Guide to image editing and production of figures for scientific publications with an emphasis on taxonomy

Image editing for scientific publications

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> *"One picture is worth ten thousand words."*
> Confucius (Chiu Kung, 552–479 B.C.)

Abstract

Figures for scientific publications go through various stages from the planning, to the capturing of images, to the production of finished figures for publication. This guide is meant to familiarise the reader with the main image-editing software used by professional photographers. The guide's focus is on digital photo editing and the production of figures using Adobe Photoshop to produce publication-quality figures for scientific publications. This guide will be of fundamental use for the academic public, especially taxonomists and others who work with images. Besides, it should be useful for anyone interested in becoming familiar with the basic tools of image editing.

Key Words

Introduction

Within the world of scientific writing, the iconic phrase of Confucius is particularly true. A good researcher knows that the power of synthesis makes all the difference in scientific works, since, with the use of a single figure, it is possible to condense information contained in several paragraphs (Mello 2012; Mudrak). Often core ideas that we want to convey in a paper can be expressed better as figures than as text or tables. Journals usually value conciseness and the efficient use of space in the article by the authors, which can be achieved by synthesising data and even ideas through the use of graphic resources (e.g. figures) to reduce the space used without losing the quality of the information to be transmitted. Digital photography is a powerful tool that can enhance our ability to commu-nicate with our peers and the public (Ratner et al. 1999). Furthermore, figures are usually one of the first things that a reader sees when deciding to read a paper (Mudrak).

In academia, we incorporate images and figures in most scientific output formats such as lectures and work-shops, handouts, papers, books, websites and other free or commercially-distributed digital teaching resources (Lewis 2016); therefore, it is essential to know how to create good figures for diverse scientific purposes (Mello 2012). Papers are the main means of communication and dissemination of research (Fry et al. 2009) and most international journals have specific, strict requirements for figures, which require technical knowledge needed for publication-ready figures.

Especially in taxonomic works, the figures contained in publications are also part of the results. They must also

be of good quality, clear and self-explanatory and they must comply with the requirements of the journal.

From the use of figures in a taxonomic work, it is possible to illustrate and catalogue taxa, detail their morphological characteristics and, based on these figures, to create a database combining the graphic information with character descriptions, creating value for researchers in various fields of science, such as anatomy, cytology, ecology, morphology, taxonomy and systematics.

Figures are arrangements of photographs or illustrations for publication, consisting either of one or several components (composite figures). Photographs, which are often the method of choice for illustrating organisms and their structures, are often indispensable. Photographs show the real appearance of organisms, such as the colouring of a bird or insect, the texture of a reptile's skin or the grooves and sutures on the integument of an arthropod. Photographs arranged into figures serve to emphasise structures, such as the venation of the wings of a dipteran or the shape of the chelicerae of an arachnid or gather a set of similar species or characters for comparison. Illustrations (drawings) are also quite important (not just) for taxonomy; however, the increasing sophistication and speed of scientific photography is leading to a replacement of drawings by photographs even in classical strongholds of the former, such as depictions of aedeagi in entomology (see, for example, Riedel et al. 2013). Figures are a practical method of presenting photographs within publications; they bring together several images of related taxa or structures (Mello 2012). All figures must be of high quality and well presented and the organism must be recognisable (Mello 2012). According to Holzenthal (2008), the characteristics of morphological structures are often so complex that they cannot sufficiently or fully be described in words and figures are therefore necessary in formal taxonomic descriptions and identification keys.

When an image is captured by the photographic equipment, the file is saved by the camera in a "raw" format. Any adjustments of brightness, contrast and white balance must be done subsequently and, frequently, researchers are not aware of (the need to apply) editing methods or perhaps even lack any interest in this topic. If such steps are omitted on the often slightly darker "raw" image, this makes it difficult to see details and colouring; in addition, the photograph may contain more information than needed (e.g. an entire animal instead of just a particular character), which would better be removed by crop adjustment in order to focus on what we want to show; finally, some photographs can generally have undesirable elements that can distract the reader from an important feature. This type of image needs editing before preparing a figure and submitting the manuscript.

Editing of images for scientific publications is increasingly encouraged and plays a key role in the development of multimedia presentations (Corl et al. 2002) and is generally beneficial when done for appropriate reasons (Richardson et al. 1995). The brightness, contrast, saturation, colour temperature, colour correction, minor tonal enhancement in problem areas and sharpness of images can be adjusted and, in some cases, images can even be "cleaned up" by removing undesirable elements such as dust spots, scratches or shadow or light spots or change the framing in order to highlight the subject and remove excess background or elements that are irrelevant for your purpose (Richardson et al. 1995; Corl et al. 2002; Gross and Thapa 2012). However, under no circumstances should such manipulations alter the scientific content. Photographic misrepresentation constitutes scientific fraud and must be avoided; maintaining the visual impression and the quantitative and qualitative relationships within images is crucial (Perniciaro 1993; Richardson 1995; Corl et al. 2002; Cromey 2010, Blatt and Martin 2013, Mudrak). A "raw" copy of each edited image should thus always be saved and any respective guidelines of the journal(s) to which you intend to submit your manuscripts should be considered, combined with the application of common sense when editing, thus avoiding exaggerated modifications that may affect reality. (Richardson 1995; Corl et al. 2002; Rossner and Yamada 2004; Cromey 2010; Gross and Thapa 2012; Blatt and Martin 2013).

Figures for scientific publications go through various stages from the planning, to the capturing of images, to the production of finished figures for publication. The aim of the present work is to familiarise researchers and students (even if they have no previous knowledge) with Adobe Photoshop, the main image-editing software used by professional photographers, to ensure that readers can easily find and use the tools and settings described and to use this software's digital photo editing capacity to produce publication-quality figures for scientific publications.

Methods

Example images were taken using a Leica M165C stereomicroscope with a DFC295 camera attached, with image capture settings applied from the Leica Application Suite (LAS) version 4.2 software. For a uniform and more efficient illumination, a geodesic light dome was used according to Kawada and Buffington (2016). Multiple images taken through the depth of field were stacked using Helicon Focus version 5.3, which generates a single image of combined focus. The images were edited using Adobe Photoshop version CC 2018 on a Dell Inspiron 15 7000 model 7572 notebook, equipped with NVIDIA GeForce MX150 dedicated graphics with 8 GB DDR5 RAM and Windows 10 Home x64.

The name of each tool, its function and shortcuts were used according to the Photoshop User Guide (Photoshop 2018). Keyboard shortcuts are shown in parentheses "( )" and show both the differences between the Windows and MacOS operating systems.

Results

The use of Photoshop in this work is divided into five steps:

1. Image editing: here we will edit photographs by changing brightness, contrast or colour.
2. "Cleaning" the photo: this step consists of removing any non-essential elements of the image that somehow harm or leave the image aesthetically ugly.
3. Removing the background: this step can be considered optional, since not all images should have their background removed.
4. Preparation of the final photograph: in this step, we will get the photo as it should be in the figure, then we will put the scale and some important information that the image should contain.
5. Producing a multi-image figure: in this step, the edited photos will be combined into a single composite figure (traditionally called a plate in taxonomic publications) that will be used in a scientific publication, containing all the necessary elements.

After starting Photoshop, the input screen appears (Fig. 1). There you will have an automatic toolbar on the left side and on the right side that you can select from the "Window" tab in the upper bar.

To open images in Photoshop go to "File > Open" or (Ctrl/Cmd + O). It will open a browser window and then select which photo you want to edit.

Quick View of default Photoshop workspace

The Document window displays the file you are working on. Document windows can be tabbed and, in certain cases, grouped and docked. The Control Panel displays the options for the currently selected tool. The Tools panel contains tools for creating and editing images, artwork and page elements. The related tools are grouped together (Fig. 2). Panels help you monitor and modify the work. Examples include "History", "Brush", "Layers", "Character" and "Paragraph", but other panels can also be added. Panels can be grouped, stacked or docked.
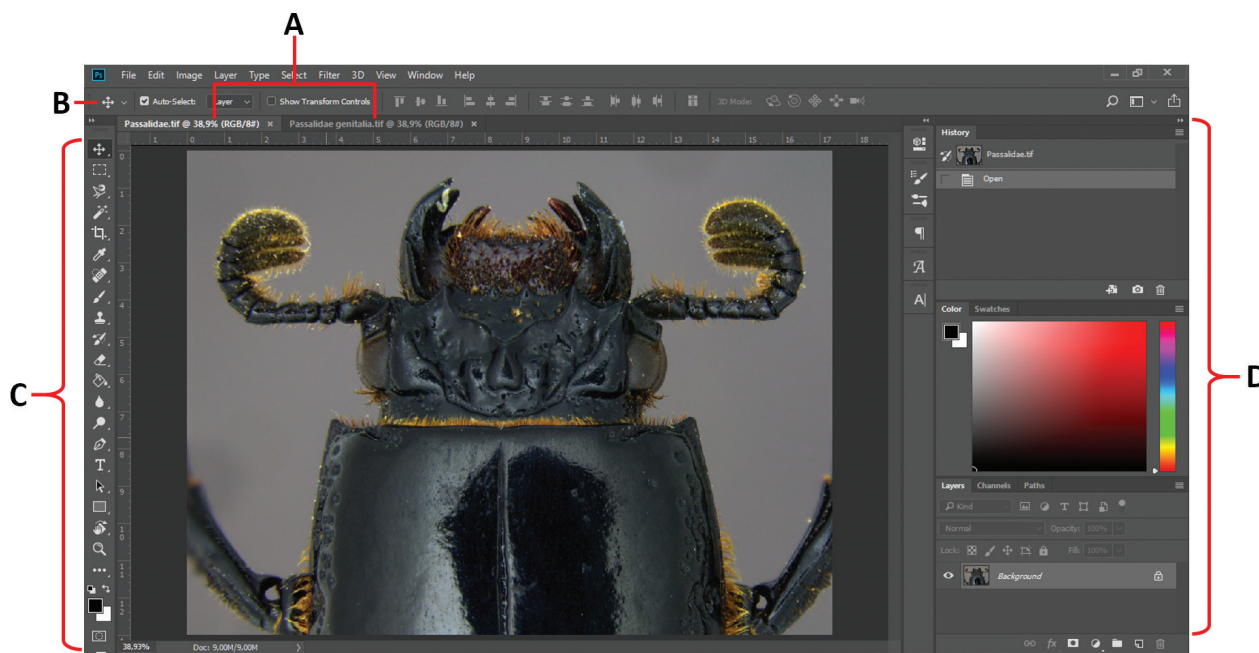

Figure 1. Default Photoshop workspace. **A**. Tabbed document windows; **B**. Control panel; **C**. Tools panel; **D**. Panel groups in vertical dock.
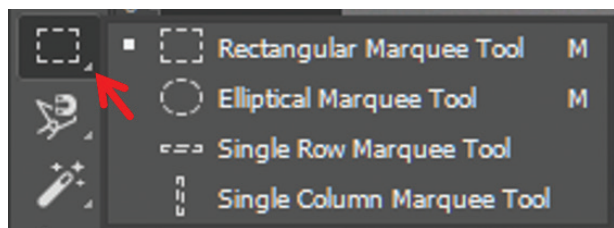

Figure 2. Closed selection tool group expanded to show all hidden tools. Hidden tools can be shown by right-clicking the indicated arrow.

Tools

When starting Photoshop, the *Tools panel* appears on the left side of the screen. You can expand some tools to show the hidden tools. A small triangle at the bottom right of the Tools icon indicates the presence of hidden tools. View information about any tool by positioning the pointer over it. The tool name appears in a tooltip below the pointer.

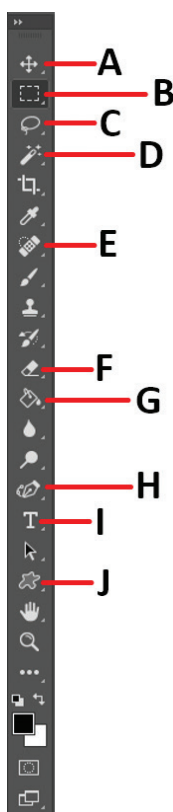This guide will cover the following tools (Fig. 3): A. Move tool, with which you can move objects, selections,

Figure 3. Tools panel. **A**. Move tool; **B**. Marquee tools; **C**. Lasso tool; **D**. Magic Wand; **E**. Spot Healing Brush tool; **F**. Eraser tool; **G**. Paint Bucket tool; **H**. Pen tool; **I**. Type tool; **J**. Shape tool.

layers and guides; B. Marquee tools make rectangular, elliptical, single row and single column selections; C. Lasso tool makes freehand, polygonal (straight-edged) and magnetic (snap-to) selections. D. Magic Wand tool creates selections from similar or contiguous areas; E. Spot Healing Brush tool used for correction of imperfections in photos, uses a sample or pattern to correct imperfections in the image; F. Eraser tool erases pixels and restores parts of an image to a previously saved state. G. Paint Bucket tool fills similarly-coloured areas with the foreground colour. H. The Pen tools allow the drawing of smooth-edged paths. I. The Type tools create type on an image (in it are various formatting types of text). J. Shape tool group draws shapes and lines in a normal layer or shape layer by creating custom shapes selected from a list of custom shapes.

