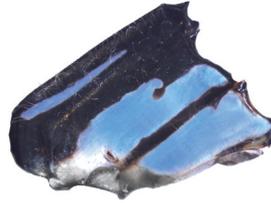


98



A. oculata
COSTA RICA, El Rodeo

99



A. oculata
MEXICO, Tebanca

100



A. oculata
MEXICO, Guiracoba River

International Dragonfly Fund - Report A Journal of the International Dragonfly Fund

1-20

Rosser W. Garrison

Preparation of preserved field collected dried damselfly specimens for character illustrations (1-7)

Imaging preserved damselflies for scientific publications (9-20)

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Preparation of preserved field collected dried damselfly specimens for character illustrations

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Introduction

Describing new species of odonates almost always involves illustrating primary and secondary reproductive structures that may ordinarily be hidden in preserved field collected material. It is therefore important to illustrate these structures (pronotum, mesostigmal plates, genital ligula, male caudal appendages) so that all characters may be seen. In this paper, I outline procedures I use to relax, manipulate and later preserve specimens for illustration or photographing purposes. We have been using this method on hundreds of field-collected specimens (some quite old) of damselflies of the neotropical genus *Argia* for many years now. The same procedures will work for many other genera as well. Previous studies by Calvert (1931: 16) have used a different procedure on pinned specimens than the one presented here as follows:

"The procedure which I have adopted to enable repeated examination of the penis [genital ligula] in dried specimens [of *Palaemnema*] is as follows: After the specimen has been softened in an ordinary relaxing jar and, preferably, pinned and bristled, the head of the pin is thrust into a bit of plasticene on a microscopic slide and the insect, ventral side up, brought into the field of a binocular microscope. The distal end of the penis, while still soft, is carefully withdrawn from under the vesicle but not detached. A bit of white paper, 2 to 3 mm. long and, say, half as wide, pointed at one end, is introduced, pointed end first, by means of a fine forceps between the penis and the ventral surface of the abdomen. A still smaller bit of paper is then slipped between the reflexed terminal filaments and the straight part of the penis so that the filaments may be kept completely ventrad of the latter. The specimen may then be set aside to dry and, after a day or longer, the bits of paper may be carefully withdrawn for better examination of the details, but it is not advisable to try to reinsert them without relaxing the specimens again, as I have found to my sorrow. Specimens which I have prepared in this manner have remained with the penis as available for examination after nine years as when first dried and without any need of a second relaxation. Those with more delicate fingers than mine will doubtless succeed with smaller bits of paper than I have used and which are much larger than need be. It is much to be regretted that certain students of penes, who have examined many specimens in our American museums, have not prepared their objects of study in some such manner as here described, so that their findings can be checked up without the necessity of again relaxing the individuals which they employed."

The procedure outlined here pertains to papered field collected specimens since most Odonatologists do not pin their specimens anymore.

Materials needed

1. Relaxing chamber for both damselflies and dragonflies. I use glass petri dishes-top and bottom (Fig. 1) – cutting a round piece of absorbent paper (brown bathroom paper towels work well) and placing the round paper disc on the bottom and adding some water to hydrate the chamber.
2. 0.10 and 0.20 minuten pins (Fig. 2) and (optional) a foam bottomed box to keep them (Fig. 3).
3. Small balsa wood blocks (Fig. 4).



Fig. 1. Relaxing chamber.



Fig. 2. Minuten pins.

NOTE: *Successful results depend on properly preserved specimens* (See further below in red text concerning compressed specimens). Place the dried specimen in the chamber and use tiny paper towelette fragments soaked in water to place on top of various parts of the specimen; usually over the genital pocket and caudal appendages of the male and over the base of the head/prothorax of the female. Relaxing can take just an hour or so for damselflies, longer for dragonflies.

Once relaxed, remove the specimen and conduct various manipulations with the aid of a pair of watch forceps. For females, simply turn the head in a horizontal position (it may not stay, that is, it might revert back to its original vertical position) and carefully move the prothorax forward in order to expose the mesostigmal plates. *Some specimens may be laterally compressed thus distorting the correct morphology of certain structures. Once these specimens are relaxed, remove the specimen and, grasping the specimen at the posterior segments between the fingers, carefully press against the 9th and 10th abdominal segments in the opposite direction (that is, dorsoventrally) in order to more correctly assume the normal positions of the terminalia. It will be harder to do this for the thorax if it is laterally compressed.* Next, cross-pin the specimen against the small balsa block using 0.20 minuten to firmly brace the thorax as shown in Fig. 5. Use a small strip of glassine paper to cover the wings using 0.20 minuten (as shown in Fig. 4)



Fig. 3. Small foam-bottomed box to hold minutens.



