

## Imaging preserved damselflies for scientific publications

Rosser W. Garrison

Plant Pest Diagnostic Center, California Department of Food and Agriculture  
3294 Meadowview Road, Sacramento, CA 958321488, U.S.A.  
Email: argiavivida@gmail.com

### Introduction

Preserved damselflies (Insect order Odonata: Suborder Zygoptera) are stick-like insects that do not normally render themselves for illustrating color details suitable for scientific publications. Many of the colors in life include shades of blue and green and suffer from postmortem preservation. Especially disappointing is the lack of preservation of the delicate eye colors that are seen in life. In this article, I will show how one can obtain images that mimic as close as possible the life-like colors of specimens that are suitable for publication in scientific papers. The illustrations below (Figs. 1, 2) are field photographs of a male *Argia translata*. Note the purple coloring with the white highlight from the sun on the eyes as well as the dusty looking appearance of the thorax. I will attempt to translate a dried preserved male to make it look like the males in the photographs. For scientific comparison, I will be illustrating only the head, thorax and abdomen. I will include the wing bases but will be deleting the legs. The legs in this case show now important diagnostic characters so they are omitted here to save space.

**Fig. 1. A perched male of *Argia translata*. In this older male, the eyes are partially darkened and the thorax is very dark.**



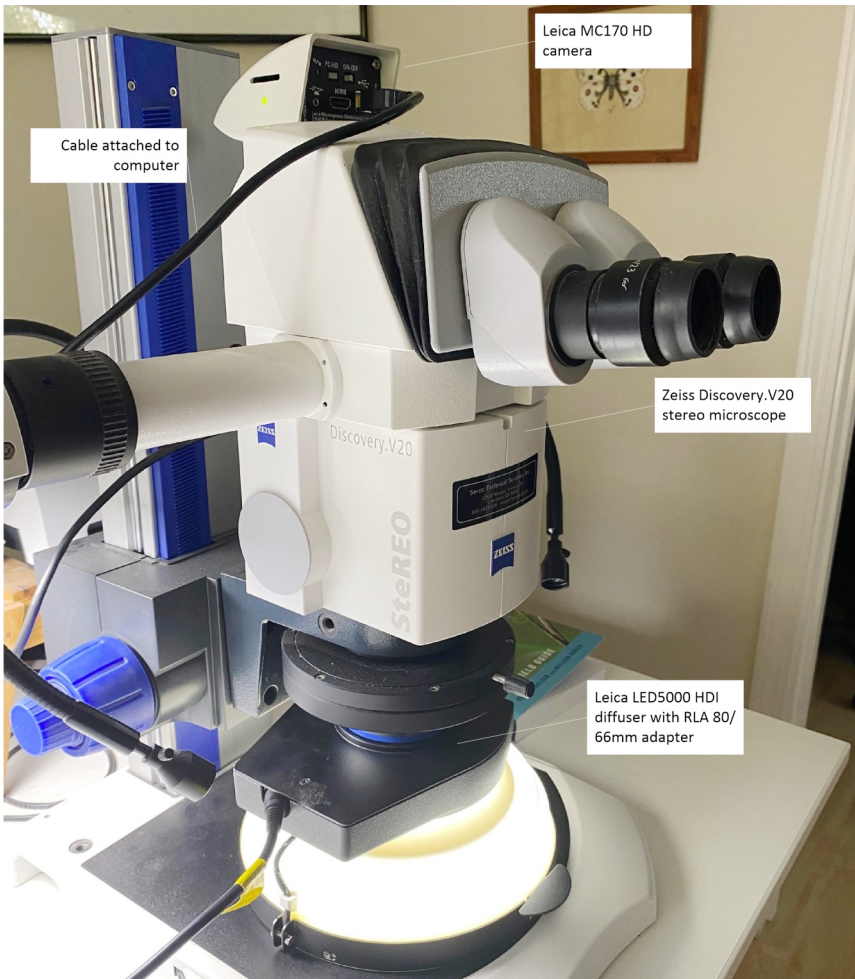
**Fig. 2. A perched male of *Argia translata*. In this younger male, the eyes are purple and the thorax is not as darkened as in Fig. 1.**



## Equipment needed

- A good binocular stereo dissecting microscope (in this example, a **Zeiss Discovery.V20**, Fig. 3).
- A camera attached to the microscope and connected to a DOS computer (in this example, a **Leica MC170 HD**).
- A diffuser mounted on the objective (in this example, a **Leica LED5000 HDI** with a **RLA 80/66** mm adapter).

