Received: 29 March 2017 | Accepted: 29 June 2017

DOI: 10.1111/2041-210X.12853

APPLICATION

Methods in Ecology and Evolution BRITISH ECOLOGICAL SOCIETY

# patternize: An R package for quantifying colour pattern variation

Steven M. Van Belleghem[1,2,3] | Riccardo Papa[2,4] | Humberto Ortiz-Zuazaga[5] | Frederik Hendrickx[6,7] | Chris D. Jiggins[1] | W. Owen McMillan[8] | Brian A. Counterman[3]

[1]Department of Zoology, University of Cambridge, Cambridge, UK

[2]Department of Biology, Center for Applied Tropical Ecology and Conservation, University of Puerto Rico, Rio Piedras, Puerto Rico

[3]Department of Biological Sciences, Mississippi State University, Starkville, MS, USA

[4]Molecular Sciences and Research Center, University of Puerto Rico, San Juan, Puerto Rico

[5]Department of Computer Science, University of Puerto Rico, Rio Piedras, Puerto Rico

[6]Terrestrial Ecology Unit, Biology Department, Ghent University, Gent, Belgium

[7]Royal Belgian Institute of Natural Sciences, Brussel, Belgium

[8]Smithsonian Tropical Research Institute, Apartado, Panama

**Correspondence**
Steven M. Van Belleghem
Email: vanbelleghemsteven@hotmail.com

**Funding information**
Directorate for Biological Sciences, Grant/Award Number: DEB-1257839; Center for Information Technology, Grant/Award Number: 5P20GM103475-13; NSF, Grant/Award Number: DEB-1257839; NIH, Grant/Award Number: 5P20GM103475-13

Handling Editor: Jana Vamosi

## Abstract

1. The use of image data to quantify, study and compare variation in the colours and patterns of organisms requires the alignment of images to establish homology, followed by colour-based segmentation of images. Here, we describe an R package for image alignment and segmentation that has applications to quantify colour patterns in a wide range of organisms.
2. patternize is an R package that quantifies variation in colour patterns obtained from image data. patternize first defines homology between pattern positions across specimens either through manually placed homologous landmarks or automated image registration. Pattern identification is performed by categorizing the distribution of colours using an RGB threshold, *k*-means clustering or watershed transformation.
3. We demonstrate that patternize can be used for quantification of the colour patterns in a variety of organisms by analysing image data for butterflies, guppies, spiders and salamanders. Image data can be compared between sets of specimens, visualized as heatmaps and analysed using principal component analysis.
4. patternize has potential applications for fine scale quantification of colour pattern phenotypes in population comparisons, genetic association studies and investigating the basis of colour pattern variation across a wide range of organisms.

**KEYWORDS**
colour patterns, heatmap, image registration, image segmentation, landmarks

## 1 | INTRODUCTION

Natural populations often harbour great phenotypic diversity. Variation in colour and pattern are of the more vivid examples of morphological variability in nature. Taxa as diverse as spiders (Cotoras et al., 2016; De Busschere, Baert, Van Belleghem, Dekoninck, & Hendrickx, 2012), insects (Katakura, Saitoh, Nakamura, & Abbas, 1994; Williams, 2007), fish (Endler, 1983; Houde, 1987), amphibians and reptiles (Allen, Baddeley, Scott-samuel, & Cuthill, 2013; Balogová & Uhrin, 2015; Calsbeek, Bonneaud, & Smith, 2008; Rabbani, Zacharczenko, Green, Abbani, & Acharczenko, 2015), mammals (Hoekstra, Hirschmann, Bundey, Insel, & Crossland, 2006; Nekaris & Jaffe, 2007) and plants (Clegg & Durbin, 2000; Mascó, Noy-Meir, & Sérsic, 2004) display natural variation in pigment or structural colorations. The distribution of colours in specific patterns play an important role in mate preference (Endler, 1983; Kronforst et al., 2006), thermal regulation (Forsman,


© 2017 The Authors. Methods in Ecology and Evolution © 2017 British Ecological Society

Ringblom, Civantos, & Ahnesjö, 2002), aposematism (Rojas, Valkonen, & Nokelainen, 2015) and crypsis (Nosil & Crespi, 2006) and represent evolutionary adaptations that in many cases have promoted diversification within lineages.

Measuring phenotypic variation in organismal colour patterns can provide insights into their underlying developmental and genetic architecture (Klingenberg, 2010). However, precisely quantifying colour pattern variation is challenging. Consistent comparisons of colour patterns from images requires the (1) homologous alignment and (2) colour-based segmentation of the images. Homologous alignment can be performed by transforming one image onto another. This transformation can be obtained from manually placed homologous landmarks or advanced image registration techniques, which can be stored and utilized to align colour patterns extracted from the images. Image segmentation concerns the categorization of pixels by colour. Previously, examples of colour pattern quantification have been extensively developed for *Heliconius* butterflies (Color Pattern Modelling [CPM] in Le Poul et al., 2014) and primates (Allen, Higham, & Allen, 2015). However, these applications are currently not easily accessible for use in other organisms. Similarly, advanced solutions are available for biomedical image analysis (Schindelin, Rueden, Hiner, & Eliceiri, 2015; Schindelin et al., 2012; Modat, McClelland, & Ourselin, 2010), but are not tailored towards quantifying colour pattern variation.

Here, we present `patternize`, an approach to quantification of colour pattern variation from 2D images using the `R` statistical computing environment (R Development Core Team, 2013). The package provides utilities to extract, transform and superimpose colour patterns as well as downstream analysis (Figure 1). The provided `R` functions combine single transformation and colour extraction approaches. While transformations are obtained from manually placed homologous landmarks (`patLanRGB()`, `patLanK()` or `patLanW()`) or automated image registration (`patRegRGB()`, `patRegK()` or `patRegW()`), colour-based segmentation of the patterns is performed using threshold RGB (Red, Blue and Green) values (`patLanRGB()` or `patRegRGB()`), unsupervised classification of pixels into a set of clusters (`patLanK()` or `patRegK()`) or watershed transformation (`patLanW()` or `patRegW()`). By extracting and aligning colour patterns from image data of large numbers of samples, `patternize` provides quantitative measures of variation in colour patterns that can be used for population comparisons, genetic association studies and investigating dominance and epigenetic interactions of colour pattern variation in a wide range of organisms. We demonstrate the utility of the package with *Heliconius* butterflies and more challenging examples from guppy fish, Galápagos wolf spiders and salamanders.

