Dear editor,
After much delay, for which we apologize, we submit a revised version of our manuscript. Please find below a detailed point-by-point reply to the comments from the editor and reviewers. We hope this new version will be acceptable for publication in PCI Journal. Thank again for the great editing and reviewing.
Pierre-André Crochet, on behalf of all authors.

Response to editor

I have carefully read the pre-print titled Color polymorphism and conspicuousness do not increase speciation rates in Lacertids and obtained two expert reviews. I would first like to apologize for the exceptionally long time it has taken to provide a decision. The work was originally being handled by another recommender who decided to abandon the process as they were unable to secure any review. In total 30 invitations were sent out to secure two reviews. This is why the process was exceptionally long and I apologize for this.
No need to apologize and we fully appreciate the quality of the editing process of PCI

Nonetheless, you will see that both reviewers did an excellent job and provide very good suggestions to improve the work.
We certainly agree, and as a result we have 1) included the comparison with Brock et al, which was previously mainly in sup mat, in the main text (mat and met, results and discussion) and 2) have considerably extended the discussion to address several points raised by reviewer 1. In fact, although we disagree with many of the comments from reviewer 1 (as can be seen in our response letter), we found all these comments to be highly relevant. They all addressed important issues that needed to be better discussed in our MS and we hope that this new version has been improved by taking these comments into account.

I must agree with the comment that much greater clarity is required regarding the coding of the coloration, especially since you found this to be one of the reasons for the discrepancy between previously published resutls and your own. I tend to agree with the reviewer regarding the fact that coloration that is not in the same body region cannot be taken as polymorphism, as it could be argued that colors in different body regions could potentially play different roles in signalling or intra-sexual competition.
See our responses to reviewer 1 (below). We have modified several sections of the text to clarify these points.

Secondly, I also agree with the surprise expressed by one reviewer regarding the choice of phylogeny used. Nearly 30% of the species that you analysed were inserted into the tree based on taxonomic information, which is rather high. How many would need to be inserted in the other published phylogeny? If a smaller number why not use that one?
We have extensively addressed this concern in our response to reviewer 1 (below) and in the discussion (l 398 and below). We hope our strategy is now more convincing.

Finally, a comment of my own has to do with sampling. In the Introduction you state the family is split into two main clades, Gallotiinae, which contains a few and often insular species, and Lacertinae, which contains most lacertids. Then, in the last paragraph of the Introduction you state you used "the coloration data of all the species of this family, to address two questions". However, in the Methods you state that you removed strictly insular species. This suggests you have rather sub-sampled Gallotiinae and thus also that you don't really have a good representation of "all species from the family".
This is correct. We have amended this sentence (now line121-122). Thanks for this comment.

Response to reviewer 1

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)[1] and even to a study testing the morphic speciation hypothesis in birds (Hugall and Stuart-Fox 2012)[2] (Line 320-322).

To be fair, we initiated this work long before we became aware of the study of Brock et al. and finding out that another team had done the exact same study as us came as a bit of a shock, especially for the first author (TdS) who was at the time completing his PhD. Anyway, it has always been our aim to publish our own results, even if we had only repeated Brock's et al. results, as we believe in the value of repeating scientific results in biology. Please also note that we test for the influence of sexual colorations on diversification, something that was absent from Brock et al. We agree that lines 126-128 do not convey this idea and we have slightly amended it (now lines 132-135).

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.
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 [1] lies in in the way of coding polymorphism.

True, but there is another source of disagreement between our results and Brock et al' results. The SDD models did not perform properly in any analysis, as 6 of the 8 estimated parameters were at the boundary of the allowed interval in our HISSE model (all four extinction rates and two transition rates are zero, using Brock et al.'s data and character states). This precludes any conclusion on the role of polymorphism on diversification.
When we applied more robust methods (STRAPP and Fiss), we found that polymorphism did not affect speciation rates, whichever data and species used (including Brock et al' coloration – as stated lines 362). We have made this clearer now in the main text.

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[1–3] 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.

We also define colour polymorphism as "the presence of discrete colors in the same trait (in the same body region) within an interbreeding population", so we agree with the reviewer here, as should now be clearer lines 161-164 (see also lines 167-172). We also agree that polymorphism sensu stricto also implies that such variation is genetically determined (see Jamie and Meier 2020 TREE 35, 795-808 for ex.) but we disagree that the morphic speciation hypothesis requires the morphs to be determined by a single genomic region in all species under consideration. In fact, the morphic speciation hypothesis

has not been framed in reference to any type of genetic basis of the phenotypic variation and some of its funding papers even allow for the possibility that the changes in morph frequency have no genetic basis (West-Eberhard 1986 PNAS 83, 1388-1392). However, as stressed by Jamie and Meier again, "Polymorphisms are expected to have a simple genetic basis": "If a polymorphism were based on multiple unlinked genes", mating among morphs would erode the distinctness of each morph. To sum up, we define polymorphism the same way as the reviewer, which implies that our polymorphisms must all have a simple genetic basis, even if we don't necessarily agree that testing the morphing speciation hypothesis implies selecting only variation that have be determined by the same single or two tightly genes that would control polymorphism in all species.