The RLA adapter fits over the 66mm objective in order to allow for attachment of the LED5000 HDI diffuser apparatus (Fig. 3).



**Fig. 3. Microscope setup for photography of a preserved damselfly.**

### LASV 4.13 camera software

An image stacking software such as **Helicon Focus** (<https://www.heliconsoft.com/>), **Zerene Stacker** (<https://www.zereneystems.com/cms/stacker/softwaredownloads>) or **CombineZP** (<https://combinezp.software.informer.com/>).

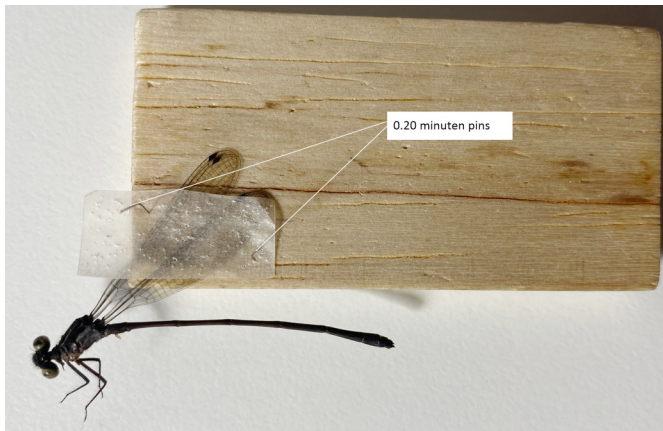
The last one of these is a free open-source staking program. In this example, I will be using Helicon Focus.

An imaging software program such as **Adobe Photoshop** (used in this example) or any other available from the web.

I will use a preserved damselfly that has undergone standard preservation techniques. Most entomologists who collect damselflies and dragonflies preserve specimens by soaking them in acetone. This is accomplished by placing the specimen in a glassine envelope and soaking in pure acetone for 12-24 hrs. before removing and allowing to dry. The acetone dissolves internal fatty tissues and prevents decay of the specimen though several of the colors seen in life are altered. Although preserving in acetone is not necessary for imaging purposes, specimens so preserved will allow for a better specimen with which to work resulting in less rendering work via Photoshop.