Fig. 4. Small balsa blocks (note the prepared specimen on upper right).

Male caudal appendages prepared for illustration purposes are relaxed as above or submerged in tap water and cleaned thoroughly with a fine paint brush as these structures are often encrusted with detritus or with water-soluble shellac-like exudates, both of which obscure important details. Once relaxed, the cerci and paraprocts should be movable and can be manipulated and pinned using 0.10 minutens (Figs. 6-8).

The genital ligula may be a species-specific character and its exposure allows for a more reliable means of identification when coupled with morphology of the male caudal appendages. Its disadvantage stems from its hidden nature; this structure must be teased from under the overlying vesica spermalis in order to be seen.



Fig. 5. Thorax of a damselfly braced with minutens.

The following procedure for its extrusion and examination in dried specimens can be performed under a binocular dissecting microscope: First apply a small wet piece of paper towel or cotton on the venter of S2-3 in order to soften the tegument and allow movement of the vesica and extraction of the genital ligula. Once softened, insert a pair of watch forceps anteriorly into the genital pocket and gently but firmly grasp the genital ligula shaft (base) and with a gentle rocking motion both forward and backward gradually flex the ligula within the genital pocket. Next insert the forceps under the ligula carefully flexing it away from under the vesica. Using a pair of watch forceps, fasten the specimen against a small piece of balsa wood by cross pinning with 0.20 mm minutens and applying a small rectangular piece of glassine paper over the wings using minutens to affix it to the wood. After the specimen is firmly attached to the wood, use 0.10 minutens to carefully flex the distal segment to about 45° away from the basal segment in order to hold the extended terminal segment in place so that the ental and entolateral surfaces can be observed and illustrated (Fig. 9).



Fig. 6. Male caudal appendages (*Palaemnema* sp.) braced with 0.10 minutens.

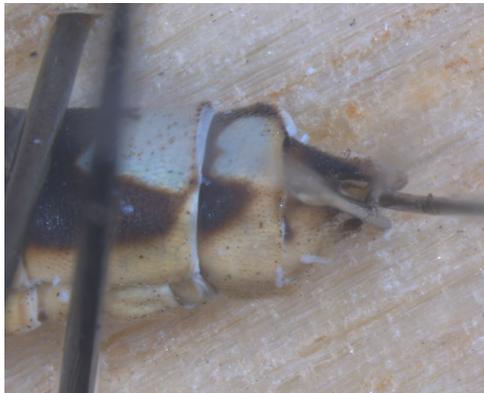


Fig. 7. Male caudal appendages (*Nesobasis* sp.) braced with 0.10 minutens, lateral view.

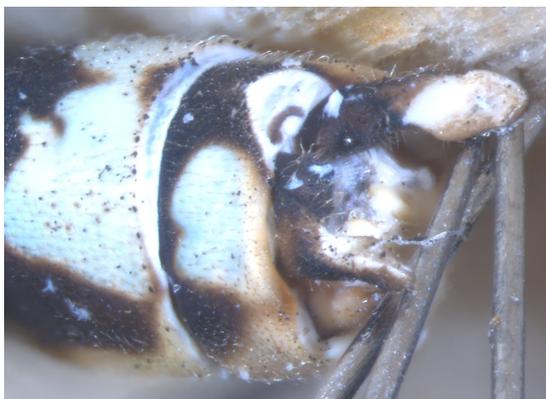


Fig. 8. Male caudal appendages (*Nesobasis* sp.) braced with 0.10 minutens, dorso-posterior lateral view.



Fig. 9. Genital ligula of a damselfly (*Nesobasis* sp.) braced with minutens.



Fig. 10 Prepared specimen to be placed in acetone bath.

Using a regular insect pin as a handle by pinning it at a right angle to the opposite side of the balsa block (Fig. 10), place the prepared specimen in an acetone bath for an hour or two (Fig. 10). The pinned specimen can then be removed and set on a substrate and allowed to dry (Fig. 11). The minutens are then carefully removed and the specimen can then be cross-pinned again with minutens on a balsa block and positioned under the microscope for illustrative (drawing or photographic) purposes.

