

Repeated evolution of cooperative breeding and life history traits in Lake Tanganyika cichlids

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Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The manuscript "Convergent evolution of cooperative breeding and life history traits in Tanganyikan cichlid fishes" reports a phylogenetic analysis of associations between life-history traits and cooperative breeding in a rapidly radiating clade of fishes. They claim that cooperative breeding is associated with body size and clutch size, and argue that these patterns are ultimately driven by predation risk. The questions are interesting, the approach is valid, and the study system is ideal for answering these questions. The work is novel and has the potential to advance the field of social evolution. However, I have serious concerns about the analysis and interpretation of the data, and I would like to see significant changes before recommending publication.

Major comments:

1. I don't believe any of the conclusions regarding predation. At best, the analysis based on body size of prey fish is an indirect way of determining predation likelihood. Many other factors presumably influence the predation pressures on any given fish species. Plus, even if body size was correlated with predation and cooperative breeding, that doesn't mean predation is linked to cooperative breeding; there are lots of things linked to body size besides predation.

However, even if you did accept the authors' logic, the data don't support the conclusions anyways. The authors argue that all of the cooperatively breeding fish fit in the size range of prey fish, whereas not all of the non-cooperatively breeding fish fit in that range. This is strictly true. But 80% of the non-cooperative breeders also fit in that range, so it's hard to buy that this size difference is linked to a broad predation difference between these two groups of fish. Digging deeper into Figure 3a, although it's true that some prey fish are up to 90mm long, the vast majority of prey fish are actually under 32mm long (see also line 177). When you compare this to the CB and NCB distributions in Figure 3b, it's actually the NCB that have more species with this smaller body length (5 vs. 1). So, if anything, I would argue that NCB fish are more likely to experience heavy predation. Bottom line, the authors should remove any conclusions related to predation unless they are able to add more compelling evidence.

2. There's some confusion, mainly in the introduction, about whether the authors are examining the evolutionary causes of cooperative breeding or the evolutionary consequences of cooperative breeding. It seems, from the abstract (lines 29-31) and introduction (lines 54-62), that the authors are trying to explain likelihood of a lineage developing cooperative breeding based on its life-history characteristics. At other times (lines 95-97) it seems like the authors are hypothesizing about how life-history traits will evolve once a species develops cooperative breeding. The actual analysis (PGLS) reflects the latter; the authors use breeding status as the independent variable and use it to predict life-history characteristics.

Both questions are interesting, and I appreciate that it may be difficult to tease apart causality given the current data. However, I think the authors need to be more explicit about which they are testing, because they are very different questions. It looks like from the discussion (which is more clear on this point) that body size is being interpreted as a cause of cooperative breeding while egg and clutch size are considered as consequences; perhaps the directionality of the PGLS should be adjusted to match that.

3. I would recommend some reorganization of the introduction, in particular the final paragraph (lines 81-109). This paragraph somewhat veers off course into a lot of interpretation which is better left to the discussion. Please consider making this paragraph more concise by simply stating the hypotheses and possibly a brief description of the approach and/or results.

4. It feels like the authors are missing an opportunity to generate more robust analyses by only using the body size measures of the fish they caught themselves. According to Fig. 1, there are a lot of missing data points for the life history traits – over half of cooperatively breeding fish! While I get that for clutch size and egg size they may want to use their own collected data to make sure those metrics are standardized, it feels like body size could be re-analyzed using existing data on these fishes. This would be very easy to do, I think, and could provide stronger support for the authors' conclusions.

5. I'm not sure about the appropriateness of converting continuous traits to discrete traits using arbitrary thresholds to run an analysis of transition probabilities (Figs 2a, 4a). I'm not an expert in this area, but it looks to me like BayesTraits has lots of methods for analyzing continuous traits but none of them involve just making the traits binary. This would seem to both throw away valuable information and lead to likely misclassification. Is this a common practice in the field? If so, please provide some citations justifying this approach, which is not at all intuitive.

Also, what does it accomplish that the PGLS doesn't? If body size and cooperative breeding are correlated, doesn't it necessitate that these transition probabilities have to be different? It feels unnecessary to me, in addition to being problematic.

Minor comments:

Line 29: Remove the words "other than parents"

Lines 95-97: Haven't read Arnold and Owens (1998) but why would decreased mortality lead to reduced opportunity to establish new breeding territories? And what does this have to do with clutch size or cooperative breeding?