## 2 | ALIGNMENT

Superimposing colour patterns to quantify variation in their expression requires the homologous alignment of the anatomical structures they occur in. Image transformations for this alignment can be obtained from landmark based transformations or image registration techniques.

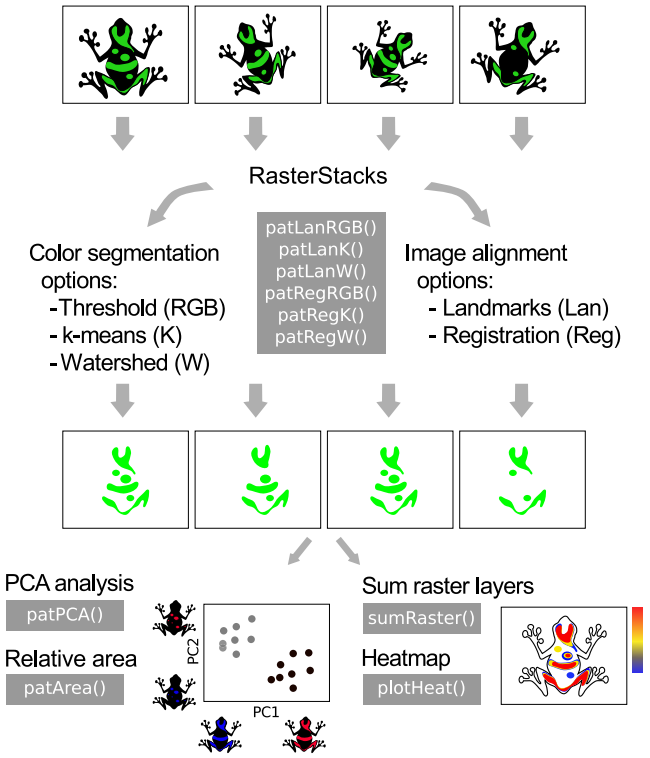


**FIGURE 1** Overview of main `patternize` functions and functionality. Images can be aligned using homologous landmarks (Lan) or automatic registration (Reg), which aligns images using common intensity patterns. Colours can be extracted using an RGB threshold (RGB), *k*-means clustering (K) or by identifying watershed lines (W). The resulting extracted patterns can be summed and visualized as heatmaps or used for principal component analysis and calculating the relative area of the colour patterns

### 2.1 | Landmark based transformations

Landmark based transformations use discrete anatomical points that are homologous among individuals in the analysis. Non-rigid, but uniform transformations from one set of "source" landmarks to a set of "target" landmarks such as *affine* transformations include translation, rotation, scaling and skewing (Hazewinkel, 2001). Additionally, non-uniform changes in shape between the source and target landmarks can be accounted for by storing the transformation as if it were "the bending of a thin sheet of metal," the so-called *thin plate spline* (TPS) transformation (Duchon, 1976). Both the affine and TPS transformation can be calculated from sets of landmarks (Figure 2a). We implemented these landmark transformations using utilities provided by the `R` package `Morpho` (Schlager, 2016). Landmarks can be transformed using an arbitrarily chosen reference sample or an average landmark shape obtained from a set of samples. The average landmark shape is obtained by means of Procrustes superimposition of the samples (Goodall, 1991).

### 2.2 | Image registration

Alternative to landmark based methods, fast and accurate image registration techniques are available for calculating a transformation from

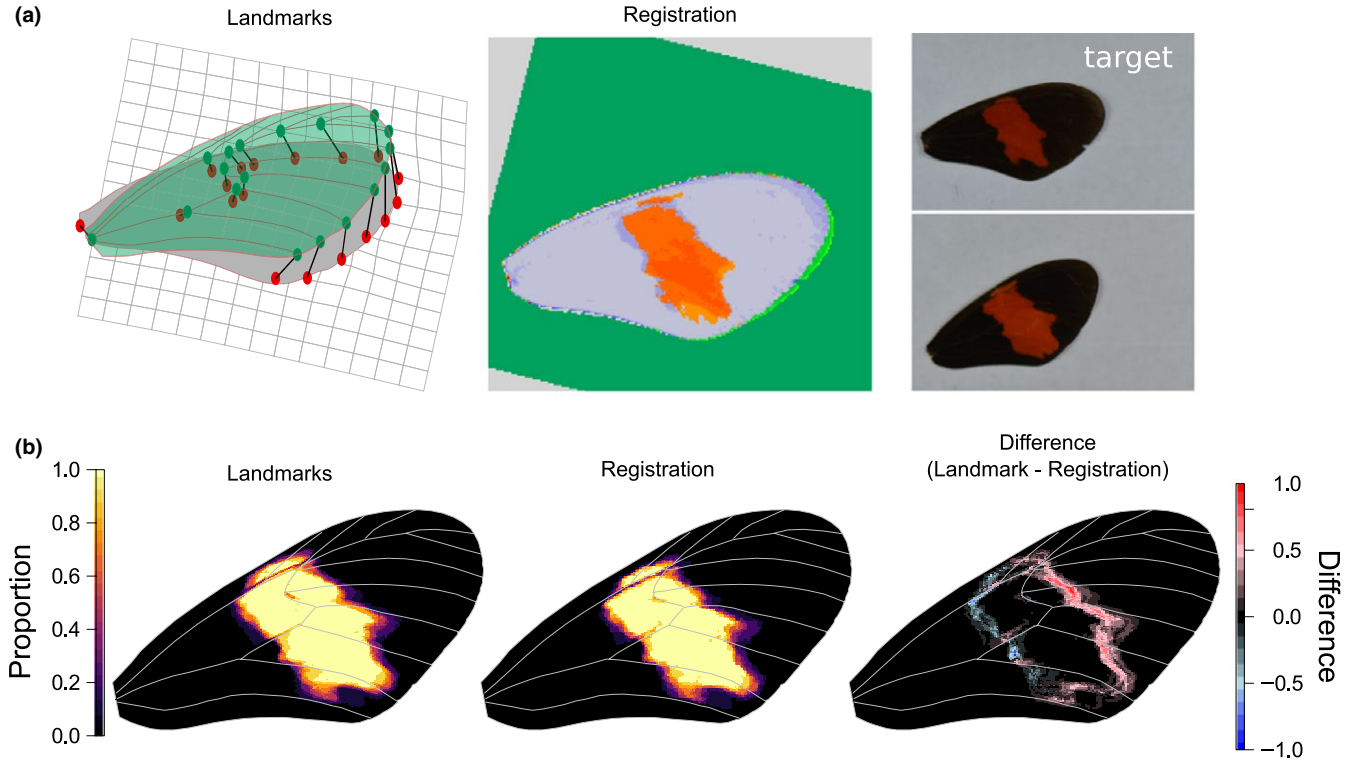


**FIGURE 2** Comparison of image transformation using landmarks or automated registration for quantification of colour pattern variation. (a) Illustration of transformation strategies of a source (green) image to a target (grey) image. The thin plate spline (TPS) transformation from the source to target landmarks is illustrated by the deformed grid and can be used to transform the image or extracted colour pattern. Image registration attempts to find common patterns in images and align the source (green) image to the pixel coordinate system of the target (grey) image. Note the extracted colour pattern in red. (b) Example comparison between landmark approach for colour pattern alignment for ten butterfly wings of male *Heliconius erato hydara*. For the landmark approach, we used TPS transformation. For the image registration approach, we used affine transformation and 75% of sub-volumes included as inliers

