largely overlooked this component (Brehm, 2025; Stella and Kleisner, 2022). Most research on colour traits and image-based analyses has focused on the visible spectrum, specifically the RGB channels (where R = red, G = green, and B = blue), leaving UV reflectance underrepresented despite its ecological relevance.

Traditional manual methods for measuring morphological traits in entomology are often time-consuming and prone to human error (Fountain-Jones et al., 2015). In Lepidoptera, this is further complicated by the fragility of wing structures, which are easily damaged. To address these challenges, recent studies have explored automated approaches for detecting, classifying, and measuring traits such as wing and body size, colour, and colour pattern using computer vision and machine learning (Feng et al., 2015;Høye et al., 2021; Manoukis and Collier, 2019; Palma et al., 2023). Image-based landmarking as a non-invasive alternative can also enable precise quantification of morphological traits without directly manipulating specimens (Eshghi et al., 2024; Le et al., 2020). These techniques reduce reliance on manual measurements, allowing for high-throughput, large-scale analyses with improved accuracy and reproducibility (Høye et al., 2021; Manoukis and Collier, 2019; Palma et al., 2023). Pioneering work by Batista et al. (2010) demonstrated the use of texture, colour, and shape features for automated species identification. More recently, researchers have integrated automatically derived traits into species identification pipelines, i.e., computational workflows that process specimen images to automatically assign specimens to species (Chiranjeevi et al., 2024; Keasar et al., 2024). Such measurable traits can improve classification performance by providing explicit, biologically meaningful information to the model (Jain et al., 2024; Korsch et al., 2021). These traits are particularly useful for part-based fine-grained species classification, where specific morphological regions are analysed separately (Korsch et al., 2019, 2021, 2022), as well as for deep learning approaches. i.e. machine learning concept based on artificial neural networks; Dargan et al., 2019; Janiesch et al., 2021), when image information is combine with metadata, such as in the MetaFormer framework (Diao et al., 2022).

Python has become the dominant programming language for developing image analysis techniques due to its advanced image processing frameworks, comprehensive and extensive scientific libraries/tools, broad user base, and active developer community, ensuring its adaptability for new applications (Van Rossum and Drake, 2009). Several pipelines have already been developed for Lepidoptera image analysis. For instance, *MothSeg* (Rodner, 2016) provides Python-based tools for segmenting dorsal and ventral views, extracting colour metrics (hue, saturation, intensity), and measuring overall shape characteristics (Jaimes Nino et al., 2019). However, it provides limited options for body measurements and requires relatively long processing times (3–5 min per image). Another pipeline, *Mothra* (Wilson et al., 2023), automates specimen detection, scale adjustment, wing measurement (e.g., forewing length), image orientation detection, and sex identification based on sexual size dimorphism. Nevertheless, *Mothra* lacks colour analysis and supports only a restricted set of morphological traits.

In this paper, we present LEPY, a free and openly available Python-based tool for the automatic segmentation and analysis of morphological and colour traits in Lepidoptera. LEPY builds upon existing tools by integrating quantitative colour metrics, including UV reflectance, improved image segmentation using deep learning, and a calibration step for more accurate morphological measurements. In addition, LEPY generates structured output files for storing trait data, and includes a visualisation module to highlight morphological traits and display colour density distributions. To demonstrate its utility, we applied LEPY to specimens from two ecologically distinct moth families, Sphingidae and Saturniidae, sampled along an elevational gradient in the Peruvian Andes.

2. Materials and methods

2.1. Implementation details

We developed LEPY as an open-source pipeline hosted on GitHub (https://github.com/tzlr-de/LEPY), and test it under Python 3.9, 3.11 and 3.12. We recommend setting up a virtual Python environment such Anaconda (Anaconda, 2023) and installing the packages listed in the requirements file. For the detailed installation instructions, we refer to the README.md in the code repository.

LEPY runs as a command-line program. Users provide the directory containing the image and a configuration file defining processing parameters via command-line arguments. The repository provides a default configuration file, which performed well for our dataset. LEPY processes RGB images in JPG or TIF format. To include UV information alongside RGB, images must be in TIF format because UV photographs are captured in raw format and require substantial re-exposure during processing (Adobe Photoshop Camera Raw). Exporting to TIF preserves bit depth and colour fidelity and produces smoother gradients in the histograms, whereas JPG exports introduce artefacts such as steps or jagged edges. 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 Fig. 3). LEPY is designed to operate on standardised images of mounted Lepidoptera specimens taken under consistent conditions, including good lighting, a neutral and homogeneous background, and a uniform pixel-to-millimeter scale across all images (with the scale-detection module verifying correct scale placement), with any labels removed before photography (Brehm, 2025; Fig. 3).

Using the UV channel, LEPY generates two derived images: an *RGB-UV mixed* image and a *GB-UV* false-colour image. 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 $I = 0.299*R + 0.587*G + 0.144*B$, corresponding to the sensitivity of a human eye. Because this weighting cannot be directly extended to the UV channel, we apply a neutral approach in which all four channels were equally weighted with 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 space, resulting in the *GB-UV* image.

LEPY processes images in four major steps: (1) pixel-accurate

segmentation of specimens, (2) localisation of points of interest (POI), (3) detection and analysis of the scale bar, and (4) computation of morphological and colour traits based on the scale bar information and the POI (Fig. 1).

For image segmentation (automatic separation of the specimen from the background), LEPY offers several methods that users can select in the configuration file (config.yml). One option is the backgroundremover package (Nader, 2025). It utilises a U-NET-based neural network (Ronneberger et al., 2015), originally trained on biomedical image datasets (electron microscopy and light microscopy) for general background removal. This solution works well with our homogeneous light-grey background (see Brehm, 2025), as the specimen can be usually easily distinguished from the background. LEPY also includes other background separation methods, e.g., GrabCut (Rother et al., 2004), Otsu's thresholding (Otsu, 1979), but the backgroundremover package produced most stable results, especially for specimens with bright colouration. Finally, we have also exploited recent work on terrestrial arthropod segmentation in images and integrate the flatbug model (Svenning et al., 2025) as a candidate segmentation model. The flatbug segmentation model incorporates a YOLOv8 backbone (Jocher et al., 2023) for detection and instance segmentation and is well suited for our segmentation task due to the specific insect training datasets that have been utilized.

After post-processing the binary mask returned by the segmentation model (that is, a black-and-white image indicating which pixels belong to the specimen and which belong to the background; Gros et al., 2021; Nanni et al., 2020), LEPY fills minor holes and extracts the specimen contour. Only the largest connected contour was retained under the assumption that it encloses the specimen. Based on this binary representation, LEPY estimates eight points of interest (POI; Fig. 2A), which separate the body from the wings and enable the computation of sizes-related traits. All POI are derived solely from the geometry of the binary mask; colour, texture, or intensity information is not used at this stage.

We build on and extended the *Mothra* pipeline (Wilson et al., 2023), which estimates the tips and bases of both forewings. Wing tips are defined as the contour points on each wing that maximise the Euclidean distance from the estimated specimen centre, ensuring a consistent definition even for asymmetric wing shapes. The bases of the forewings are defined as inner contour points located between the wing and the body. These points are identified by searching for inward contour locations separating the wing region from the central body region within a constrained area near the specimen centre, rather than by relying on a single curvature extremum. This approach allows the wing–body junction to be detected even when the boundary is gradual.

LEPY additionally estimates the upper and lower tips of the body by analysing the vertical extent of the body region defined between the two wing bases. Fine structures such as hairs are included in the binary mask and are therefore treated as part of the specimen outline (Fig. 2). While these definitions work robustly for the majority of specimens, we have noted that the placement of wing bases can be ambiguous for some morphologies, as is also the case during manual annotation.

