

Dear authors and editor, I have finished my revision of the paper entitled: “Color polymorphism and conspicuousness do not increase speciation rates in Lacertids”. The main goal of the study was to test the effect of conspicuous colorations and/or color polymorphism on speciation rates of lacertid lizards (Lacertidae). A second objective of the study was to evaluate the evolutionary histories of conspicuous colorations and color polymorphism in Lacertidae. As I understand (lines 126-128), the author’s main motivation for sharing their results is because they found an opposite result (no association between color polymorphism and speciation rates) to a previous study addressing the same main question in the same group (Broke et al., 2021)<sup>1</sup> and even to a study testing the morphic speciation hypothesis in birds (Hugall and Stuart-Fox 2012)<sup>2</sup> (Line 320-322). Certainly, the non-association between color polymorphism and speciation rates founded in this work, opens up the opportunity to debate the generality of the morphic speciation hypothesis (Line 26-29). However, I have two major concerns about the methods design, as I describe below.

### **Major concerns.**

**1. Coding color polymorphism.** The authors mention (Supplementary Methods) that the difference of State Dependent Diversification model’s results between their study and Brock et al. study<sup>1</sup> lies in the way of coding polymorphism. Yet, the authors do not provide a detailed definition (with citations) of color polymorphism (see lines 50-52, and lines 154-155), and it is not entirely clear how they coded color polymorphism in lacertids. The definition of color polymorphism in previous studies testing the morphic speciation hypothesis<sup>1-3</sup> have in common that a color polymorphism implies the presence of discrete colors in the same trait (in the same body region) within an interbreeding population, and that the color polymorphism is genetically determined by different alleles of a single gene or two tightly genes. Furthermore, ventral color polymorphism (white/yellow/orange) in the same population of different lacertid species is linked to two small regulatory regions (sepiapterin reductase [SPR] and beta-carotene oxygenase [BCO2])<sup>4</sup>. Under that theoretical framework, it makes perfect sense to me to use only throat coloration to code color polymorphism in lacertids and other lizards (as previously was performed<sup>1,3</sup>). By contrast, I disagree with “coding polymorphism as the presence of discrete states on any conspicuous coloration” (Line 351-352). The blue/green colors, used to code as polymorphic species to *Adolfus alleni*, *A. jacksoni*, *Darevskia chloragaster*, and *Gallotia atlantica* (Table S2), for example, are not associated with the SPR/BCO2 regulatory regions. Further, blue/green colors can occur even in the same individual, associated with its ontogeny or body temperature. The same male of the fence lizard *Sceloporus undulatus*, for example, shows ventral blue coloration at 29°C, but green coloration at 25°C<sup>5</sup>. So, I encourage to the authors to use a clearer code of genetically determined color polymorphism.

**2. Ultrametric tree.** The authors decided not to use the most recent maximum clade credibility (MCC) tree for lacertids (Broke et al. 2021)<sup>1</sup> to perform their phylogenetic analyses, because that phylogenetic tree “was built with only five genes and some clades were at odd with all the previous researches” (Supplementary Methods). Instead, the authors chose another phylogenetic tree (Garcia-Porta et al. 2019) as backbone phylogeny and randomly added 85 species (30% of the species used in the study) not included in the original phylogenetic tree. What are the advantages of running the phylogenetic analyses on a phylogenetic tree where the 30% of the species were added randomly, over a phylogenetic tree built with five genes? How do randomly added species impact the results, taking into account, for example, that the stochastic character mapping is dependent of the character states on the tips? I

suggest the authors to rerun the analysis on the phylogenetic tree of Garcia-Porta et al. (2019) without randomly adding more species, and also rerun the analysis on the MCC tree of Broke et al. (2021). Finally, to account for phylogenetic uncertainty, the authors can rerun the analyses across individually sampled trees from the posterior distribution.

### Minor comments

**Line 79-84.** It is not clear to me how disassortative mating could maintain intrapopulation polymorphisms (and drives slow speciation rates) in the scenario where the population previously lost a morph, during the colonization of a new area (as is explained in the lines 62-66).

**Line 116.** There is not data for all the species (in the author's database), see for example *Gallotia goliath*

**Line 139-141.** The authors removed strictly insular species of their data acquisition list because they believe that geographic isolation is more important than conspicuous colorations and/or color polymorphism for speciation. However, the colonization of islands are ideal scenarios for the loss of one morph and rapid phenotypic divergence (morph speciation). I suggest that the authors also include strictly insular species in their analyses.

**Line 159-162.** These sentences are not clear to me. Can the same species present at the same time non-conspicuous and conspicuous colors?

**Line 163-164.** Here the authors describe that there are not polymorphic species without conspicuous coloration, but in the line 159-160, they say that some species are polymorphic with non-conspicuous color, which is confusing.

### References

1. Brock, K. M., McTavish, E. J. & Edwards, D. L. Color Polymorphism is a Driver of Diversification in the Lizard Family Lacertidae. *Syst Biol* **71**, 24–39 (2021).
2. Hugall, A. F. & Stuart-Fox, D. Accelerated speciation in colour-polymorphic birds. *Nature* **2012** *485:7400* **485**, 631–634 (2012).
3. Corl, A., Davis, A. R., Kuchta, S. R. & Sinervo, B. Selective loss of polymorphic mating types is associated with rapid phenotypic evolution during morphic speciation. *Proc Natl Acad Sci U S A* **107**, 4254–4259 (2010).
4. Andrade, P. *et al.* Regulatory changes in pterin and carotenoid genes underlie balanced color polymorphisms in the wall lizard. *Proc Natl Acad Sci U S A* **116**, 5633–5642 (2019).
5. Ligon, R. A. & McCartney, K. L. Biochemical regulation of pigment motility in vertebrate chromatophores: a review of physiological color change mechanisms. *Curr Zool* **62**, 237–252 (2016).