The Options Bar or Control panel

The Photoshop Options bar changes according to each selected tool, making it very dynamic, so it is important to realise what properties each tool has. Here, we will use a lot of tools that behave like brushes, so it is important to point out that brush tools count on the increase of the radial dimension and shape and this can be done through the properties bar (Fig. 4). You can also increase the size of the brush by clicking "]" and to decrease click "[".
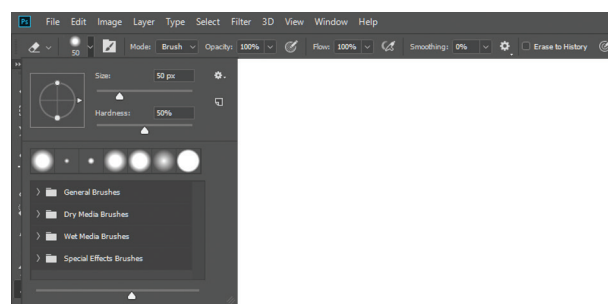
Figure 4. Options bar of a brush-type tool which can change the stroke size and hardness.

Adjustment Panels

This area is fully adjustable; however, here we will focus on the use of four panels (Fig. 5): A. History, passes to any recent image state created during the running session. Each time a change is applied to an image, the new image state is added to the panel. You can also work from that state. You can also use the History panel to exclude image states and create a document from a state or snapshot. To display the History panel, choose Window > History. B. Colour, displays the current foreground and background colour values. To display the Character panel, choose Window > Colour. C. Character, provides options of formations characters (some options of formation also are available in the options bar). To display the Character panel, choose Window > Character. D. Layers, are like sheets of stacked acetate. Through the transparent areas of a layer, you can see the layers below. You move a layer to position the content on the layer, as if you were sliding an acetate sheet on to a stack. You can also change the opacity of a layer to make the content partially transparent. To display the Layers panel, choose Window > Layer.
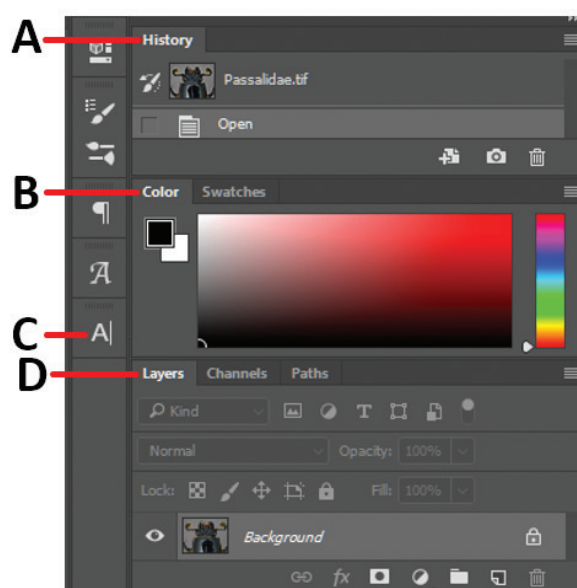

Figure 5. Docked adjustment Panels. **A**. History panel; **B**. Colour panel; **C**. Character panel; **D**. Layers panel.

Photo editing

The native Adobe Camera Raw filter in Photoshop CC is similar to the Develop module in Lightroom (so it can be used in the same way) and lets you adjust the colour and tonal range of your photos, as well as crop photos and make other corrections.

The panels on the left side of Camera Raw allow you to select the photos, select and view them at various stages of editing and apply general presets. The centre of the panel provides a viewing and working area.

To open the Camera Raw filter (Fig. 6), go to "Filter > Camera Raw Filter" or simply by clicking on the Shift + Ctrl/Cmd + A shortcut. Wait a few moments to open the filter window with the selected photo. Now starts the image editing process. By default, Camera Raw Filter displays panels to make global photo adjustments on the right side of the window. You can make adjustments in any order, but a common approach to using these panels is to start at the top and continue to the bottom and alternating the positions (left / right) of each setting.

If necessary, adjust the angle and framing of the photo to leave in the centre what is most important and what should be highlighted. To do so in the "Tool panel" (Fig. 7), click on "Tool transform (Shift + T)" (Fig. 7) which will pop up the tool settings panel. After making the desired settings, click on the "Hand Tool (H)" to exit.

There is no "cake recipe" in this step. Each photo should be treated as unique because the settings that are made on the camera reflect how images should be edited. One suggestion is to perform detailed control using the sliders provided for each setting.

However, there are ways to know when a photo is well edited or if it needs some tweaking. The first point is that scientific photos should portray the reality of the specimens or at least approach it. This issue can be solved mainly in colour-coded commands, since cameras often do not portray the colouring of specimens correctly. It certainly saves time if the white balance is already set on the camera *before* taking the photo(s); please refer to the camera user manual for the necessary steps. The second issue to consider is the good lighting of the photo. This is undoubtedly one of the greatest difficulties since not all have photographic equipment that already have good diffusers or lighting systems. Thinking about this, Kawada and Buffington (2016) have developed a system of lighting that is cheaper than most lighting systems that accompany most of the stereomicroscopes currently sold. However, even after performing a good illumination measurement, some photos can come out with more or less backlit areas. Here comes the main editing tip: always try to compensate more or less illuminated points to leave the photo in its maximum homogeneous state in the matter of illumination. In addition to being homogeneous, the photo should be clear enough so that the structures are visualised in the best possible way, besides presenting a good contrast so as to have a notion of the tridimensionality of the structure such as sutures, grooves, bristles, tubercles or depressions.

With the "Basic" panel (Fig. 8), you can adjust the white balance, tonal scale and colour saturation of a photo, including clarity and vibration. "Temperature" adjusts the white balance of the photo. Move the slider to the left to make the photo appear colder and to the right to warm up the photo colours. "Tint" adjusts the white balance to compensate for a shade of green or magenta. Move the slider to the left (negative values) to add green to the photo; move it to the right (positive values) to add the magenta. "Exposure" sets the brightness of the overall image. Adjust the slider until the picture looks good
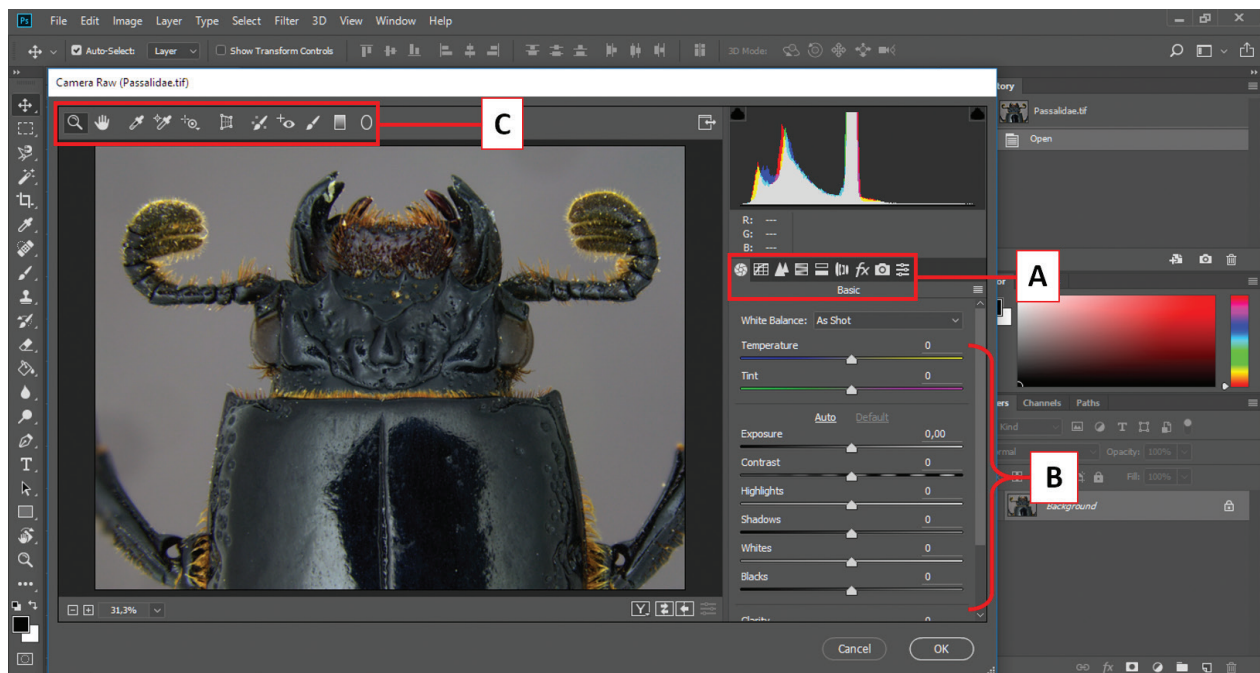

Figure 6. Camera Raw dialogue box. **A**. Image adjustment tabs; **B**. Adjustment sliders; **C**. General Tools panel.
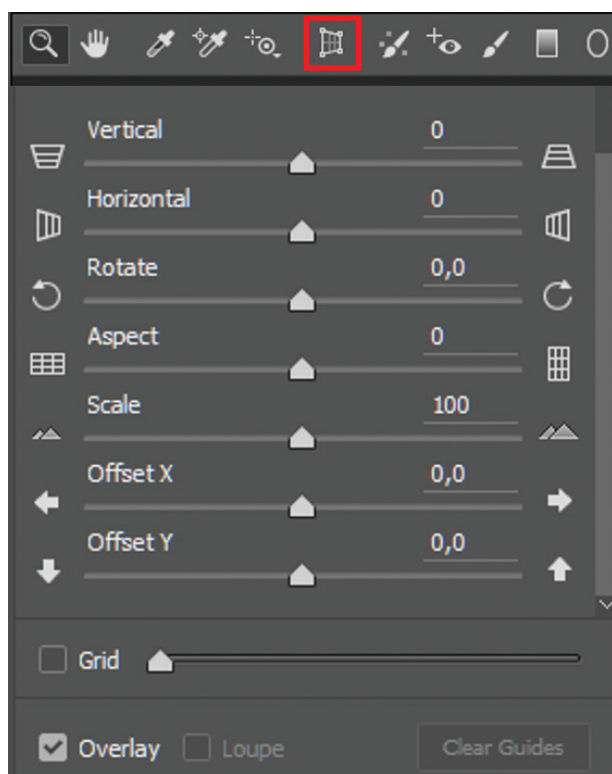
Figure 7. Tool transform panel.

and the picture has the desired brightness (this is the main lighting regulator of your photo). "Contrast" increases or decreases the contrast of the image, affecting mainly the mid-tones. When the contrast is increased, the areas of the half-dark image become darker and the areas of the half-light image become lighter. The tones in the image are affected inversely as you reduce the contrast. "Highlights" adjusts areas of image brightness. Drag to the left to darken highlights and retrieve highlight details "off". Drag to the right to emphasise highlights by minimising cropping. "Shadows" adjusts dark areas of the image. Drag to the left to darken shadows by minimising the clipping. Drag to the right to lighten shadows and retrieve shadow details. Highlights and Shadows are the most commonly-used commands to homogenise the light and dark areas of the photo. Usually, both commands are adjusted in opposite ways, with Highlights with negative measures and Shadows with positive measures. "Whites" adjusts the white cut-out. Drag to the left to reduce the clipping in highlights. Drag to the right to increase the highlights clipping. Increased trimming may be desirable for specular enhancements, such as metal surfaces. "Blacks" adjust black cut-out. Drag to the left to increase the black clipping (turns more shadows to pure black). Drag to the right to reduce the shadow clipping. Moving the slider to the right increases the areas that turn black, sometimes creating the impression of increased image contrast. The greatest effect is in the shadows, with minor changes in mid-tones and highlights. "Clarity" increases the depth of an image by increasing local contrast. To maximise the effect, increase the setting until you see haloes next to the details of the image edge and then slightly reduce the set-
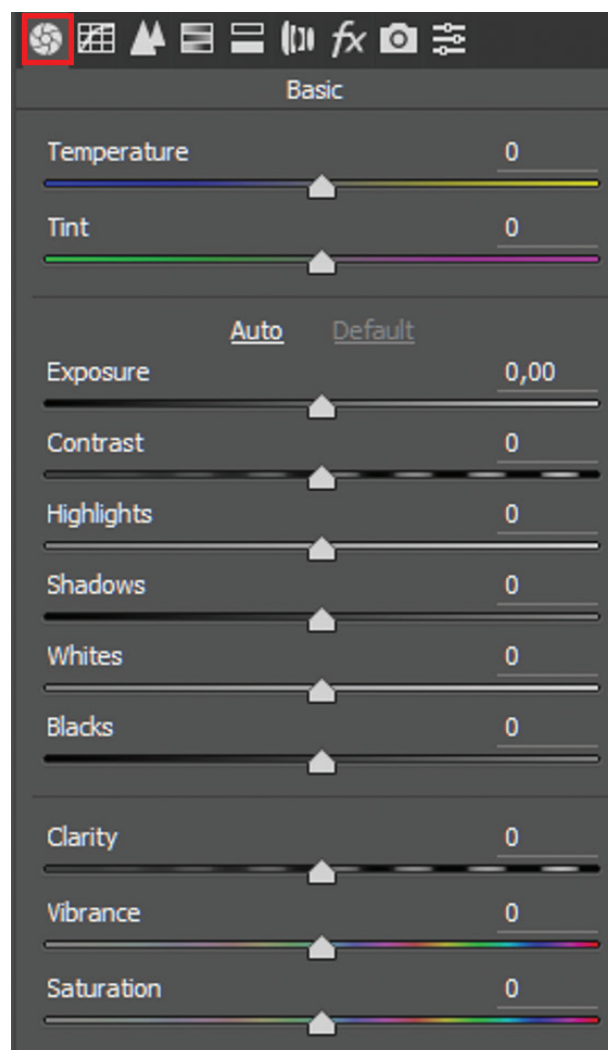
Figure 8. Basic Panel with possible white balance and lighting adjustments.

ting. "Vibrance" adjusts saturation so that the clipping is minimised as colours approach full saturation, changing the saturation of all colours with lower saturation with less effect on colours with more saturation. "Saturation" adjusts the saturation of all image colours uniformly from -100 (monochrome) to +100 (double saturation).

