We are grateful to the recommender, Eric Pante, for considering and handling our manuscript, as well as the reviewers Kevin Sánchez and Aglaia Antoniou for providing thorough and helpful feedback. We have edited the manuscript accordingly, and provide additional information and justification to some comments. One point raised by the reviewers was that the proportion of original data used in this manuscript was not clearly stated. We have made changes, as detailed below, to explain that, and have modified a mistake regarding our sampling size (in the previous version, we mistakenly wrote that we used 296 ingroup samples, when the true sampling size is 325). Finally, we have added an appendix table providing Genbank accession numbers for each individual sequence.

Replies to the recommender's and reviewers' comments are given in ***blue bold italic.*** The line numbers refer to the **tracked changes version**.

Recommender (Eric Pante):

[…] I have a comment additional to those of the referees : on line 367 you state that S-Yah has a basal position within Moroccan populations. (1) there is no indication in the methods that the tree is rooted;
In the methods (lines 223-227), we stated that "two representatives of the following lineages […] were included as outgroups […]". We now have slightly reworded that section to increase its clarity. We have also edited the caption of Figure 1A to further clarify the rooting strategy there. The nDNA tree represented on Figure 1B, however, is not rooted, and we also clarified that in the related caption and in the Methods (lines 235-239).

(2) rephrasing is needed: when using the term 'basal' please explicit to what the clade is basal to ('basal to all other Moroccan clades'?).
We edited that sentence as suggested (now lines 418-419).

Reviewer 1 (Kevin Sánchez):

1. **Title and Abstract**
◦ Ln 53: I found some trouble with the word "safely"; is there any synonym?
We changed it to "reliably".

2. **Introduction**
◦ Lns 104-106: … and ILS.
True, ILS can generate discordance, but ILS is generally not expected to generate biogeographical discordance patterns. This has been added lines 106-107.

◦ Ln 169: change "could results" to "could result".
Done.

3. **Materials and methods**
◦ Ln 186: State here that your are using a subset of those 392 sequences, not all of them. i.e. 296 samples, or 306 including outgroups.
We have extensively re-written the part explaining the origin and composition of the dataset (see also the replies to reviewer 2's comments). See lines 167-169 and 190-204.

◦ Ln 221: Why not to use the multilocus dataset to calibrate the phylogeny? (e.g. with starBeast).

We refrained from calibrating a nuclear phylogeny because of the lack of phylogenetic signal. We spent a substantial amount of time trying to get a species tree from the nine nDNA markers using *BEAST but did not manage to reach proper convergence and obtained variable topologies across runs. Moreover, the position of the Iberian populations as sister to the "Rif" lineage is not supported in our nDNA phylogeny (see figure S5). Because of that, we argue that any attempt to calibrate the nDNA phylogeny would yield unreliable results. This has been added to the methods section (lines 286-291)

◦ Ln 218: Why didn't you also estimate branch supports with ultrafast bootstrap?
We preferred alrt over ultrafast bootstrap as it is faster for datasets with large numbers of samples. Given that our phylogenetic analyses are used only as a preliminary step to infer population structure rather than to draw strong conclusions, we estimated that it was not important to do in-depth investigations of branch support.

◦ Ln 239: How did you know that only exons were included in the nuclear dataset?
This was a mistake; the nuclear genes are a mixture of intronic and exonic sequences. We have removed references to exons.

◦ Lns 275-276: To ease the reading with figure 1, state which subgroups are included in each phylogeographic unit, e.g. Rif includes N-Rif and S-Rif, Mid-Atl includes Mid-Atl1 and Mid-Atl2, etc.
Done.

◦ Ln 284: You missed an "r" in the web link.
Corrected.

◦ Ln 292: "two-step approach", remove "s".
Corrected.

◦ Lns 306-307: Please clarify that you are including adjacent Atlantic lineages to test for IBD in Inland lineages. Also state that O-Gha is included in S-Atl, because it does not appear in the map.
We clarified this point (see lines 353-354). The colors on Figure 1 represent the mitochondrial clades, where O-Gha (locality 24) is included in S-Atl. However, when considering the nuclear data O-Gha appears as rather distinct, and we decided to consider it as a different unit for the IBD tests. All populations (including O-Gha) can be found on the map by using the numbers provided in Table 1 and displayed in Fig. 1C. This has now been clarified in the corrected legend for Fig. 1C.