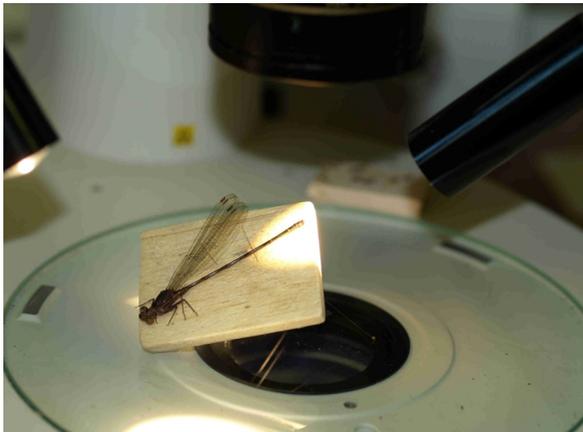
Rendering is done by tracing the outlines of the various parts with a mechanical pencil using the fine focusing knob of the microscope to clearly see and illustrate all necessary parts (Figs. 12–14). The outline and shading (stippling) are rendered with a Rapidograph pen (Fig. 15). Once the ink is dry, the pencil markings are

erased and the inked figure is then scanned via a flatbed scanner at 1,200 DPI (Fig. 16). All of our figures were drawn on Staedtler 4.25 x 5.50-inch sheets of 25% Rag Chiffon Tela Vellum.

Fig. 11. Prepared specimens removed from acetone bath and set aside to dry.



Fig. 12. A prepped male specimen (*Argia funcki*) under a dissecting microscope. The balsa block is positioned using two insect pins (not visible in this photo).



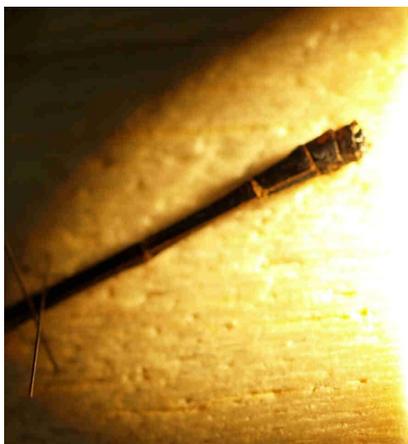


Fig. 13. Close-up of male appendages of *Argia funcki*.



Fig. 14. Close-up of male appendages (not stacked) of *Argia funcki* ready for illustrating.



Fig. 15. Illustration of appendages of *Argia funcki* using a camera lucida with illustration in Rapidograph pen (in yellow).

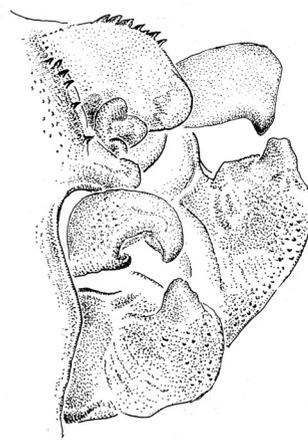


Fig. 16 The finished illustration of appendages of *Argia funcki*.

Acknowledgment

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

Reference

Calvert, P.P. 1931. Generic characters and species of *Palaemnema* (Odonata: Agrionidae). Transactions of the American Entomological Society 57: 1-110.

Imaging preserved damselflies for scientific publications

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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).