The "HSL / Grayscale" panel (Fig. 9) adjusts the individual colour gamut of the photo. For example, if a red object looks too vivid and distracting, you can adjust it using the Red Saturation slider. Note that all red blots in the photo will be affected. The settings made in the HSL / Grayscale panel produce similar results, so we will only use the "Luminance" setting. Click the header of the HSL / Grayscale panel and then "Luminance". Choose which colour you want to edit and drag the sliders or enter values in the text boxes to the right of the sliders to change the brightness of the colour gamut.

It is very important to be careful in this part of the editing, because modifying image colours very differently from the original can be seen as fraud in many scientific publications and even harm the readers of your publication. For example, if you say that a bird has a yellowish
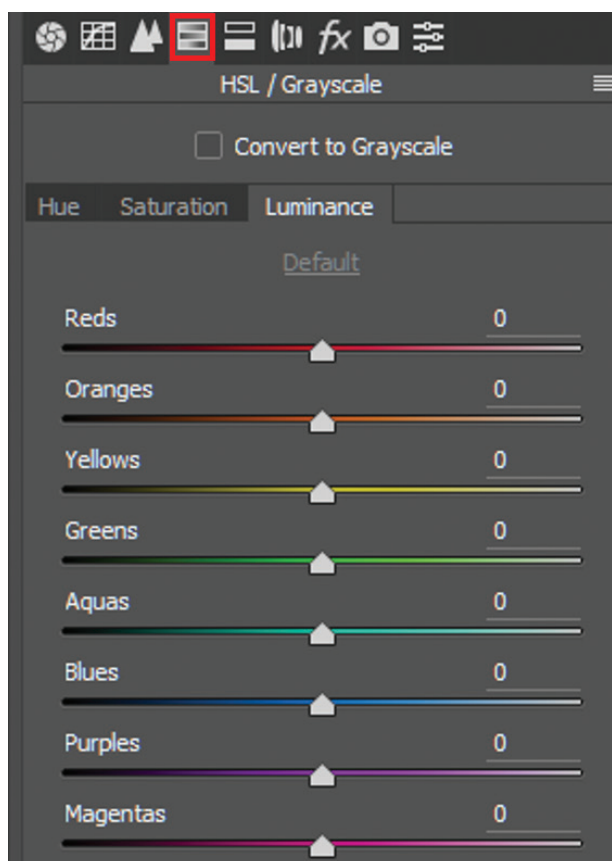
Figure 9. HSL / Grayscale panel with luminance adjustment.

colour, you should leave those tones with little modification, as yellow may turn orange, thereby conflicting with what you state in your publication. Common sense is recommended at this stage.

After editing the image, if you are not satisfied with the result, re-do any of the previous steps no matter what order you follow. However, if the result pleases you, just click "OK" and the image will return to the Photoshop workspace.

Image cleaning

Many scientific photos have spots, smudges, spots of light or shadow that, although they do not impair (in some cases) the interpretation of the characters, make the image visually and aesthetically less attractive. It is common in the study of insects that the images contain pieces of other organisms, dust, dirt particles, amongst other elements, that can be considered unwanted. Removal of these elements is often seen as "over-pricing" or "wasting time"; however, in most international journals, image (and overall figure) quality is taken into account and is often one of the reasons reviewers request that a manuscript is reviewed again. However, as outlined above, it is crucial to avoid modifying the original image(s) beyond removing distractions from the relevant object or increasing their aesthetic value. Any measures that change the object in an arbitrary way may constitute image fraud or be regarded as such (Perniciaro 1993; Richardson 1995; Corl et al. 2002; Taylor 2003a; Cromey 2010, Blatt and Martin 2013). While intentional image fraud in taxonomy is rare, it may be done unintentionally by, for example, selectively sharpening or even 'repairing' parts of an image using the tools mentioned below. Useful advice on how to avoid this can be found, for example, at https://www.aje.com/en/arc/avoiding-image-fraud-7-rules-editing-images/. The first rule is particularly important: always keep an unmodified copy, i.e. the 'raw' version of your image coming from the camera.

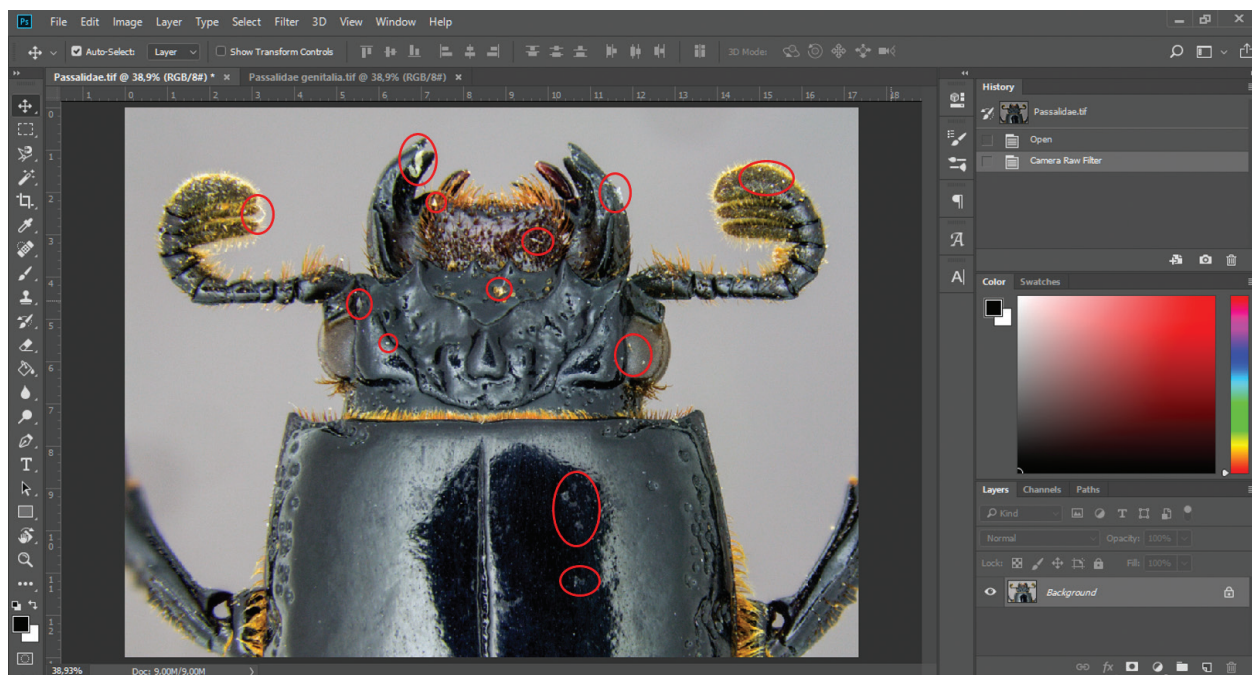In the picture below (Fig. 10), some elements can be considered unwanted, such as dust particles, remnants

Figure 10. Unwanted elements in the image with highlighted dust particles, remnants from the insect collection and shadow spots.

from the insect collection and shadow spots. To remove these elements, you can use the Spot Healing Brush tool (J). It is recommended to use a large zoom so that you perfectly see which areas you need to remove from the image and adjust the brush size to not much larger than the diameter of what you want to clean. To zoom in, use Alt + Scroll up (when using the mouse) or Ctrl/ Cmd + (when using the keyboard); to zoom out, use Alt + Scroll down (when using the mouse) or Ctrl/Cmd – (when using the keyboard). With the brush set, paint each spot that you find undesirable in the photo. Note that when you click or scroll on the screen, the brush that marked the areas will be darker and soon the selected spots will disappear.

Again, it is important to avoid unintentional deletion or modification of structures in depicted specimens. After this step, the image will be clean and aesthetically more acceptable since the reader will be able to view in a simpler way without dirt stains or points of light and unnecessary shadows, which can disrupt the interpretation of the structures in the bidimensional plane (Fig. 11).

Removing image background

After removing all undesirable elements from the image, it's time to improve the aspect of the background. The best way to do this is to eliminate the original background of the image by leaving it transparent or by placing another background, which usually has a different colour. In many cases, just blurring the background is not enough or the researcher would like to add a new colour to the background of the image. For this, you must delete the desired area using three tools: "Magic Wand", "Lasso" and

"Eraser". Note in the image below (Fig. 12) that there are spots that can (and should) be removed, as they are not desirable and will not add anything positive. In addition, the background is not homogeneous besides presenting a part of the elytra of the specimen.

After cleaning the spots, it's time to remove the background of the image by using two similar methods: Magic Wand or Lasso. You may feel at ease to use just one method or both. 1. Select the "Magic Wand" tool (W); 2. Unlock the image layer by clicking on the padlock icon next to the layer name; 3. Click the region you want to delete. Note that one area has been selected (Fig 13). To select more areas, hold the Shift button and click on more areas to be deleted; 4. After selecting the largest possible region to wish to delete, click the "Del" or "Delete" button. This will remove the entire region that was previously selected. If you want to deselect the area and start again, click Ctrl/Cmd + D or click with the right button of the mouse on the selected area and choose "deselect". As the Magic Wand tool selects similar regions, you will hardly have a well-defined selection when working with less homogeneous regions. So do not worry about selecting small or very specific areas as they will be deleted in the next step.

To use the Lasso Tool, do the following: 1. Select the "Lasso" tool (L); 2. Unlock the image layer by clicking on the padlock icon next to the layer name; 3. Contour it in the region to want to keep so that it is selected; 4. Right-click and select "select inverse" (Shift + Ctrl/Cmd + I); 5. After selecting the region (Fig. 14) you want to delete, click the "Del or Delete" button. This will remove the entire region that was previously selected. The remaining areas can be easily erased with the "Eraser" tool. If you do not want to use the Magic Wand or Lasso tools and keep selecting the areas to be deleted, you can go
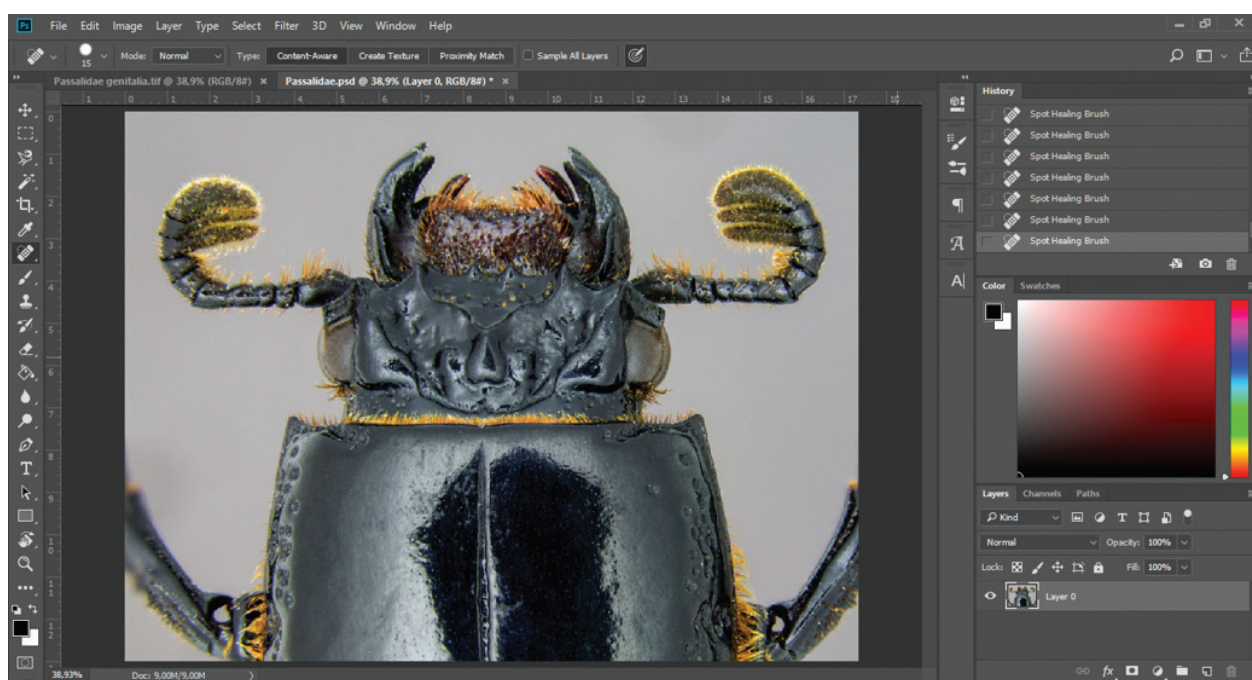
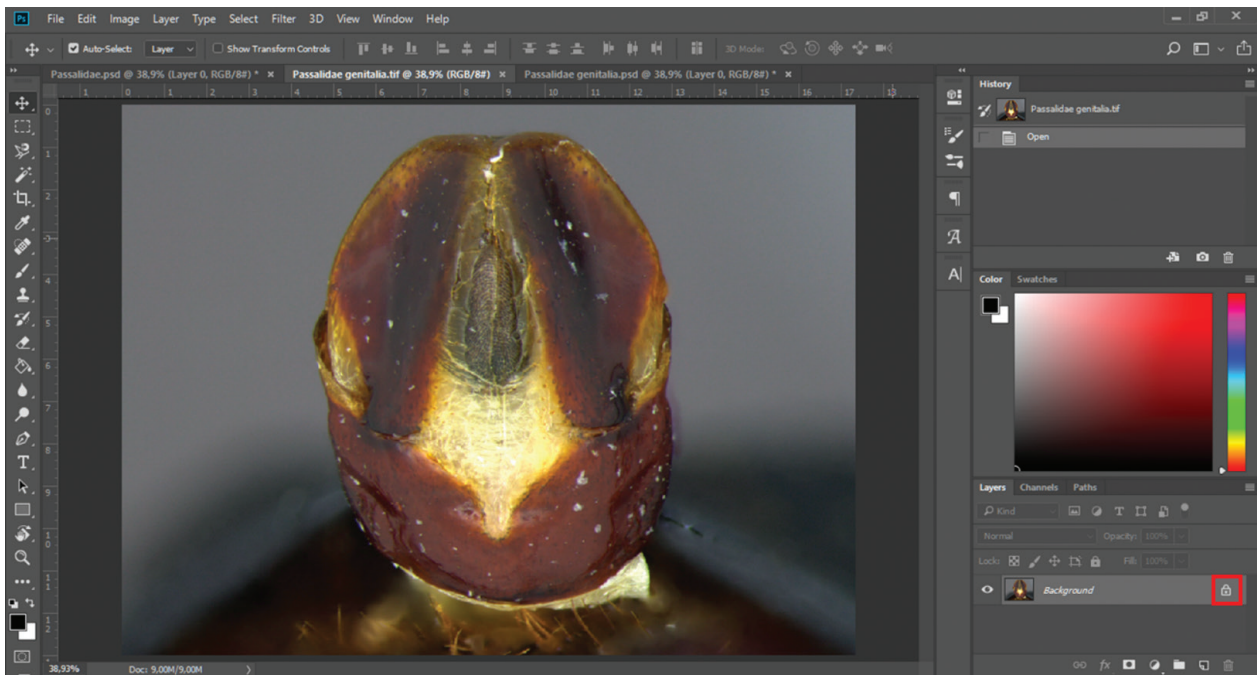

