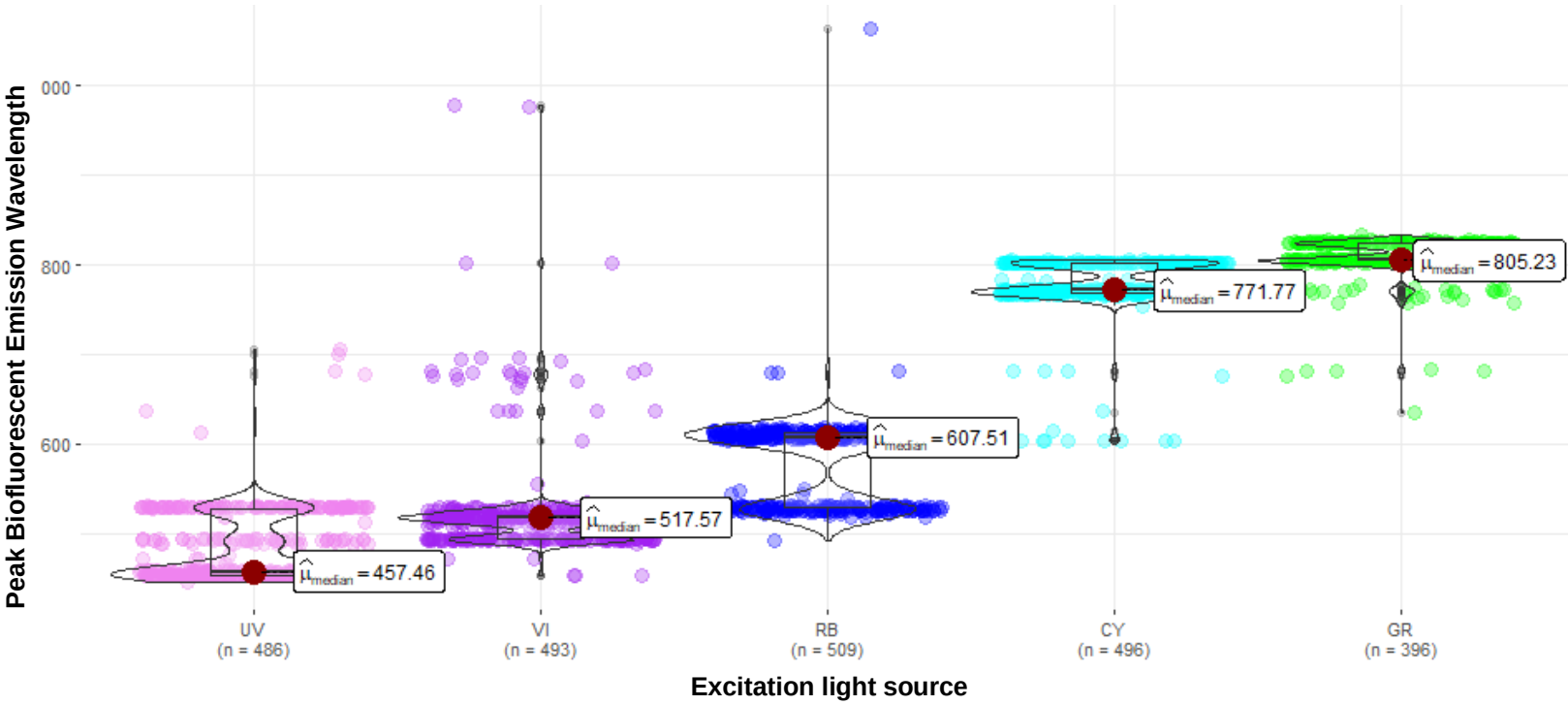
Supplementary Materials for the manuscript:

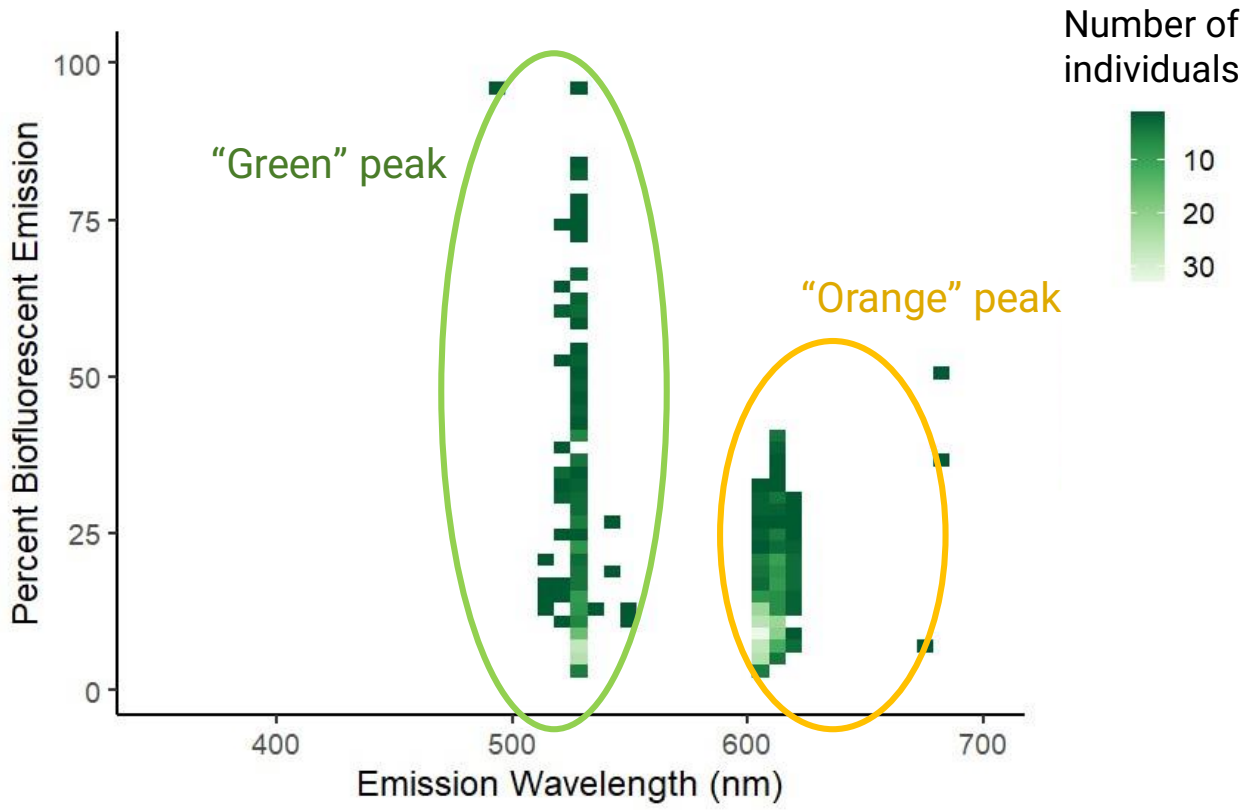
*Evidence for ecological tuning of anuran biofluorescent signals*

Courtney Whitcher, Santiago R Ron, Fernando Ayala-Varela, Andrew Crawford, Valia Herrera-Alva, Ernesto Castillo-Urbina, Felipe Grazziotin, Randi M Bowman, Alan R Lemmon, & Emily Moriarty Lemmon. (2024). *Nature Communications*.