Lines 134-149: I appreciate the authors' forthrightness in discussing this piece of ambiguous data and its effects on their results. Could the authors extend this to see if changing the status of *N. niger* would also affect the results of the PGLS? I suspect not, but it would be nice to see that confirmed.

Lines 265-277, Table 1: Should clutch size be included as a covariate in the PGLS analysis of egg size and social status? Given the predicted trade-off controlling for clutch size may help isolate possible relationships with egg size.

Lines 286-288: The PPA is neat but if the authors have a hypothesis about diet and trophic state shouldn't the $\delta^{15}\text{N}$ stuff be in the PGLS as well? I can't follow why the authors only bring up this data here, especially since they discuss diet in lines 124-129.

Line 360: Confusing wording here. Should this say "> 5"?

Reviewer #2

(Remarks to the Author)

With great interest have I read the manuscript by Satoh et al. on the evolution of co-operative breeding in cichlids. I found it to be an enjoyable read, the questions are interesting, and the analyses and results make a lot of sense to me. There is quite some speculation throughout, but it is clearly communicated as being speculative, and the arguments are well sourced from the literature. I should note however, that I am not familiar enough with the ecology of these species to really critically evaluate some of the arguments made. I think there are some parts of the manuscript that can be more clear, especially the path analysis, which I'll explain below.

There's not much information presented about the path analysis. PPA, as proposed by Von Hardenberg & Gonzalez-Voyer, is intended to be confirmatory; i.e. comparing the evidence between causal hypotheses. But it is unclear what the hypotheses are. The authors mention that they compared 109 models (which is a lot! more than the number of species), but there is no information on why/how these models were chosen. Can the authors please clarify their reasoning here, and also include analytical details, such as what model of evolution was assumed, and what estimation method for the binary pglms was used.

The interpretation of the model with most support was also not very clear to me. The authors argue that the causal model supports the role of predation, but this variable is not in the model. I'm not sure if the "no effect" arrows are really helping the interpretation here, these paths aren't present at all in the final model, correct?

Table 1 can do with a more detailed legend. What are the superscript numbers on the lambda parameters? What is the C.I. column? Can the degrees of freedom be added?

Figure 1: Perhaps I missed it, but it is never spelled out what the numbers and arrows in this figure refer to. I assume the independent transitions to CB?

38: PPA does not reconstruct ancestral states or provide any temporal information, so it cannot reveal that cooperative breeding evolved first in smaller species. One can think of path analysis models are trying to describe what would happen in the case of an intervention. That is, if e.g. a species would become smaller, it would be more likely to become a cooperative breeding. If the authors want to make claims about what evolved first and in what species, they need to use evidence from other analyses.

156: Variations should be variation.

174: I think "preys" should be "preyed"?

231: Can the authors double-check that it is indeed a log10 BF of 40, and not just the BF? That would make the alternative hypothesis 10 duodecillion times more likely than the null, which seems rather extreme.

238: "life history hypothesis" is such a generic term, that it would be helpful what is meant by that sensu ref [10].

266: Since the authors are using Bayes Factors here, they can interpret that BF to mean strong to very strong evidence in favour of the null.

289: Cite Von Hardenberg and Gonzalez-Voyer 2013, *Evolution* 67(2): 378-87

294: This needs more explanation. Predation is not in the path model, so this interpretation isn't straightforward.

349: This reference to table 1 is not correct.

356: I have an inkling, but can the authors please clarify their reasoning behind removing ovary mass from the body size measure? E.g. predators don't care about this distinction, and body size is used a lot in this paper in relation to predation.

360: Double check if this is indeed supposed to say less than 5.

362: Is it not possible to estimate egg volume? E.g. one can use the volume of an ellipse: $V = (\pi * L * W * H) / 6$, and plug in the values that already have been measured. It seems to me that it is the volume of the egg (or the log volume) that matters.

367: What about tissue shrinkage? Would the eggs kept in formalin not be measured to be smaller?

396: It is phytools, not phytool.

421: This section would be easier to read if it is specified earlier that it is all about BayesTraits Discrete (BayesTriats has more models). Also, please clarify the priors used.

428: <2 includes <0, it's more clear to give intervals [0, 2], [2, 5], [5, Inf].

440: I assume this refers to Pagel's lambda model? Why was this model of evolution chosen, were others considered? Was the same model of evolution assumed for the path analysis?

460: It's probably the CICC that was used, which is the CIC with correction for small sample sizes.