Figure 11. Image after editing and cleaning.

Figure 12. Image containing dust particles where the background is not completely homogeneous. Highlight the padlock icon that can lock or unlock the layer.
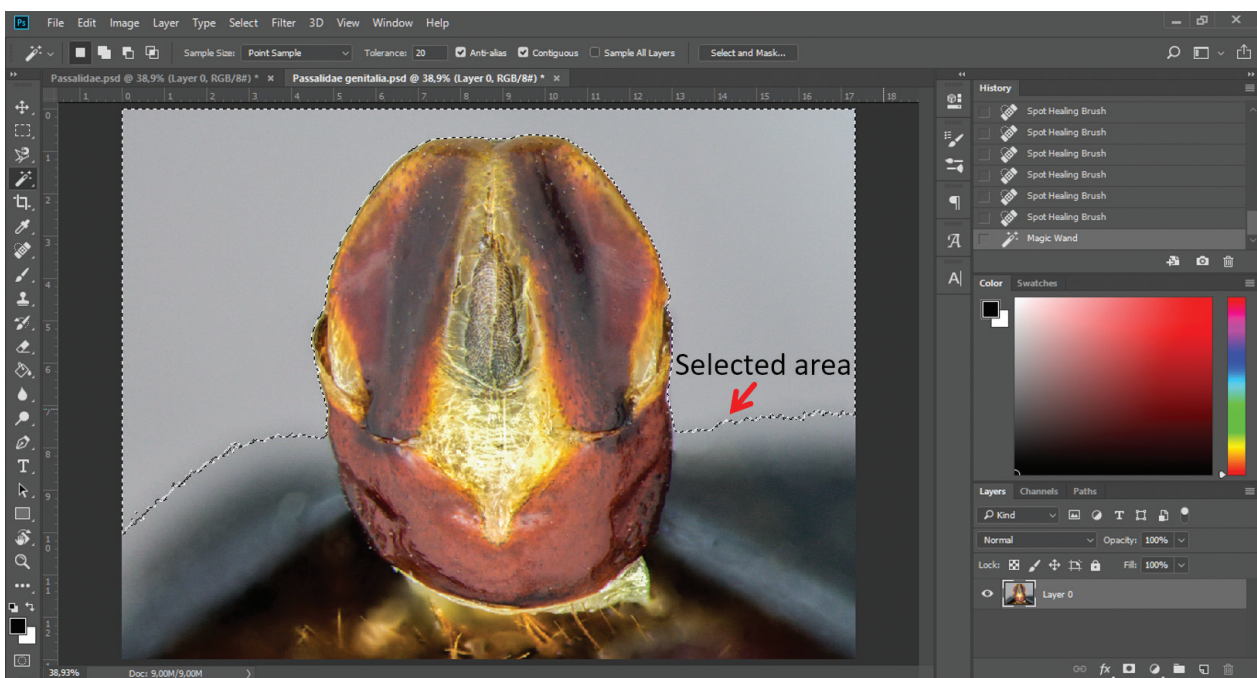

Figure 13. Most homogeneous area that can be selected using the Magic Wand tool.

straight to that next step and erase all areas of the image background with the Eraser tool.

Click the "Eraser tool" (E) and select the first tool in the group, adjust the size of the brush so that it is an ideal size and move throughout the region you want to erase. The most important tip for working with the Eraser tool is the size and hardness of the brush. It is recommended that you use an increased zoom along with a medium hardness by about 30 to 50%. Another use of the Eraser tool is the "Background eraser tool" to delete more homogeneous areas, as it can clear large areas of the background with just one click.

After removing the background of the image (Fig. 15), you can select a new background colour. To do this, follow these steps: 1. Create a new layer by clicking "Create a new layer" button (Fig. 16) in the layers panel or by pressing Ctrl/Cmd + Shift + N.
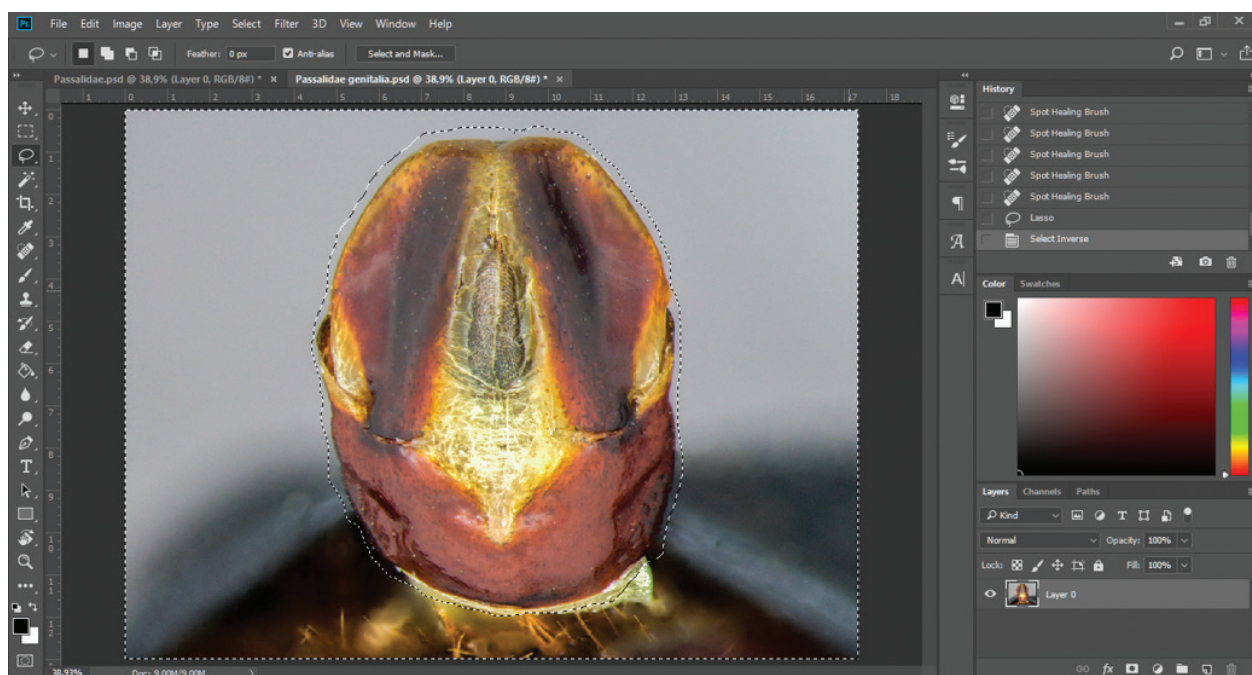
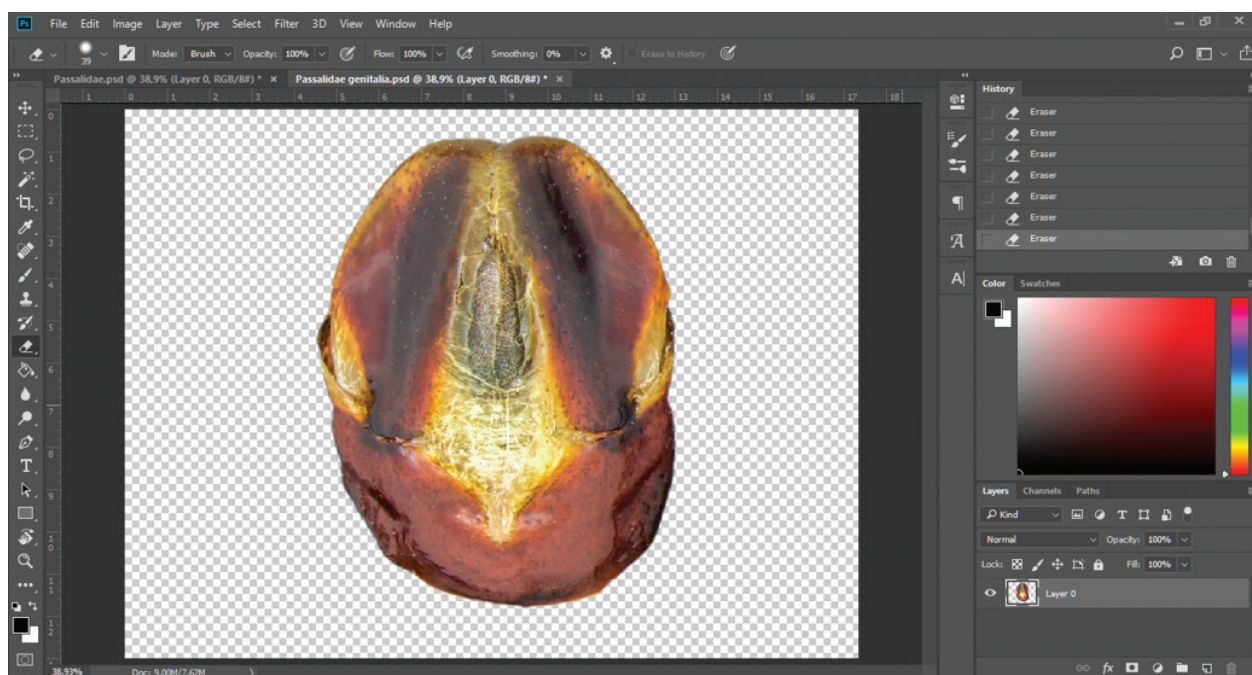**Figure 14.** Selected area with the Lasso tool being inverted to be deleted.

Figure 15. Background completely removed using the Eraser tool.

A very important detail, but what makes a difference when working in Photoshop, is to pay attention to which layer you are working on and which layer should be on top or under. In this case, the new layer with the new background will be below the layer that corresponds to the structure being edited. 2. Select the "Paint Bucket tool" (G) and then choose which colour you want your new image background to have. To do this, click the "Colours" tool and select the desired foreground colour (Fig. 17). 3. After selecting the desired colour, select the

new layer that was created and click on the screen with the Paint Bucket tool (Fig. 18).

Preparation of the final photo

After removing undesirable elements in the image and making the appropriate background, it is time to finalise the image by adding elements that will compose the photo that will be present on the figure. Such elements
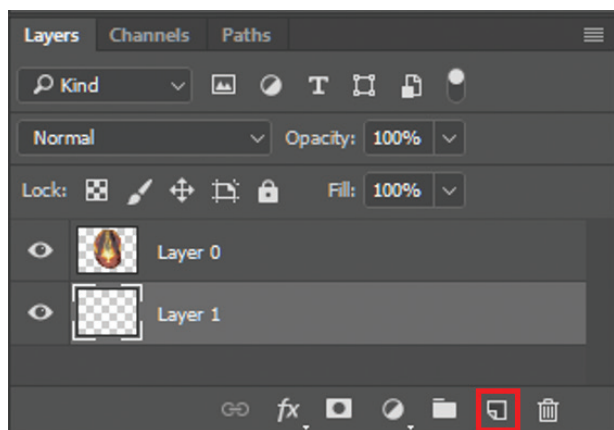
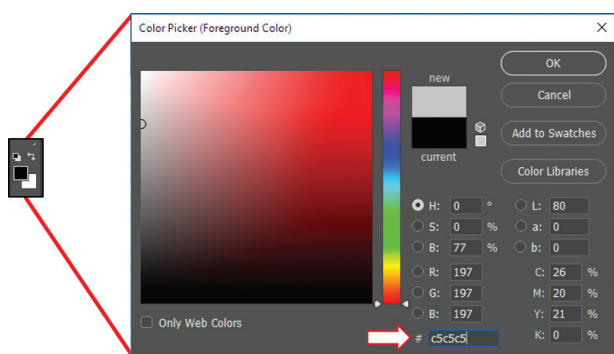**Figure 16.** Layer panel with the "create new layer" icon highlighted.