## Supplementary Figures

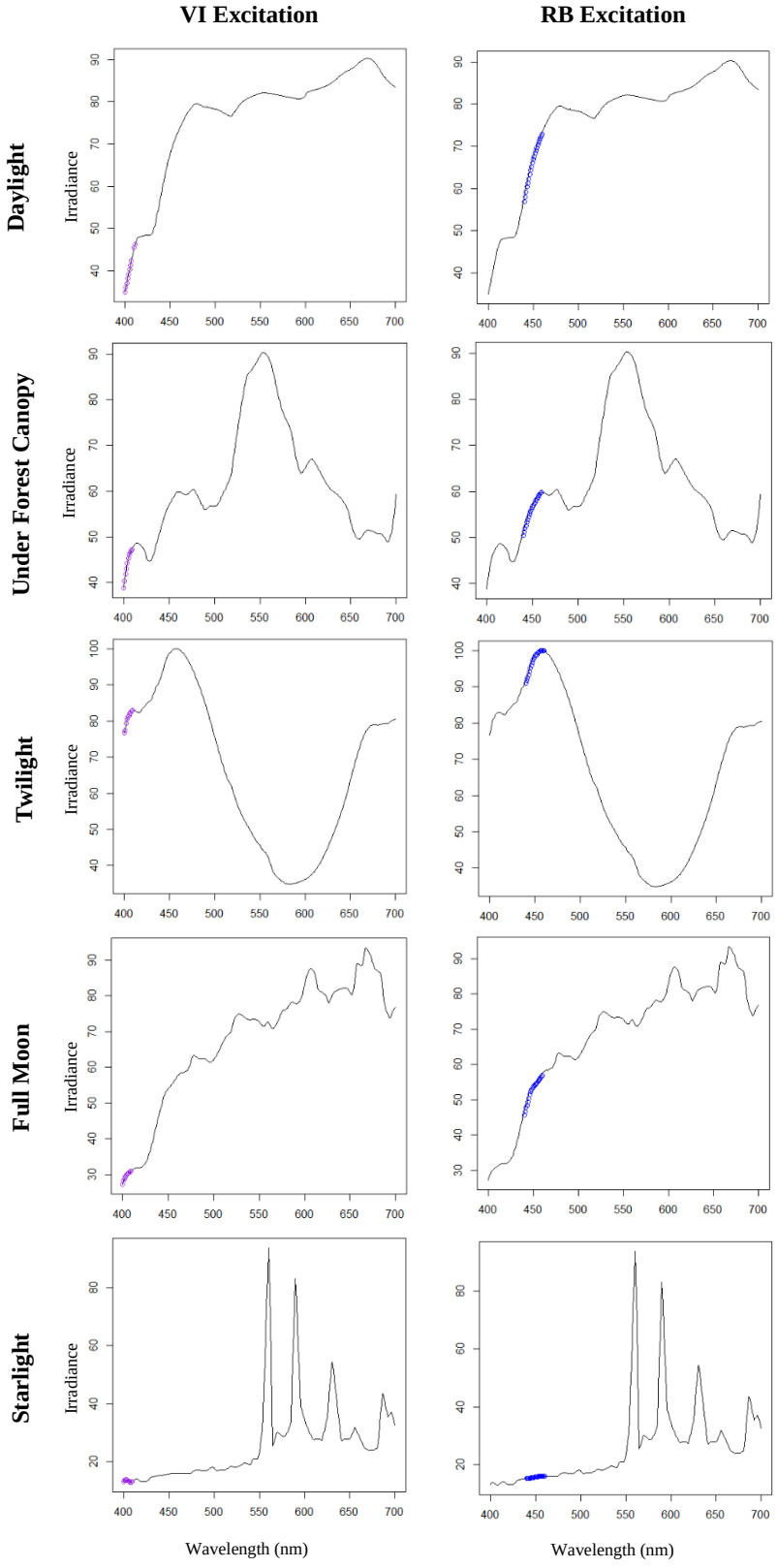



**Supplementary Figure 1. Peak biofluorescent emission by excitation source.** The wavelength of biofluorescent emission by excitation light source: UV – Ultraviolet (360-380 nm), VI – Violet (400-415 nm), RB – Royal blue (440-460 nm), CY – Cyan (490-515 nm), and GR – Green (510-540 nm). Each point represents the peak biofluorescent emission wavelength of a single individual under the particular excitation light source.