Reviewer #3

(Remarks to the Author)

In this study, Satoh and co-workers examine the evolution of cooperative breeding in cichlid fishes from Lake Tanganyika. This is an exciting topic, as the lamprologine cichlids from Lake Tanganyika are a textbook example for cooperative breeding in fish. Through character state reconstructions, the authors confirm the previous finding that cooperative breeding has evolved several times in the Lamprologini. They provide a large (but far from complete) dataset on cooperative breeding in Lamprologini and estimates for two life history traits, and it is obvious that they have spent quite some effort into collecting these data in the field. Unfortunately, the manuscript does not do justice to the interesting research question of broad relevance and the efforts in the field by the authors as well as their expertise on the cichlids. The analyses performed are standard at best, and much of the manuscript is more like story-telling and full of speculation, with many of the statements not being supported by data or analyses (which would nevertheless be possible).

One problem that I have is that the authors use only a subset of species (see below) and that they pick out a few traits and/or phenotypic axes. All lamprologine cichlids, also the non-cooperative breeders, perform brood care, but I do not see the diversity of "breeding types" reflected in their analyses, potentially leading to circularities. For example, the authors focus on

shell dwellers but do not take into account other types of "protection against predators", such as digging sand tunnels, living below rocks or in caves, or breeding in crevices. Since shell dwellers have the property of being small (otherwise they would not fit into *Neothauma* shells), body size might simply be a confounding factor in their analyses. It would be important to see what would happen to a pPGLS when also other breeding types are included. Similarly, while the authors speculate about such confounding factors (see e.g. L298FF), they do not formally test for it. While I fully understand their focus on cooperative versus non-cooperative breeders, I do not see how some of the predictions can be applied to this strict distinction. For example, why should cooperative breeders have stronger predation than non-cooperative ones, especially at their juvenile age (e.g. LINE 178FF)? And if large predators are included in the noncooperative group, and clutch size is correlated to body size, then of course noncooperative ones have larger clutch sizes, as a confounding effect of body size.

According to Ronco et al. (their reference 16), there are 100 species of lamprologine cichlids in Lake Tanganyika. In the present study, they analyzed 74 of those (for cooperative versus non-cooperative breeders) and much less than that for most of the other comparisons. At the same time, the authors show that their analyses are highly sensitive to the character state of single species (notably, they present the case of *N. niger*). In the light of the latter observation, I am wondering how credible their results are on the whole. What if some of the *Telmatochromis* species are cooperative? Wouldn't that increase the probability for "just" 3 origins of cooperative breeding? In any case, it would be important to know the rationale behind their selection of subsets of species for the respective analyses. Perhaps the authors could also comment on the riverine lamprologines that they kept in their analyses (Fig. 1), any suggestion/prediction on those?

I also have an issue with the sample sizes used for some of the analyses, in particular the female body size analyses (And why only females??? There is only one species with a substantial male dimorphism, for all the other species an analysis of males would work well!). Judging from the methods section (L368), 155 females have been examined for 39 species. According to table S1, only 1-8 individuals per species (typically less than 4) were measured, which, in my opinion, is not sufficient to measure intra- and inter-specific variation (and way below current standards in the field). At minimum, these measurements should be presented, for example, in a figure with box-blots per species. The statement "female body size was smaller in cooperative breeders than in noncooperative breeders" (L164F) is problematic - what if you repeat this analysis without the lepidiolamprologine clade (large predators that might strongly influence all analyses that include female body size)? If the authors are interested in what small species do (see statement in L167, then they should only analyze small species that suffer from predation). In general, I find it problematic that the data of this study have not been made available to the reviewers, and I strongly suggest to the authors to make their primary data (and not just tables derived from analyzing them, such as Table 1) available publicly before submitting their work.

Another aspect that is entirely missing from this manuscript is that the other half of the cichlid species in Lake Tanganyika are mouthbrooders, which is not even mentioned in the manuscript. While I do not ask the authors to include those in their study, it should be at least discussed what can be expected in those (in the light of predation pressure, clutch size, etc.).

I have not checked it for each and every analysis that the authors have performed, but by default, all analyses presented should be phylogenetically corrected (and not all are). Everything else does not make sense in this case. In general, the material and methods section is rather vague, program versions are often not provided, and links to the scripts and datasets used are entirely lacking, making it impossible for this reviewer to judge the validity of their analyses. Any revised version should contain a link to datasets and scripts used, etc.