Figure 17. Colour tool with open colour selection panel. Highlight the dialogue box where you can write the code for each colour you want to get.

are important in taxonomic works because they make the structure more evident, indicate regions of interest or represent dimensions of the anatomical piece, such as the

scale. Let's divide this step into two: pointing structures and applying the scale in the photo.

Pointing structure

In scientific publications, the disclosure of certain structures, regions or details in the photo are indispensable for the researcher to be able to pass on the correct information to the reader and to make the image appreciation and interpretation easier. That is why it is very common to use certain devices that highlight certain regions in the image, such as the use of arrows or polygons. This step can also be done during the preparation of figures, that is, when each photo is added to the figure, the highlighting tools are applied.

1. In the Shape tools group (U), select the one that suits you best (Fig. 19). If it is an arrow, select "Custom Shape Tool"; 2. The properties bar will show the possible settings for each selected tool (Fig. 20). In this example, we will use a polygon (circle) and a Custom Shape tool (arrow); 3. After selecting the desired shape, set it up properly. In the example, we will leave the form "ellipse" without fill, with a simple red dash of 2 pts. With Shift pressed, click with the left mouse button where you want to put the shape and set the desired size; 4. If the shape is not in the desired location, use the "Move" tool (V) and position it correctly; 5. Create a new layer (Ctrl/Cmd + Shift + N), select the arrow shape present in the vector library in the toolbar's custom toolbar and adjust it so that it has a white fill and a simple red stroke with 2 pts. With Shift pressed, left-click where you want to place the shape and set the desired size. If you want to adjust the size or rotation of the shape, click Ctrl/Cmd + T
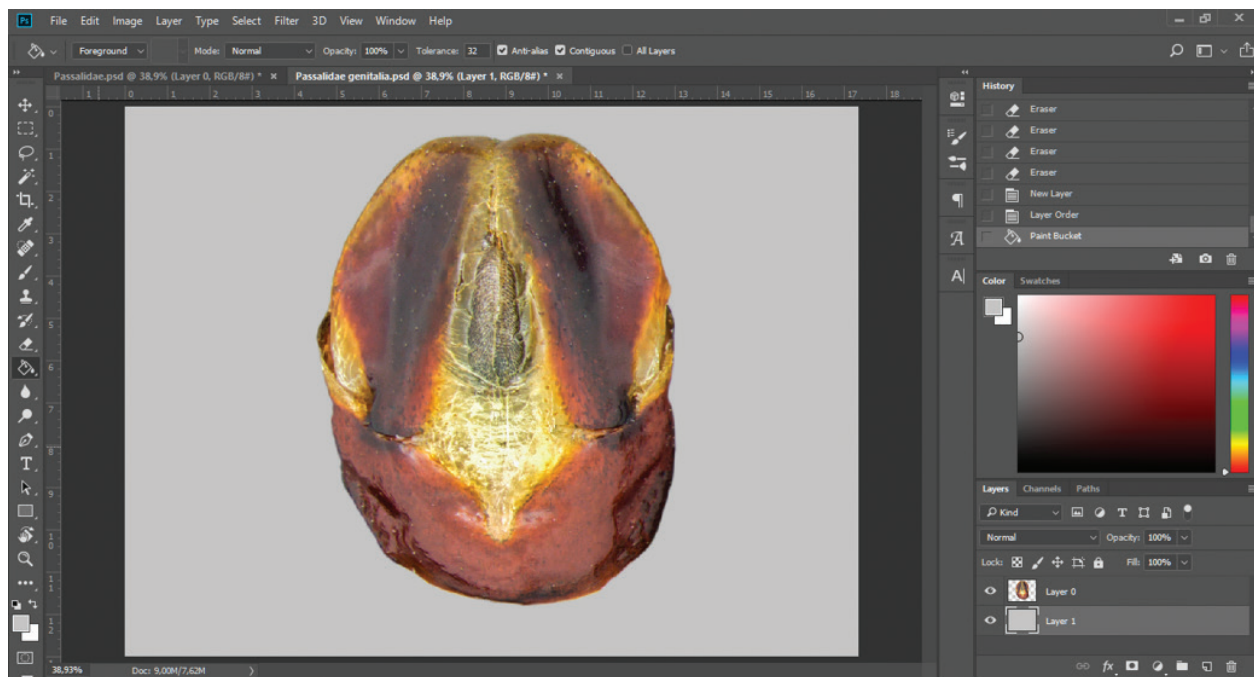

Figure 18. Image with completely erased background and a new coloured layer serving as a completely new homogeneous background.
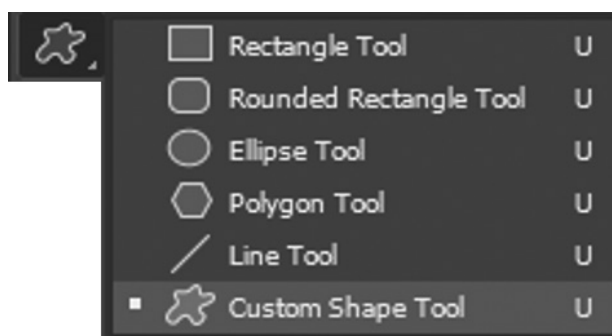
Figure 19. Shape tool group expanded to show all hidden tools.

to activate the free transform. After finishing the desired settings, click Enter (Fig. 21).

Scale bar

Another element present in scientific figures is the scale bar. The scale bar reflects the mathematical relationship between the dimensions of the object in the real state and that of the image that represents it. It is one of the essential elements of images of minute organisms or structures, along with orientation and legend.

Usually, taxonomists, such as entomologists, arachnologists and malacologists, use special equipment to take pictures with, for example, a stereomicroscope coupled to a camera with extended and stacked focus. This equipment with their (usually proprietary) software already provides the scale bar along with the final image, otherwise the scale has to be inserted manually. The scale bar that is provided by the software or manually may also not be suitable for the target journal, as the publisher provides detailed instructions for making it. Here's how to add the scale bar to comply with the journal's pattern.

There are two very simple ways to create scale bars, one using selection tools and another using the pen tool. In this example, we will use a photo that was already exported with the scale bar, but it is not in the pattern required by the journal. First open the image containing the scale bar in File > Open or Ctrl/Cmd + O, then create a new layer (Ctrl/Cmd + Shift + N). First let's follow the process using the selection tool (Fig. 22). 1. Use the Rectangular Marquee tool (M) to bypass the scale size of the original photo; 2. Using the Paint Bucket tool (G), select the desired colour for the scale bar (in this example, black was used), then paint the new layer; 3. Deselect (Ctrl/Cmd + D); 4. Copy the scale bar (Ctrl/Cmd + C) then paste (Ctrl/Cmd + V) the new layer into the previously edited photo; 5. Position the scale bar in the desired loca-
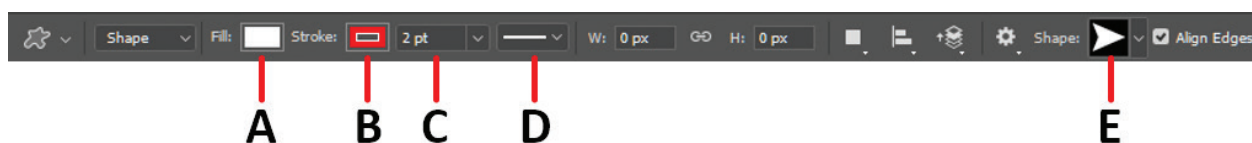

Figure 20. Shape tool properties bar. **A**. Sets the shape fill colour; **B**. Sets the stroke colour of the shape; **C**. Sets the stroke thickness; **D**. Sets the stroke type; **E**. If it is a custom shape, it defines which shape is chosen.
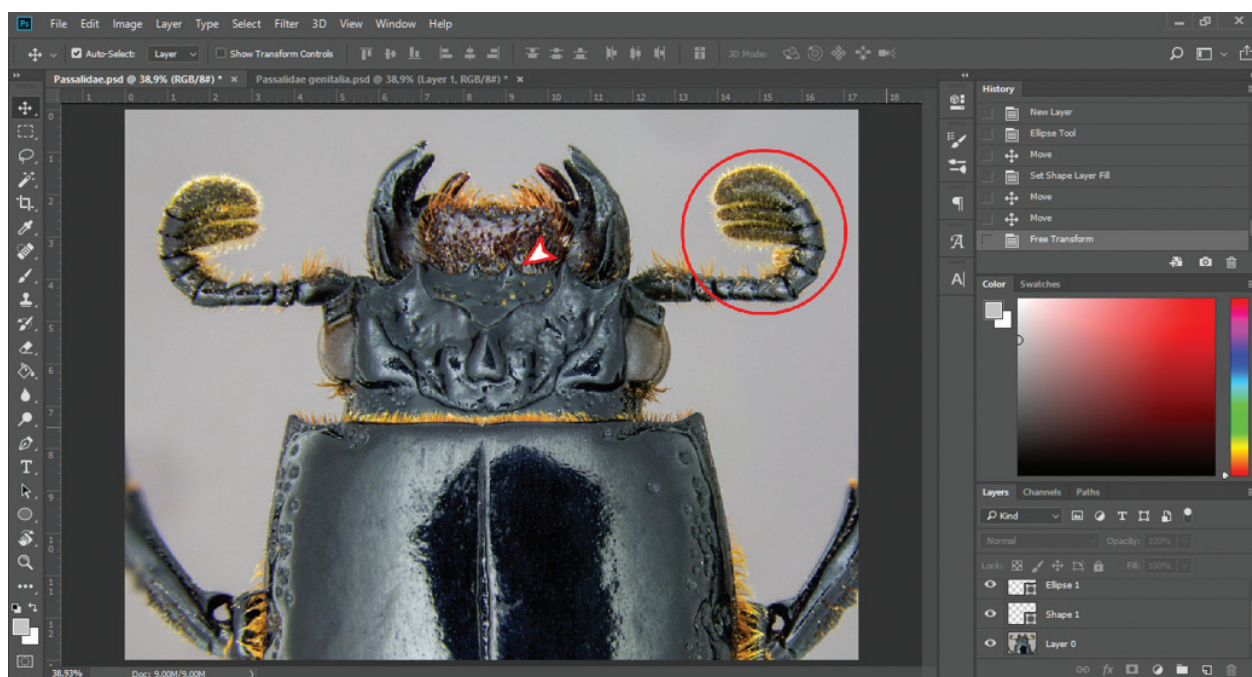

Figure 21. Image containing evidence elements.

tion using the Move tool (V). In the second way (Fig. 23): 1. Use the Pen tool (P); 2. In the tool options panel, select the thickness and colour that you want to obtain for the scale bar; 3. Click the beginning of the scale bar and, with Shift pressed, click at the end of the scale bar; 4. With the Move tool selected, copy the new scale and paste it into the previously edited photo; 5. Position the scale bar in the desired location using the Move tool.

The next step is optional, because some journals do not accept the text of the scale to be plotted together with the image and require it to be placed in the legend. However, let's deal with this step, because some allow or even require such formatting. 1. Select the Horizontal Text Tool (T) and click on the image; 2. In the properties bar, change the text formatting according to your wish (Fig. 24). In this example, we will use the font Times New Roman, size 24 pts, in black colour; 3. Enter the desired text and then use the Move tool to move the text of the scale to the desired location; 4. With the Move tool selected, click Ctrl/Cmd + T to activate the free transform option. With
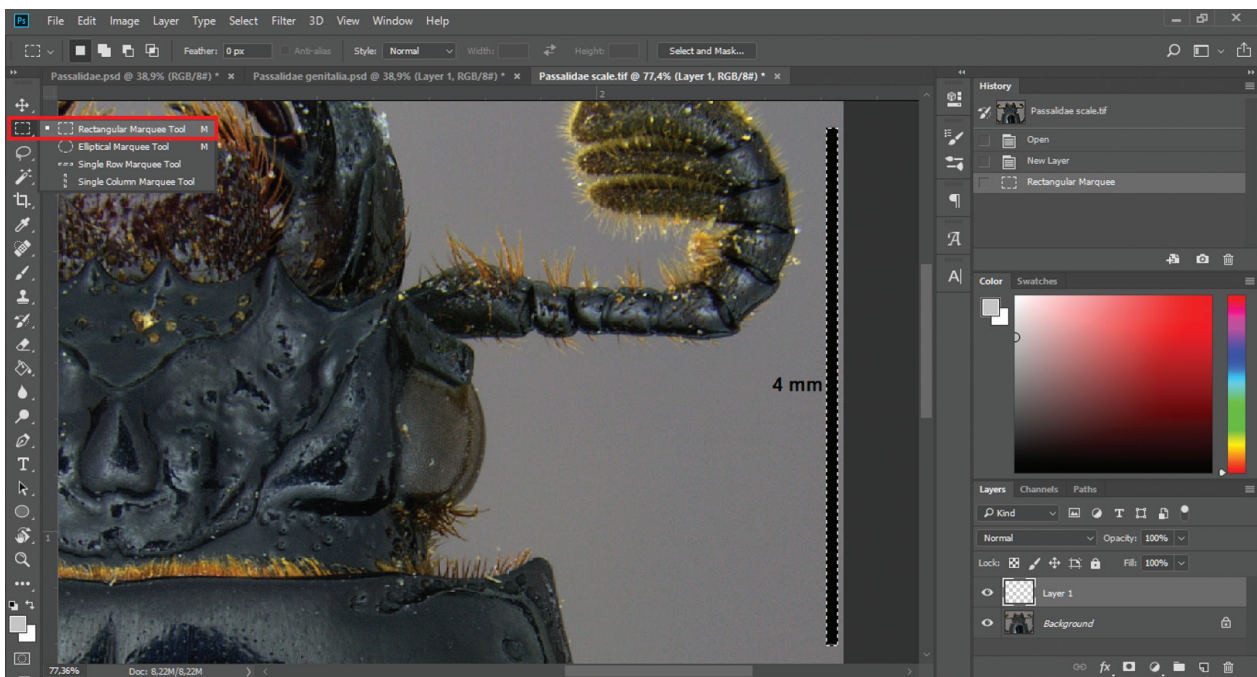