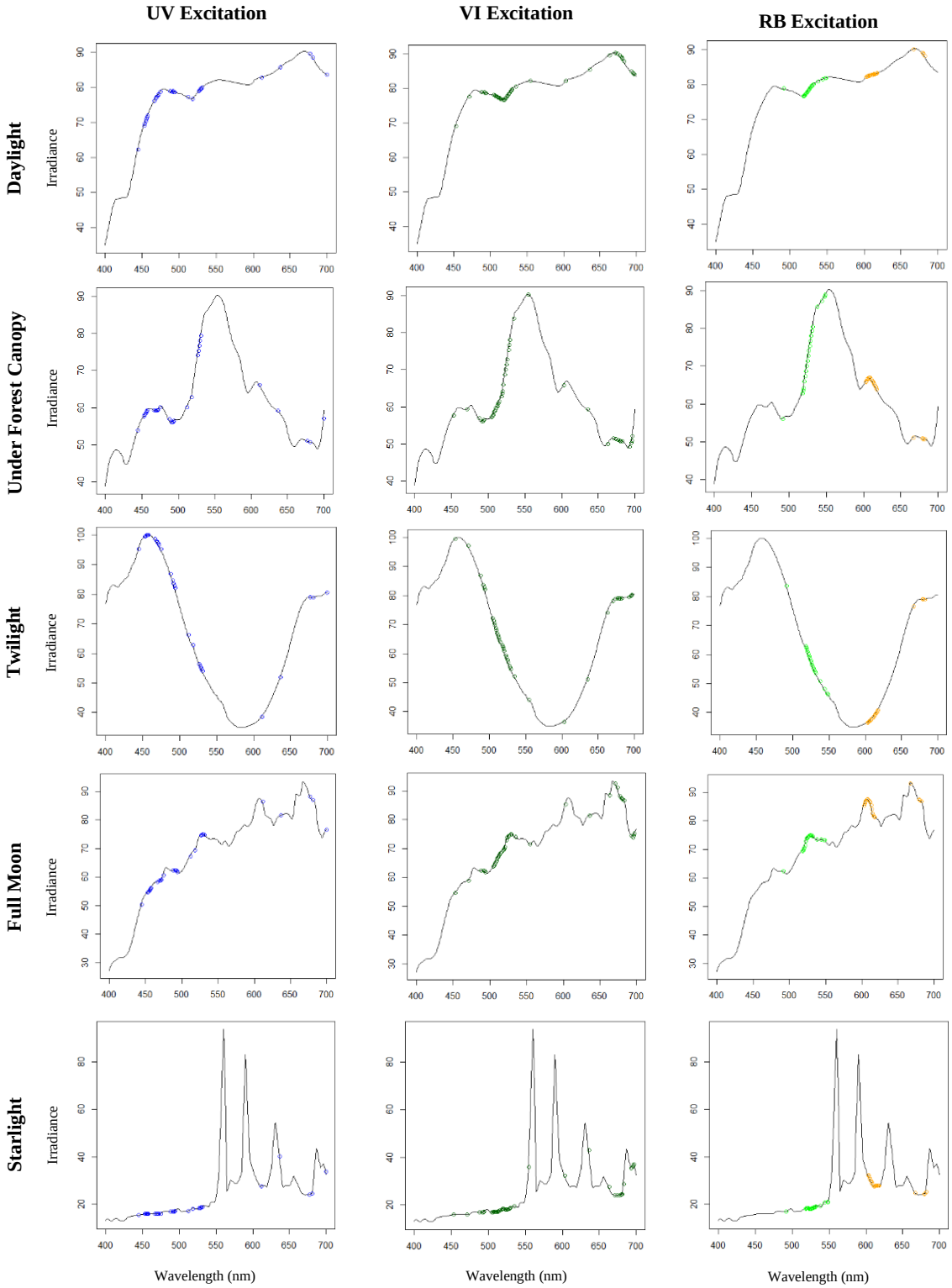



**Supplementary Figure 2. Peak biofluorescent emission wavelengths from blue (440-460 nm) excitation light.** The percent biofluorescent emission by wavelength, where each point is colored by the number of individuals with a maximum percent biofluorescent emission recorded for that wavelength and intensity under the blue (440-460 nm) excitation light source. Lighter green corresponds to more instances of that wavelength and intensity emission. Note the peak emission wavelengths produced by blue light excitation are centered around ~527 nm and ~608 nm. Hence, we refer to these groupings of emission peaks as the "green" and "orange" peaks respectively.

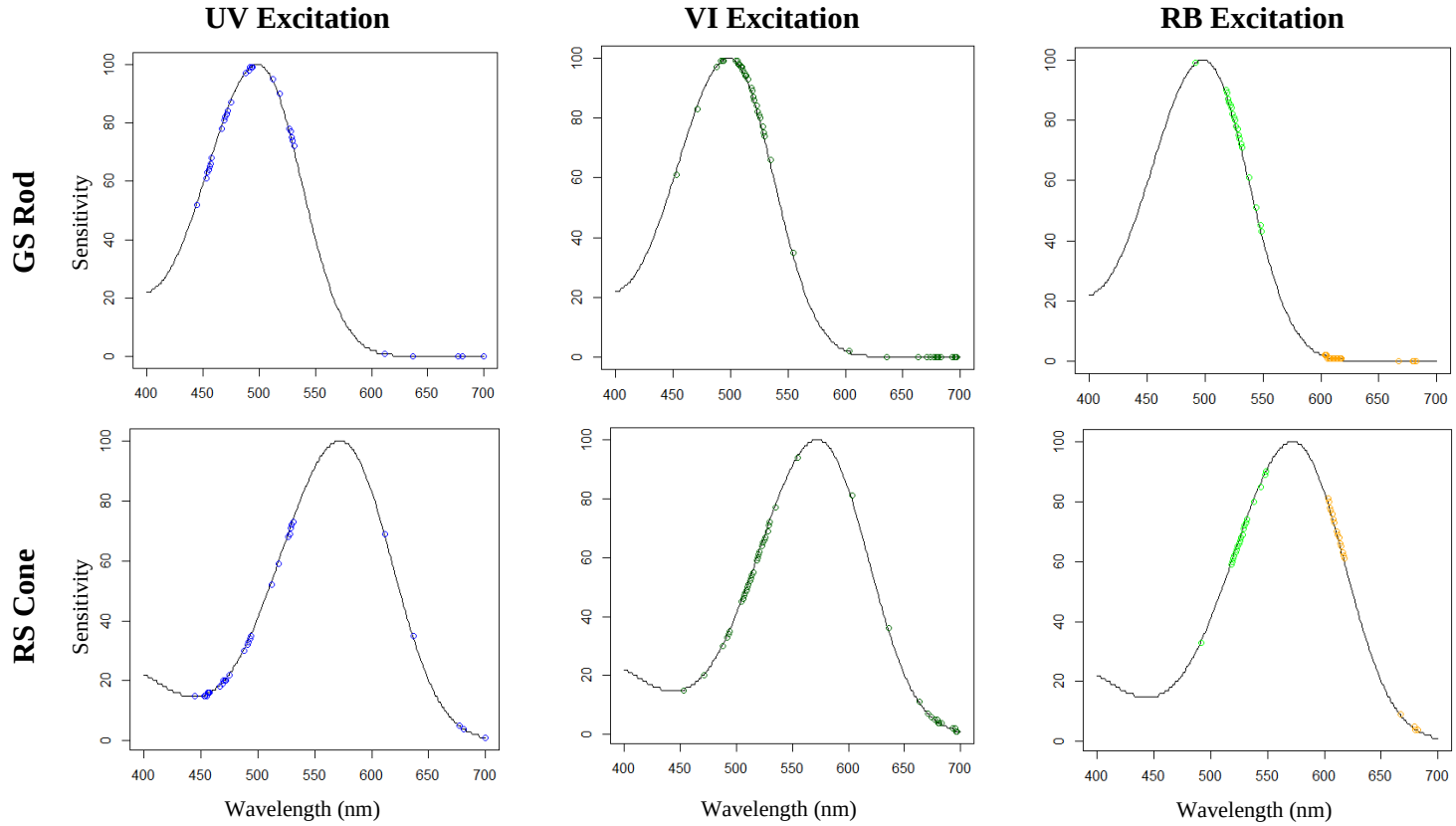
**Supplementary Figure 3. Excitation wavelengths plotted by light environment for each excitation source.** Violet (VI) and Blue (RB) light fluorescent excitation wavelengths compared to irradiance spectrum (black line; digitized with permission from Cronin et al. (2014)) of each light environment. In each panel, the excitation wavelengths of light source are presented as colored circles. Plots visualize the relative irradiance values of the corresponding excitation wavelengths in each light environment. Visualization of potential matching for Criterion 1.