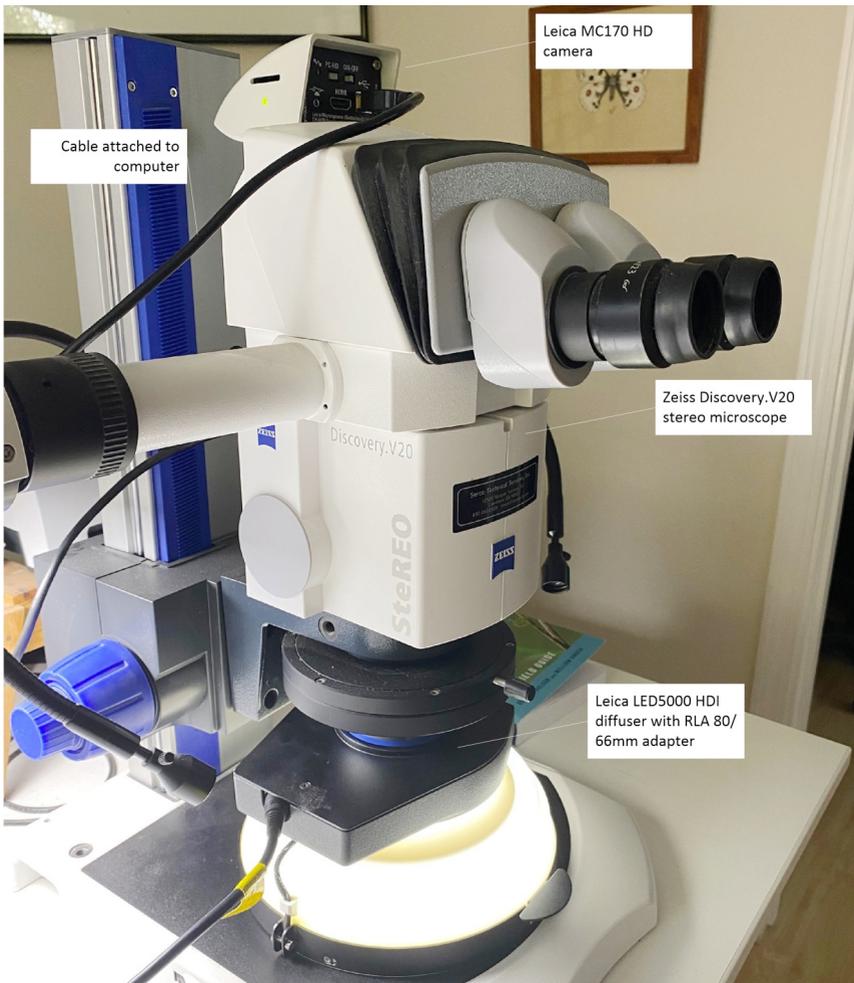


Fig. 3. Microscope setup for photography of a preserved damselfly.

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.

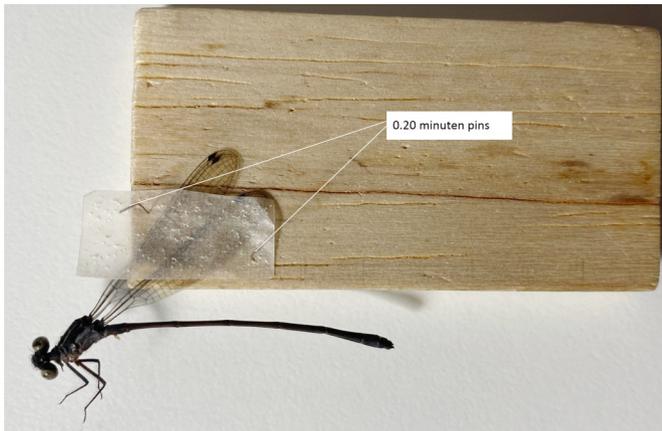


Fig. 4. Prepared specimen of a male *Argia translata* ready for photography.

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.