To extract scaling information from the scale bar present in the image, LEPY uses the *scalebar* pipeline (Korsch, 2025), a reworked and improved version of the *MothSeg* pipeline (Rodner, 2016). The package locates the scale bar by template using a simple pattern-matching method (OpenCV., 2025), by resizing a template image at different scales (ranging from 2.5% to 100% of the original template size in 2.5% steps) across the left 15% area of the image. As a template, LEPY uses a single image of a chessboard reference (see Fig. 3) with a pre-computed ratio of 290 pixels per mm (a scale bar template is supplied in SI1; if printing, ensure it is at 100% for accurate measurements). The scale bar was designed in Adobe Illustrator and printed professionally (Druckhaus Gera, Germany). It includes two reflectance standards (95% and 10%) purchased from Sphere Optics, Herrsching, Germany (Brehm, 2025; Troscianko and Stevens, 2015). Our method also provides a quality score for the template matching step, allowing users to filter out results affected by incorrect or missing scale bar detection. For analyses that do not require absolute size information (such as those focused solely on colour analysis), scale bar detection can be omitted altogether. The final scale is computed and returned using this pre-computed scale and the resize factor matched best during pattern matching. 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.

After estimating the eight POI and the image scale, LEPY quantifies various morphological and colour traits. For morphological measurements, it assesses thorax width, body length, and forewing length (Fig. 2A). By segmenting the mask into distinct body and wing regions,

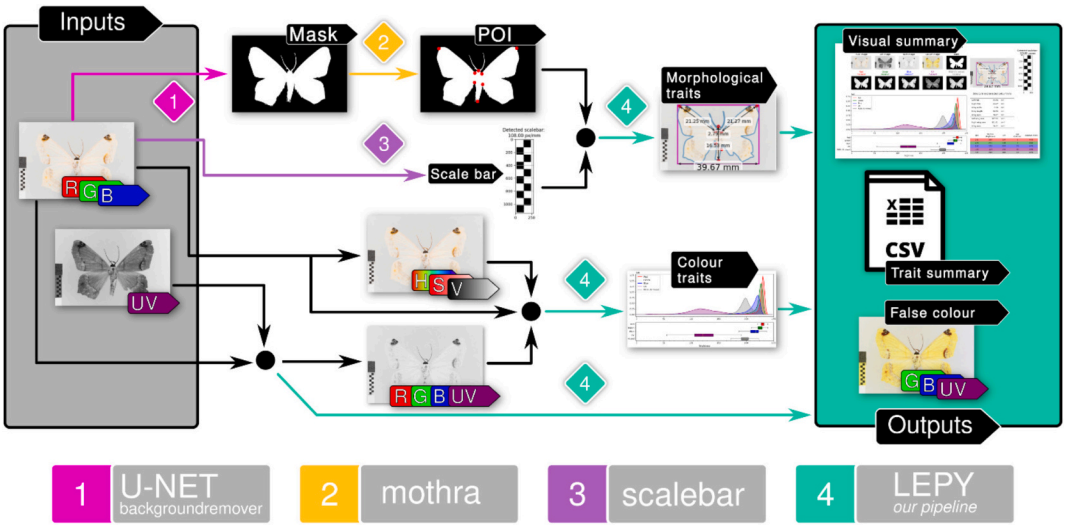

Fig. 1. LEPY Pipeline. Input images are processed in multiple steps, including a CNN-based pixel-accurate segmentation of the specimen, the localisation of points of interest (POI) using the *Mothra* pipeline, the detection and processing of the scale bar using the scalebar package, and finally the computation of various morphological and colour 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.

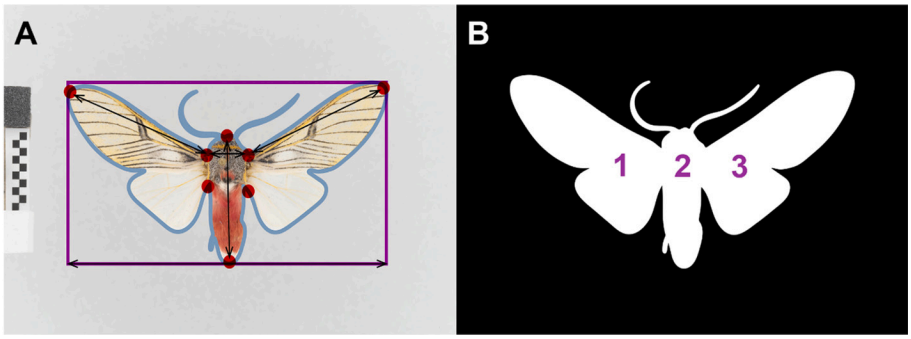

Fig. 2. A: Points of interest (POI, shown as red dots) detected on a binarised moth image, highlighting the body and length of both forewings, and the thorax width generated by the pipeline. B: The binarised moth image is segmented into three sections: 1. the left forewing and hindwing, 2. the body, and 3. the right forewing and hindwing. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

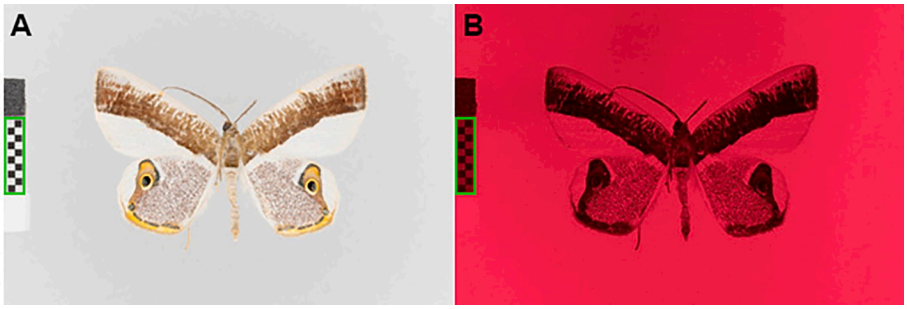

Fig. 3. Example of a pair of original images of a moth (Geometridae: *Opisthoxia* sp.), taken with a modified camera, UV lens and respective lighting and filtering (Brehm, 2025). A: normal RGB photograph. B: UV photograph in which the red channel is the most sensitive to UV. A 10 mm scale bar with chessboard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

LEPY also calculates their respective areas (Fig. 2B). These measurements are recorded in the visual summary generated for each image and the corresponding .csv file (see 2.2). For the colour traits, LEPY analyses each channel of the RGB (red, green, and blue) image separately. LEPY also 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. Using the segmentation mask estimated in the previous image-processing step, LEPY calculates the mean, median, minimum, and maximum pixel values for the RGB-UV and HSV. For all four channels (RGB-UV), LEPY calculates the 25% quartile (Q25), 75% quartile (Q75), the interquartile range (IQR) which represents dynamic range / contrast, Shannon index, luminance, and chromaticity (descriptions detailed in SI2). In the visual summary, we also add density and box plots for each channel and the *RGB-UV mixed* image (Fig. 4).

2.2. Pipeline outputs

The LEPY pipeline generates a dedicated output 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 newly 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 morphological and colour traits stored in separate columns (for detailed descriptions, see SI2).

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 morphological and colour traits as the corresponding row in the CSV summary file, and (4) a visual summary of the main morphological and colour 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 morphological trait measurements and performance on an example dataset

To evaluate LEPY's robustness across a wide range of body sizes, we analysed hundreds of standardised images of moths from several families, including Arctiinae, Geometridae, Saturniidae and Sphingidae. This ensured that the validation covered specimens with diverse morphological traits. The segmentation module was implemented using several binarization approaches (e.g., U-Net, flatbug, GrabCut, Otsu). For the validation and performance analyses presented here, we used the U-Net-based method.