4. **Results**
◦ Lns 350-351: You could add the branch leading to the IM clade in the figure.
Done.

◦ Lns 352-360 and Figure 1: The 11 lineage names could be added to the phylogenetic trees to ease the reading of the text and the map.
Done.

◦ Ln 361: instead of "lineages 3 and 6" use the name of these groups.

Corrected.

◦ Ln 400: Add “the” before “latter”.
Done.

5. **Discussion and Conclusions**
◦ Ln 550: “extent” instead of “extant”.
Corrected.

◦ Figure 5: You could use a different color for each contour, to ease
We tried to implement the reviewer’s request, but we found that the figure became quite difficult to read as it incorporated more information, which diverted the attention from highlighting IBD gradients. Hence, we decided to leave Figure 5 as it was.

Reviewer 2 (Aglaia Antoniou):

In my opinion, there are some minor issues that the authors need to address in order to make their study and ms clearer and easier for the reader to follow. In terms of language, there are typos throughout the ms while it would be significantly improved if someone proficient in English would make edits (some parts of the manuscript are not clearly written e.g. lines 146-149 rendering the reasoning of the authors not easy to follow). Furthermore in various parts of the ms appropriate references are missing (e.g. lines 146-149).

We have modified this part and added some references (see now lines 141-151).

Specific comments:

Title

I believe that the title is promising of a more theoretical/methodological approach that the one followed in this study (e.g. of proposing a new way of discriminating between IBD and phylogeographic barriers underlying vicariance events). Therefore I believe that the title is not an accurate description of the conducted study and does not clearly reflect its contents.

We edited the title, hopefully making it a more accurate description of the manuscript’s content.

Abstract

The abstract is concise and presents the main findings of the study.

Introduction

Introduction explains the motivation and the questions that this study attempts to address. However it would be important to make clear that that this is a sort of meta-analysis study and refer to the importance of meta-analysis.

Most of the samples used in this study have never been used in a previous publication, so most of this work is in fact entirely original. Likewise, only phylogenetic analyses overlap

those of Miralles et al. (2020), but ours include more samples. The confusion was probably our fault as we did not clearly explain the differences between the samples used here and the samples used in Miralles et al (2020, see also comments from reviewer 1). We have tried to clarify this in this revised version (lines 167-169 and 190-204).

Materials and Methods

At the Materials and Methods section, there is a lack of justification why mtDNA data (displaying the higher resolution groupings) were not used to test IBD in a comparative framework with those of nuDNA.

Tests of IBD rely on the decrease of relatedness between individuals as geographic distance individuals increases. Single-marker measures of relatedness are not very accurate and as a consequence, mitochondrial DNA divergence is not an effective way to test for IBD, as shown empirically and by simulations by Audzijonyte & Vrijenhoek (2010, Evolution 64-8: 2369–2384) and Teske et al. (2018, Scientific Reports 8:8448, DOI:10.1038/s41598-018-25138-9). In addition, most squamates seem to exhibit male-biased dispersal. We have added this information lines 326-331.

Furthermore, (L186) it is not clear which samples were used in the study i.e. Miralles et al. 2020 molecular analyses involved 128 samples and not 392.

As we stated above, the previous version of the manuscript was indeed unclear on this aspect. We apologize for that and are thankful to the reviewer for pointing it out. We have edited the first section of the Methods to try to explain this more clearly (lines 190-204).

Furthermore it appears that the study focused on IM clade of Miralles et al. 2020 and not on the Moroccan populations as stated in L195. If this is not the case then the authors need to justify why Moroccan samples from Tizi n' Tichka and Siroua (WHA in Miralles et al. 2020), Isli and Tislit (EHA in Miralles et al. 2020) were not included in the analyses.