Figure 22. Method for creating a new scale using selection tools. Highlight the Closed Selection Tools group.
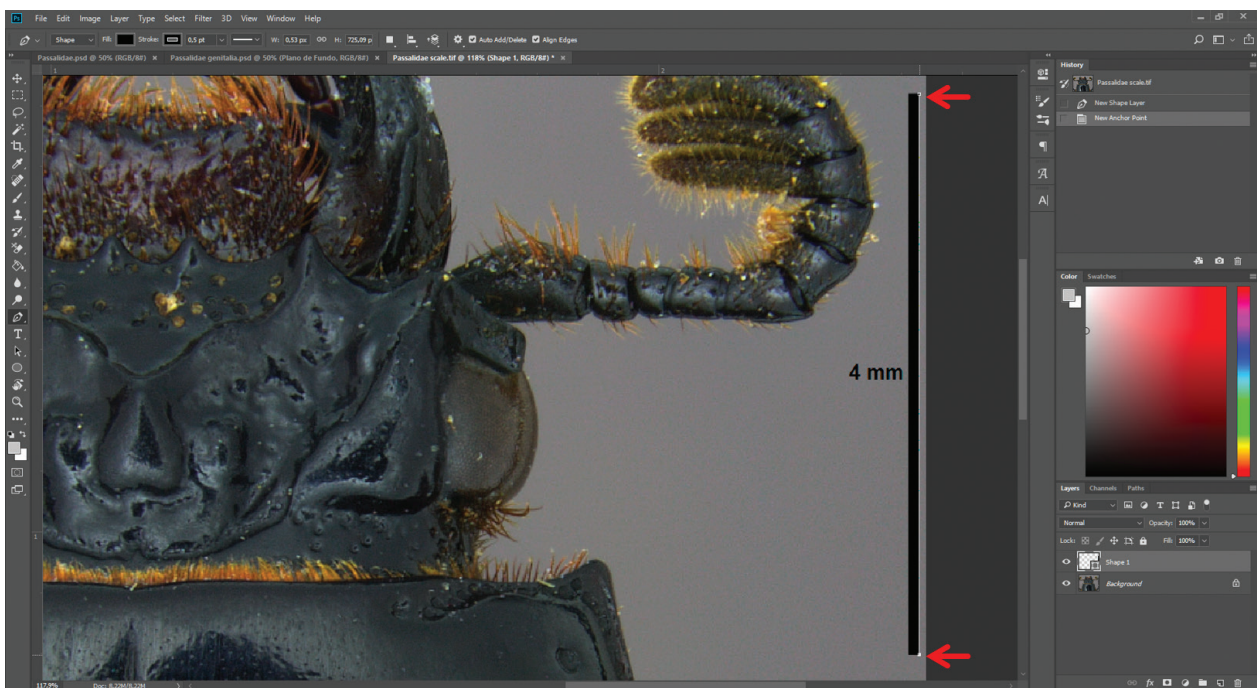

Figure 23. Method for creating a new scale using pen tool. Highlight the beginning and end of the pen tool click.

Figure 24. Type tool properties bar. **A**. Font type; **B**. Font style; **C**. Font size; **D**. Font orientation; E. Font colour.
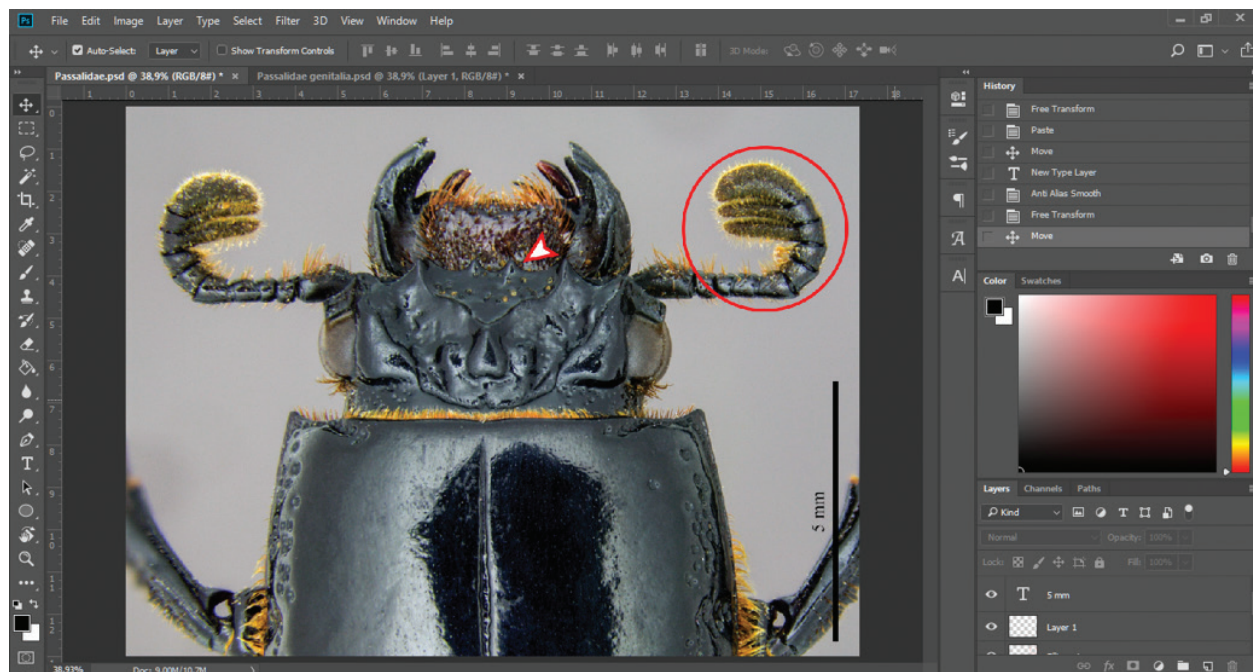

Figure 25. Image containing evidence elements and scale bar with numbering.

the Shift button held, rotate the text using the mouse to the desired direction and finally press Enter (Fig. 25).

Now the image is already completely edited, "clean", with the elements that show structures and with the scale. Save this image to continue editing other images or to start composing the figure. To save, click on File > Save As or Ctrl/Cmd + Shift + S; choose the folder in which you want to save the image and select the Photoshop file type whose extension is .psd then click Save. The image is saved and ready to be used again when needed. The file format .psd is not mandatory, since you can choose many other formats including the widely used .jpg, .png or .tif formats. However, it is strongly advised to use the Photoshop or .tif format with the 'layers' option on to facilitate the later editing of separate elements composing the final photo, such as highlighting markers and scale bars. You should also note that some file formats include the commonly used .jpg format-reduced file size through image compression. This always involves a reduction of quality, even when the highest available quality (thus the lowest reduction in file size) is chosen. Quality will be reduced every time a .jpg file is saved and this can significantly diminish the quality of your final image. Such file formats should not be used for saving images that are likely to be further processed (see below in the section on saving a figure for more details on file formats and compression).

Composing the figure

In scientific publications, journals give preference to the presentation of the images by means of composite figures or plates, instead of single photos, since this way of presenting their results contains more information in a smaller space, besides gathering in the same image photos of several structures of the same organism or the same structure belonging to several different taxa.

Here, I present a quick and easy method of composing figures, which can contain multiple images in a single figure. Initially, one must be careful in what resolution and size the journal requires for the images. First, it is necessary to understand that most journals currently publish their papers in two ways: online and printed, that is, there are two different resolutions: video display and print display.

Video projection is a relatively low-resolution system and print is a relatively high-resolution system, which means that most new monitors have a resolution of 96 dpi (however, a resolution of 72 dpi must be assumed if you want images for distribution online), whereas modern printers print at a minimum at 300 dpi; consequently, the video projected image derived from a 72 dpi digital image file looks pretty much the same as the projected image of a 300 dpi file. In contrast, there is a very noticeable difference in the quality of a print derived from

a 72 dpi digital image file compared with a print of a 300 dpi image file (LaBerge and Andriole 2003). Second, the resolution describes the information or detail in an image per unit length. Images with higher resolution contain more discernible fine detail (LaBerge and Andriole 2003). When printed, a high-resolution image contains more pixels than a lower resolution image. For example, an image of 1 inch by 1 inch, with a resolution of 72 dpi contains a total of 5184 pixels (72 pixels wide × 72 pixels high = 5184). The same 1 by 1 inch image, with a resolution of 300 dpi, contains a total of 90,000 pixels. Higher resolution images typically reproduce more details and more subtle colour transitions than lower resolution images. However, increasing the resolution or size of a lower resolution image will only spread the original pixel information over a larger number of pixels and will hardly improve its quality, causing a pixel distortion effect called 'pixelation', that is, large and visible pixels.

A copy of any image or figure should be kept at the original resolution or a reasonable maximum resolution of 1,200 dpi if the file is otherwise far too large. It is always possible to reduce the resolution to 300 dpi, but it is not possible to enlarge a 300 dpi image or figure to 600 dpi without a noticeable loss in quality. Keep in mind that you may start off by submitting your manuscript to a journal that only requires 300 dpi pixel images, but could end up at a journal demanding a higher quality. The same is true for the maximum size of figures, which should not be reduced in the original figures to meet the demands of a specific journal. Commonly, most journals require at least a resolution of 300 dpi; however, the maximum image size is variable. Here, I will adopt the common size of an A4 sheet and 300 dpi resolution.

In the Photoshop workspace: 1. Create a new document (Ctrl/Cmd + N) and choose the desired options that are available in the dialogue box that will open (Fig. 26); 2. After setting the new document as desired, click on "Create", then a new document will open. This step should be done after you have edited all the photos that will compose the figure, because here we will just organise them and list them as desired. 3. Open all the images that will compose the figure. Note the layer panel of each image and see that all the layers that make up the photo are present (Fig. 27), but they move independently of each other. In this step, this is not desirable, since each element that composes the photo should be treated as part of the photo. One way that keeps all layers intact, so we can tweak them when we want, is by linking them all into a layer group. 1. Select all the layers you want with the Ctrl/Cmd button held and click on each layer; 2. Click on "link layers" (Fig. 27A); 3. After that, create a group by clicking on "create a new group" (Fig. 27B). So that the group of layers is created, all layers must be unlocked, without the padlock on the side. 4. After the group is created, rename it with the image name by double-clicking
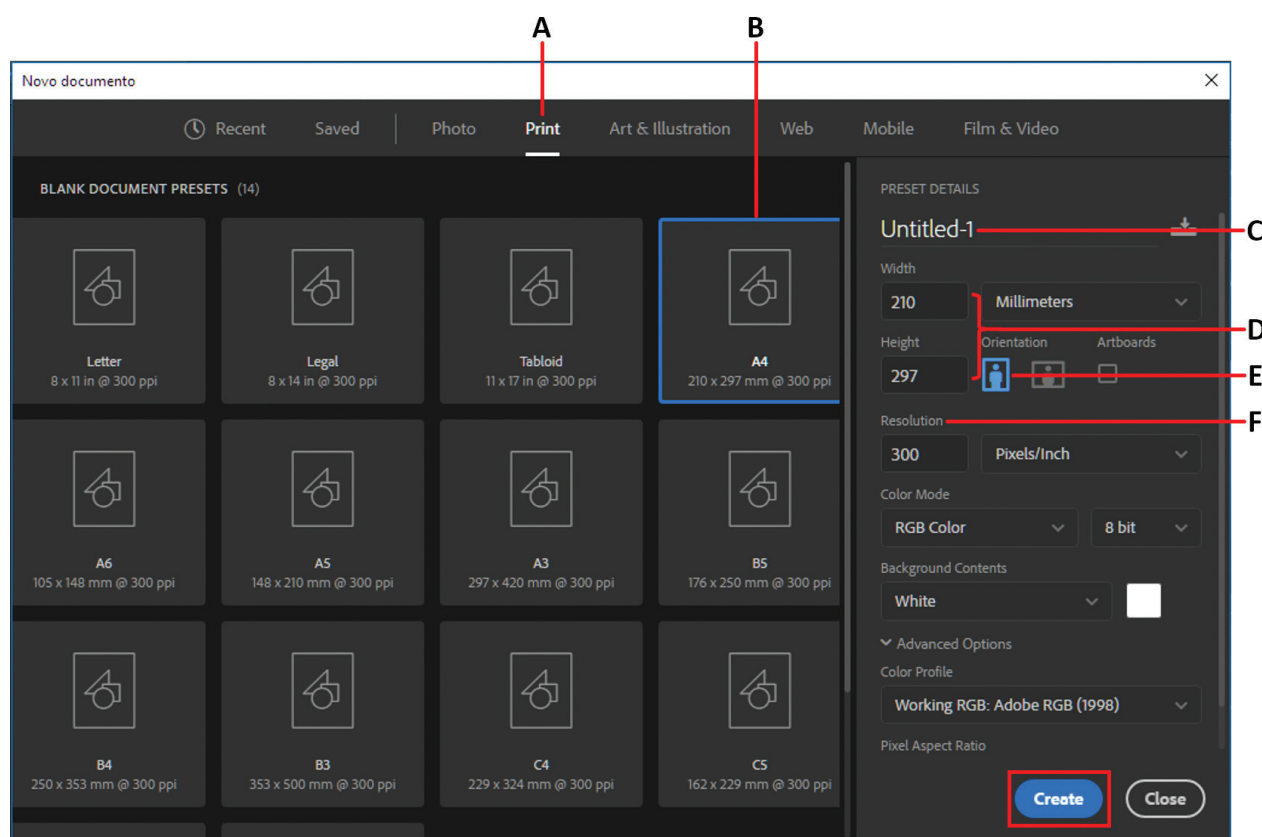

