Supplementary Information

The following Supplementary Information contains the experimental methods used to generate the data presented in Figures 1 & 2 of "Comment on Amézquita et al. (2017) "Conspicuousness, color resemblance, and toxicity in geographically diverging mimicry: The pan-Amazonian frog *Allobates femoralis*". The John Carroll University Institutional Animal Care and Use Committee (IACUC protocols 1101, 1700) approved the methods used in the study.

Experimental Methods.

Allobates femoralis alkaloid analysis. Two individuals of *Allobates femoralis* (Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul, MCP 10199–200) were collected by hand from Igarapé Esperança, Reserva Extrativista Riozinho Liberdade, Cruzeiro do Sul, Acre, Brazil and euthanized in the field by pithing. Following death, the skin was immediately removed from each frog and stored in individual 4 mL glass vials with Teflon-lined lids containing 4 mL of 100% methanol. Each skin/methanol extract was subjected to acid-base fractionation (see methods below) prior to analysis using GC-MS (see *Chemical Analysis*).

Alkaloid fractionations were prepared by adding 50 µL of 1 N HCl to 1 mL of the original skin/methanol extract. This combined methanol extract was carefully concentrated with nitrogen gas to 100 µL and then diluted with 200 µL of deionized water. This solution was then extracted four times, each time with 300 µL of hexane. The aqueous layer was then made basic with saturated $NaHCO_3$, followed by extraction 3 times, each time with 300 µL of ethyl acetate. The combined ethyl acetate fractions were dried with anhydrous Na_2SO_4, evaporated carefully with nitrogen gas to dryness, and then reconstituted with methanol to 100 µL.

Benzocaine uptake experiment. One wild-caught individual of *Melanophrynsicus moreirae* (Itatiaia National Park, Serra da Mantiqueira, Rio de Janeiro, Brazil, 22°23′05.88S, 44°40′41.83W), one captive-bred individual of *Dendrobates auratus*, and one wild-caught individual of *Lithobates clamitans* were euthanized in the lab by oral administration of 10 µL of liquid Orajel ® (Church & Dwight Co., Inc., Ewing, New Jersey, USA) containing 20% benzocaine, delivered using a micropipette. Following death, the skin was immediately removed from each frog and stored in individual 4 mL glass vials with Teflon-lined lids containing 1 mL of 100% methanol. Each skin/methanol extract was directly analyzed using GC-MS (without acid-base fractionation, as in Amézquita *et al*. 2017), following the methods described below.

Chemical Analysis. Each skin extract was examined using Gas Chromatography-Mass Spectrometry (GC-MS) using a temperature program from 100 to 280˚C at the rate of 10˚C per minute with helium as a carrier gas (1 ml/min). The GC-MS was a Varian 3900 GC coupled with a Varian Saturn 2100 T ion trap MS with a 30 m x 0.25 mm i.d. Varian Factor Four VF-5 ms fused silica column. All samples were analyzed using both electron impact mass spectrometry (EI-MS) and chemical ionization mass spectrometry (CI-MS), with methanol as the ionizing reagent. The relative amount of benzocaine and tricyclic **265S**, pumiliotoxin **267C**, allopumiliotoxin **323B**, and bufotenine for *M. moreirae* was calculated by comparing peak areas using a Varian MS Workstation V.6.9 SPI.