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])[4]. 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 [1,3]).

This is where we disagree. Clearly, not all polymorphism in Lacertidae is linked to throat coloration. As an example, consider the photos below of adult males from a single population of *Podarcis lusitanicus* (included in *P. guadarramae* in Brock et al. and in our study as the species was not recognised at the time):

We interpret this as an example of the "classical" red / white polymorphism in *Podarcis* that is only expressed on the belly (and not the throat) in this species (and the closely related *P. guadarramae*). We don't see any reason why a test of the morphic speciation hypothesis in Lacertidae would require ignoring such polymorphism and treat this species as monomorphic, as has been done in Brock et al.

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[5]. So, I encourage to the authors to use a clearer code of genetically determined color polymorphism.

Again, we disagree here that "*blue/green colors can occur even in the same individual, associated with its ontogeny or body temperature"* in Lacertidae. We are well aware of changes in coloration within individuals in Agamidae or Phyllodactylidae (we are not familiar with Phrynosomatidae, the family of the genus *Sceloporus*) but we have never seen any change in ventral coloration within individuals in adults Lacertidae. Coloration can change ontogenically (but this is true for throat coloration as well, which is not expressed in juveniles as in adults) but we have restricted our definition of polymorphism to distinct coloration in the same sex and age class (lines 156-158), excluding seasonally variable coloration (in many Podarcis species, males are green in spring but brown in autumn). We maintain that the variation in ventral coloration of e.g. *D. chlorogaster* is better treated as polymorphic. Adult males in spring can have yellow, green or yellow/green underparts (see photos below). For us, treating this species as monomorphic because we are unsure of the genetic determinism of the colour variation is "more wrong" than treating it as polymorphic. As a side comment, we are not aware of any work linking the ventral polymorphism in Darevskia (many species of Darevskia were treated as polymorphic in Brock et al) to variations in the SPR/BCO2 regulatory regions…

Adult males of Darevskia chlorogaster*, all from the same population in the Talysh Mountains of Azerbaidjan, in late May. It is clear that males can be green (left), green/yellow (centre) or yellow (right). Green males seem to have consistently paler throat (greenish white) than yellow males (photos by the authors).*

To sum up, we agree with the reviewer on the definition of what constitutes a polymorphism but we do not agree that testing the morphic speciation in Lacertidae is better done by restricting polymorphism to throat colour polymorphism determined by variation in a single regulatory region.
In addition, we cannot find any evidence that all the colour polymorphism coded and used by Brock et al. are really determined by the same gene regions as in *Podarcis*: to our knowledge, the determinism of throat colour polymorphism in the genera *Darevskia* or *Anatololacerta* or *Iberolacerta* (among others) remains unstudied.

2. Ultrametric tree. The authors decided not to use the most recent maximum clade credibility (MCC) tree for lacertids (Broke et al. 2021)[1] 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?

This comment sounds like we chose one incomplete but well supported tree against one complete but less well-supported tree. This is not the case: Garcia-Porta et al's tree include 244 species while Brock et al's tree include 263 species (both including parthenogenetic or insular species that we have excluded), out of 295 species in our dataset. Using Brock et al.'s tree, we would have 72 species to add to their phylogeny (species in our dataset but not in Brock et al's trees) against 85 using Garcia-Porta et al.'s tree. Among these species to add to one or the other phylogeny, many were added by us using other previously published phylogenies devoted to particular genera or species-groups. The species that we added randomly (randomly placed within their genus) are currently 68. Using Brock et al.'s phylogeny, this number would decrease to 57, since the species included in our dataset AND present in Brock et al. AND added randomly in our study (ie not using another study as source of phylogeny) are only 11 (see Table S1).
To sum up, switching from Garcia-Porta et al's tree to Brock et al's tree, the number of randomly added species would decrease from 68 to 57. Considering that Garcia-Porta et al's backbone is based on genomic data while Brock et al's tree, based on a small amount of sequence data, has obtained some spurious relationships, we clearly prefer to retain our analyses.

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.