Figure 26. Create new document dialogue box. **A.** Guide containing some presets with document sizes; **B.** Selected document; **C.** The name of the file to be created; **D.** File dimensions (can be expressed in millimetres, centimetres, inches or pixels); **E.** Orientation of the document (portrait, landscape or square); **F.** Resolution of the document to be created.
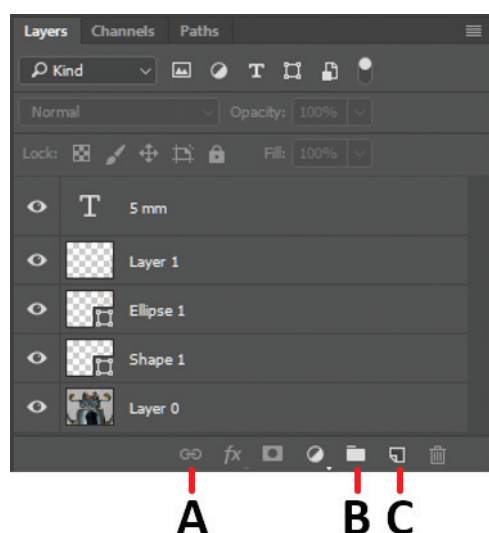
Figure 27. Layer panel. **A**. Icon to link layers; **B**. Layer group creation icon; **C**. Layer creation icon.

thus distort proportions. After all the images have been re-sized, it is time to organise them so that all the elements are well arranged on the figure. A practical way is from the use of "guides". The guides are horizontal and vertical lines that demarcate certain areas within a document. To create a guide, just click and drag on one of the rulers of the document (if the rulers are not visible, click View > Rulers or Ctrl/Cmd + R). Once you have created the guides, adjust them to position all the images on the figure (Fig. 29).

Since the figure is a set of images, they must be numbered so that there is no confusion at the moment of quoting them within the body of the text. Some authors prefer to leave the text "loose" within the image, while others prefer to place the text inside a polygon (or any other shape) for better visualisation. See how to proceed: I suggest creating a new group of layers called "Letters". In this group will be the layers that will be part of the numbering of each image inside the Figure 1. With the shape tool (U), select the "Ellipse tool" and create the ellipse of
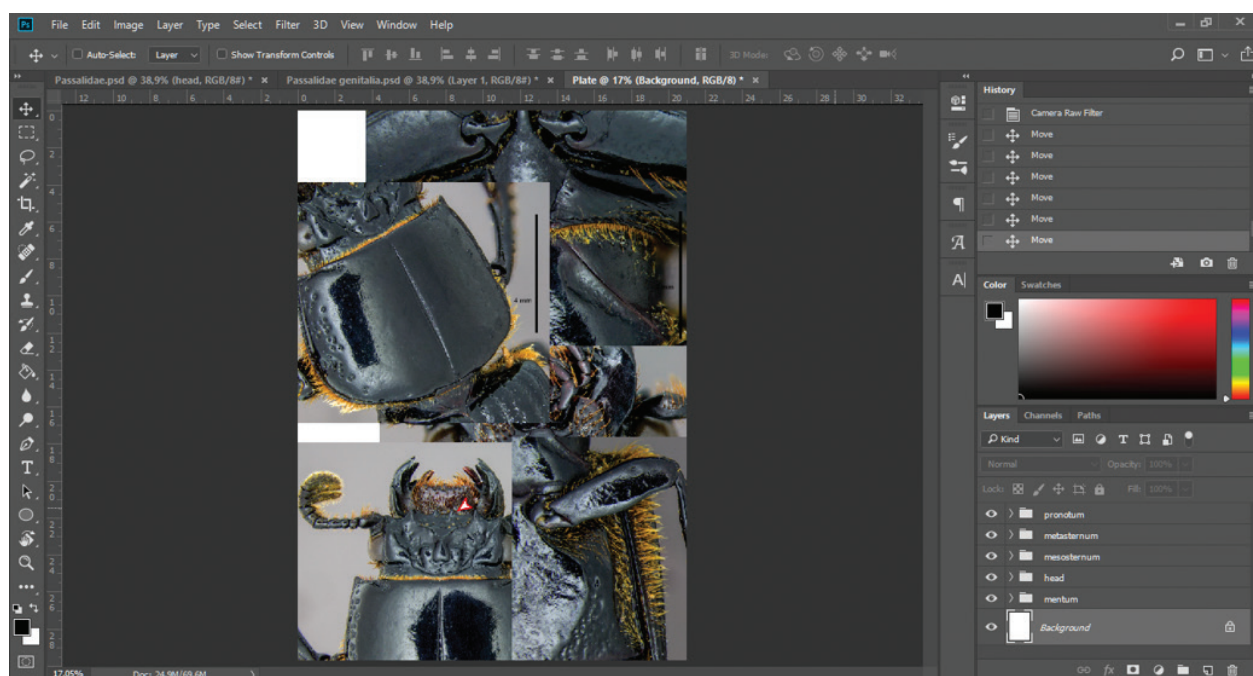

Figure 28. Window with new document containing images that will make up the figure.

on its name. 5. Once have linked all the layers of all the images intended for your figure, with the Move tool activated, copy the layer group and paste it into the new document created to compose the figure (Fig. 28).

Note that the images are in disproportionate and undesirable sizes for the figure, so it is recommended that you adjust the size of the images as you drag them on to the figure. To do this, simply click on Ctrl/Cmd + T and resize them in a desirable way and then press Enter to confirm the modification. Again, it is important to only decrease the size of the single images and not blow them up. Keep copies of the images in their original format. Another crucial consideration is always to re-size images or figures proportionally, i.e. not just to reduce one dimension and

the desired size. It is recommended that there be a background and a stroke that differ from the image. In our example, we will use a white background and a simple black stroke; 2. With the text tool (T), write the numbering or letter corresponding to each image and position it in the centre of the ellipse. An important observation is that in the layer panel, the layer corresponding to the letter must be above the layer corresponding to the ellipse. 3. Link the two layers and repeat the same operation for all images on the figure. To save time, you can simply copy (Ctrl/Cmd + C) and paste (Ctrl/Cmd + V) the layers for each image of the figure. The group, consisting of the letters or numbers of the images, must be above all other groups of the figure so that no element overlaps the others.
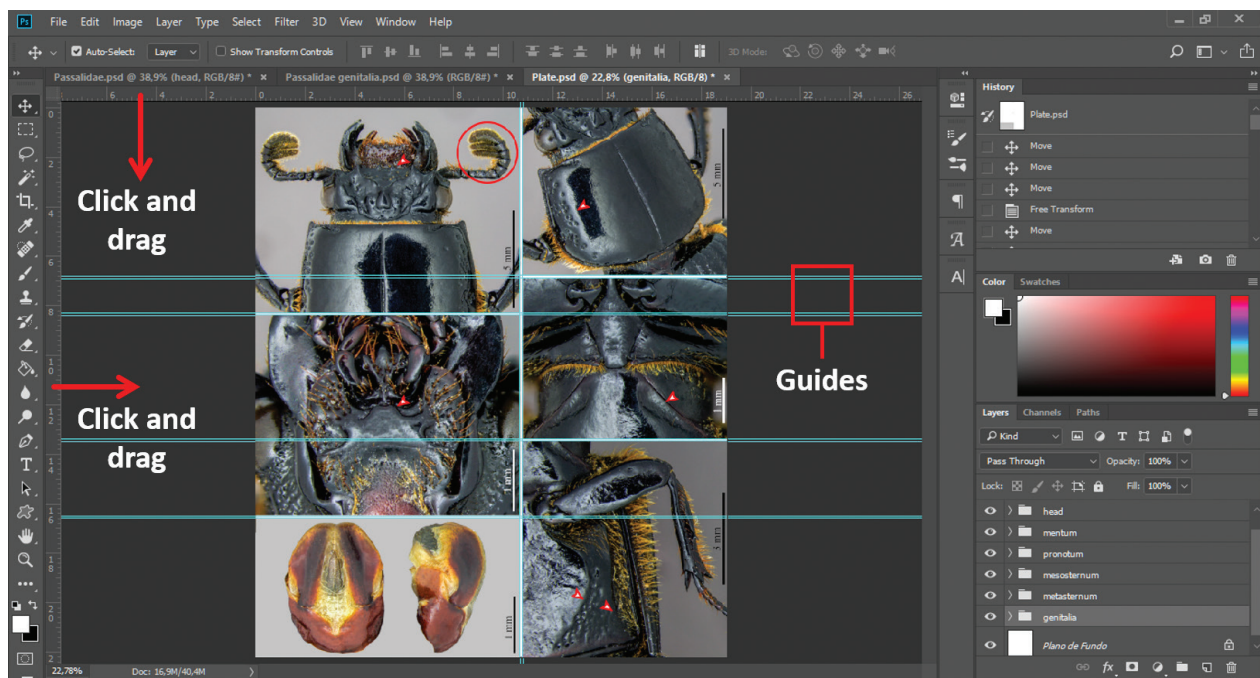
Figure 29. Photos positioned on the figure document using guidelines to adjust the position of each.

After completing all procedures, the figure is complete (Fig. 30). It is recommended to save the project in two ways: the first one as a .psd file so that all elements are maintained and you can later make changes and the second one in the format that the journal suggests, commonly in .jpg, .png or .tif format. The file size of the image (expressed in kb or mb) will increase gradually between these three formats due to the degree of compression of each one. The image file formats differ in the way they represent the picture information, in the way they compress data and in the way they are used by applications. Some formats have very specialised applications and others are more versatile (LaBerge and Andriole 2003). The file format influences the file size and image quality and choosing an appropriate file type is predominantly determined by the intended end-user image display format. Formats are broadly classified into those intended for internet use, computer display and print applications. In general, internet applications accommodate formats with small file sizes that are transported efficiently and opened quickly. Print applications require formats that provide high resolution and may require longer Internet transmission times (LaBerge and Andriole 2003). In the context of saving figures for taxonomic publications, we only need to consider the requirements of print applications.

I will comment briefly on the characteristics and use of each of the four types of image file formats, mentioned above. For more details, I suggest consulting the works of Wiggins et al. (2001), LaBerge and Andriole (2003), Graham et al. (2005) and Gross and Thapa (2012) – the following section contains a summary of these papers. The Photoshop document or PSD (.psd) is the native file format for Adobe Photoshop, which does not compress

the image and supports all subsequent Photoshop operations. This file type supports the "layer" function within Photoshop. The main disadvantage of PSD files is their large file size, particularly when multiple layers are added to the original image.

The Tagged Image File Format or TIFF (.tif) was created primarily by imaging developers of input and output devices, such as printers, monitors and scanners and, as a result, it is specifically designed to be compatible with different image processing devices. TIFF files can support a full range of image sizes, resolutions and colour depths and different compression technologies can be applied, including lossless compression with the LZW algorithm. Another useful feature of a TIFF file is that each file can contain more than one image, allowing the "layer" function within Photoshop. All these features make TIFF the standard file format for printed images. However, the large file size is the main disadvantage of this format.

The Portable Networks Graphics or PNG (.png) is a lossless compression format that is applicable for Internet use because of its small file size. PNG files use lossless compression performed by the LZW algorithm and gamma correction for cross-platform brightness consistency and RGB colour and variable transparency are supported. This flexible file format has several good features such fast image viewing, low file size and the option of maintaining transparent areas in the image (very useful resource for composing presentations for lectures), which make this file format excellent for images viewed on video devices, but it is not highly recommended as a printed image file format.

Joint Photographic Experts Group or JPEG (.jpg) is actually the compression algorithm used to create these files. It was created specifically for the storage and trans-

mission of photographic images. The main advantage of this file format is its compressibility and resultant small file size which require significantly less storage space per image and allowing faster movement in the electronic environment. Consequently, it has become a standard for photos, also within, for example, PowerPoint presentations. The type of compression performed by JPEG allows genuine image compression, opposed to LZW compression. Unfortunately, this compression is "lossy", meaning that there is loss of data in the compressed representation; therefore, users should save a JPEG file only once. This irreversible loss of data is the main disadvantage of JPEGs, as it may lead to an unacceptable level of image degradation.