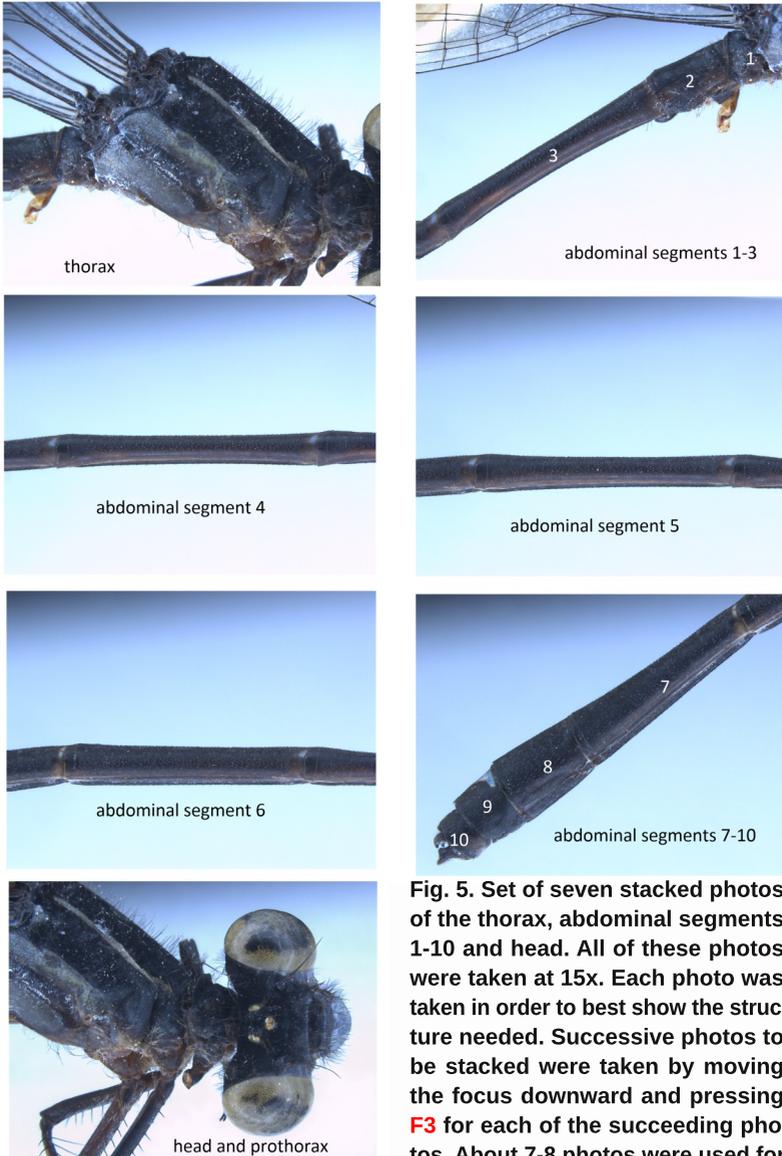


Fig. 5. Set of seven stacked photos of the thorax, abdominal segments 1-10 and head. All of these photos were taken at 15x. Each photo was taken in order to best show the structure needed. Successive photos to be stacked were taken by moving the focus downward and pressing **F3** for each of the succeeding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

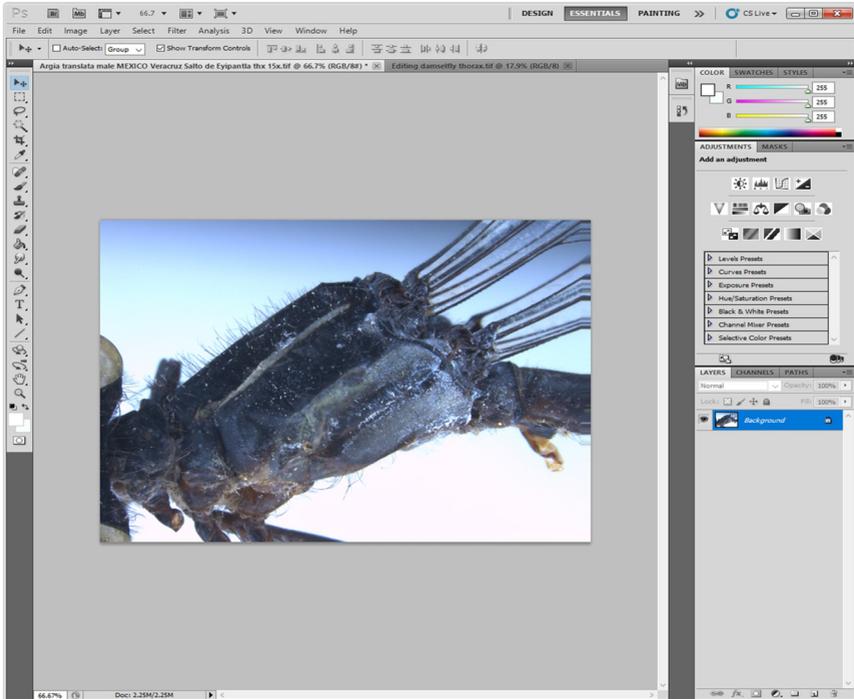


Fig. 6. Flipping the thorax.

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.