Throughout, the authors compare their results to previous studies in birds, which I find problematic, in particular, since birds have way smaller clutch sizes than most of the lamprologine cichlids; if such a comparison is intended, then I feel that the authors would need to perform similar measurements as have been done in birds (e.g. egg sizes; L268).

I also have a problem with the title of this study: Where do the authors show convergent evolution of life history traits in their study? In general, it is a matter of debate if they show "convergence" in cooperative breeding (that would basically mean that the behavior is the same in all lineages it evolved), so it would be safer to say "Repeated evolution of cooperative breeding in Tanganyikan cichlid fishes".

Finally, I feel that for the non-cichlid experts, it would be good to provide a bit more background on the study system in general and the Lamprologini in particular, and to define what are "tribes".

OTHER COMMENTS:

LINE28FF: This definition for cooperative breeding is somewhat different from the one provided in the beginning of the Introduction (LINE47FF) in that, in the abstract, individuals other than the parents "raise" offspring that is not their own, whereas in the Introduction they provide care to them, i.e., they "contribute to raising" them. To me, these are two different scenarios, please clarify and provide a consistent definition throughout.

LINE36: Should be "from the field" or from "field observations".

LINE65: If you use "significant", then I'd expect a P-value to back this up.

LINE105: Reference format is different to the other references.

LINE120: The phylogenetic hypotheses used is not for the "whole tribe" but for the entire radiation.

LINE134F: This sentence is somewhat awkward ("multiple occurrences of evolution"). I guess it should read "...of multiple gains and losses of cooperative breeding", but then I think that "multiple" is a bit of an over-exaggeration.

LINE137: Should be "insufficiently".

LINE139F: Maybe better "in which" instead of "when".

LINE167FF: Why "or" here? What about *N. multifasciatus*? This species is highly social (perhaps the most social of all lamprologines according to some authors) AND lives in shells.

LINE176: Remove "-" before "mm".

LINE198: What is a "simple" substrate? Is rock more "simple" than gravel or sand?

LINE203: You mean "maintenance by parents"?

LINE210: Should be "temporalis".

LINE216: I do not get the logic here: First, what is the difference between "small species" (and please define this) versus "lineages with small body size" (please define)? And how can you know in which environment they evolved? This cannot be answered by the type of character state reconstructions done by the authors, and I doubt that it is possible with the available data to reconstruct how large the species was, in which cooperative breeding evolved some millions of years ago, and in which environment it lived. All this is pure speculation.

LINE220: Remove "a".

LINE252: According to this logic, shell dwellers should have an even lower predation pressure and, hence, a higher survival rate, because juveniles are protected in the shell; similarly, tunnel diggers should have a higher survival rate. All these are testable predictions. And what about mouthbrooders???

LINE280: Please say "two life history traits".

L288/296: It should be "trophic" instead of "tropic". How did you deal with the N-values. Did you use species means?

L298FF: "We believe...". Please provide a formal test for the influence of larger fish eaters on these results.

L319: What is novel to this study then? It has been shown before that cooperative breeding evolved more than once and that cooperative breeding is associate to life history traits.

L327: "*Neothauma*" should be in italics.

L336: I do not see the point of overruling what is known from the literature. If it is known that *N. multifasciatus* also breeds on rocks, then I do not see the point of making them "obligate shell brooders" just for this study. Please repeat the analyses with defining them as facultative.

LINE 376F: What about catfishes?

LINE379: These coordinates are very vague.

LINE388FF: I do not get this section. First of all, the phylogeny of Ronco et al. includes all cichlids from Lake Tanganyika (not just the lamprologines). Second, the phylogeny of Ronco et al. is available as tree file, so what have the authors "reconstructed" with RAxML? Which data have they used to do so? Finally, the phylogeny of Ronco et al. is time-calibrated, so what's the logic of dating it with BEAST2?

L406FF: All these analyses should be corrected for phylogeny, please redo.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

I am reviewing the manuscript "Repeated evolution of cooperative breeding and life history traits in Tanganyikan cichlid fishes" for the second time. I apologize for the delay in completing the review. I initially had significant comments about both the analyses, writing, and interpretation. I now feel that the paper is much improved, but I still have comments about the interpretations regarding predation as well as interpretations regarding constraint. I also point out some grammatical issues

that the authors can start to work on cleaning up as this gets closer to acceptance.