a source to target image based on either intensity patterns or features such as points, lines or contours present in the images (Goshtasby, 2005; Figure 2a). We use a computation efficient intensity-based image registration technique implemented in the NiftyReg image registration library (Translational Imaging Group (TIG) 2016) and made available in `R` through the `RNiftyReg` package (Clayden, Modat, Presles, Anthopoulos, & Daga, 2017). This methodology calculates the global transformation of an image by finding correspondences between sub-volumes of the two images (Modat, McClelland, et al., 2010; Modat, Ridgway, et al., 2010). Correspondence is assessed using intensity-based similarity measures and used to calculate the transformation parameters through a least trimmed square (LTS) regression method (Modat, McClelland, et al., 2010; Modat, Ridgway, et al., 2010). The number of corresponding sub-volumes to be included or considered as outliers in the calculation of the transformation can be varied by the user. The global transform calculated by NiftyReg can be rigid (i.e. including translation, rotation and scaling) or affine (i.e. translation, rotation, scaling and skewing).

## 3 | COLOUR PATTERN EXTRACTION

Studying variation in colour patterns requires the correct identification of the colour boundaries. `patternize` provides functionality to categorize the distribution of colours using either an RGB threshold, *k*-means clustering or watershed transformation.

### 3.1 | RGB threshold

Colour boundaries can be extracted from images or the trait of interest using an RGB threshold (Figures 2, 3). By selecting pixels within a specified colour range (specified as RGB value and offset) we provide a basic image segmentation approach that works well for extracting distinct colour patterns. Additionally, for distinct colour patterns, the RGB value can be iteratively recalculated as the average for the extracted colour pixels. This latter approach permits patterns to be easily combined when extracted from sets of images that may have been taken under different light conditions resulting in differences in intensity and contrast.

### 3.2 | *k*-Means clustering

We implemented an unsupervised approach for colour-based image segmentation using *k*-means clustering (Figures 4, 5) (Hartigan & Wong, 1979). This algorithm assigns pixel RGB values to *k* clusters by iteratively assigning each pixel in the image to the RGB cluster that minimizes the distance between the pixel and the cluster centres.

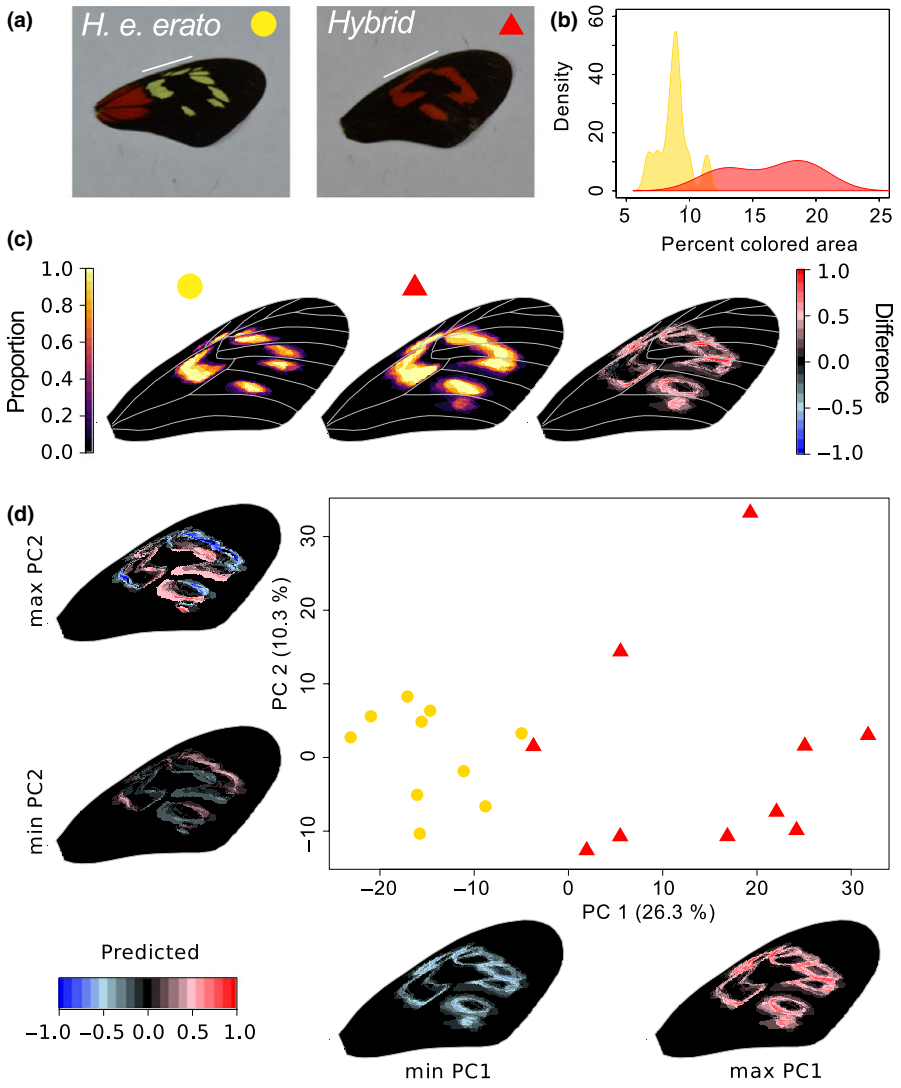


**FIGURE 3** Example of image registration and threshold colour extraction in the forewing band area of *Heliconius erato erato* ($n$ = 10) and hybrid ($n$ = 10) butterflies (French Guiana). (a) Example of original images with a white line indicating the forewing band area. The hybrid represents a naturally occurring backcross in a hybrid zone with *Heliconius erato hydara* (see Figure 2) that results in red colour expression in the forewing band. (b) Density plot showing the probability to find a sample with a certain percentage of coloured area in the wing expressing yellow in *H. e. erato* and red in the hybrid. (c) Visualizing the variation in colour pattern expression in a heatmap shows a consistently larger pattern in the hybrid phenotypes (*H. e. erato*: left, hybrid: middle, hybrid minus *H. e. erato*: right). (d) Principal component analysis confirms that the main axis of variation (PC1) is related to size of the pattern (yellow or red in *H. e. erato* and hybrids, respectively) and separates the *H. e. erato* and hybrid samples. The second principal component (PC2) axis highlights more complex shape differences in the forewing band among the samples as demonstrated by the shape changes of the colour patterns along the principal component axis