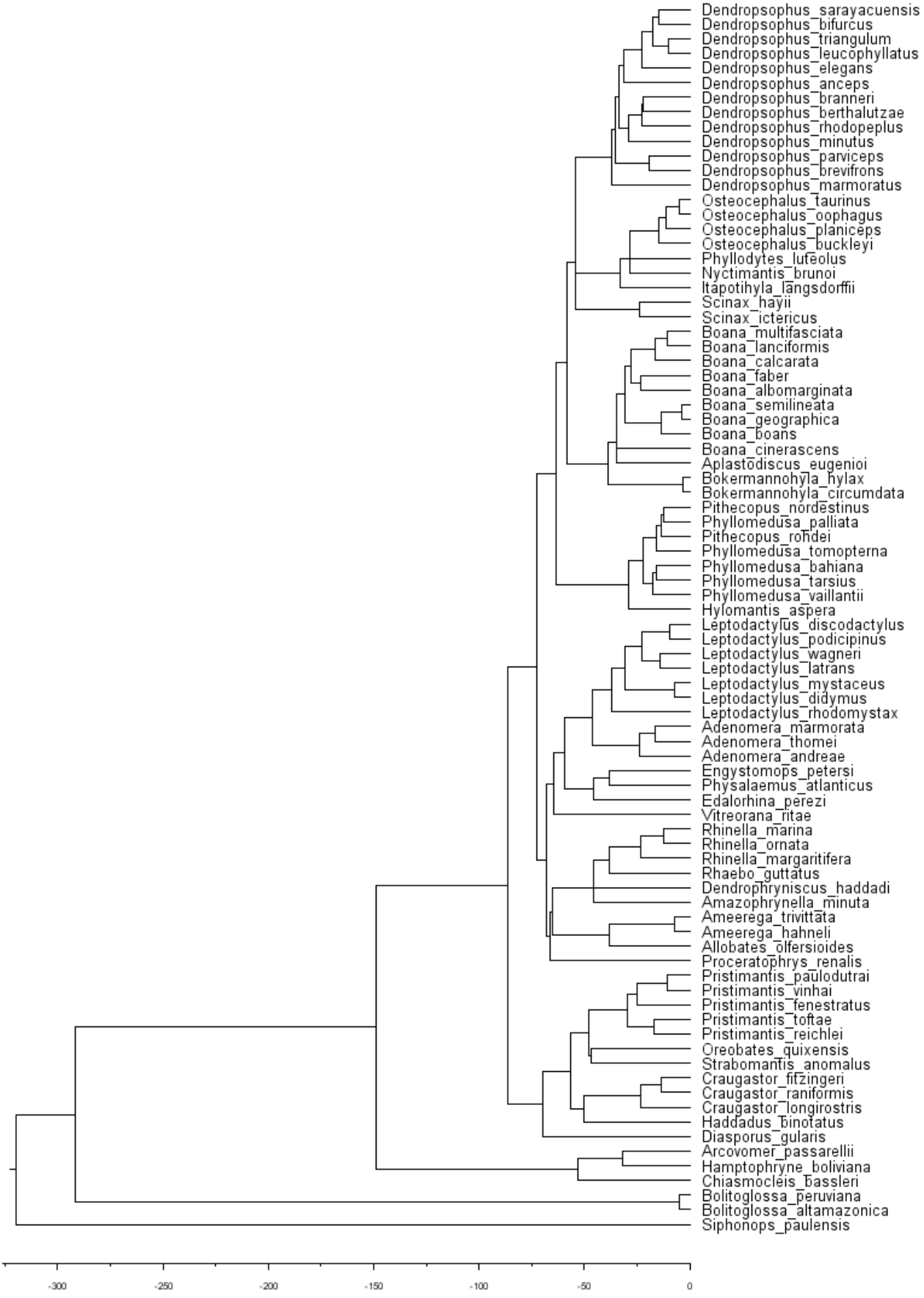
**Supplementary Figure 4. Emission wavelengths plotted by environment for each excitation source.** Ultraviolet (UV), Violet (VI), and Blue (RB) light induced fluorescent emission wavelengths compared to irradiance spectrum (black line; digitized with permission from Cronin et al. (2014)) of each light environment. In each panel, the emission wavelengths of fluorescence under that light source are presented as colored circles. Plots visualize the relative irradiance values of the corresponding emission wavelengths in each environment. Visualization of potential matching for Criterion 2.