Major Comments:

1. I initially commented that I was not convinced of a relationship between predation risk and cooperative breeding. In this revision, the authors still claim to be testing a relationship between predation risk and cooperative breeding using body size as a proxy. I still don't find this compelling. The authors should feel free to speculate that predation might be the mechanism by which body size and social status are related, but they should not state so directly that they are testing predictions about this (e.g. lines 90-93). Body size is related to so much more than predation risk.
2. I'm a bit confused about the use of the word "constraint" throughout this manuscript. The authors seem to be suggesting that predation is an "ecological constraint" on social evolution – but at the same time they are arguing that predation drives social evolution. I'd suggest they stop using that word; there's no evidence here for anything constraining social evolution, just that there may be certain ecological factors that select for cooperative breeding behavior.
3. I'm finding the 15N analysis and Figure 5 a bit confusing and more importantly pretty tangential to the exciting questions and analyses in the paper. I guess I can live with it, but the authors could consider simplifying and shortening this paper a bit by just removing the whole analysis. In my opinion, it doesn't contribute anything.

Minor Comments:

Lines 57-58: It feels a bit obvious that "evolutionary processes" mediate the development of cooperative breeding systems. Could you elaborate on a specific type of evolutionary process that these citations have revealed to be involved in this evolutionary innovation?

Lines 193-205: See major comment 2 above. This is also a weird argument to make, that ecological factors are restricting the evolution of cooperative breeding. If the authors are arguing that shell-brooding is another solution to the problem of high predation, then a more straightforward explanation is that here, just like in large species, there is little selection for cooperative breeding. The words "constraint" and "restrict" imply that the trait would be beneficial but there is some other factor, genetic, developmental, or environmental, that prevents the trait from evolving. I don't see any need to invoke that concept here (or anywhere).

Figure 1: Can the authors increase the contrast on the scales for the quantitative traits on the phylogeny? For total length especially, all the little green boxes look the same color to me – so I can't use that to evaluate the key result that body size is phylogenetically correlated to social system.

Grammatical Comments:

Line 61: Remove the "However" at the start of the sentence.

Line 65: Should say "...single breeding event".

Lines 75-78: Rewrite as "Lamprologine chichlids, most of which are endemic to Lake Tanganyika, have diverse social and mating systems ranging from biparental care, polyandry, polygyny, polygynandry, and highly complex cooperative breeding systems."

Line 81: Remove the space after species.

Lines 81-84: Awkward. Rewrite as "The combination of a well-resolved phylogenetic tree, their monophyletic origins, and their diverse ecological and social systems make lamprologines a suitable model system for comparative analyses of social evolution."

Lines 109-110: Change to "... although the loss of cooperative breeding was not found."

Line 114: Remove "the depiction of the social system in"

Line 116: change "the cooperative breeder" to "a cooperative breeder"

Line 117: remove "the"

Line 167: What is the "362" doing here?

Line 231: Remove space before citation 45.

Reviewer #2

(Remarks to the Author)

I would like to start my review by expressing my dissatisfaction with the response letter. Many of my comments simply have not received any response at all, making it unclear if or how these have been addressed in the revision. I notice several comments from Reviewer 1 have also gone without response. The responses which are present are often terse and give little information on the changes made or the intent behind them. The authors have also re-ordered my comments, which is a minor point but adds to the confusion as it seems at least one of my comments has disappeared altogether:

"362: Is it not possible to estimate egg volume? E.g. one can use the volume of an ellipse: $V = (\pi * L * W * H) / 6$, and plug in the values that already have been measured. It seems to me that it is the volume of the egg (or the log volume) that matters."

As just one example of a terse response, I had asked: "440: I assume this refers to Pagel's lambda model? Why was this model of evolution chosen, were others considered? Was the same model of evolution assumed for the path analysis?" To which the authors respond: "We have changed our PGLS to BPMM. PPA continues to use Pagel's lambda model." So this doesn't answer why this model of evolution was chosen, or whether alternatives were considered. It also ignores what the assumed model of evolution of the BPMM is. I am not helped by the fact that the marked up manuscript does not have all changes marked. Overall, the response letter is really minimally useful to me, and makes my job as a reviewer more difficult

than necessary.

I would kindly ask the authors to take care when writing their response letter to 1) reproduce my comments verbatim and in the same order, and 2) clearly respond to each point individually. When changes have been made, clearly indicate where I can find those, and what motivated the changes. If a comment is no longer relevant, or if the authors disagree with comment, please also indicate this clearly.