It is correct that the scope of our sampling corresponds to the IM clade defined by Miralles et al. (2020). This does not include all Moroccan populations historically included under the name* Acanthodacylus erythrurus*, as the two High Atlas clades (WHA and EHA) have been described by Miralles et al. (2020) as distinct species (*A. montanus *and* A. lacrymae*) which are clearly separated from* A. erythrurus *by barriers to gene flow. Hence, we did not take them into account in the present manuscript, and do not consider them as Moroccan populations of* A. erythrurus*. Again, we clarified this (see lines 198-201).

There seems to be some misunderstanding regarding the data.

L200 "The samples were grouped based on their mitochondrial lineages" in order to run PHASE to infer individual genotypes. Please provide a justification why you preferred to run PHASE on each group separately. Given the drawbacks of mtDNA in defining evolutionary units (also stated in the introductory sessions by the authors) I find this rather problematic.

Phasing algorithms assume that the samples are drawn from a single population at Hardy-Weinberg equilibrium. Hence, phasing all our samples at once could introduce errors, especially in the case of barriers to gene flow between some populations, or simply Isolation By Distance gradients (both of which we observe in our data). We agree that using

mitochondrial groups for phasing could be problematic in a case where distantly related individuals share similar mitochondrial haplotypes (e.g., in the case of mitochondrial introgression between two sampled populations). However, we do not observe this in our dataset (i.e., individuals sharing similar mtDNA haplotypes are always closely related in the nDNA data, although the contrary is not true) and we verified it prior to phasing. Hence, it seems to us that the main risk of our approach would be to split populations into smaller units, which might reduce the phasing accuracy. Since PHASE provides a phasing likelihood, this drawback can be accounted for. We included this justification to the manuscript, (lines 220-225).

L234-235 I am not sure what the authors mean by "conducting independent analyses of the same data" since STRUCTURE performs "independent" analyses for each K and each replicate run within each K. Were the resulting solutions bi- or multi-modal? Does any make biological sense? Do the different groups/clusters contain samples with high membership coefficients (q-values)? This in my opinion is important information that needs to be communicated.

We have expanded and partly reformulated the section describing the STRUCTURE analyses (lines 261-265).

L234-239 this belongs to the Results section.

While we agree that this section describes results, we believe that it is important to justify our general approach for the nDNA analyses, and the criteria applied to define evolutionary units, before the rest of the methods. Considering that the manuscript already contains many analyses and results, we find it clearer to present these preliminary results (otherwise not used in the remaining of the manuscript and to draw conclusions) in the Methods rather than in a dedicated paragraph in the Results section.

L257 "an alternative approach" Please clarify to which approach(es) this constitutes an alternative

We added clarifications (lines 296-297).

L258 Maybe here it is Factorial Correspondence Analysis (FCA)?

FCA is based on a single variable with several modalities. MCA (multiple correspondence analyses) is the same but with several variables. So, a "FCA" based on 9 loci (i.e., 9 variables), as here, is an MCA. This is explained (in French unfortunately) here : https://kimura.univ-montp2.fr/genetix/.

DeepL translation gives (after correction) "Factorial Correspondence Analysis (FCA, Benzécri (1973); Lebart, Morineau, and Tabard (1977) Greenacre, (1984), Manella, 1987, Escofier and Pagès (1990)) is a type of canonical analysis that is particularly well suited to describe the associations between two qualitative variables (analysis of a contingency table crossing the modalities of the two variables). Subsequently, the properties of this method led to its use on other types of tables, in particular disjunctive tables crossing individuals (objects) and the modalities of several variables (descriptors). This is known as a multiple correspondence analysis (MCA) in which each individual normally presents the value 1 once and only once for a single modality for each variable (full disjunctive table). Here, we

extend this analysis to a 0/1/2 table corresponding to a coding more appropriate to data from the genetics of diploid organisms as proposed by She et al. (1987) or Duplantier et al. (1990)"

L274 the justification of this decision is lacking

We added a sentence to justify this decision (lines 314-316).