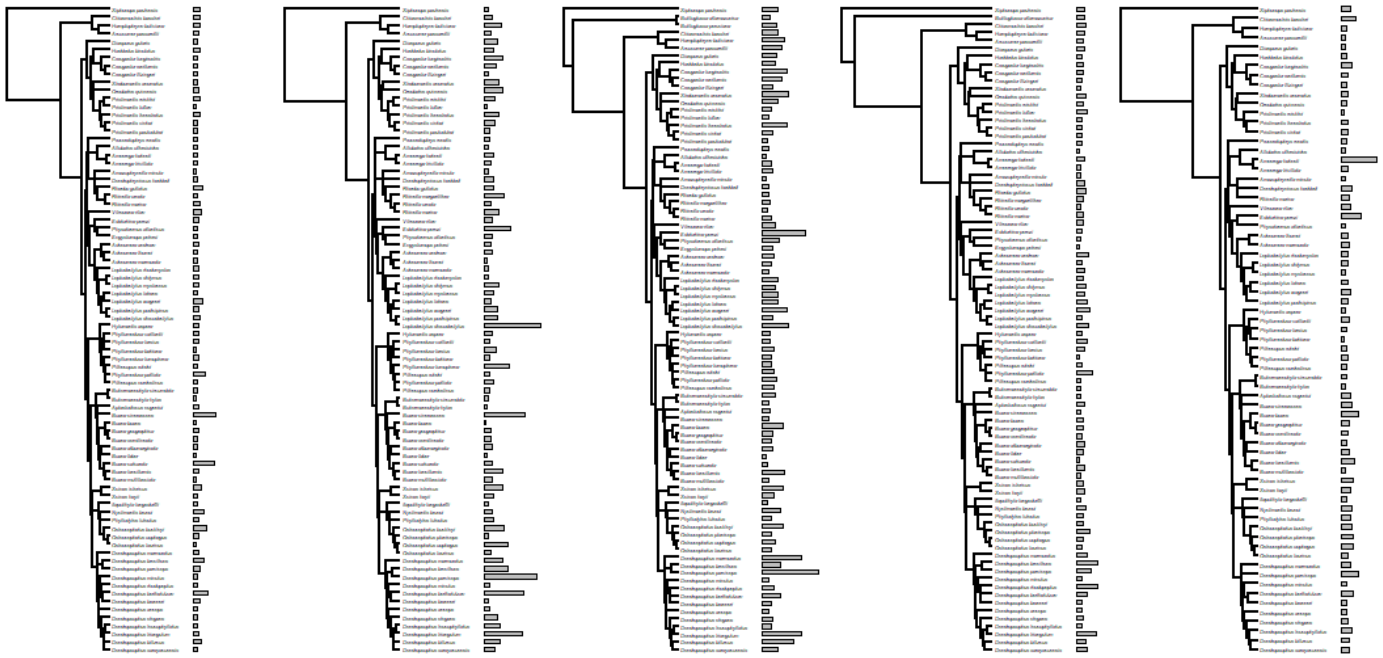
**Supplementary Figure 5. Emission wavelengths plotted by photoreceptor sensitivity for each excitation source.** Ultraviolet (UV), Violet (VI), and Blue (RB) light induced fluorescent emission wavelengths compared to sensitivity spectrum (black line) of each photoreceptor. In each panel, the emission wavelengths of fluorescence under that light source are presented as colored circles. Plots visualize the photoreceptor sensitivity values of the corresponding emission wavelengths induced by each excitation source. Visualization of potential matching for Criterion 3.



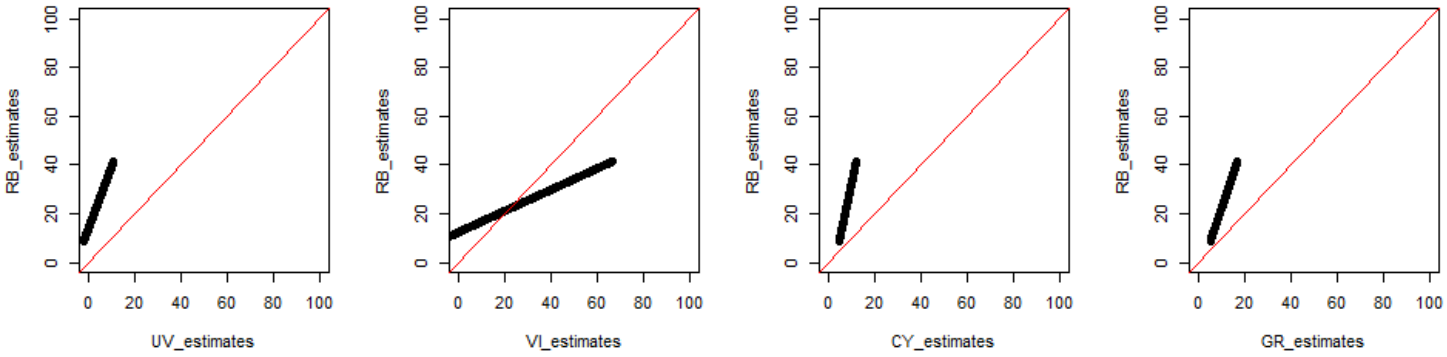
**Supplementary Figure 6. Amphibian phylogeny obtained from Timetree.org.**