Below, I first give my general view on the revision, with more specific comments following.

Overall, I don't find the revised version of the manuscript more convincing than before. In particular, the new ancestral state reconstructions suggest many more independent transitions than before, but the authors are not very self-critical of this result (also see below). Secondly, they now present phylogenetic regressions of a range of variables (including breeding mode) on body mass, clutch size and egg size. However, their conclusions are all about breeding mode, which was not used as an independent variable. While in a simple $Y \sim X$ regression the direction of the regression can be swapped more or less indiscriminately, this is not the case for multiple regression, nor is it the case for phylogenetically informed regression. I therefore believe these models to be inappropriate for the goal the authors have in mind.

Specific comments:

From my previous review: What estimation method for the binary pglms was used in the PPA?

From my previous review: The interpretation of the model with most support was also not very clear to me. I'm not sure if the "no effect" arrows are really helping the interpretation here, these paths aren't present at all in the final model, correct?

From my previous review: Is it not possible to estimate egg volume? E.g. one can use the volume of an ellipse: $V = (\pi * L * W * H) / 6$, and plug in the values that already have been measured. It seems to me that it is the volume of the egg (or the log volume) that matters. This is more appropriate than estimate egg size as is currently done.

Line 69: "However, only a few studies have focused on the relationship between cooperative breeding and life history traits in vertebrates except for birds and mammals due to the limited accumulation of knowledge." This reads as saying that there have been few studies on this because there have been few studies, which is tautological.

108: Several of these independent transitions are supported by a single species. How sensitive is this result to uncertainty in the phylogeny? My understanding is that the analyses did not consider this.

143: While this is far from clear from the text here, my understanding is that these analyses use body size as the independent variable, not cooperative breeding. So they suggest that body size evolution is affected by CB, but I do not believe they provide evidence that CB is affected by body size, which was the stated aim of this paper.

151: "As consequence of analysis using data from FishBase, we found that the marginal correlations between social system and body size (total length) among lamprologini cichlids ($n = 61$ species) even when *N. niger* as classified as a non-cooperative breeder, cooperative breeder, and undescribed species (Fig. 3c; Supplementary S4-S6)." I don't follow what this sentence is trying to say. What is meant by the marginal correlations? Marginal to what? Do the authors mean this in a statistical sense, like after adjusting for other effects, or do mean that the effects are weak? I would recommend rephrasing. Why are there no statistics reported from the GLMM, like was done in 148 for the BPMM?

406: Can the authors comment on what the multiple tips per species means for their analyses? How have they assigned trait data to these tips? Are any the species not monophyletic?

408: Can the authors provide appropriate references for the ARD and ER models, as well as the "equal" and "FitzJohn" models? I am not quite sure what the authors mean by the latter, do mean the prior for the state at the root, i.e. the flat vs FitzJohn priors? How do these choices affect their estimate of the number of transitions? I don't believe it is appropriate to compare the results of models using different priors with AIC, but I could be mistaken. Can the authors provide a reference for this approach?

Figure 1: The figure legend leaves me with many questions. Many of the species abbreviations are not unique, which makes the figure confusing. Are these the multiple populations of the same species mentioned in the text? One tip is labeled "Neo sp.", which seems like it represents more than 1 species? Why are some species names underlined? What does it mean for a node to be "unpredictable"? It is not clear to me what panel B adds to this figure.

Figure 2: The length of the plotted tails of these distributions are quite arbitrary. Can the authors please add the limits of the credible interval? The x-axis reads "Z-score", but it seems this is not true for the binary predictors?

Figure S8: Why are there multiple models for some combinations of hypotheses (e.g. APA, PPP, PAP) but not others (e.g. APP)? Is AAA the same as the null model?

Version 2:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

I am reviewing the manuscript "Repeated evolution of cooperative breeding and life history traits in Lake Tanganyika cichlids" for the third time. I had concerns in my second review about the presentation of figures and about some of the interpretations, particularly regarding predation risk and evolutionary constraints. I feel the authors have done a good job of responding to these comments, and I find the manuscript more compelling and much easier to follow. Another reviewer had substantial comments on the analyses - I don't have the same methodological expertise as that reviewer, so I can't evaluate all of their changes, although it appears the authors have made considerable efforts to address those concerns as well. Taking all of this together, I would recommend accepting the manuscript.