In summary, you should always opt for a format using an algorithm (such as LZW) for lossless data compres-
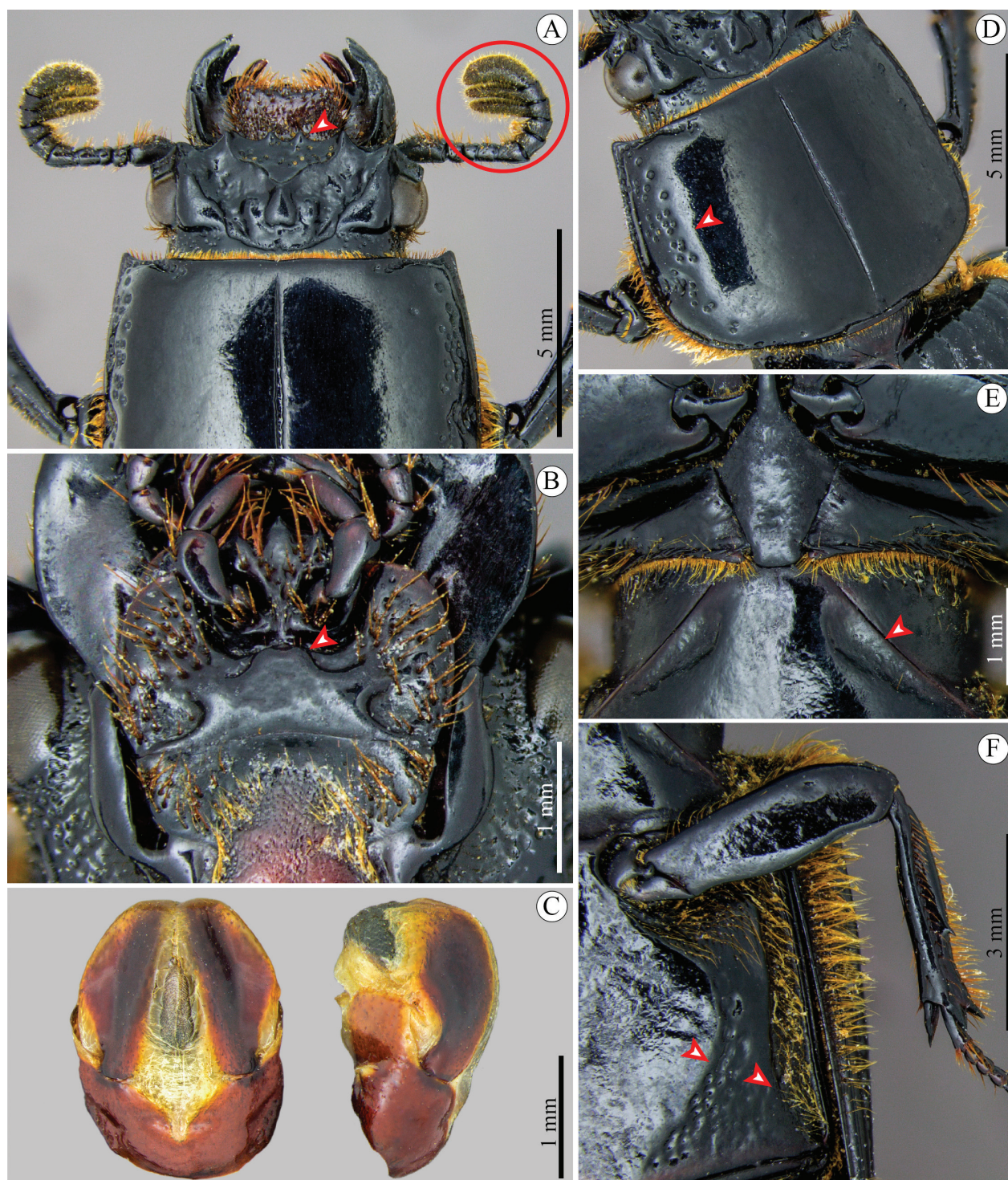

Figure 30. Finished figure containing all edited and cleaned images, evidence elements, scale bars and numbering

sion that will not affect the quality of the figure. If the journal requires the submission of figures in .jpg format, keep a copy in a format meeting the former requirement.

Discussion

As previously mentioned, the images presented in scientific publications are part of the results obtained by the researcher, mainly in taxonomic works, since the correct identification of the taxon depends on the description of this taxon along with good images that illustrate the diagnostic characters. With the advent of new technologies to represent specimens and their structures, there are publications containing techniques that promote different means of graphic representation, mainly by illustrations (Coleman 2003, 2006, 2009; Holzenthal 2008; Tereshkin 2013). Although there are publications that address graphic representations through photographs (Ray 1999; Savazzi 2011), as well as editing techniques using other types of image editors (Lababede 2004a, Lababede 2004b; Solomon 2009) or Adobe Photoshop (Corl et al. 2002; Taylor 2002, Taylor 2003a, Taylor 2003b, Taylor 2003c; Chalazonitis et al. 2003; Caruso and Postel 2006; Kirsch and Geller 2006; Gross and Thapa 2012), these works are focused mainly on the medical field, especially radiology, there being as yet no manual for using these image editing tools for taxonomy, as well as for composing figures for scientific papers in the Adobe Photoshop in its current version.

Unlike the illustrations that demand personal skills, specific abilities and considerable time expenditure (Tereshkin 2013), scientific photographs make the methodological process quicker and simpler. A structure that could take an entire morning to be represented by drawing, instead could be photographed in minutes and, in a few more minutes, edited in a good way. However, the editing of the images and the composing of the figure are often left out of the publishing process and the researcher does not worry about spending such time. Therefore, it is important that more works approach other techniques for capturing, editing and manipulating images aimed at the scope of taxonomy, to encourage and enhance the use of images that compose scientific papers, generating greater visibility and reliability.

Conclusion

Thinking about improving images for scientific publications and saving time, this guide provides a simple and quick way for anyone to edit photos to make them more attractive, both aesthetically and scientifically. The information provided here might be useful in many areas of the academic world, such as medicine, biomedicine, biology or chemistry, as well as for any person interested in such means as undergraduate, masters or doctoral students or even researchers or for any type of

academic paper, such as dissertations, theses, scientific articles, event banners, oral presentations or explanatory folders.

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References

Blatt M, Martin C (2013) Manipulation and misconduct in the handling of image data. Plant Physiology 163(1): 3–4. https://doi.org/10.1104/pp.113.900471

Caruso RD, Postel GC (2002) Image editing with Adobe Photoshop 6.0. Radiographics 22(4): 993–1002. https://doi.org/10.1148/radiographics.22.4.g02jl32993

Chalazonitis AN, Koumarianos D, Tzovara J, Chronopoulos P (2003) How to optimize radiological images captured from digital cameras, using the Adobe Photoshop 6.0 program. Journal of Digital Imaging 16(2): 216–229. https://doi.org/10.1007/s10278-003-1651-1

Coleman CO (2003) "Digital inking": How to make perfect line drawings on computers. Organisms Diversity and Evolution 3(4): 1–303. https://doi.org/10.1078/1439-6092-00081

Coleman CO (2006) Substituting time-consuming pencil drawings in arthropod taxonomy using stacks of digital photographs. Zootaxa 1360(1): 61–68. https://doi.org/10.11646/zootaxa.1360.1.4

Coleman CO (2009) Drawing setae the digital way. Zoosystematics and Evolution 85(2): 305–310. https://doi.org/10.1002/zoos.200900008

Corl FM, Garland MR, Lawler LP, Fishman EK (2002) A five-step approach to digital image manipulation for the radiologist. Radiographics 22(4): 981–992. https://doi.org/10.1148/radiographics.22.4.g02jl33981

Cromey DW (2010) Avoiding twisted pixels: ethical guidelines for the appropriate use and manipulation of scientific digital images. Science and Engineering Ethics 16(4): 639–667. https://doi.org/10.1007/s11948-010-9201-y

Fry J, Oppenheim C, Creaser C, Johnson W, Summers M, White S, Butters G, Craven J, Griffiths J, Hartley D (2009) Communicating knowledge: how and why researchers publish and disseminate their findings. Supporting paper 2: Report of focus groups findings. The Research Information Network (RIN) and the Joint Information Systems Committee (JISC) http://www.jisc.ac.uk/media/documents/publications/communicatingknowledgereport.pdf [Accessed on: 13/03/2020]

Graham RN, Perriss RW, Scarsbrook AF (2005) DICOM demystified: a review of digital file formats and their use in radiological practice. Clinical radiology 60(11): 1133–1140. https://doi.org/10.1016/j.crad.2005.07.003

Gross JA, Thapa MM (2012) Software aids for radiologists: Part 1, Useful Photoshop skills. American Journal of Roentgenology 199(6): W704–W713. https://doi.org/10.2214/AJR.11.8433

Holzenthal RW (2008) Digital Illustration of Insects. American Entomologist 54(4): 218–221. https://doi.org/10.1093/ae/54.4.218

Kawada R, Buffington ML (2016) A scalable and modular dome illumination system for scientific microphotography on a budget. PLoS ONE 11(5): e0153426. https://doi.org/10.1371/journal.pone.0153426

Kirsch J, Geller BS (2006) Using Photoshop filters to create anatomic line-art medical images. Academic Radiology 13(8): 1035–1037. https://doi.org/10.1016/j.acra.2006.04.011

Lababede O (2004a) Digital image editing using PowerPoint: part 1, introduction to the image-manipulation commands. American Journal of Roentgenology 183(3): 847–851. https://doi.org/10.2214/ajr.183.3.1830847

Lababede O (2004b) Digital image editing using PowerPoint: part 2, practical applications of the image-manipulation commands. American Journal of Roentgenology 183(3): 853–858. https://doi.org/10.2214/ajr.183.3.1830853

LaBerge JM, Andriole KP (2003) Digital image processing: a primer for JVIR authors and readers: part 1: the fundamentals. Journal of Vascular and Interventional Radiology 14(10): 1223–1229. https://doi.org/10.1097/01.RVI.0000106744.94154.B3

Lewis PJ (2016) Guidelines for the scholarly use of images. Academic Radiology 23(6): 675–681. https://doi.org/10.1016/j.acra.2016.02.001

Mello M (2012) Como fazer figuras para artigos científicos. Sobrevivendo na ciência: um pequeno manual para a jornada do cientista. https://marcoarmello.wordpress.com/2012/03/13/figuras [Accessed on: 13/03/2020]

Mudrak B (2020) Avoiding Image Fraud: 7 Rules for Editing Images. American Journal Experts. https://www.aje.com/arc/avoiding-image-fraud-7-rules-editing-images/ [Accessed on: 13/03/2020]

Perniciaro C (1993) Electronic manipulation to enhance medical photographs. Mayo Clinic Proceedings 68(12): 1220–1221. https://doi.org/10.1016/S0025-6196(12)60080-9

Photoshop (2018) Adobe Photoshop CC User Guide. https://helpx.adobe.com/pdf/photoshop_reference.pdf [Accessed on: 13/03/2020]

Ratner D, Thomas CO, Bickers, D (1999) The uses of digital photography in dermatology. Journal of the American Academy of Dermatology 41(5): 749–756. https://doi.org/10.1016/S0190-9622(99)70012-5

Ray S (1999) Scientific photography and applied imaging. Routledge, 559 pp. https://doi.org/10.4324/9780080516707

Richardson ML, Frank MS, Stern EJ (1995) Digital image manipulation: what constitutes acceptable alteration of a radiologic image? American Journal of Roentgenology 164(1): 228–229. https://doi.org/10.2214/ajr.164.1.7998545

Riedel A, Sagata K, Surbakti S, Tänzler R, Balke M (2013) One hundred and one new species of *Trigonopterus* weevils from New Guinea. ZooKeys 280: 1–150. https://doi.org/10.3897/zookeys.280.3906

Rossner M, Yamada KM (2004) What's in a picture? The temptation of image manipulation. The Journal of Cell Biology 166(1): 11–15. https://doi.org/10.1083/jcb.200406019

Savazzi E (2011) Digital photography for science: Close-up photography, macrophotography and photomicrography. Lulu Enterprises, Raleigh, North Carolina, 704 pp.

Solomon RW (2009) Free and open source software for the manipulation of digital images. American Journal of Roentgenology 192(6): W330–W334. https://doi.org/10.2214/AJR.08.2190

Taylor GA (2002) Initial steps in image preparation. American Journal of Roentgenology 179(6): 1411–1413. https://doi.org/10.2214/ajr.179.6.1791411

Taylor GA (2003a) Removal of dust and scratches from electronic image files. American Journal of Roentgenology 180(6): 1533–1536. https://doi.org/10.2214/ajr.180.6.1801533

Taylor GA (2003b) Improving image contrast. American Journal of Roentgenology 180(2): 329–331. https://doi.org/10.2214/ajr.180.2.1800329

Taylor GA (2003c) Sharpening the image. American Journal of Roentgenology 181(1): 43–45. https://doi.org/10.2214/ajr.181.1.1810043

Tereshkin A (2013) Guide to preparing scientific illustrations in Entomology on an example of Ichneumonidae (Hymenoptera). Linzer Biologische Beiträge 45(2): 1047–1277.

Wiggins RH, Davidson HC, Harnsberger HR, Lauman JR, Goede PA (2001) Image file formats: past, present, and future. Radiographics 21(3): 789–798. https://doi.org/10.1148/radiographics.21.3.g01ma25789