### Specimen Preparation

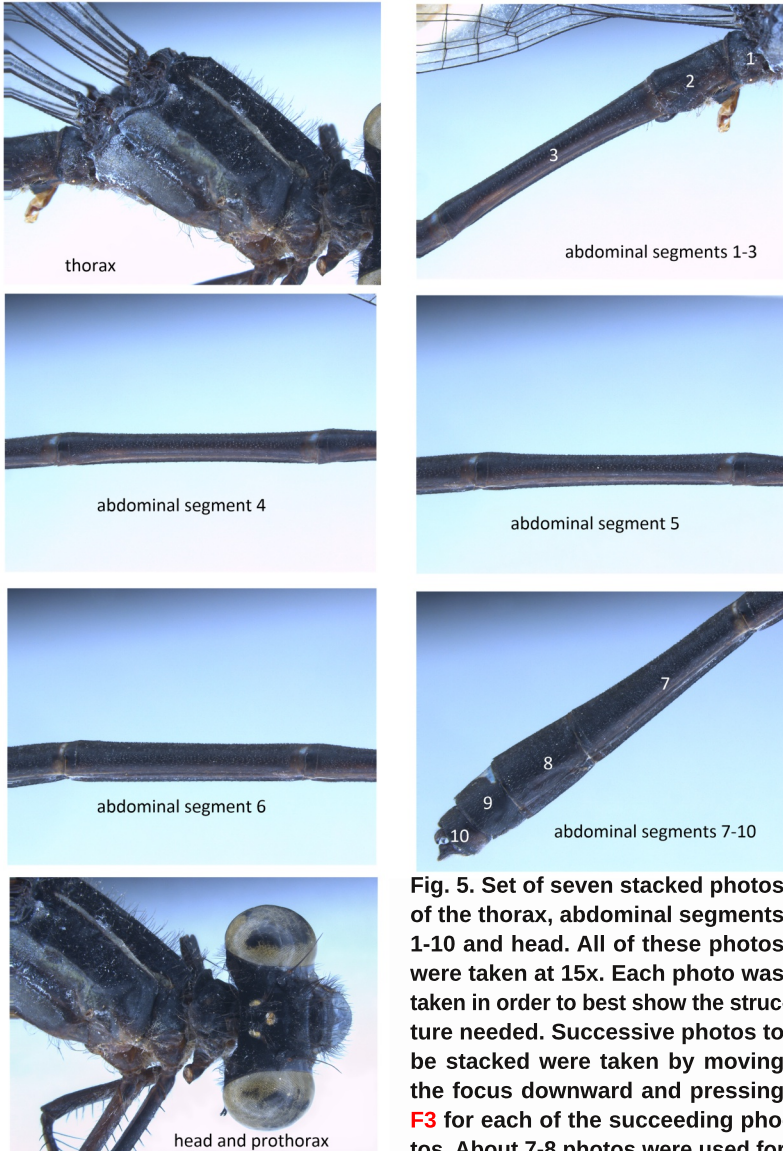
A preserved damselfly is attached to a small (e. g. 50 x 30 x 5mm) balsa wood block using a small strip of glassine paper fixed using two 0.20 minuten pins (the latter available from entomological supply houses, e. g., BioQuip Associates, <https://bioquipinc.com/product-tag/minuten-insect-pins/>) as shown in Fig. 4.



**Fig. 4. Prepared specimen of a male *Argia translata* ready for photography.**

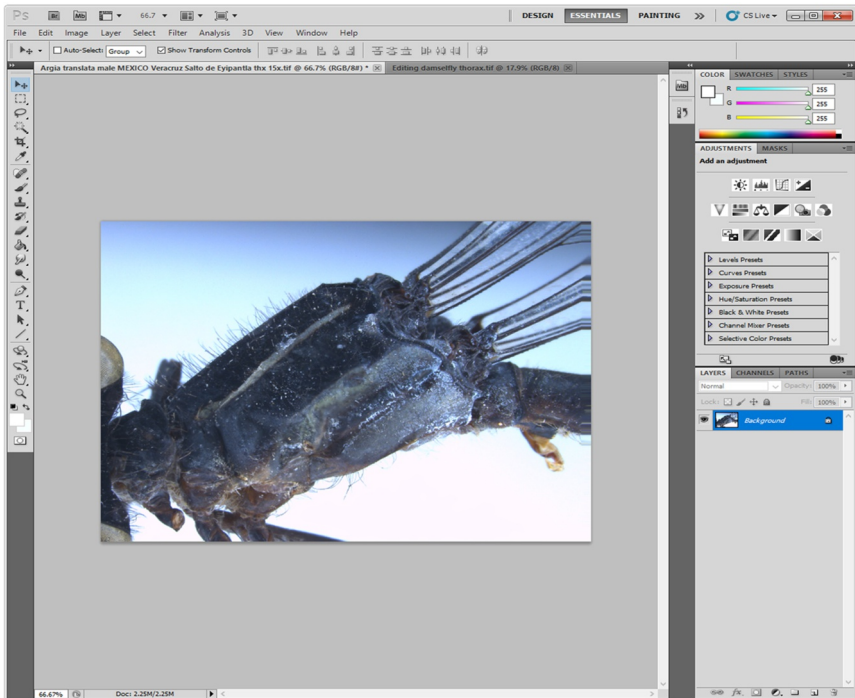
### Methodology

NOTE: The following image editing and manipulations require knowledge of an imaging software. In this example, I provide step by step procedures for editing the thorax using Adobe Photoshop 5. For the purposes of this exercise, I have designated all key board strokes (e. g., **Shift, Ctrl**) in blue. All choices from the Adobe Photoshop program (e. g., **Ruler Tool, Image**, etc.) are in red.