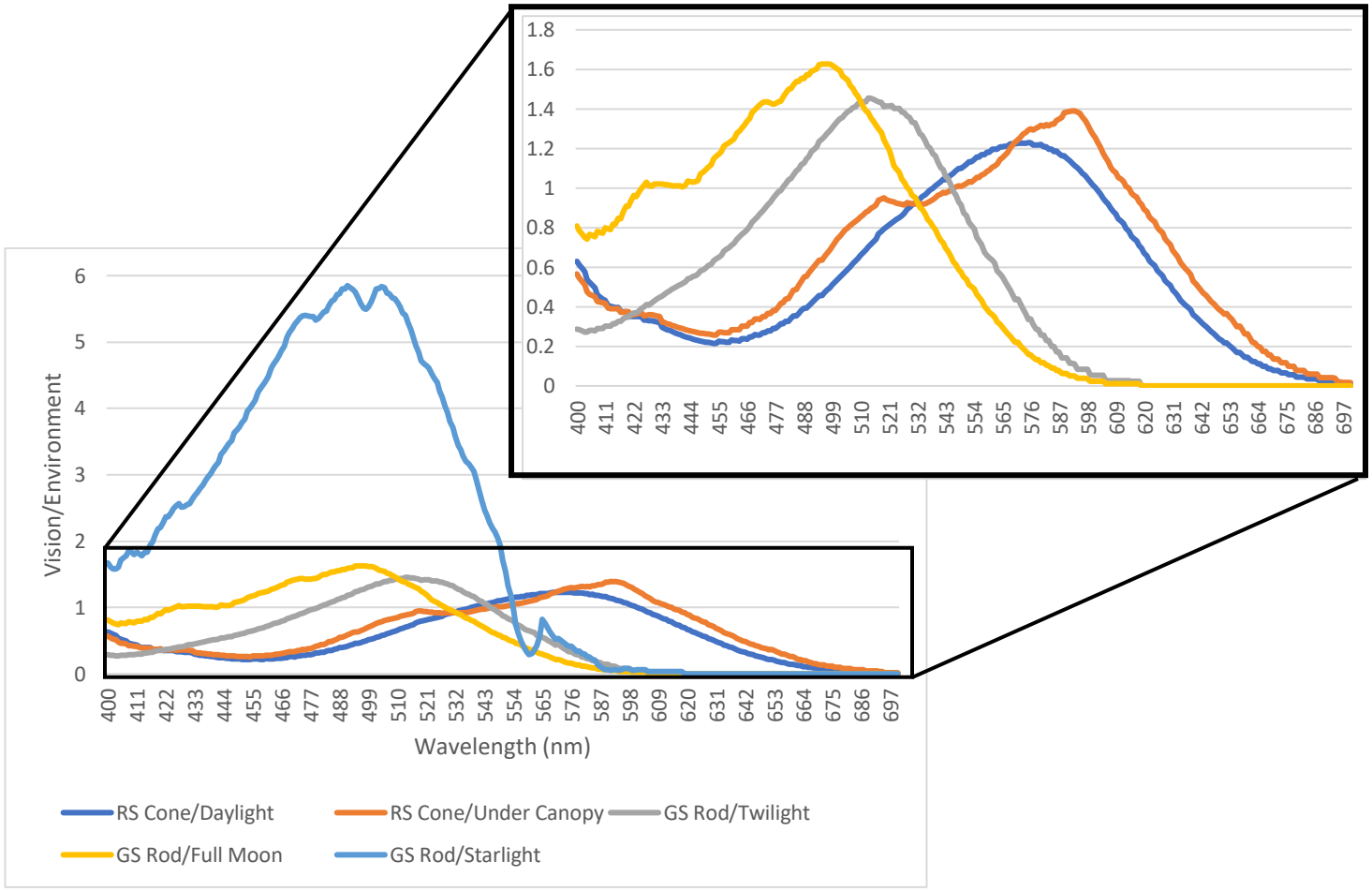
**Supplementary Figure 7. Visualization of Phytools Input for Ancestral State Reconstruction of Percent Biofluorescent Emission by Excitation Source.** Trees are in order of excitation source, left to right: UV – Ultraviolet (360-380 nm), VI – Violet (400-415 nm), RB – Royal blue (440-460 nm), CY – Cyan (490-515 nm), and GR – Green (510-540 nm). Bars to the left of each tip represent the average percent biofluorescent emission for that species.