Cluster centres are recalculated each iteration by averaging all pixels in the cluster until convergence. We implemented *k*-means clustering using the `R` package `stats` (R Development Core Team, 2013). Clusters are first obtained from a reference image and then used as initial cluster centres for the *k*-means clustering of the subsequently analysed images. This allows the program to match clusters that represent the same colour pattern in different images. For *k*-means clustering, the number of clusters must be defined manually. For organisms with less distinct pattern boundaries, this is best done by testing different numbers of clusters and choosing a number that best assigns pixels to colour patterns.

### 3.3 | Watershed transformation

The watershed transformation is a powerful tool for image segmentation (Figure 6; Beucher, 1991). The concept of watershed treats the image as a topographic map by calculating a gradient map with high values in parts of the image where pixel values change abruptly (Figure 6b). Subsequently, a flooding process propagates pattern and background labels guided by the gradient map. Continuing the flooding until pattern and background labels meet, determines the watershed lines (ridges in the topography) that are used to segment the image (Figure 6c). We implemented the watershed algorithm with utilities from the `R` package `imager` (Barthelme, 2017) that is based on the image processing library CImg (Tschumperle, 2004). In our implementation, the pattern and background labels are chosen by manually identifying at least one pattern and one background pixel (at least one for each separate pattern and background element). This manual assignment helps the user to overcome potential differences in image lightning, glare or overlap between pattern and background RGB values.

## 4 | OUTPUT

The main `patternize` functions generate a list of extracted colour patterns from each image stored as a `raster` object (Hijmans, 2016). These extracted patterns can be summed and visualized as heatmaps or used to calculate the relative area of the colour patterns. To better characterize variation in colour patterns among samples, we implemented linear principal component analysis (PCA).

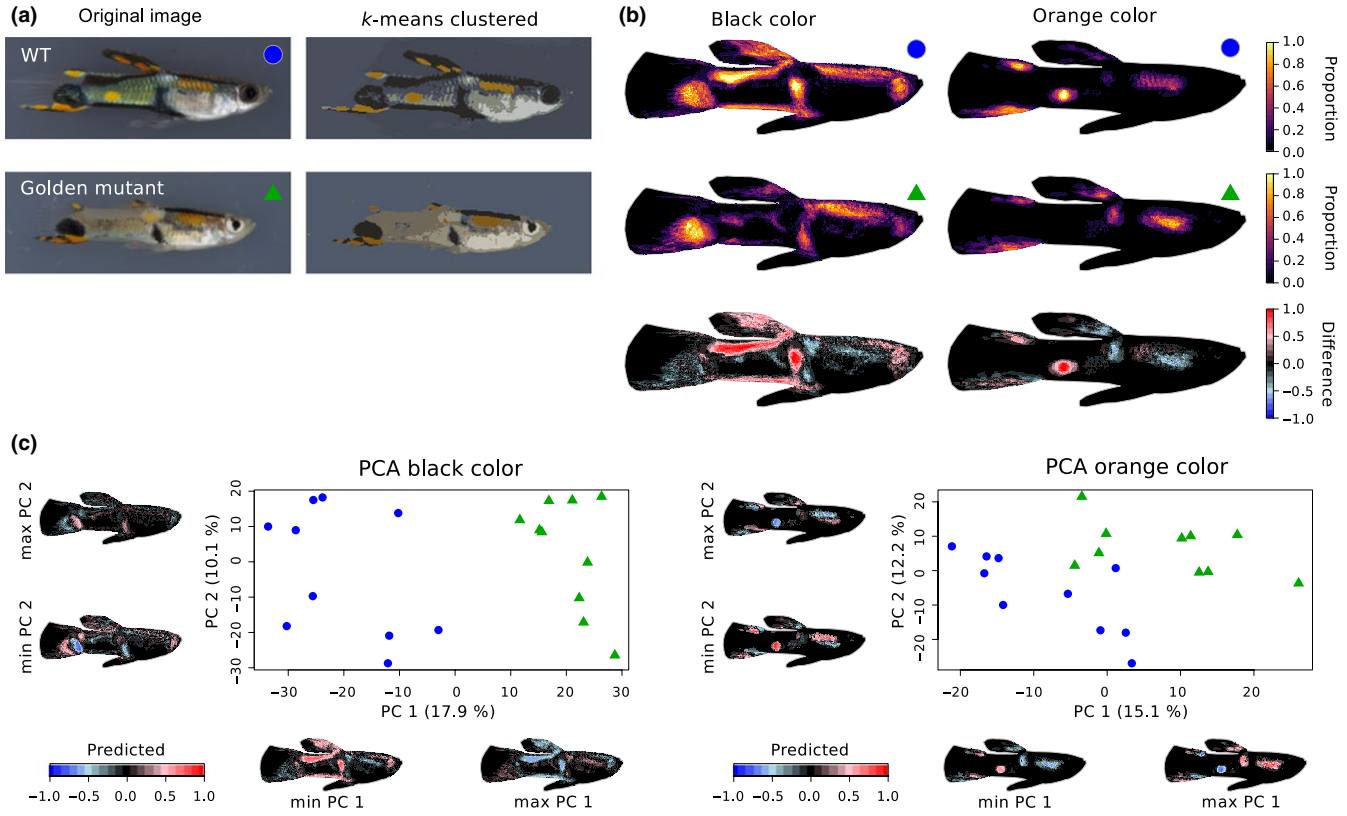


**FIGURE 4** Example of image registration and *k*-means clustering of colours in guppies (*Poecilia reticulata*). (a) Original image of a wild type (WT) and *golden* mutant guppy and their *k*-means clustered representation (clusters = 7). (b) Heatmaps and difference between WT ($n$ = 10) and golden mutant ($n$ = 10) for black and orange colour clusters. (c) Principal component analysis of the pixel matrices obtained for the black (left) and orange (right) colour clusters. Images were obtained with permission from Kottler et al. (2013)

For an extracted colour pattern, PCA can be performed on the binary representation of the aligned colour pattern rasters obtained from each sample (Figures 3–5). In this matrix, pixel coordinates that have the colour of interest in a sample have a value of one, whereas pixel coordinates without the colour have the value zero assigned. The variance–covariance matrix obtained from the binary matrix for a colour is suitable for PCA, which allows visualizing the main variations in colour pattern boundaries among or between groups of samples, as well as the predicted colour pattern changes along the principal component (PC) axis (Johnson & Wichern, 2007). In the visualization of the predicted colour pattern changes, positive values present a higher predicted expression of the pattern, whereas negative values present the absence of the pattern. Note that parts of the colour patterns that are expressed in all considered samples have a predicted value of zero, as these pixels do not contribute variance for the PCA analysis.