**Fig. 5.** Set of seven stacked photos of the thorax, abdominal segments 1-10 and head. All of these photos were taken at 15x. Each photo was taken in order to best show the structure needed. Successive photos to be stacked were taken by moving the focus downward and pressing **F3** for each of the succeeding photos. About 7-8 photos were used for

stacking of the head and thorax (due to greater depth of field) and about 4-5 for each set of abdominal segments. **NOTE: It is important that all photos be taken at the same magnification.** Each stacked photo will need to be edited using an imaging software (in this case, Adobe Photoshop).



**Fig. 6.** Flipping the thorax.

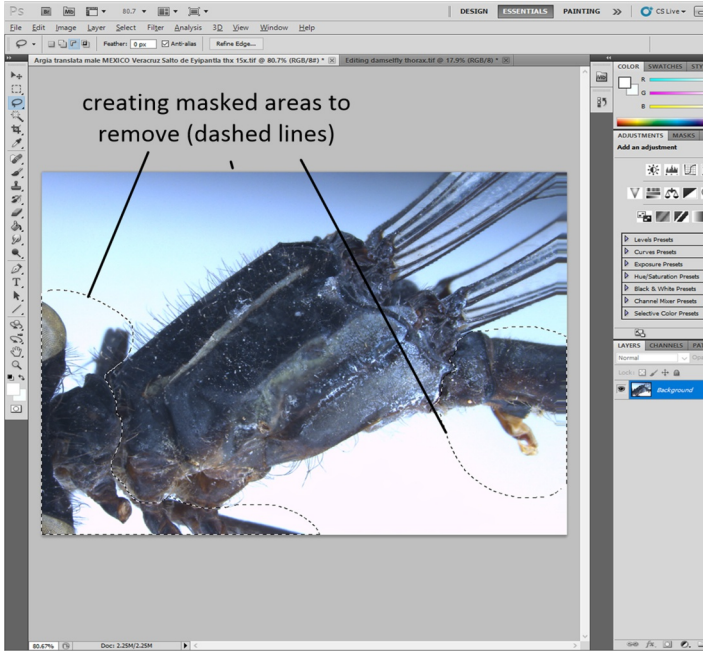
In order to orient the specimen from left to right, select **Image**, select **Image Rotation**, select **Flip Canvas Horizontal** (Fig. 6). The images of abdominal segments (hereafter "S") 1-3 and S7-10 will need to be rotated so that they extend from left to right. There are two ways to do this.

- 1). Right click (with the mouse) the **Eyedropper tool**, select the **Ruler tool**. Use the mouse to draw a straight line through S1-3 (and again through S7-10). With the line drawn, select **Image, Arbitrary.....** A dialog box (**Rotate Canvas**) will appear providing you will the following details (Angle xx [a number will be shown here]; °CW or °CCW) and click **OK**. The image should be horizontal.
- 2). Alternatively, select the **Lasso Tool** making sure that the **Feather** is at **0 px**. Draw a polygon (mask) around the parts that you wish to rotate (if you make a mistake, press **Ctrl D** to remove the dotted polygon and start over again). Select the **Move Tool (V)**. You will see a rectangle with handles at each of the four corners as well as at the middle of each side. Moving the mouse cursor to one of the side handles you will see a small curved arrow (if this does not appear, press **Ctrl**) and rotate the image to the horizontal position.