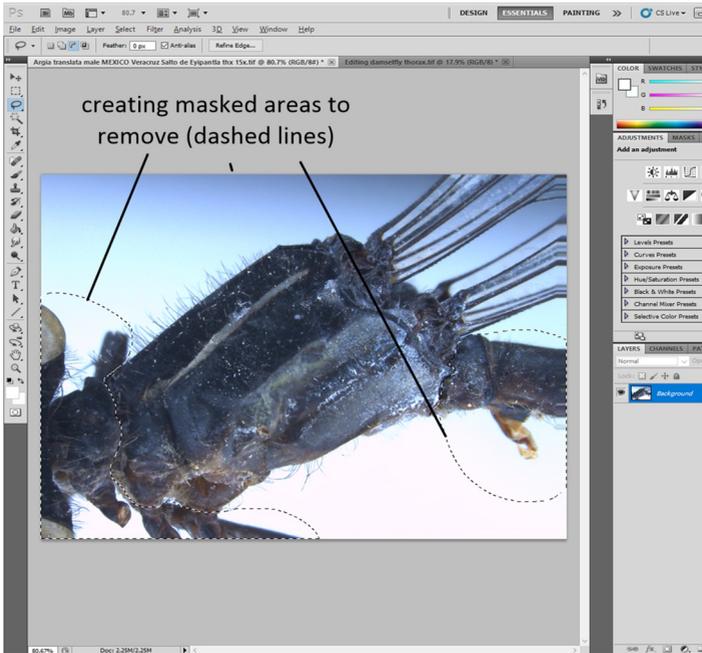
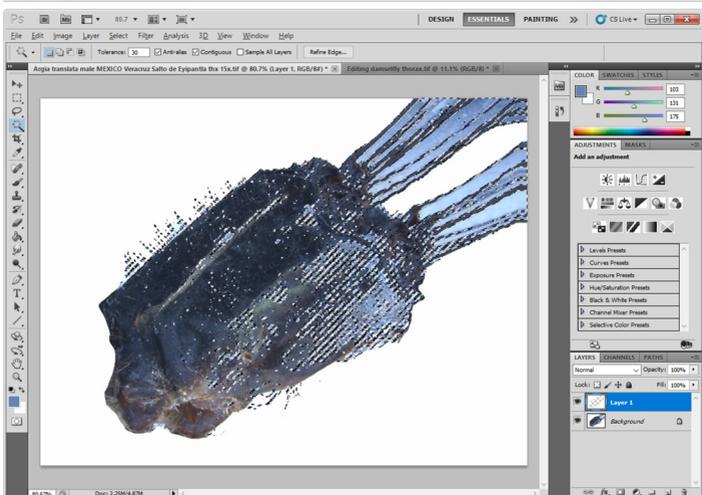


Fig. 8. Removing background of thorax 1.



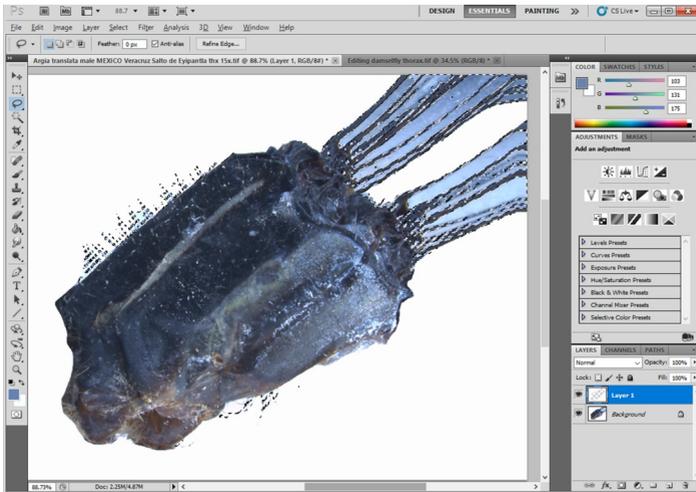


Fig. 9. Removing background of thorax 2.

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).

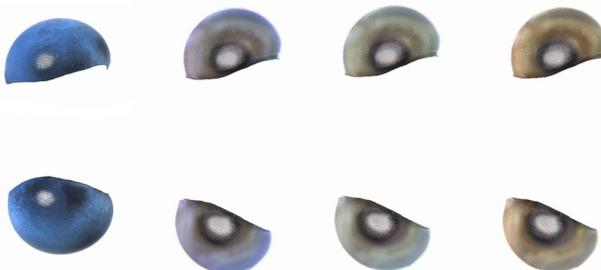


Fig. 13. Sets of eyes rendered using Photoshop. Each eye set was rendered using tools in Photoshop as described above.