## 5 | EXAMPLES

### 5.1 | RGB threshold pattern extraction in *Heliconius* butterflies

We demonstrate the utility of image alignment and RGB threshold extraction in the forewing band area of *Heliconius erato* populations (Figure 2). *Heliconius* butterflies from the Neotropics display great diversity in forewing band shape, which is mainly defined by expression of the *wntA* gene (Martin et al., 2012; Van Belleghem et al., 2017). Expression of red pigments in the wing scales is on its turn defined by expression of the *optix* gene (Reed et al., 2011). Comparison of the landmark and image registration approach applied to the red forewing band variation in *Heliconius erato hydara* shows that both methods perform well (Figure 2b). The TPS transformation used in the landmark approach resulted in a better fit to the internal structures of the wing (i.e. wing veins). The slight offset between the colour pattern and vein position in the image registration approach likely resulted from a bias in the linear transformation towards aligning the outline of the wing and not including non-uniform changes in shape within the wing.

Next, we performed automated image registration and RGB threshold colour extraction on the same forewing band area of *H. erato erato*. In this region of the wing, *H. e. erato* lacks *optix* expression and, thus, red scales. However, naturally occurring hybrids between *H. e. erato* and *Heliconius erato hydara* show *optix* expression in the forewing band area (Figure 3). With this example, we demonstrate the ability to compare homologous, but differing coloured pattern elements (i.e. yellow vs. red). The PCA analysis and relative area of the extracted patterns allow to differentiate the two groups of butterflies and indicate overexpression of the colour pattern in hybrids.

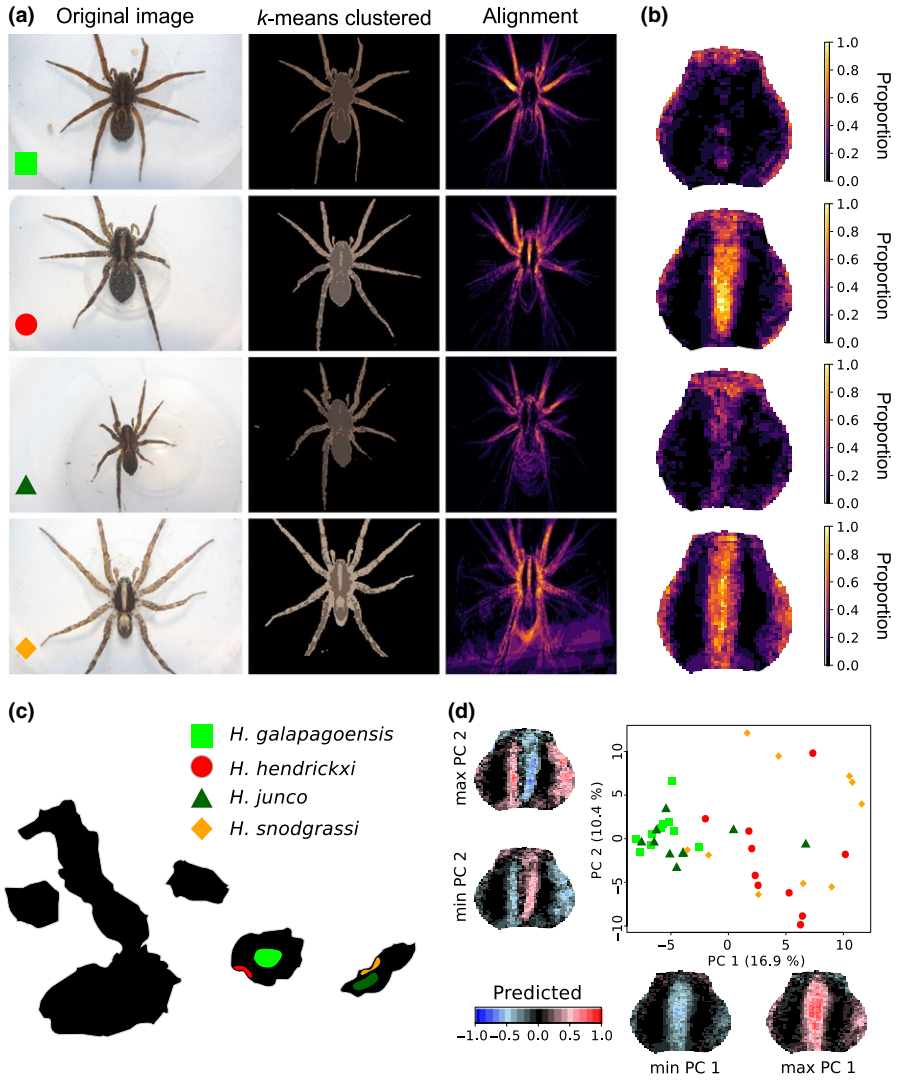


**FIGURE 5** Example of image registration and *k*-means clustering of the colour pattern of Galápagos wolf spiders (*Hogna*). (a) From left to right: example of original image (10 images were used for each species), *k*-means clustered image ($k = 3$) with removed background, and alignment of the lightest colour. (b) Heatmap corresponding to the lightest colour cluster focused on the carapace. (c) Map of the Galápagos islands with colours indicating the distribution of four *Hogna* species, two high elevation species (light and dark green) and two coastal species (red and orange). (d) PCA analysis of the pixel matrices obtained for the lightest colour cluster demonstrates that the coastal (*Hogna hendrickxi* and *Hogna snodgrassi*) and high-elevation (*Hogna galapagoensis* and *Hogna junco*) species cluster phenotypically together and share, respectively, the presence and absence of a pale median band on their carapace. Images were obtained with permission from De Busschere et al. (2012)