Results

L351-363 The description of the phylogenetic tree in this section is not in agreement with the one depicted in Figure 1. Please make the appropriate changes to Figure1A as to indicate the >90 support of the 11 clades.

We changed Figure 1A accordingly.

Figure1C: it would be helpful for the reader to indicate on the map the three deeper mtDNA groups (e.g. by encircling them). Furthermore adding the group codes to the mtDNA tree and nuDNA network will also be helpful. There is probably a shift in the numbers of the localities (S-Yah is 19 at the Figure and 20 at the Table, S-Bou is 20 at the Figure and 21 at the table etc.) with number 27 missing from the Figure.

We have added the group codes to Figure 1A and 1B. We tried to encircle the three deeper clades on Figure 1C but the map became overloaded and difficult to read. Since the deeper relationships are not a very important result, we decided to leave the map as it was. Hopefully, the inclusion of the group codes in Figure 1A makes the connection easier to make.

L426-427 this is not the case in Figure 1B where Mara is closer to the remaining than to Rif, N-Atl groups.

We clarified this lines 483-484.

Discussion

It is very important to infer the results in the light of the assumptions made by the different approaches employed (e.g. STRUCTURE and IBD, Phylogenies and the level of differentiation in the dataset, IBD and the assumption of linear relationship of genetic and geographic distances). I would like the authors to elucidate more on the fact that there are geographically distant sites that belong to the same group as well as site in close geographical proximity that belong to different groups e.g. by comparing environmental conditions of the respective areas.

This is now discussed more in-depth (lines 554-558, 563-564, 711-722).

L507-511 couldn't these results be justified by the attributes of the analysed nuclear markers (e.g. being under selection)?

Selection could indeed erode genetic diversity if strong, uniform selective pressures would apply to all populations at all sampled loci. Given that we consider 9 regions; located in genes coding for diverse functions, we believe it is very unlikely to be a major issue here. Other properties of nuclear markers compared to mitochondrial ones could result in a lower nuclear structure, such as the matrilinear inheritance of mtDNA (in case of sex-biased dispersal) and the higher effective population size of nDNA markers. These issues are addressed in the discussion (ll. 539-542, ll. 631-632). Besides, mtDNA is under selection too.

L505-507 and L513 attention must be given to the fact that STRUCTURE should be avoided when IBD holds since such datasets do not conform precisely to the structure model while it is difficult to infer K.

We have added a sentence about that lines 574-577.

There are too many redirections to the "next section" (e.g. L543-544, 546-547, 560-561) that make it difficult for the reader to follow.

We have reduced the redirections.

L558 phylogenetic analyses cannot by any means be considered as simple or simpler approaches to the ones designed to test IBD patterns. The two aforementioned approaches are better suited for addressing different questions focusing at different levels of divergence although phylogenetic methods can be considered as more flexible covering a wider range of diversification (coalescence, yule etc).

This sentence was unclear, and we apologize for that. We do not mean that phylogenetic approaches can be used to detect IBD gradients, but that spatially explicit methods (e.g., taking IBD into account) can yield more information on phylogeographic breaks compared to analyzing population splits in phylogenetic trees. We reformulated this sentence (lines 624-625).

L563 also genome-wide

We have rephrased this sentence.

L565-570 this contradicts somehow the statements made at L559-561.

We do not think these statements contradict each other. In the first statement, we say that before doing the IBD analyses, we believed that the signal of vicariance was clear (based on results from phylogenies, MCA, ect), which was apparently contradicted by the IBD analyses. The second statement proposes an explanation as to why these results were apparently contradictory. We edited the first statement to make this clearer (lines 602-604).

L641 Do those breaks reflect present distinct environmental conditions?

In most cases, they do not. We clarified it in the text (lines 711-715).

L642-644 Is there any information/hypothesis on which areas faced the harshest conditions?

Fine scale data on the paleoclimatic conditions in Morocco in the Pliocene and Pleistocene is missing as far as we know. In the manuscript, we hypothesize that mountain ranges and humid areas (coastal wetlands, river basins) were the most suited to* Acanthodactylus *lizards during arid periods, lowland plains being too arid (lines 727-732).