We validated LEPY's morphological trait measurements by comparing them with values obtained using ImageJ, a widely used software for morphometric analysis (Rasband, 2015). For this validation, we created a vector graphic (pdf) containing 31 moth shapes (SI3), generated from photographs of real specimens of Arctiinae, Geometridae, Sphingidae, and Saturniidae. We scaled these shapes to precisely known reference dimensions, which we used as ground-truth measurements and hereafter refer to as *real values*. We printed all images on 120 g paper with a neutral grey background and withheld the reference dimensions from the observers to avoid bias. We photographed the printed moths at the Phyletisches Museum Jena (PMJ), Germany, following the standardised photography and lighting protocol described by Brehm (2025).

Six observers independently measured four morphological traits

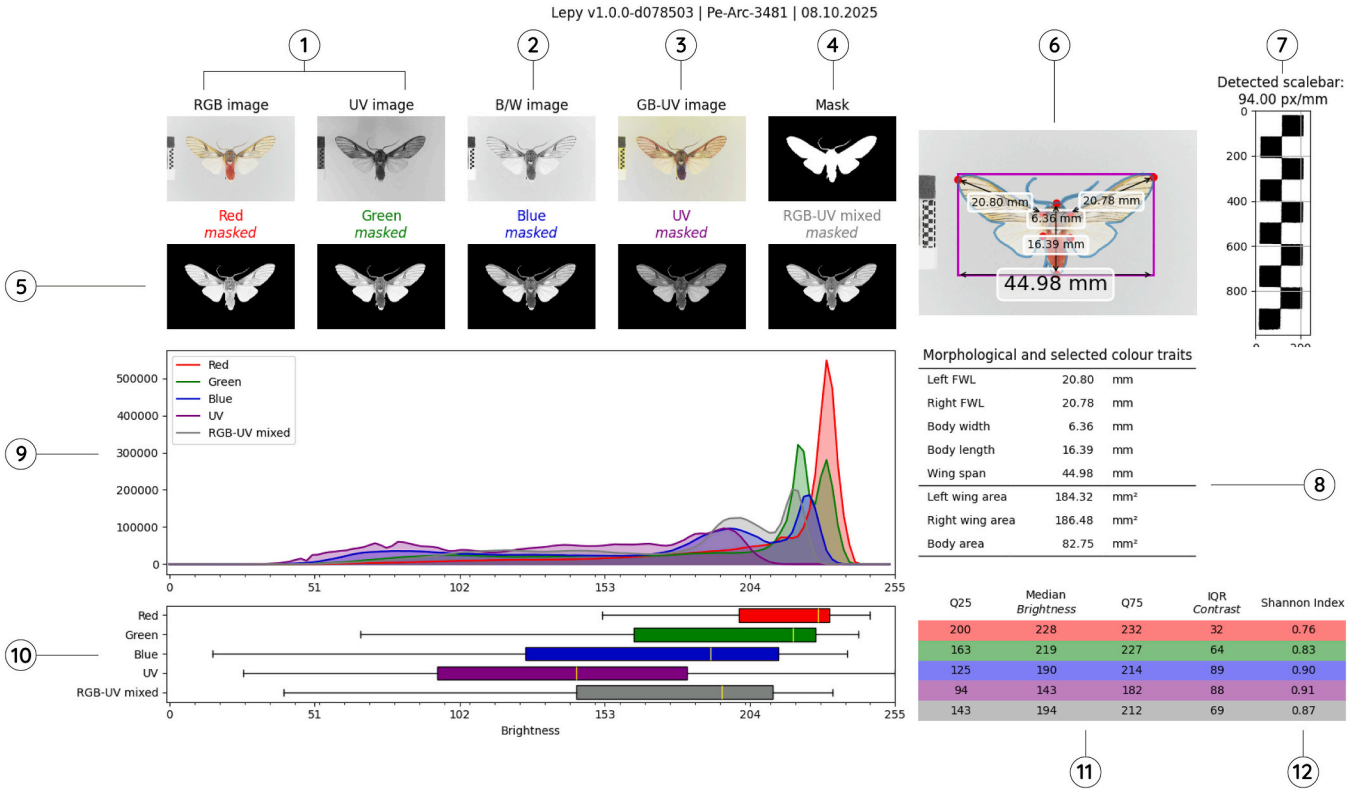

Fig. 4. Example of a visual summary generated by LEPY for a moth specimen (Arctiinae: *Idalus* sp.). The numbered elements indicate the main components of the output: (1) input images, (2) black and white image, (3) false colour image, (4) binary mask, (5) masked colour channels, (6) points of interest (POI) and length measurements, (7) scale bar, (8) morphological trait data, (9) density plots for all colour channels and their combination, (10) boxplots for all colour channels and their combination; (11) colour metric summary table, and (12) Shannon index. The density plots show that the specimen displays relatively high brightness across all channels (in decreasing order: red, green, blue, and ultraviolet). The boxplots illustrate the interquartile range (IQR) of dynamic range / contrast: the green, blue and UV channels exhibit broader ranges (higher contrast), whereas the red channel shows a narrower range, indicating lower contrast. See SI2 for further explanation of the colour metrics. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(body length, right forewing length, thorax width, and wingspan) from the photographs using ImageJ. We summarised the six manual measurements per specimen and trait using the median to minimise observer bias. We obtained LEPY measurements once per specimen (SI4).

We evaluated agreement between automated LEPY measurements and manual ImageJ measurements (median of six observers) by regressing both against the real (reference) values. We used the slope to assess scaling bias (i.e. deviations from a slope of one indicate that measurement error depends on trait size), while the intercept indicated a constant offset. We quantified systematic over- or underestimation using the mean bias (%) and the mean absolute percentage error (MAPE) relative to the reference dimensions.

We further examined whether measurement bias depends on specimen size. For each specimen, we calculated the signed percentage error relative to the reference dimension and regressed this error against specimen size. We conducted these analyses separately for each trait and method (LEPY and ImageJ median). The slope of this relationship quantified size-dependent bias, with a non-zero slope indicating that measurement error changes systematically with specimen size. We considered this effect significant when $p < 0.05$.

As an ecological case study, we analysed complete datasets of Sphingidae and Saturniidae from the Peruvian Andes, collected in 2022 and 2023 as part of the ANDIV project (Holzmann et al., 2025). Specimens were collected at 26 sites along a forest elevational gradient ranging from 250 and 3650 m a.s.l. covering habitats from Amazonian lowland rainforest to the Andean treeline. In total, we analysed 224 photographed moth specimens, comprising 109 image pairs of Saturniidae and 115 image pairs of Sphingidae (SI5).

To demonstrate LEPY's ecological application, we investigated relationships between elevation and morphological and colour traits, including forewing length, wing area, brightness (median of the four colour channels), and contrast (interquartile range of the four colour channels).

We used generalized additive models (GAMs) to test whether elevation had linear or non-linear effects on morphological and colour traits. For the primary analysis, we fitted separate models for each taxonomic family and trait, modelling trait values as a smooth function of elevation. To avoid overparameterization, the basis dimension of the smoothing term was fixed at $k = 5$ for all models, and parameters were estimated using restricted maximum likelihood (REML) as implemented in the *mgcv* package (Wood, 2023). Individual trait measurements were treated as independent observations, providing community-level estimates of trait variation along the elevational gradient.

To evaluate whether elevational responses differed between families, we additionally fitted models that included taxonomic family as a categorical factor and allowed for family-specific smooth functions of elevation (i.e. factor–smooth interactions). These models provided a complementary assessment of whether observed patterns were consistent across families or differed in magnitude or direction.