## 5.2 | Automated registration and *k*-means clustering in guppies and spiders

To assess the general utility of our application across taxa, we applied the automated registration and *k*-means clustering approach to groups with more complex body shape and colour pattern variation; guppy fish and Galápagos wolf spiders. Males of the guppy (*Poecilia reticulata*) vary greatly in their ornamental patterns that have evolved in response to both natural and sexual selection. Several mutants have been described among male guppies that affect colour pattern expression. Manually quantifying the differences in colour pattern expression among these mutations has provided valuable insights into the developmental basis and interactions of the involved genes (Kottler, Fadeev, Weigel, & Dreyer, 2013). Here, we summarized and compared the black and orange colour patterns expressed in wild type (WT) vs. *golden* mutants of *P. reticulata* males using images obtained from Kottler et al., 2013 (images were used from backcrosses obtained from *golden blue* mutant females with heterozygous males from crossing *golden blue* with inbred wild-derived Cumána populations) (Figure 4). All images were aligned to a target image using image registration and colours were *k*-means clustered into seven groups. Before *k*-means colour clustering, the background was masked using the outline of the guppy in the target image. Our analysis of the black and orange colour cluster strongly matched the description presented in Kottler et al. (2013), demonstrating the absence of a posterior orange spot in *golden* mutants backcrossed into a Cumána population genetic background and more diffuse and shifted black ornaments in the *golden* mutants.

Wolf spiders of the genus *Hogna* inhabit high elevation and coastal habitats on the Galápagos islands Santa Cruz and San Cristobal (De Busschere et al., 2010). Despite the phylogenetically close relationship of the high elevation and coastal populations within both islands, morphometric analysis, including measurements of colour intensity, have highlighted striking parallel phenotypic divergence between the high elevation and coastal species between the islands (De Busschere et al., 2012). Coastal species appear to be paler with a more conspicuous median band on the carapace compared to high elevation species. Here, we demonstrate the robustness of automated image registration by aligning the highly variable images of the wolf spiders (Figure 5).

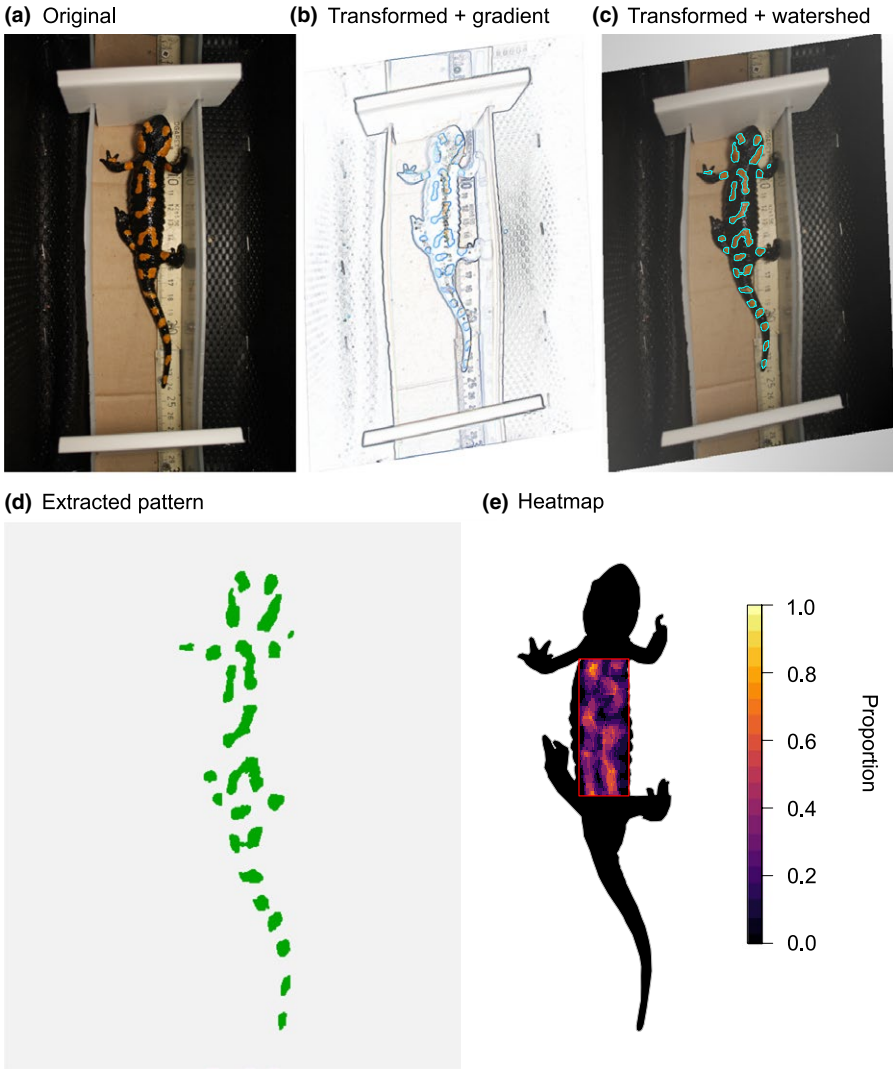


**FIGURE 6** Example of watershed transformation for colour pattern extraction in fire salamanders (*Salamandra salamandra*). (a) Original image. (b) Image gradient transformed to a reference shape using landmarks. (c) Transformed image with watershed lines highlighted. (d) Extracted patterns using the watershed lines. (e) Heatmap of orange patterns extracted from ten male fire salamanders. Areas outside the red box were masked. Images were obtained with permission from Balogová and Uhrin (2015)

By focusing on correspondence between the images, the automated image registration technique manages to align the spider's carapace, which is morphologically the most consistent part in the images. By assigning colours in the spiders to only two clusters, we show a similar pattern as described in De Busschere et al. (2012) in which the coastal species show a consistently broader and more conspicuous median band on the carapace and pale lateral bands compared to the high elevation species.

### 5.3 | Watershed pattern extraction in fire salamanders

The glare that is usually present in images of amphibians can make it challenging to correctly extract the colour patterns. Additionally, some pattern elements may be difficult to identify based on colour alone. To overcome these difficulties, we illustrate the watershed segmentation using images of fire salamanders obtained from Balogová and Uhrin (2015) (Figure 6). The fire salamander (*Salamandra salamandra*) is common to Europe and is black with yellow, orange or red spots or stripes. The watershed approach confidently identifies the orange pattern boundaries in the analysed images. Combining this colour pattern extraction approach with aligning the images allows users to identify regions in the salamander's body where spots or stripes are more consistently expressed.