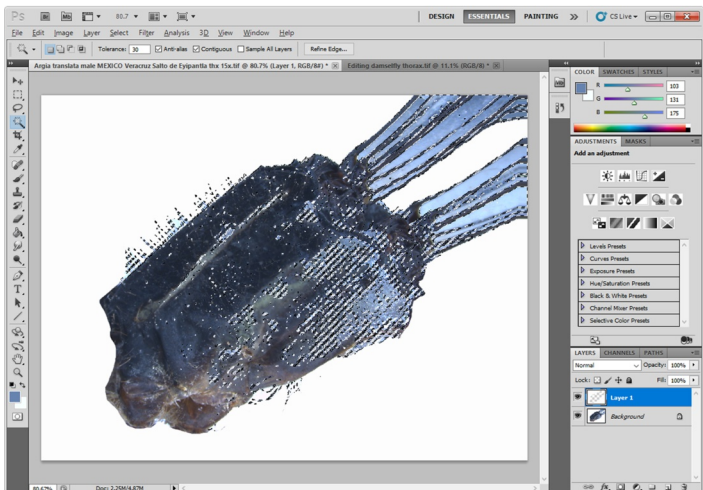
The remainder of the editing process for each of the seven photos (Fig. 5) involves: 1). removing unwanted sections from each of the seven photos noted above and then 2). removing the unwanted background (Figs. 8, 9).

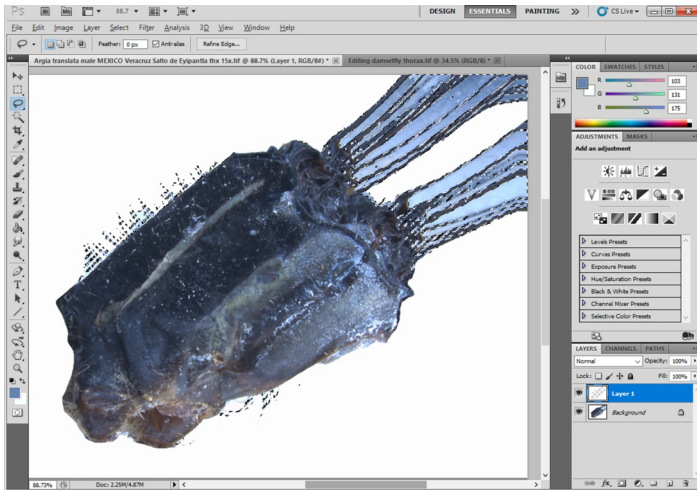
- 1). I will now remove remnants of the prothorax and abdomen from the thorax (Fig. 7). The same procedure will apply for editing all the abdominal segments as well as removing the thorax from the head. Select the **Lasso Tool** making sure that **Feather:** is at **0 px**. Very carefully draw a polygon (mask) around the parts you do not wish to keep. You can increase magnification (using the mouse wheel) to make sure your

**Fig. 7. Editing the thorax.**



**Fig. 8. Removing background of thorax 1.**





**Fig. 9. Removing background of thorax 2.**

dashed line exactly bisects between the parts you wish to keep versus those you wish to discard. If you make a mistake, press the **Shift** key (and continue pressing) to add to the parts) already drawn, or **Alt** key (and continue pressing) for parts you wish to delete. If you wish to circumscribe more than one area, be sure to press the **Shift** key while drawing supplementary polygons (Fig. 7). Once you have finished, press the **Del** key to remove the areas not selected.

- 2). There are two ways remove background colors in order to have an all-white background (Figs. 8, 9).

**A).** The first involves simply using the **Magic Wand Tool (W)** to multiply click on background areas you wish to remove. Supplementary addition of areas to remove is accomplished by holding the **Shift** key. I suggest using a **Tolerance**: of about **20 to 30** but you can experiment with this in order to achieve the best results for background removal. This method works best where the outlines are well-defined (e. g., abdominal segments) and there is no fine vestiture (e. g., hairs or bristles on head or on thorax).

**B.)** In order to preserve vestiture (hair, bristles), first, make a new Layer (**Ctrl J**). Now press **Select**, then **Color range**. Using the **eye dropper**, multiply click on the various colors of the background especially those near, not including the bristles or hair. Do not select anything in the thorax proper. You must continually hold the **Ctrl** key in order to add background colors you wish to remove. Press **OK**. Note that parts of the thorax have also been selected (dashed lines in Fig. 8) as areas to remove. Since we do not want to remove any portion of the thorax proper, select the **Lasso Tool (L)** on the left. Make sure the **Feather**: is at **0 px**. Using the **Lasso tool**, press the **Alt** key (and continue holding) while you encircle the dotted areas (masked areas) *within* the thorax as shown (Fig. 8). Now select **Layer**, **Flatten Image**, then press the **Del** key. In order to clean up any areas around parts of the thorax or head, especially areas around bristles, hairs, etc., I recommend using the **Paint Tool** on the left. Press the **Brush Preset picker** on the upper left and select **Soft Round** (upper left-hand corner). You can vary the