Finally, to assess the extent to which species-level differences contributed to overall trait variation, we fitted alternative models incorporating species identity either as a random intercept or as species-specific smooths of elevation. Because many species were represented at only one or a few elevational sites, these models were not used for primary inference on elevational trends. Instead, they served as robustness checks to evaluate how strongly interspecific differences in mean trait values influenced the apparent elevational patterns detected at the community level.

All statistical analyses were conducted in R v.4.4.0 (R Core Team, 2023).

2.4. Runtime and repeatability analysis

We evaluated LEPY's processing time using a dataset of 50 Arctiinae and Geometridae individuals representing a broad range of body sizes. To assess how image type and format influence runtime, we compared three configurations of the same dataset: paired RGB + UV images in TIF format (100 images in total), RGB images only in TIF format, and RGB images only in JPG format. Each configuration was run five times on the same computer using identical LEPY settings. We calculated the average processing time per individual by dividing the total runtime by the number of individuals. We report mean and standard deviation to assess performance consistency across repeated runs.

To assess repeatability, we quantified run-to-run variability in four representative traits (forewing length, wing area, brightness, and contrast) across five identical LEPY runs of the same dataset. For each specimen and trait, we calculated the coefficient of variation (CV) across runs.

LEPY processes images in batches, with the number of images per run limited by available system memory (RAM and, when applicable, GPU memory), particularly for high-resolution datasets. To facilitate large-scale analyses, we developed a complementary script, LEPYLoop (https://github.com/DesBoe/LepyLoop), a companion script executed in the same environment as LEPY. LEPYLoop automatically verifies RGB-UV image pairs, splits large datasets into user-define batches (we recommend ~100 images, depending on available computational resources), processes them sequentially, and finally merges all outputs into consolidated CSV and Excel files. This batch-processing approach enables efficient analysis of datasets that would otherwise exceed memory limits.

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 Fig. 4. This output visualises the most important morphological and colour traits, the different colour channels (including the UV channel), as well as statistics of each channel as density and box plots. The visual summary is both useful and practical overview, 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.

In the upper left part, the visual summary displays a series of images. From left to right in the first row, it shows the original input images, the UV (ultraviolet) channel, the classic grayscale image (B/W), 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, it shows the 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 visualisation presents the estimated points of interest (POI), the length measurements of the specimen, and the detected scale bar along with the estimated pixel scale in pixels per millimeter.

The lower part of the visual summary displays density and box plots of the four colour channels and their combination, and the *RGB-UV mixed* image on the left. On the right, it shows two tables containing the most important morphological and colour traits of the specimen. Based on the eight POI, LEPY calculates eight morphological traits: five lengths and three areas, as shown in the visual summary (Fig. 4). The colour traits include the median, Q25, Q75, IQR, and the Shannon index of the four channels and of the *RGB-UV mixed* image. LEPY uses the median pixel value as a measure of brightness for each channel. To represent dynamic range / contrast, instead of using the difference between maximum and minimum pixel values, LEPY uses the IQR because it is less sensitive to statistical outliers and therefore provides more stable results. As mentioned in Section 2.2, LEPY stores additional colour traits in the main trait summary file, which we explain in more detail in the Supplementary Information (SI2).

3.2. Validation of morphological trait measurements

LEPY's automated measurements closely match with the reference dimensions across all morphological traits and performed comparably to ImageJ (median across observers). When analysing all traits together (Table 1, Fig. 5A-D), LEPY produced a slope of 0.966 and a R-squared of 0.999, indicating an near-linear relationship with real measurements. This result confirms that LEPY accurately scaled morphological traits. The slope slightly below 1 revealed a minor systematic underestimation of about 4%, mainly in larger specimens. Two outliers were observed for thorax width (Fig. 5C) and corresponded to specimens with extreme values, which are more challenging for automated measurements, but did not influence overall model performance.

Trait-wise regressions (Table 2) confirmed that LEPY consistently reproduced real measurements across all traits. For body length, right forewing length, and wingspan, LEPY achieved a very high agreement ($R^2 > 0.99$, MAPE ~2–3%), and maintained mean bias within ±2%. For thorax width, variability was higher ($R^2 = 0.96$, MAPE ~8%).

We further tested whether measurement bias varied with specimen size (Table 3, Fig. 6A–D).

For LEPY, slopes of the regression between signed percentage error and real specimen size were small but statistically significant for most traits, i.e., body length, right forewing length, and wingspan (Table 3). These positive slopes suggest that LEPY slightly overestimated measurements in larger individuals, but the effect remained minimal ($\leq$ 2% across the full size range). For thorax width, the slope was non-significant (Table 3), indicating greater dispersion but no consistent size effect.

Across all traits, regression lines in Fig. 6A–D remained close to horizontal, indicating that measurement errors were small and consistent across all traits except for thorax width were is not significant. LEPY showed a slight size-related bias, tending to slightly underestimate measurements in larger specimens, but this effect was minor and within acceptable error limits (<4%).

3.3. Performance of LEPY in analysing traits along an elevational gradient

General performance. Specific image and specimen characteristics influenced the performance of LEPY. For example, visible antennae or legs in the images occasionally caused 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, caused POI detection errors.

Morphological traits responses to elevation. Morphological traits exhibited family-dependent responses along the elevational gradient (Fig. 7; Table 4). Forewing length increased significantly with elevation in Sphingidae, whereas Saturniidae did not exhibit a significant elevational trend (Fig. 7A; Table 4). Wing area did not vary significantly with

Table 1
Global comparison of LEPY and ImageJ (median across observers) with reference dimensions. MAPE = mean absolute percentage error.

Method	n	Slope	R^2	MAPE (%)
ImageJ (median)	124	0.993	1.000	2.37
LEPY	124	0.966	0.999	4.02

