Supplemental File 1 — Protocol for Removing the Matrix of a White-Light-Imaged Fossil Using Red and Green Fluorescence to Mask the Matrix.

This procedure is a generalized protocol for using independent fluorescent red and green images of the same photograph under different lighting regimes to generate a mask to remove the matrix surrounding a fluorescent fossil vertebrate to emphasize the vertebrate skeleton. This protocol was specifically completed using Adobe Photoshop Release 19.1.0, but these steps could be completed in other imaging software. This procedure uses masking layers, which are done differently in different software packages. We recommend interested researchers look at https://www.macworld.com/article/2150251/photo-editing-101-master-the-layer-mask.html for comparable procedures in software packages beyond Adobe Photoshop. Finally, we want to highlight that this is one of many possible techniques that can be used to remove the confounding matrix in fossil vertebrates that fluoresce. Not all fossils fluoresce, and some matrix materials fluoresce, so variations on this protocol may be necessary for some fossil imagery. This specific protocol was used to make the images in Figure 2B, D, F, H. Researchers are strongly encouraged to be creative and explore other possible uses and composition for fluorescence imagery with fossil vertebrates.

Step 1.—Take independent images of the specimen under standard reflected light, red fluorescence (under green light), and green fluorescence (under blue light). It is critical that the focus and composition of each image are completely unchanged among the three images.

Step 2.—Correct the exposure and contrast of each individual image. Typically, this involves the automatic or manual manipulation of the *Tone*, *Contrast*, *Color*, and *Levels* (these are all found under IMAGE: ADJUSTMENT in the menu bar). Begin by trying the automatic correction settings for each adjustment. If the automatic correction is satisfactory, continue to the next adjustment. If the automatic correction is undesirable, experiment with variations in the manual controls until you are pleased with the results of each particular adjustment. Pay particular attention to the *Levels* setting in the images taken under fluorescence to ensure that the majority of the black (or near black) regions of the image are truly black (RGB #000000). If the background is not truly black, this will introduce irregularities or imperfections into the final composite image.

Step 3.—Create a new file that is in the Grayscale color space that will be called "fossil.tif" that has the background contents set to "transparent" and has identical dimensions and resolution (i.e., dots per inch/cm) as the source files.

Step 4.—Open the green fluorescent image (image taken under blue light). Use the "magic wand tool" to select any region of the black area of the image. It is important that the contiguous check box is unchecked. We typically sample as a "point sample" and have the tolerance set between 20 and 40, but this will be highly image specific. Next, select *Modify: Feather* from the SELECT heading in the menu bar. We set the feather value between one and three depending on the resolution of the image (increasing the feathering number as resolution increases). Next, use the "paint bucket tool" to fill the selected area with pure black (RGB #000000). Copy the selected area of the image. This procedure smooths any sharp edges in the masked area and should copy a large black area that roughly corresponds to the matrix.

Step 5.—Return to the "fossil.tif" file and paste this black selection into a new layer.

Step 6.—Open the red fluorescent image (image taken under green light). Use the "magic wand tool" to select any region of the black area of the image. It is important that the contiguous check box is unchecked. We typically sample as a "point sample" and have the tolerance set between 20 and 40, but this will be highly image specific. Next, select *Modify: Feather* from the SELECT heading in the menu bar. We set the feather value between one and three depending on the resolution of the image (increasing the feathering number as resolution increases). Next, use the "paint bucket tool" to fill the selected area with pure black (RGB #000000). Copy the selected area of the image.

Step 7.—Return to the "fossil.tif" file and paste this black selection into a new layer (independent of the layer produced in *Step 5)*.

Step 8.—In the "fossil.tif" file, select *Merge Visible* from the LAYER heading in the menu bar to combine the matrix masks from each of the fluorescent images into a single layer that should approximately look like the fossil.

Step 9.—While still in the "fossil.tif" file, select all, and copy the image.

Step 10.—Open the white-light image (image taken under reflected light), and paste the merged layer from the "fossil.tif" file on to the white-light image.

Step 11.—If the image looks to the researcher's satisfaction, select *Flatten Image* from the LAYER heading in the menu bar to mask the matrix from the image. Alternatively, this masking layer could be retained as a *clipping mask* or be enabled as a *layer mask: from transparency* from the LAYER heading in the menu bar. Functionally, they all will result in a similar image in this case, but they have varying degrees of permanence. If the image is not to one's satisfaction, return to *Step 4* and repeat the procedures while altering the "magic wand tool" tolerance and adjusting the feathering value.

Step 12.—Once the image is approximately ready, make any final corrections to the image using the same general techniques described in *Step 2*, and crop the image to remove any vignetting around the margins of the image that are introduced by imaging through a microscope.