## 6 | CONCLUDING REMARKS AND RECOMMENDATIONS

### 6.1 | Alignment

`patternize` provides an unbiased, fast and user-friendly approach for colour pattern analysis that is applicable to a wide variety of organisms. `patternize` takes jpeg images as input, which can be downsampled to decrease computation times. While the landmark based approach is computationally slightly faster, automated image registration removes the need for labour-intensive landmark setting. Moreover, image registration reduces any variation introduced by differences in how users manually place image landmarks. However, because automated registration uses intensity patterns in the images, it

can be highly sensitive to artefacts in the background and care should be taken by standardizing the experimental setup. For cases in which the background differs starkly from the studied object, functionality is included that allows users to remove the background by providing RGB cutoff values. The package also allows users to review the image registration progress to assess the quality of the automatic registration.

## 6.2 | Colour pattern extraction

Variation in photographic conditions complicates colour pattern extraction. The option for iteratively recalculating the RGB value and defining the start clusters for *k*-means clustering from a reference image can improve colour pattern extraction under these conditions. However, setting correct RGB or cluster parameters may impact results and should be optimized for each analysis. Appropriate RGB and offset values can be obtained, for instance, by extracting RGB values from image pixels or areas of interest (e.g. use `sampleRGB()`). Using few or many *k*-means clusters may, respectively, result in grouping colours of interest or assigning multiple clusters to a single pattern of interest. Finally, in contrast to RGB threshold colour extraction and *k*-means clustering, watershed transformation takes into account the spatial proximity of pixels. Doing so, the interactive identification of pattern vs. background in the watershed transformation provides a way to extract colour patterns that is robust to variation in photographic conditions.

## 6.3 | Output

The output of the main `patternize` functions are `raster` objects (Hijmans, 2016) that provide for a wide range of downstream analyses. As demonstrated by the examples, we provide functions to intersect (mask) the extracted patterns with defined outlines, sum or subtract the patterns to plot heatmaps, calculate the relative area in which the pattern is expressed and carry out principal component analysis (PCA). Overall, we hope this `R` package provides a useful tool for the community of researchers working on colour and pattern variation in animals.

## ACKNOWLEDGEMENTS

We kindly thank Jon Clayden for help with implementing RNiftyReg, Stefan Schlager for help with Morpho, Maria Bencomo, Emily Shelby and Heather Smith for help with digitizing the *Heliconius* images and Verena Kottler, Charlotte De Busschere and Monika Balogová for allowing us to use the guppy, wolf spider and fire salamander images, respectively. S.M.V.B. and B.A.C. were funded by NSF grant DEB-1257839. H.O.Z. was supported in part by NIH grant 5P20GM103475-13. All authors declared that they have no conflict of interest.

## AUTHORS' CONTRIBUTIONS

S.M.V.B. and B.A.C. conceived the development of the package. S.M.V.B. wrote the code. S.M.V.B., R.P. and B.A.C. wrote the manuscript. H.O.Z. helped improving the code. S.M.V.B., B.A.C., F.H. and R.P. conceived data acquisition. H.O.Z., F.H., C.D.J. and W.O.M. contributed helpful comments for building the package and writing the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

## DATA ACCESSIBILITY

The package and descriptions of the functions and parameters are available as library ("patternize") on CRAN (cran.r-project.org/web/packages/patternize). The code, ongoing developments and data and code used for the examples can be accessed through GitHub (github.com/StevenVB12/patternize; github.com/StevenVB12/patternize-examples). Bug reports and feature requests can be sent using the GitHub issue tracker.

## REFERENCES

Allen, W. L., Baddeley, R., Scott-samuel, N. E., & Cuthill, I. C. (2013). The evolution and function of pattern diversity in snakes. *Behavioral Ecology*, *24*, 1237–1250.

Allen, W. L., Higham, J. P., & Allen, W. L. (2015). Assessing the potential information content of multicomponent visual signals: A machine learning approach. *Proceedings of the Royal Society B*, *282*, 20142284.

Balogová, M., & Uhrin, M. (2015). Sex-biased dorsal spotted patterns in the fire salamander (*Salamandra salamandra*). *Salamandra*, *51*, 12–18.

Barthelme, S. (2017). imager: Image processing library based on 'CImg'. R package version 0.40.2. Retrieved from https://CRAN.R-project.org/package=imager

Beucher, S. (1991). The watershed transformation applied to image segmentation. *Proceeding of 10th Pfefferkorn Conference on Signal and Image Processing in Microscopy and Microanalysis*, pp. 299–314.

Calsbeek, R., Bonneaud, C., & Smith, T. B. (2008). Differential fitness effects of immunocompetence and neighbourhood density in alternative female lizard morphs. *Journal of Animal Ecology*, *77*, 103–109.

Clayden, J., Modat, M., Presles, B., Anthopoulos, T., & Daga, P. (2017). RNiftyReg: Image Registration Using the 'NiftyReg' Library. R package version 2.5.0. Retrieved from https://CRAN.R-project.org/package=RNiftyReg

Clegg, M. T., & Durbin, M. L. (2000). Flower color variation: A model for the experimental study of evolution. *Proceedings of the National Academy of Sciences*, *97*, 7016–7023.

Cotoras, D. D., Brewer, M. S., Croucher, P. J. P., Geoff, S., Lindberg, D. R., & Gillespie, R. G. (2016). Convergent evolution in the colour polymorphism of *Selkirkiella* spiders (Theridiidae) from the South American temperate rainforest. *Biological Journal of the Linnean Society*, *120*, 649–663.

De Busschere, C., Baert, L., Van Belleghem, S. M., Dekoninck, W., & Hendrickx, F. (2012). Parallel phenotypic evolution in a wolf spider radiation on Galápagos. *Biological Journal of the Linnean Society*, *106*, 123–136.

De Busschere, C., Hendrickx, F., Van Belleghem, S. M., Backeljau, T., Lens, L., & Baert, L. (2010). Parallel habitat specialization within the wolf spider genus *Hogna* from the Galápagos. *Molecular Ecology*, *19*, 4029–4045.

Duchon, J. (1976). Splines minimizing rotation invariant semi-norms in Sobolev spaces. In W. Schempp & K. Zeller (Eds.), *Volume 571 of the series Lecture Notes in Mathematicss* (pp. 85–100). Berlin: Springer.

Endler, J. A. (1983). Natural and sexual selection on color patterns in poeciliid fishes. *Environmental Biology of Fishes*, *9*, 173–190.

Forsman, A., Ringblom, K., Civantos, E., & Ahnesjö, J. (2002). Coevolution of color pattern and thermoregulatory behavior in polymorphic pygmy grasshoppers *Tetrix undulata*. *Evolution*, *56*, 349–360.