**Opacity:** and **Flow:** selections to a particular setting. I use **100%** in both categories. Use the left bracket key ( [ ) to reduce the diameter of the brush, or the right bracket key ( ] ) to increase the brush diameter. Carefully clean up any background areas that have been missed using the methods described above (Fig. 9). Use the same methods to remove associated unwanted parts of the thorax not pertaining to the head.

### Cementing all of the parts together

This is done by selecting a new palate and naming it (in this example, *Argia translata* male 15x) in order to assemble all of the seven pieces as follows. Next, open S7-10, use the **Magic Wand tool** to select the white background, then press **Shift + Ctrl + I** (or **Select, Inverse**) to select only S7-10. Now press **Ctrl C**, select the blank palate and press **Ctrl V** to place S7-10 on the new black palate. Next, do the same with S6. To move S6 in position with S7-10, select the **Move Tool (V)** and using the mouse, carefully move S7 in position to the front end of S7. Once done, Select **Layer**, then **Flatten Image**. In order to merge the colors between S6 with S7, select the **Clone Stamp tool (S)** from the left. Using the **Alt** key, carefully select an area just to the other side of either S6 or S7 and then carefully "clone" the colors to remove any glaring differences. Practice here will make perfect, and you can also experiment changing the **Opacity:** and **Flow:** numbers. You can alternatively use the **Blur Tool** to achieve the same purpose, but I think use of the Clone tool does a better job. The remainder of the five images are assembled in the same manner. Two results are shown in Fig. 10.



Fig. 10. Cementing parts together.



Fig. 11. Macro (stacked) photo of a damselfly preserved in ethyl alcohol (ETOH).





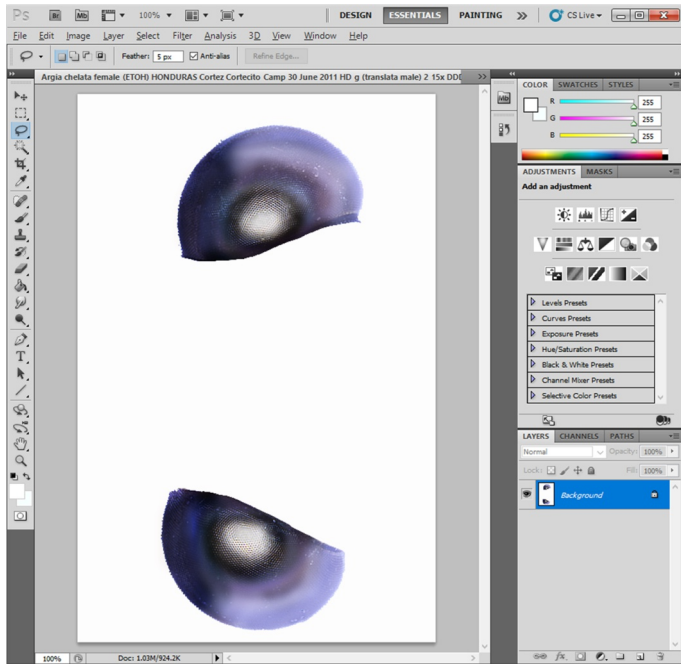
**Fig. 12. Macro (stacked) photo of a live or freshly killed damselfly with largely blue eyes.**