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

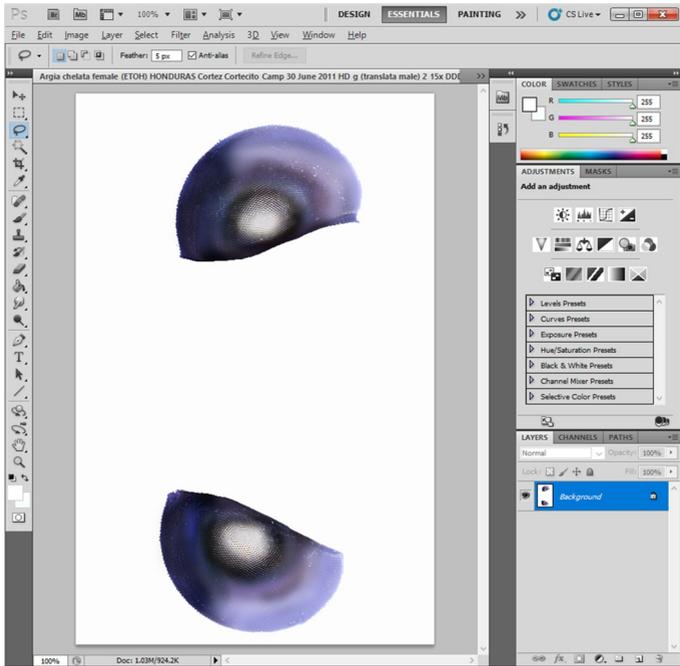


Fig. 14. Rendered (purple) set of eyes for *Argia translata*.

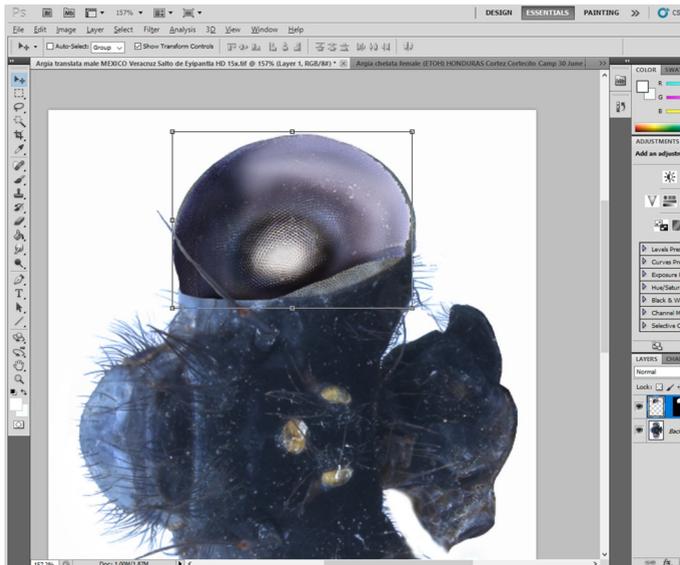


Fig. 15. Placing the rendered (purple) right eye from Fig. 14 into the head of the preserved head of *Argia translata*.

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.