Goodall, C. (1991). Procrustes methods in the statistical analysis of shape. *Journal of the Royal Statistical Society Series B*, *53*, 285–339.

Goshtasby, A. (2005). *2-D and 3-D image registration: For medical, remote sensing, and industrial applications*. Hoboken, NJ: Wiley.

Hartigan, J. A., & Wong, M. A. (1979). Algorithm AS 136: A k-means clustering algorithm. *Journal of the Royal Statistical Society Series B*, *28*, 100–108.

Hazewinkel, M. (Ed.) (2001). *Affine transformation. Encyclopedia of mathematics*. Netherlands: Springer.

Hijmans, R. J. (2016). raster: Geographic data analysis and modeling. R package version 2.5-8. Retrieved from http://cran.r-project.org/package=raster

Hoekstra, H. E., Hirschmann, R. J., Bundey, R. A., Insel, P. A., & Crossland, J. P. (2006). A single amino acid mutation contributes to adaptive beach mouse color pattern. *Science*, *313*, 101–104.

Houde, A. E. (1987). Mate choice based upon naturally occurring color-pattern variation in a guppy population. *Evolution*, *41*, 1–10.

Johnson, R. A., & Wichern, D. W. (2007). *Applied multivariate statistical analysis*, 6th ed. Upper Saddle River, NJ: Pearson.

Katakura, H., Saitoh, S., Nakamura, K., & Abbas, I. (1994). Multivariate analyses of elytral spot patterns in the phytophagous ladybird beetle *Epilachna vigintioctopunctata* (Coleoptera, Coccinellidae) in the province of Sumatra Barat, Indonesia. *Zoological Science*, *11*, 889–894.

Klingenberg, C. P. (2010). Evolution and development of shape: Integrating quantitative approaches. *Nature*, *11*, 623–635.

Kottler, V. A., Fadeev, A., Weigel, D., & Dreyer, C. (2013). Pigment pattern formation in the guppy, *Poecilia reticulata*, involves the Kita and Csf1ra receptor tyrosine kinases. *Genetics*, *194*, 631–646.

Kronforst, M. R., Young, L. G., Kapan, D. D., McNeely, C., O'Neill, R. J., & Gilbert, L. E. (2006). Linkage of butterfly mate preference and wing color preference cue at the genomic location of *wingless*. *Proceedings of the National Academy of Sciences of the USA*, *103*, 6575–6580.

Le Poul, Y., Whibley, A., Chouteau, M., Prunier, F., Llaurens, V., & Joron, M. (2014). Evolution of dominance mechanisms at a butterfly mimicry supergene. *Nature Communications*, *5*, 1–8.

Martin, A., Papa, R., Nadeau, N. J., Hill, R. I., Counterman, B. A., Halder, G., … Reed, R. D. (2012). Diversification of complex butter flywing patterns by repeated regulatory evolution of a Wnt ligand. *Proceedings of the National Academy of Sciences of the USA*, *109*, 12632–12637.

Mascó, M., Noy-Meir, I., & Sérsic, A. N. (2004). Geographic variation in flower color patterns within *Calceolaria uniflora* Lam. in Southern Patagonia. *Plant Systematics and Evolution*, *244*, 77–91.

Modat, M., McClelland, J., & Ourselin, S. (2010). Lung registration using the NiftyReg package. *Medical Image Analysis for the Clinic: A Grand Challenge, Workshop Proc. from MICCAI 2010*, 33–42.

Modat, M., Ridgway, G. R., Taylor, Z. A., Lehmann, M., Barnes, J., Hawkes, D. J., … Ourselin, S. (2010). Fast free-form deformation using graphics processing units. *Computer Methods and Programs in Biomedicine*, *98*, 278–284.

Nekaris, K. A. I., & Jaffe, S. (2007). Unexpected diversity of slow lorises (*Nycticebus* spp.) within the Javan pet trade: Implications for slow loris taxonomy. *Contributions to Zoology*, *76*, 187–196.

Nosil, P., & Crespi, B. J. (2006). Experimental evidence that predation promotes divergence in adaptive radiation. *Proceedings of the National Academy of Sciences*, *103*, 9090–9095.

R Development Core Team. (2013). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Rabbani, M., Zacharczenko, B., Green, D. M., Abbani, M. O. R., & Acharczenko, B. R. Z. (2015). Color pattern variation in a cryptic amphibian, *Anaxyrus fowleri*. *Journal of Herpetology*, *49*, 649–654.

Reed, R. D., Papa, R., Martin, A., Hinas, H. M., Counterman, B. A., Pard-Diaz, C., … McMillan, W. O. (2011). *optix* drives the repeated convergent evolution of butterfly wing pattern mimicry. *Science*, *333*, 1137–1141.

Rojas, B., Valkonen, J., & Nokelainen, O. (2015). Aposematism. Quick guide. *Current Biology*, *25*, R350–R351.

Schindelin, J., Arganda-carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., … Cardona, A. (2012). Fiji: An open-source platform for biological-image analysis. *Nature Methods*, *9*, 676–682.

Schindelin, J., Rueden, C. T., Hiner, M. C., & Eliceiri, K. W. (2015). The ImageJ ecosystem: An open platform for biomedical image analysis. *Molecular Reproduction & Development*, *82*, 518–529.

Schlager, S. (2016). Morpho: Calculations and visualisations related to geometric morphometrics. R package version 2.3.1.1. Retrieved from http://cran.r-project.org/package=Morpho

Translational Imaging Group (TIG). (2016). NiftyReg. Retrieved from https://sourceforge.net/projects/niftyreg/

Tschumperle, D. (2004). The CImg library. Retrieved from http://cimg.sourceforge.net. The C++ Template Image Processing Library.

Van Belleghem, S. M., Rastas, P., Papanicolaou, A., Martin, S. H., Arias, C. F., Supple, M. A., … Papa, R. (2017). Complex modular architecture around a simple toolkit of wing pattern genes. *Nature Ecology & Evolution*, *1*, pii: 0052.

Williams, P. (2007). The distribution of bumblebee colour patterns worldwide: Possible significance for thermoregulation, crypsis, and warning mimicry. *Biological Journal of the Linnean Society*, *92*, 97–118.

**How to cite this article:** Van Belleghem SM, Papa R, Ortiz-Zuazaga H, et al. `patternize`: An `R` package for quantifying colour pattern variation. *Methods Ecol Evol*. 2017;00:1–9. https://doi.org/10.1111/2041-210X.12853