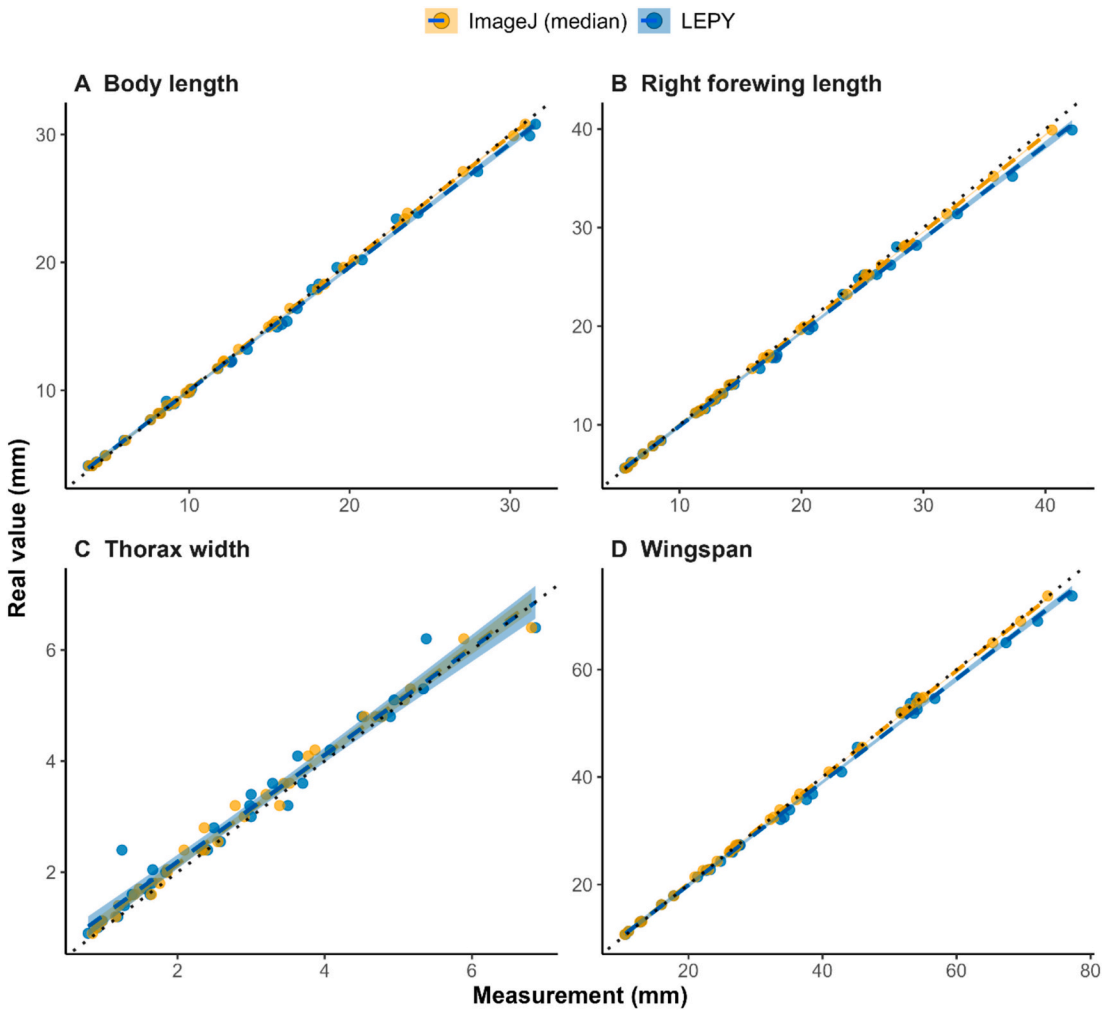

Fig. 5. Comparison of LEPY and ImageJ (median) measurements against real values for four traits: (A) body length, (B) right forewing length, (C) thorax width, (D) wingspan. Each point represents one specimen–trait combination. The x-axis shows the measured value obtained by either LEPY (blue) or ImageJ (yellow), while the y-axis shows the corresponding reference dimension for the same specimen. Blue and yellow points associated with the same specimen therefore share the same y-value. Dashed lines indicate linear regression fits; dotted lines indicate the 1:1 relationship (y = x). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Trait-wise validation metrics for LEPY and ImageJ (median across observers; n = 31 for all traits). MAPE = mean absolute percentage error. FWL = Forewing length.

Method	Trait	Slope	R^2	MAPE (%)	Mean Bias (%)
LEPY	Body length	0.964	0.998	2.68	0.13
LEPY	Right FWL	0.95	0.998	2.93	2.474
LEPY	Thorax width	0.96	0.965	8.08	−6.426
LEPY	Wingspan	0.959	0.998	2.398	1.586
ImageJ (median)	Body length	0.991	1	1.099	−0.696
ImageJ (median)	Right FWL	0.983	1	1.205	1.072
ImageJ (median)	Thorax width	0.979	0.989	6.388	−5.416
ImageJ (median)	Wingspan	0.991	1	0.788	−0.291

Table 3
Regression slopes of signed percentage error versus moth size, testing for size-dependent bias (n = 31 for all traits). FWL = Forewing length.

Method	Trait	Slope (% per mm)	R^2	p-value	Significance
LEPY	Body length	0.241	0.293	0.002	**
LEPY	Right FWL	0.133	0.224	0.007	**
LEPY	Thorax width	1.798	0.076	0.134	
LEPY	Wingspan	0.069	0.242	0.005	**
ImageJ (median)	Body length	0.123	0.273	0.003	**
ImageJ (median)	Right FWL	0.042	0.191	0.014	*
ImageJ (median)	Thorax width	1.581	0.184	0.016	*
ImageJ (median)	Wingspan	0.035	0.394	0	***

elevation in either family, although Sphingidae showed a marginal elevational trend (Fig. 7B; Table 4). Across the gradient, Saturniidae consistently exhibited larger wing areas than Sphingidae, reflecting pronounced family-level differences in this trait (SI6).

Models incorporating species identity substantially improved model fit for both morphological traits (SI7). However, species-level extensions did not reveal consistent elevational responses within species, indicating

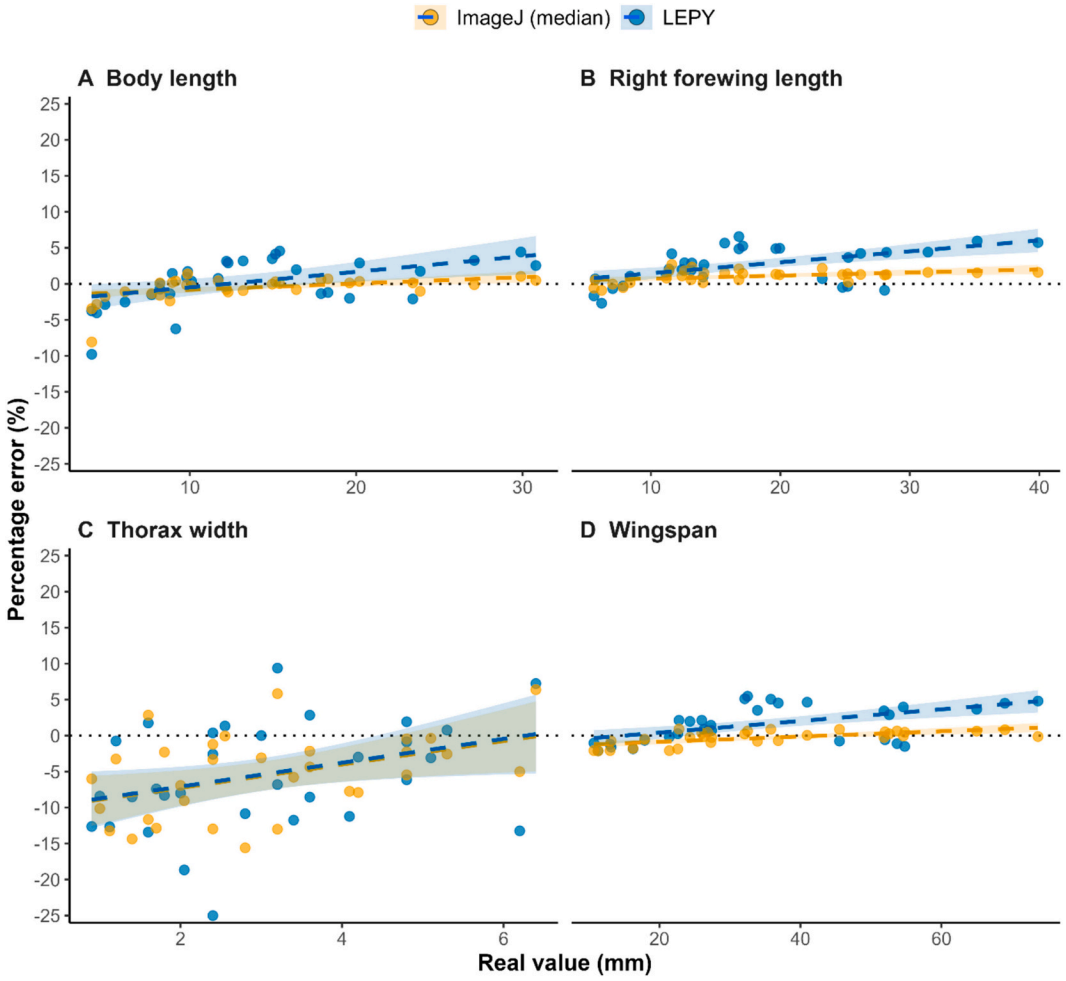

Fig. 6. Signed percentage error (%) versus real specimen size (mm) for LEPY and ImageJ (median): (A) Body length, (B) Right forewing length, (C) Thorax width, (D) Wingspan. Dotted lines represent zero error; shaded areas show 95% confidence intervals.