To assess how randomly added species impact the results, we did all our analyses (and thus base our conclusions) on 100 trees (100 repetitions of the random inclusion process) on which all subsequent analyses were done (as explained lines 181-182, 198-199, 244-246). We are thus confident that our conclusions are robust to this process. We also rerun our analysis without the semi-randomly added species and the results were the same.
It should be noted that in their article, Brock et al. also repeated their analysis on the tree of Garcia-Porta et al. 2019 without founding any change in their results. This is the reason why we did not repeat our analysis on the tree built by Broke et al. (2021). This observation also supports the idea that the differences of results between with our works come from the methods and package used to investigate the speciation rates rather than differences of phylogenetic trees. This is not detailed in the methods, results and discussion in this new version.

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).

Lines 62-66 and lines 79-84 belong to different sections. Lines 62-66 belong to a section where we explain the principles of the morphic speciation model as formulated by its proponents, while lines 79-84 belong to a section where we provide alternative arguments that counter the morphic speciation hypothesis. Disassortative mate preferences based on color morph is known to have the potential to hamper ecological specialization by enhancing the homogenization of genomic backgrounds; ultimately preventing ecological speciation, as reported in *Heliconia numata* (Chouteau et al. 2017).
We modified lines 75-89 (of the revised version) to better reflect this articulation and clarify this section.

Line 116. There is not data for all the species (in the author's database), see for example Gallotia goliath
True. As a matter of fact, G. goliath is almost certainly extinct (it is currently only known from fossils and subfossils). We were unable to find information in coloration for several species, and other species were excluded as explained in Mat and Met. We have modified this sentence (now lines 121-122).

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 (morphic speciation). I suggest that the authors also include strictly insular species in their analyses.
In Lacertids, no pair of closely-related species occur within one island: there is no reported instance of intra-island speciation. Insular species thus all diverged in full allopatry and speciation is the result of a long geographical isolation after island colonisation or isolation.
In such cases, we do not believe that geographic isolation is more important that coloration for speciation: we observe that species status of insular species of Lacertidae is assessed based on divergence time from mainland relatives: insular populations that are not long-diverged are treated as subspecies, irrespectively of their phenotypic divergence. For example, the extremely variable insular populations of *Podarcis lilfordi* or *P. pityusensis*, that differ tremendously in coloration from islets to islets, are all treated as conspecific because their isolation is recent, while many species have been raised to species rank on the basis of their divergence time only. In other words, the process that leads to insular species recognition is a long divergence in allopatry, and is totally independent of phenotypic changes. The way insular populations are ranked as species or subspecies is thus mostly independent of their phenotypic evolution. We thus maintain that insular species must be excluded, because their speciation (at least as currently assessed) is independent of their phenotypic divergence and only depends on the timing of their isolation on islands. We do not believe that this isolation time can be affected by their amount of phenotypic divergence.
We thus maintain that strictly insular species (species endemic to islands) should be excluded.

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.
Sorry if this was confusing. We have now reformulated this section (now lines 168-172) and hope it is now clear.

Response to reviewer 2

In this manuscript, the authors investigate if increased rates of speciation can be associated with conspicuous body colouration and colour polymorphisms. Using the Lacertid lizards as their study

system, they find that these traits are not related to speciation. They also show that these colouration patterns were gained and lost several times across the phylogeny, hinting at the evolutionary lability of colour traits.
Overall, the manuscript is well written. In particular, the hypotheses stated in the introduction are clear, and the diversification analysis was well described. The result found in this study showing a lack of an effect of colour polymorphisms on speciation rates is contradictory to findings in a similar recent study. The authors provide a thorough comparison of the methodology used in both studies, and discuss at length the reasons for the differing results. This is presented nicely in the supplementary methods and results. It is fairly uncommon to see this type of discussion and I think it is well thought out. The overall results are discussed within a broad context, for example the discussion of pre and post-zygotic isolation is interesting, but also importantly the discussion doesn't try to stretch the results too far. I suggest some minor points that could be added or edited for clarity.
Thanks a lot for the positive feedback and for the suggestions for improvement.

Some photos or a figure showing the variation in colouration in the lacertid lizards would be nice, particularly showing an example of conspicuous colouration. This would also help to clarify lines 150-151, where a 'species was considered as having conspicuous coloration if…the side was not white'. In some other species and habitats, a bright white colour would be considered as conspicuous so there could be some extra explanation here.
The definition of conspicuous as neither white nor grey only applies to ventral coloration and is quite common in lizard studies. But we agree that some examples would be nice and we now provide in Figure S1 some examples of polymorphisms and conspicuous colorations.

In the final paragraph of the introduction, it could be made clearer what type of data is being used (i.e. using photographs, genetic data, population level?).
Lines 121-122 have been modified to include this information (which is also provided in mat and met in the "Data acquisition" section).

Some small typos on lines 51 (of as), 63 (loses), 65 (in a reproductive), 91 (macroevolutionary) and 340 (research).
Thanks for noticing these. Corrected.