The eyes are likely the most difficult to render adequately because it is difficult to preserve the fine multifaceted structures of each eye as well as any highlights (Fig. 11). I have at one time or another photographed a set of eyes from a preserved (Fig. 11) or live (Fig. 12) damselfly (*Argia*) in order to document the shape and overall structure of the eye pattern and use as a model. The following instructions pertain to these new images of eyes noted above. Depending on species, use of the color palate and other attributes will be necessary in order to achieve the correct results. The methods for doing so involve trial and error and working with supplementary layers, using each individual layer to achieve the correct result. In order to apply different layers to a masked area again, using a **Lasso Tool** for instance, do the following: Press **Ctrl J** to make a new layer. Now use either the **Lasso Tool** (or, even better, right click the **Magic Wand Tool (W)** and select **Quick Selection Tool (W)**). Using a method that best suits your needs, carefully select one of the compound eyes as shown (Fig. 13). Now choose **Layer, New, Layer via Copy (Ctrl + J)**. Now, we can open a new image-in this case we have already manipulated a set of eyes. Use of various methods of coloration, etc. can be achieved by selecting **Layer, New Adjustment Layer**, and choosing how next to modify the eye colors. These include choosing **Levels...**, **Hue/Saturation...**, **Channel Mixer...** and/or **Color Balance...** and working with each in order to achieve the best results. Try to match as close as possible the color and type to the photograph of the eyes in life of the target species. I have already made a set of colored eyes from which to choose for a target species. Some examples are shown in Fig. 13. Once the correct eye color and pattern have been achieved, select **Layer, Flatten Image** to save (be sure to rename this as a new image).

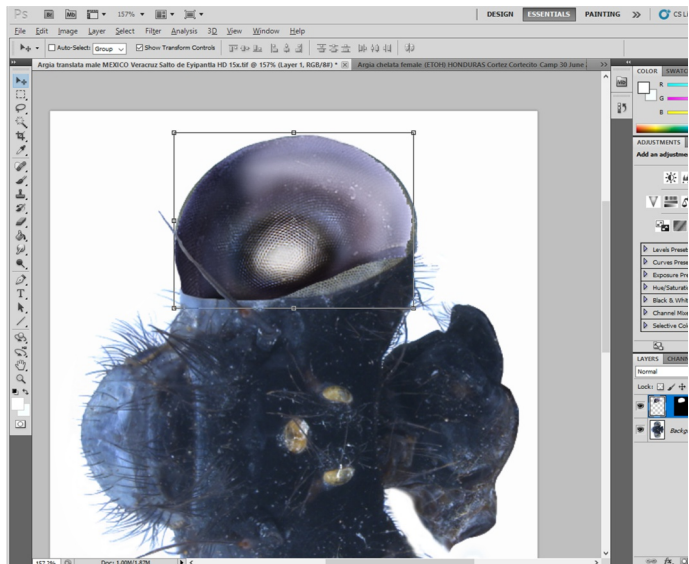


**Fig. 13. Sets of eyes rendered using Photoshop. Each eye set was rendered using tools in Photoshop as described above.**

In order to replace the old preserved eye with the new corrected eye, do the following: From the rendered set of purple eyes (Fig. 14) select the **Magic Wand Tool (W)** and click the



**Fig. 14.** Rendered (purple) set of eyes for *Argia translata*.



**Fig. 15.** Placing the rendered (purple) right eye from Fig. 14 into the head of the preserved head of *Argia translata*.

white background. While holding the **Ctrl** key, choose the Lasso Tool and draw an outline (mask) around the left (bottom) eye. Now press **Ctrl + Shift + I** (or **Select** and **Inverse**) to select only the right eye. Press **Ctrl + C** to copy the entire right eye (Fig. 14). Now open the other image of the preserved head (Fig. 15; head properly oriented and cleaned originally displayed in Fig. 5). On this image, select the **Quick Selection Tool** (right click the **Magic wand Tool**) and carefully select only the preserved left eye. The **Quick Selection Tool** will generally select the area within the eye ignoring the contrasting (e. g., white background vs. eye margin) areas. If the selection also includes parts of the head, these areas can be removed by selecting the **Lasso Tool**, and setting **Feather:** at **0 px**. Now, while holding the **Shift + Alt**, carefully remove the unwanted area by drawing a polygon (mask) within the head proper (not including any of the eye) being most careful to distinguish between the inner eye margin and the remainder of the selected head. Once done, only the entire right eye should be masked (you can easily check if this is true by pressing **Q** on the keyboard to see in masked (= white area).