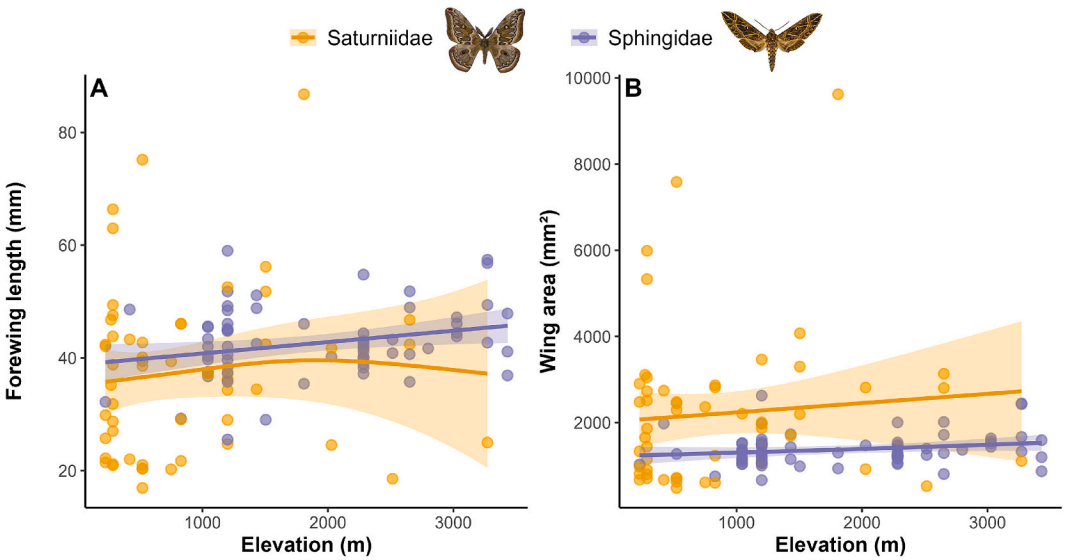

Fig. 7. Elevation–morphology relationships in Andean moths. (A) Relationship between elevation and forewing length and (B) wing area for Sphingidae (purple) and Saturniidae (orange). Points represent individual specimens. Solid lines show family-specific generalized additive model (GAM) predictions, and shaded areas indicate 95% confidence intervals. Forewing length displayed family-specific elevational patterns, increasing significantly with elevation in Sphingidae but showing no consistent elevational trend in Saturniidae. Wing area did not vary significantly with elevation in either family, although Sphingidae showed a marginal elevational trend. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
Summary of generalized additive models (GAMs) testing the effect of elevation on morphological and colour traits, fitted separately for Sphingidae (Sph) and Saturniidae (Sat). For each trait- family combination, we report the effective degrees of freedom (edf), F-statistics, *p*-values for the elevation smooth, and the proportion of deviance explained by the model. Ns = Not significant.

Trait	Family	edf	F	p-value	Deviation explained (%)	Interpretation
Forewing length	Sat	1.400	0.194	0.714	2.33	Ns
Forewing length	Sph	1.000	5.322	0.024	7.37	Significant elevational response
Wing area	Sat	1.002	0.424	0.517	0.84	Ns
Wing area	Sph	1.074	2.502	0.098	4.63	Marginal elevational trend
Brightness	Sat	1.000	3.364	0.072	6.19	Marginal elevational trend
Brightness	Sph	1.001	1.682	0.199	2.46	Ns
Contrast	Sat	1.713	1.204	0.294	6.30	Ns
Contrast	Sph	1.000	0.244	0.623	0.36	Ns

that improved model performance primarily reflected differences in mean trait values among species rather than shared elevation-driven trends.

Colour trait responses to elevation. Colour traits showed limited elevational variation (Fig. 8; Table 4). Brightness declined marginally with elevation in Saturniidae, while Sphingidae showed no detectable elevation response (Fig. 8A; Table 4). Contrast (dynamic range) did not change significantly with elevation in either family (Fig. 8B; Table 4). Family identity strongly structured colour traits: Sphingidae consistently exhibited lower brightness values than Saturniidae, whereas contrast did not differ between families (SI6).

Including species identity also improved model fit for both colour traits (SI7). Nevertheless, species-specific smooths did not indicate coherent elevational responses across species, suggesting that variation in brightness and contrast was dominated by interspecific differences rather than by consistent elevation-related change within species.

3.4. Runtime and repeatability

LEPY processed each individual within seconds, with runtime determined mainly by the number of images analysed. When both RGB and UV photographs were included, LEPY required on average ~ 21 s per individual (mean per image = 10.5 ± 0.06 s). In contrast, processing only RGB images took ~16 s per individual, with mean per image of 15.9 ± 0.48 s for TIF files and 15.7 ± 0.12 s for JPG files. Thus, including UV images therefore roughly doubled the total runtime per individual, reflecting the increased number of input images, whereas image format (TIF vs JPG) had no measurable effect on processing speed.

LEPY is fully deterministic; therefore, repeated executions on identical image datasets produced identical trait values for all specimens and traits (forewing length, wing area, brightness, and contrast), resulting in zero within-specimen variance across runs.

4. Discussion

4.1. Capabilities and validation of LEPY

LEPY provides a powerful and efficient framework for extracting morphological and colour traits from standardised images of moths and butterflies. It represents a significant improvement over previous pipelines such as *Mothseg* and *Mothra* by combining high accuracy, reproducibility, and processing speed within a single automated workflow.

LEPY achieved high accuracy across morphological traits and performed comparably to manual measurements obtained in ImageJ. When all traits were combined (slope = 0.966, R^2 = 0.999), LEPY showed a near-linear relationship with reference dimensions (real values), confirming precise scaling of morphological measurements. The slope slightly below one indicates a minor underestimation of about 4%. However, regressions of signed percentage error against specimen size revealed a limited positive trend, showing that this underestimation decreased with increasing size and occasionally shifted toward slight overestimation in the largest specimens. This pattern likely reflects scale-bar resolution differences in images of large moths, where the scale-bar occupies a smaller portion of the image, rather than calibration errors. Overall, this small size-related bias appears biologically