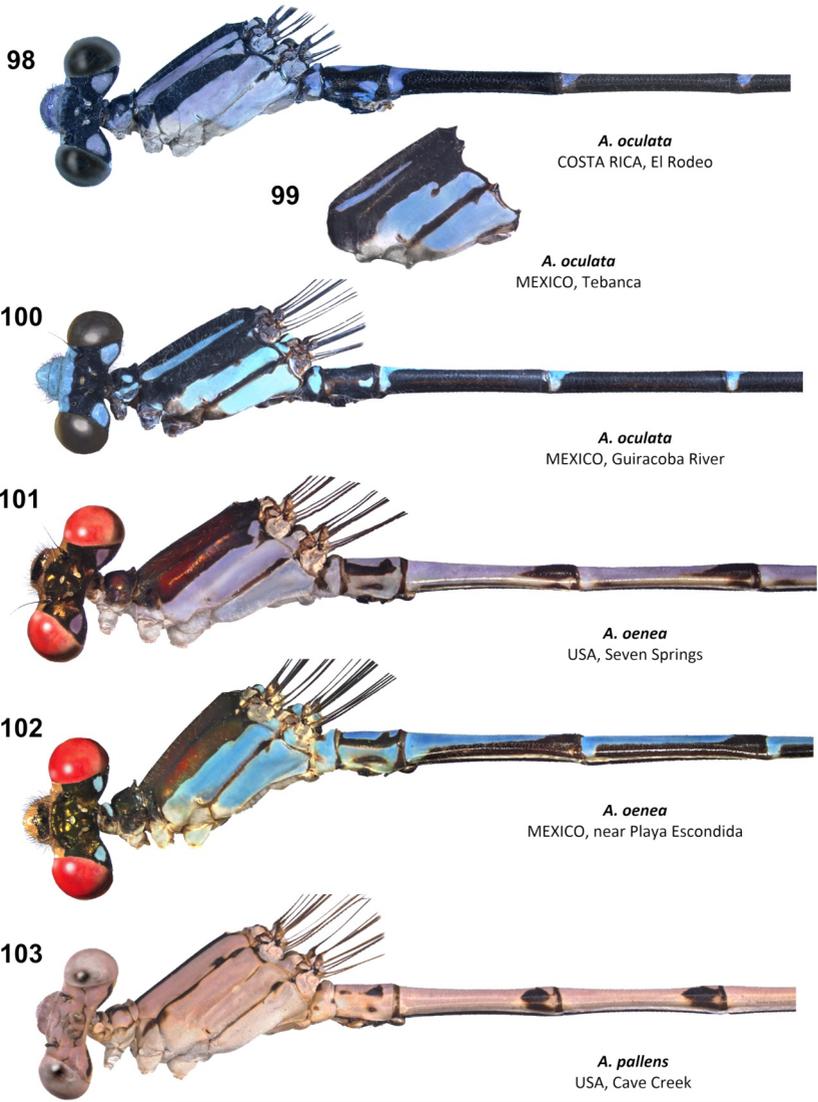


Fig. 16. A finished plate ready for publication.

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Manuscripts submitted to the journal should preferably be in English; alternatively German or French will also be accepted. Every manuscript should be checked by a native speaker of the language in which it is written; if it is not possible for the authors to arrange this, they must inform the Editorial Board on submission of the paper. Authors are encouraged, if possible, to include a version of the abstract in the primary language of the country in which their study was made.

Authors can choose the best way for them to submit their manuscripts between these options: a) via e-mail to the publisher, or b) on a CD, DVD or any other IBM-compatible device. Manuscripts should be prepared in Microsoft Word for Windows.

While preparing the manuscript authors should consider that, although the journal gives some freedom in the style and arrangements of the sections, the editors would like to see the following clearly defined sections: Title (with authors names, physical and e-mail addresses), Abstract, Introduction, Material & Methods, Results, Discussion, Acknowledgments and References. This is a widely used scheme by scientists that everyone should be familiar with. No further instructions are given here, but every author should check the style of the journal.

Authors are advised to avoid any formatting of the text. The manuscripts will be stylised according to the font type and size adopted by the journal. However, check for: a) all species names must be given in italic, b) the authority and year of publication are required on the first appearance of a species name in the text, but not thereafter, and c) citations and reference list must be arranged following the format below.

Reference cited in the text should read as follows: Tillyard (1924), (Tillyard 1924), Swezey & Williams (1942).

The reference list should be prepared according to the following standard:

Swezey, O. & F. Williams, 1942. Dragonflies of Guam. Bernice P. Bishop Museum Bulletin 172: 3-6.

Tillyard, R., 1924. The dragonflies (Order Odonata) of Fiji, with special reference to a collection made by Mr. H.W. Simmonds, F.E.S., on the Island of Viti Levu. Transactions of the Entomological Society London 1923 III-IV: 305-346.

Citations of internet sources should include the date of access.

The manuscript should end with a list of captions to the figures and tables. The latter should be submitted separately from the text preferably as graphics made using one of the Microsoft Office products or as a high resolution picture saved as a .jpg .tif or .ps file. Pictures should be at least 11 cm wide and with a minimum 300 dpi resolution, better 360 dpi. Line drawings and graphics could have 1200 dpi for better details. If you compose many pictures to one figure, please submit the original files as well. Please leave some space in the upper left corner of each picture, to insert a letter (a, b, c...) later. Hand-made drawings should be scanned and submitted electronically. Printed figures sent by the post could be damaged, in which case authors will be asked to resubmit them.

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