Reviewer #4

(Remarks to the Author)

I was asked by the editor to assess if the authors adequately responded to the concerns of Reviewer#2. This reviewer was not available to re-review the MS.

I will start by saying that I enjoyed reading this paper. It is an interesting study on a contentious clade. The results seem robust. The authors' interpretation that predation on small species led to cooperative breeding which then constrains group size (smaller clutches) rightly deserves further exploration as the authors state. The fact that group size is constrained is so different from social insects where group size tends to increase through evolutionary time in some clades. Figure 1 is great and the authors provide good descriptions of statistical models including clear statements of predictor and response variables.

R2 was not pleased with the authors' previous response and raised the following main issues (from my reading):

- The new ancestral state estimations have resulted in more origins of cooperative breeding. R2 wanted the authors to discuss this and how phylogenetic uncertainty might change things. To me, the authors have done as good a job as possible in addressing this comment:

- + They explain at length how one species (*N. pulcher*) affects reconstructions, including further analyses classifying this species in different ways.

- + They consider how changes in tree topology might affect their reconstructions. They are not able to use different phylogenies presumably because the original study describing the tree did not provide a posterior distribution of trees (I have not checked this).

- + They are careful to say that their results may change with the inclusion of more data, which is true of most comparative studies.

- Swapping response / predictors in the authors' statistical models confounds / confuses things. R2 wanted the authors to address this, which they have done by:

- + Explaining that this was initially due to a convergence issue, which was resolved when they changed R package, and were able to make social system their response variable.

- + In their other models, they deliberately keep social system as an explanatory variable, given their hypotheses.

- That examining egg volume is more appropriate than egg size and that the authors should explain why they chose Pagel's lambda. The authors replied by:

- + Approximating egg volume and using this in their analyses.

- + Explaining that most of the R packages they use have only incorporated Brownian motion to date.

Overall, I think the authors have responded well to R2's comments.

I was not asked to provide my own review of the paper. However, several points do crop up, which relate to some of R2's comments, which I would like the authors to consider in their future work. And a couple of points that can be easily fixed in the present work.

Modelling issues:

- + Report the phylogenetic signal in your traits.

- + When using multiple regression, interpreting the intercept and other parameter estimates can be difficult. In some of your models, you have one fixed effect with five levels (breeding habitat) and another that is continuous (log body size). The intercept here is the mean value of Y for the first level of breeding habitat when log body size = 0. The other coefficients for breeding habitat are then differences from this baseline. Are these contrasts biologically meaningful? I guess that the reference level was chosen appropriately. An alternative would be to interact the fixed effects.

- + In phylogenetic multiple regressions, only phylogenetic signal in the response is considered. This means that swapping which trait is the response (eg. between your PGLS vs BPMM models) changes the interpretation. This is true for the path analysis as well.

- + Using multi-response BPMMs can help avoid this. The paper cited below provides an excellent description of the issues and the solutions to the problem. Unfortunately such models are data hungry.

Reproducibility issues:

- + Unfortunately, the Dryad link didn't work so I couldn't check the code, underlying data and whether or not the authors uploaded a README file.
- + The data in Table S1 seems incomplete, as this only includes breeding system information.
- + Line 357, life history data in Table 1... but Table 1 is a results table?
- + In general, comparative studies struggle with reproducibility, especially when data are compiled from existing sources because any subjective decisions regarding, eg. intraspecific variation or sex differences in trait values, are not made plain. Having a data extraction file detailing where exactly the value comes from and any subjective decisions would be wonderful.

Referencing issues:

- + Lines 55 to 57 in the main text - good to cite Boomsma's monogamy hypothesis here and other classical theory.
- + There are quite a few comparative studies now from other clades which have tried to tease apart direct and indirect fitness benefits which could be cited.

Halliwell et al. 2025. Multi-response phylogenetic mixed models: concepts and application. Biological Reviews.

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Reviewers' comments:

Reviewer #1 (Remarks to the Author):

The manuscript "Convergent evolution of cooperative breeding and life history traits in Tanganyikan cichlid fishes" reports a phylogenetic analysis of associations between life-history traits and cooperative breeding in a rapidly radiating clade of fishes. They claim that cooperative breeding is associated with body size and clutch size, and argue that these patterns are ultimately driven by predation risk. The questions are interesting, the approach is valid, and the study system is ideal for answering these questions. The work is novel and has the potential to advance the field of social evolution. However, I have serious concerns about the analysis and interpretation of the data, and I would like to see significant changes before recommending publication.