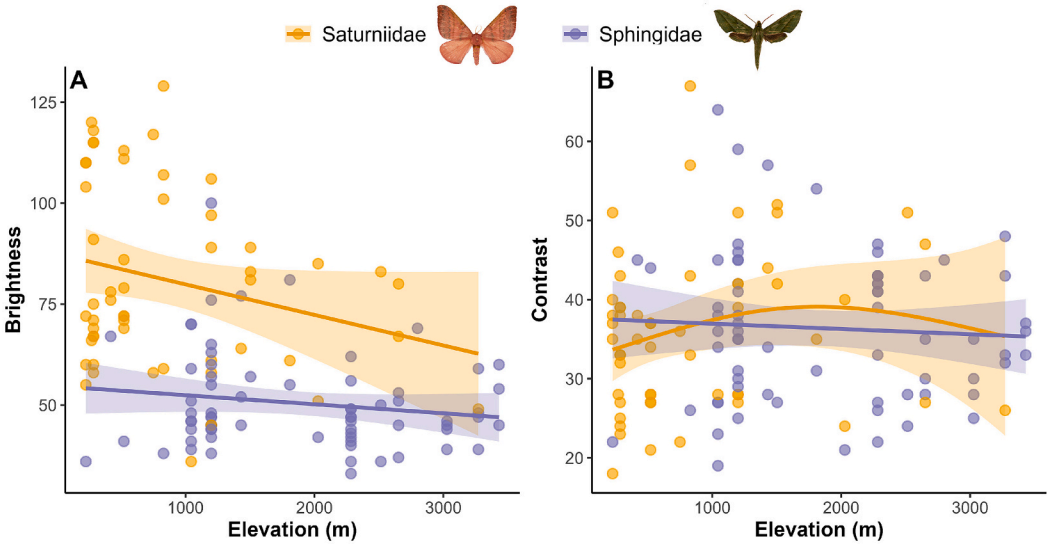

Fig. 8. Elevation–colour relationships in Andean moths. (A) Brightness (median of four colour channels; values range from 0 to 255) and (B) dynamic range (contrast; interquartile range of four colour channels; values range from 0 to 128) plotted against elevation for Sphingidae (purple) and Saturniidae (orange). Points represent individual specimens. Solid lines and shaded areas indicate family-specific GAM predictions and 95% confidence intervals. Brightness showed a marginal decline with elevation in Saturniidae, whereas no comparable elevational pattern was detected in Sphingidae. In contrast, dynamic range (contrast) showed no significant elevational trends in either family. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

negligible and does not compromise LEPY's measurement reliability.

Trait-wise analyses further confirmed that LEPY consistently reproduced real morphological values across traits. For body length, right forewing length, and wingspan, LEPY achieved R^2 values exceeding 0.99 and MAPE of approximately 2–3%, with mean bias values close to zero. Thorax width showed higher variability ($R^2 = 0.96$, MAPE ~8%), likely reflecting the difficulty of consistently identifying thoracic boundaries in two-dimensional images rather than limitations in LEPY's segmentation algorithm. These results demonstrate that LEPY reliability quantifies morphological traits across species with diverse body shapes and sizes.

Because LEPY operates deterministically, identical image inputs yield identical segmentation masks and trait values. In this study, the U-Net–based background removal produced identical outputs under identical inputs, ensuring complete algorithmic repeatability of all measured traits.

Beyond accuracy and reproducibility, LEPY substantially improves processing efficiency. Manual measurements in ImageJ typically require approximately one minute per specimen, whereas LEPY processes each individual in 15–21 s, depending on the number of images analysed. Because morphological and colour traits are extracted simultaneously, LEPY reduces total processing time by three- to fourfold compared with manual workflows. Moreover, using LEPYLoop, analyses of large datasets (e.g. 1000 individuals or more) can be completed unattended overnight, making LEPY particularly well suited for high-throughput ecological and evolutionary studies.

The validation and ecological case study along an elevational gradient further demonstrated LEPY's analytical capabilities. By quantifying multiple morphological and colour traits in a single workflow, LEPY revealed that these variables can lead to contrasting ecological interpretations. For example, a longer forewing length does not necessarily indicate a larger wing area, and this relationship varies among taxa. While many studies rely on a single measurement such as wing length, this approach is only appropriate for morphologically homogeneous groups (e.g., Brehm et al., 2019). LEPY's ability to extract multiple, independent morphological and colour traits allows researchers to explore complex trait-environment relationships and patterns of morphological adaptation.

The integration of UV information represents a significant advancement. Although UV patterns play important roles in communication, camouflage, and mating signals (Pinna et al., 2021; Prabhulinga et al., 2022), they have been largely ignored in digitisation workflows so far (Brehm, 2025). LEPY's four-channel approach (RGB + UV) enables full incorporation of UV reflectance through quantitative analysis and false-colour visualisation. This addition opens new opportunities to study visual signalling and aposematism. For instance, many butterflies and Arctiinae moths are well known for their aposematic and UV-reflective colour patterns, which function in warning displays and sexual communication (Lyytinen et al., 2004; Stella and Kleisner, 2022; Yeager and Barnett, 2021). Including UV data therefore provides biologically relevant information that complements RGB-based analyses and reveals traits invisible to the human eye but critical for understanding ecological and behavioural functions (Cronin and Bok, 2016; Goldsmith, 1994; Prudic et al., 2007).

In the elevational dataset, individuals from both Sphingidae and Saturniidae became darker at higher elevations, while wing contrast remained constant. These results highlight LEPY's ability to capture trait-environment relationships across ecologically relevant gradients. Future improvements could focus on refining segmentation for complex body regions, improving scale-bar detection in large specimens, and developing models for interpreting spectral data.

Overall, LEPY provides a scalable and reproducible solution for extracting quantitative morphological and colour traits from standardised images. It offers a robust foundation for studying phenotypic diversity, ecological adaptation, and macroecological patterns, while facilitating the creation of open-access trait databases for Lepidoptera (e.g., *Lep Traits*; Shirey et al., 2022).

4.2. Ecological relevance and potential applications of LEPY

LEPY supports a wide range of ecological and evolutionary research in Lepidoptera, including studies of morphology-function relationships, trait evolution, phenotypic variation across space and time, and responses to environmental changes (Clusella-Trullas and Nielsen, 2020; Duarte et al., 2017; Henriques et al., 2022). Morphological traits such as body size are closely linked to key ecological functions, including dispersal capacity, which in turn influence species' sensitivity to habitat fragmentation and environmental change (Mattila et al., 2008). By enabling automated and standardised extraction of morphological and colour traits from specimen images, LEPY provides a scalable framework for detecting trait-environment relationships across populations and communities (Koneru and Caro, 2022; Mikitová et al., 2022).

In our elevational case study focusing on Sphingidae and Saturniidae, morphological traits showed taxon-dependent responses to elevation. Forewing length displayed contrasting family-level patterns. Sphingidae exhibited a significant and approximately linear increase with elevation, whereas Saturniidae showed no consistent elevational response. Wing area followed a similar pattern, with Sphingidae exhibiting a marginal increase along the elevational gradient, while Saturniidae showed no significant elevational effect despite a weak increasing tendency. Notably, Saturniidae consistently displayed larger wing areas than Sphingidae across the gradient.

These results suggest that elevation plays a taxa-dependent role in shaping morphological size traits, while family identity explains a larger proportion of the observed variation. This pattern accords with previous studies showing that body size-elevation relationships in moths are highly heterogeneous and context dependent, varying across taxa, regions and environmental gradients (Heidrich et al., 2018; Papandreou et al., 2023). For example, Brehm et al. (2019) reported increasing forewing length with elevation along a nearly 2900 m gradient in Costa Rica, whereas Brehm and Fiedler (2004) observed a negative size-elevation relationship in Geometridae moths from Ecuador across a shorter gradient exceeding 1600 m. Such discrepancies underscore that size-elevation relationships depend on taxonomic composition, geographic context, and the extent of the elevational gradient rather than reflecting a universal ecological rule.

Where elevational increases in forewing length occur, they may reflect adaptations to flight constraints in montane environments, where reduced air density can favour larger wings to maintain efficient flight performance (Brehm et al., 2019; Classen et al., 2017). However, the relatively small proportion of variance explained by elevation in our models highlights that additional factors, such as phylogenetic history, taxon-specific functional constraints, and ecological niche differentiation play a dominant role in shaping morphological trait variation at the community level (Heidrich et al., 2021).