Next, select Edit, **Paste Special, Past Into** (or **Alt + Shift + Ctrl + V**). You will see that the new purple eye is placed within the masked area of the preserved head (Fig. 15) but you will need to enlarge the copied image so that it fills the masked area of the preserved eye. Select **Shift** and click on one of the comers to move the inserted eye in order to best fill the masked area. In Fig. 15, one would need to move the lower right-hand handle in order to "complete" the fill. Note: selecting the **Shift** key while moving a selected handle ensures that the eye is enlarged or reduced proportionally. Be sure to select **Layer, Flatten Image** afterwards before moving on to the right (top) eye.

Rendering a good image for publication may take a couple of hours or more depending on the preserved state of the animal. Poorly preserved specimens will require more work and trial and error with both stacked macrophotography and Photoshop. You will need to do trial and error with both lighting and rendering to achieve the best results. An example of a plate ready for publication is shown in Fig. 16.

## Acknowledgment

I thank Natalia von Ellenrieder for her review of this manuscript.

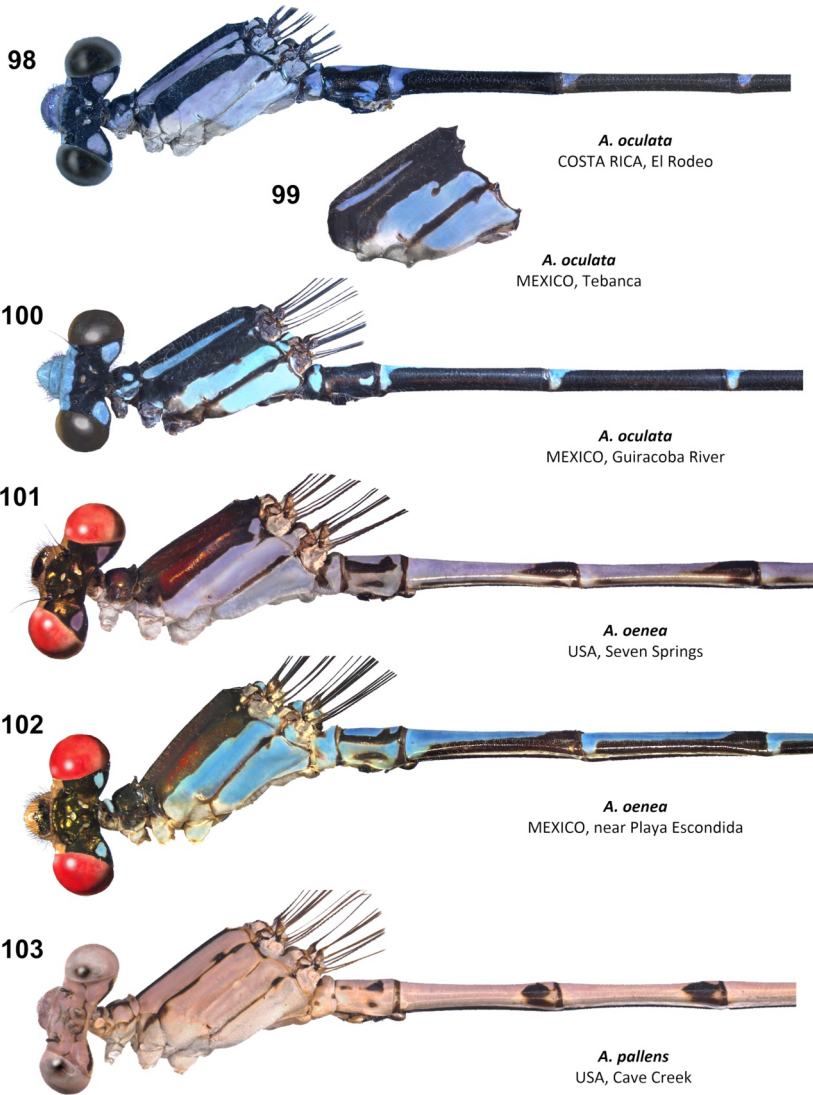


Fig. 16. A finished plate ready for publication.