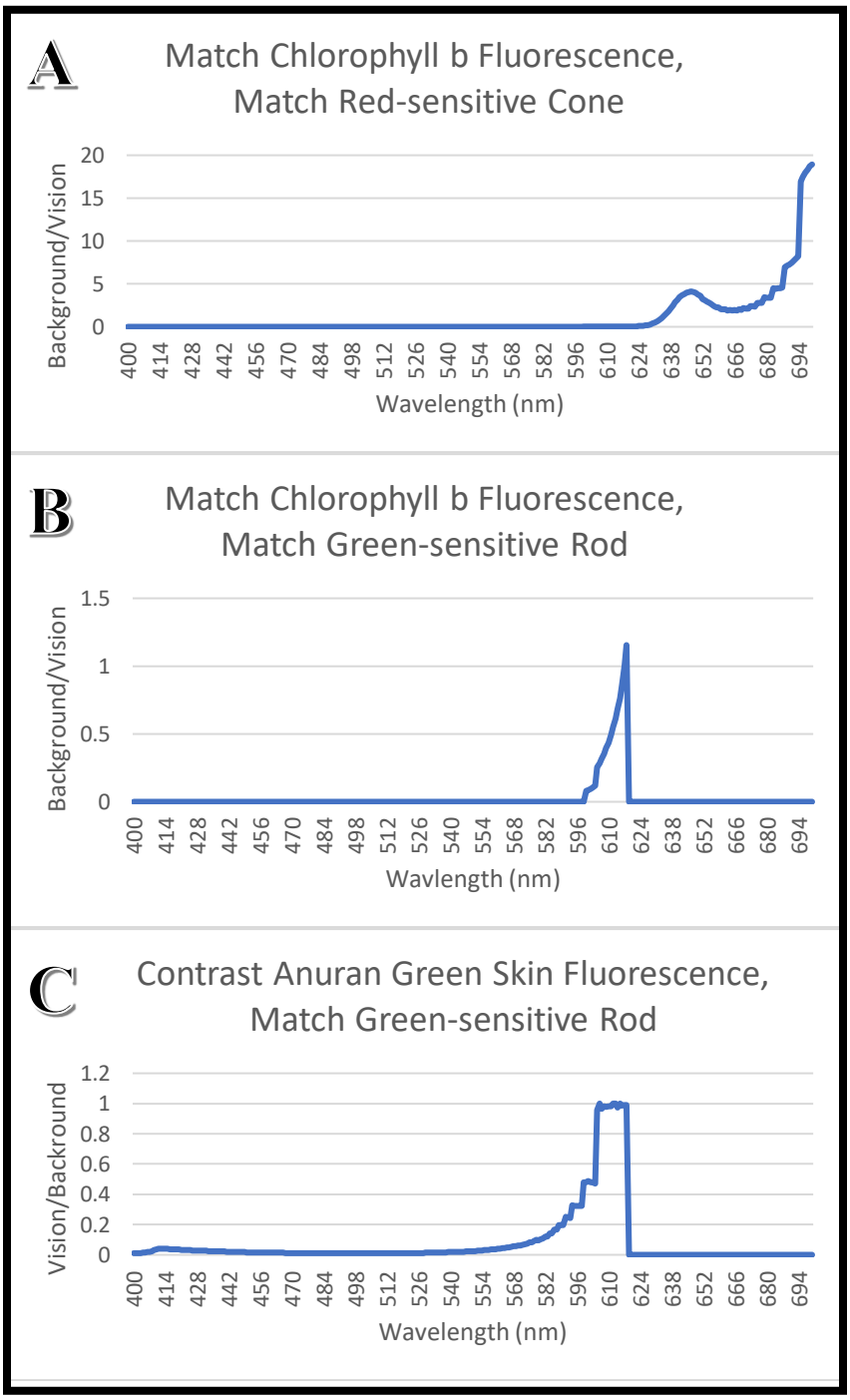
**Supplementary Figure 8. Comparison of estimates of ancestral percent biofluorescent emission by excitation source from 1,000 mcmc iterations.** All ancestral biofluorescent intensity estimates under RB (440-460nm) excitation light (y-axis) are compared to all estimates under each of the four other excitation light sources, panels left to right: UV – Ultraviolet (360-380 nm), VI – Violet (400-415 nm), CY – Cyan (490-515 nm), and GR – Green (510-540 nm). Blue excitation (440-460nm) produced a significantly greater percent fluorescent emission than any other excitation light source in 85.25% of the percent biofluorescent emission trait estimates for the amphibian most recent common ancestor.



**Supplementary Figure 9. Trade off spectra for each of the five light environment and photoreceptor visual sensitivity conditions.** Lines represent the division of the vision sensitivity/environmental irradiance spectra. The wavelength at the peak of each curve represents the wavelength which maximizes match with visual sensitivity and contrast with the background environment. These peak wavelength values were used to set the theta (constrained optimum) parameter in the phylogenetic evolutionary models. The inset figure to the right depicts a zoomed in version of four criteria for additional detail.



**Supplementary Figure 10. Three additional tradeoff spectra for which the blue light-induced orange fluorescent signal was modeled against.** Lines represent the division of the background environment and visual sensitivity spectra. The spectrum in panel A depicts the wavelengths that maximize matching both the peak emission of the fluorescent chlorophyll background environment and the peak Red-sensitive cone visual sensitivity. The spectrum in panel B depicts the wavelengths that maximize matching both the fluorescent chlorophyll b background environment and the Green-sensitive rod visual sensitivity. The spectrum in panel C depicts the wavelengths that maximize contrast with the anuran green skin fluorescence and matching the visual sensitivity of the Green-sensitive rod visual sensitivity. These peak wavelength values were used to set the theta (constrained optimum) parameter in the phylogenetic evolutionary models.



**Supplementary Figure 11. Example of potential ecological significance of blue light-induced orange fluorescence in *Osteocehpalus buckleyi*.** Fluorescent spectra (A) of green biofluorescence from throat (gray line) and orange biofluorescence from flank/vent (blue line). Note that the orange fluorescent peak closely matches the wavelength of highest contrast against the green fluorescent ventral skin (as in the bottom panel of Supp. Fig. 10). (B) Hypothesized ecological significance scenario for blue light-induced fluorescence in this species, where contrast of orange fluorescence of throat against the green fluorescent background of the body may be driving evolution of fluorescent emission wavelengths.