Colour traits showed a similarly taxon-structured but limited response to elevation. Brightness showed pronounced family-level differentiation, with Sphingidae consistently darker than Saturniidae across the elevational gradient. Within families, brightness showed a marginal decline with elevation in Saturniidae, whereas Sphingidae did not display a statistically supported elevational response, despite an apparent decreasing trend. This pattern is consistent with previous evidence that darker colouration may be favoured in colder, high-elevation environments, potentially due to thermoregulatory advantages, even in nocturnal species (Fiedler and Brehm, 2021; Heidrich et al., 2018). In contrast, dynamic range (contrast) did not vary systematically with elevation in either family and explained little variation overall, suggesting that brightness represents the primary colour dimension associated with elevational gradients in these moth families, whereas contrast likely reflects alternative selective pressures such as signalling, camouflage, or phylogenetic constraints (Fiedler and Brehm, 2021; Heidrich et al., 2018; Heidrich et al., 2021).

Models incorporating species identity substantially improved statistical fit, reflecting strong interspecific differences in mean trait values. However, species-specific smooths did not reveal consistent elevational patterns and should be interpreted cautiously. Many species were represented by few individuals and occurred at only one or a small number of neighbouring elevational sites, reflecting strong species turnover along the gradient. Under these conditions, species-level smooth terms are likely to capture sampling structure rather than genuine within-species elevational responses. We therefore treat species-level models primarily as exploratory robustness checks and base ecological inference on family-level analyses, which are more robust to strong species turnover along the elevational gradient.

Future research integrating LEPY-derived trait data with phylogenetic information will be essential for disentangling the relative contributions of environmental filtering and evolutionary history in shaping trait variation along environmental gradients (Shrestha et al., 2014). Beyond ecological inference, LEPY's ability to automatically extract biologically meaningful morphological and colour traits from images also offers substantial potential for advancing trait-based species identification workflows. When combined with machine learning approaches, including neural network classifiers, such traits can complement pixel-based image features by explicitly incorporating information on size, shape, and colour, attributes routinely used by taxonomists but often poorly captured by conventional image-only models. This integration may substantially improve identification accuracy, particularly for taxa that are difficult to distinguish based solely on wing patterns or raw pixel information.

4.3. Current limitations and future directions of LEPY

Despite its strengths, LEPY has some limitations that offer opportunities for future improvement. For instance, LEPY currently requires a specific chessboard pattern for calibration (available for printing in SI1) and adapting the tool to different scales or formats would require additional programming. In most cases, such adaptation appears feasible if researchers standardise their scale references (e.g., a uniform 10 mm black scale bar).

In addition, the accuracy of measurements depends on reliable scale bar recognition, which may be affected by image quality or incorrect placement. Poorly prepared specimens, visible legs, or pale or translucent wings can lead to segmentation errors and inaccurate detection of points of interest. These issues primarily affect the extraction of morphological traits. At the same time, colour trait analysis is also sensitive to image quality and calibration accuracy, for example, errors in scale bar detection or variable lighting conditions can influence colour quantification. We therefore strongly recommend a high degree of standardisation of photographs in terms of background, exposure, and light quality (Brehm, 2025). Nevertheless, LEPY may still provide useful measurements from non-standardised image collections, particularly for clearly visible morphological traits when scale references are present and lighting conditions are reasonably consistent. However, reduced standardisation is likely to increase segmentation uncertainty and may affect trait accuracy, especially for colour and UV-based metrics. We therefore encourage users to conduct small pilot tests when applying LEPY to heterogeneous datasets. While such challenges are common in automated image analysis, LEPY's visual summary of results allows users to quickly identify potential errors and, if necessary, replace them with manual measurements.

Future extensions of LEPY could include more fine-grained trait extraction, such as measuring fore- and hindwings separately or distinguishing thorax and abdomen in terms of size and colouration. However, in pinned Lepidoptera specimens, subtle overlap between wings and ambiguous body boundaries between thorax and abdomen can complicate consistent segmentation from dorsal images alone. These limitations arise from specimen preparation and posture rather than from the pipeline itself and would make such measurements sensitive to segmentation uncertainty. Moreover, the traits currently implemented in LEPY correspond to those most widely used and biologically relevant in the Lepidoptera literature, for example forewing-related measurements, which typically dominate wing area and strongly influence flight performance (Jantzen and Eisner, 2008).

Recent developments in foundation models for image segmentation, such as SAM (Kirillov et al., 2023), offer promising avenues for addressing some of these challenges in future versions of LEPY. While such models have demonstrated strong performance across diverse domains, including biomedical and microscopy applications (Ma et al., 2024; Marks et al., 2025), their use for fine-scale morphological segmentation of Lepidoptera specimens may still require careful prompting, task-specific configuration, or additional post-processing to obtain analysis-ready masks. Emerging approaches that combine segmentation with semantic or texted-guide information, such as Moondream (Moondream Team, 2024), GroundedSAM (Ren et al., 2024), SAM3 (Carion et al., 2025), or Moonshine (Jeffries et al., 2024), may further reduce this effort and facilitate more direct integration. For a broader overview of recent foundation models for image segmentation, see Zhou et al. (2024).

Finally, LEPY is currently more applicable to standardised datasets, where specimens are prepared and photographed under consistent conditions. Applying the pipeline to more heterogeneous image collections would require further adaptation. A promising direction for future work is the extension of LEPY to other insect groups beyond Lepidoptera. Species with more pronounced three-dimensional body structures are expected to require complementary imaging techniques, such as focus stacking or multi-angle photography, which in principle could be integrated into the pipeline. Owing to its modular and flexible design, LEPY offers a solid foundation for such developments and is well positioned for expansion to a broader range of insect taxa.

5. Conclusions

LEPY is a free and openly available tool that addresses key challenges in Lepidoptera trait research by automating the analysis of morphological and colour traits, including ultraviolet (UV) information, across large specimen images datasets. Our results show that LEPY performs effectively in quantifying morphological and colour traits variation and examining how environmental gradients influence patterns in Lepidoptera communities. By integrating trait and ecological data, LEPY provides valuable insights into how environmental factors shape trait distributions within insect assemblages.

LEPY offers flexibility as a major advantage. It can process regular RGB images, making it accessible for researchers working with standard photographic material, and extends functionality by integrating UV images. Incorporating UV information represents a key strength, as this feature is rarely available in other existing tools for trait analysis.

LEPY also has strong potential as a tool for large-scale trait data generation. Its capacity to analyse traits in a fully automated and consistent manner makes it well suited for building big data resources in Lepidoptera research. Such datasets can significantly support comparative studies, facilitate the integration of morphological and colour traits into biodiversity monitoring programs, and support broader efforts in ecological and evolutionary research.

CRediT authorship contribution statement

Yenny Correa-Carmona: Writing – review & editing, Writing – original draft, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Dennis Böttger:** Writing – review & editing, Validation, Software, Methodology, Formal analysis, Conceptualization. **Dimitri Korsch:** Writing – review & editing, Validation, Software, Methodology, Formal analysis, Conceptualization. **Kim L. Holzmann:** Writing – review & editing, Validation. **Pedro Alonso-Alonso:** Writing – review & editing, Validation. **Andrea Pinos:** Writing – review & editing,

Validation. **Felipe Yon:** Writing – review & editing, Validation. **Alexander Keller:** Writing – review & editing, Validation, Funding acquisition. **Ingolf Steffan-Dewenter:** Writing – review & editing, Validation, Funding acquisition. **Paul Bodesheim:** Writing – review & editing, Validation. **Marcell K. Peters:** Writing – review & editing, Validation, Funding acquisition, Formal analysis. **Gunnar Brehm:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition, Data curation, Conceptualization.

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Declaration of competing interest

The authors have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoinf.2026.103680.

Data availability

Data are accessible through Zenodo and Github repositories

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