Response: First and foremost, we would like to extend our sincere apologies for the delay in resubmitting our revised manuscript. In response to the thoughtful and constructive feedback provided by you and the other reviewers, we conducted additional fieldwork, which unfortunately led to a two-year delay. We are truly grateful for your valuable comments and sincerely hope that the revisions we have made meet your expectations.

Major comments:

1. I don't believe any of the conclusions regarding predation. At best, the analysis based on body size of prey fish is an indirect way of determining predation likelihood. Many other factors presumably influence the predation pressures on any given fish species. Plus, even if body size was correlated with predation and cooperative breeding, that doesn't mean predation is linked to cooperative breeding; there are lots of things linked to body size besides predation.

However, even if you did accept the authors' logic, the data don't support the conclusions anyways. The authors argue that all of the cooperatively breeding fish fit in the size range of prey fish, whereas not all of the non-cooperatively breeding fish fit in that range. This is strictly true. But 80% of the non-cooperative breeders also fit in that range, so it's hard to buy that this size difference is linked to a broad predation difference between these two groups of fish. Digging deeper into Figure 3a, although it's true that some prey fish are up to 90mm long, the vast majority of prey fish are actually under 32mm long (see also line 177). When you compare this to the CB and NCB distributions in Figure 3b, it's actually the NCB that have more species with this smaller body length (5 vs. 1). So, if anything, I would argue that NCB fish are more

likely to experience heavy predation. Bottom line, the authors should remove any conclusions related to predation unless they are able to add more compelling evidence.

Response: Our manuscript continues to explore the relationship between predation pressure and social systems, a working hypothesis originally proposed in light of previous studies indicating a link between social complexity and predation pressure (e.g., Groenewoud et al. 2016 PNAS). However, your comments are well taken, and we fully acknowledge the concerns raised. Indeed, our data demonstrate a correlation between body size and social systems rather than a direct measurement of predation pressure.

Accordingly, we have exercised caution in interpreting these findings and have explicitly noted the need for future research to critically re-evaluate this hypothesis and its associated uncertainties (e.g., L 201-205; L274-276). In particular, we have substantially revised the discussion of stomach content analysis, as you correctly point out that its relevance to social systems remains uncertain. Although we continue to report stomach content data, its role in the manuscript is now limited to assessing whether predation pressure is predominantly directed toward smaller fish (L158-164).

2. There's some confusion, mainly in the introduction, about whether the authors are examining the evolutionary causes of cooperative breeding or the evolutionary consequences of cooperative breeding. It seems, from the abstract (lines 29-31) and introduction (lines 54-62), that the authors are trying to explain likelihood of a lineage developing cooperative breeding based on its life-history characteristics. At other times (lines 95-97) it seems like the authors are hypothesizing about how life-history traits will evolve once a species develops cooperative breeding. The actual analysis (PGLS) reflects the latter; the authors use breeding status as the independent variable and use it to predict life-history characteristics.

Both questions are interesting, and I appreciate that it may be difficult to tease apart causality given the current data. However, I think the authors need to be more explicit about which they are testing, because they are very different questions. It looks like from the discussion (which is more clear on this point) that body size is being interpreted as a cause of cooperative breeding while egg and clutch size are considered as consequences; perhaps the directionality of the PGLS should be adjusted to match that.

3. I would recommend some reorganization of the introduction, in particular the final paragraph (lines 81-109). This paragraph somewhat veers off course into a lot of interpretation which is better left to the discussion. Please consider making this paragraph more concise by simply stating the hypotheses and possibly a brief description of the approach and/or results.

Response: We thank you for the points you have made. We believe that our manuscript has been improved in many places so as not to confuse the readers.

4. It feels like the authors are missing an opportunity to generate more robust analyses by only using the body size measures of the fish they caught themselves. According to Fig. 1, there are a lot of missing data points for the life history traits – over half of cooperatively breeding fish! While I get that for clutch size and egg size they may want to use their own collected data to make sure those metrics are standardized, it feels like body size could be re-analyzed using existing data on these fishes. This would be very easy to do, I think, and could provide stronger support for the authors' conclusions.

Response: We obtained subject cichlid body size data from Fisbase and analysed it by correlation analysis. A total of 61 cichlids were included in these analyses. Thank you for your useful suggestions.

5. I'm not sure about the appropriateness of converting continuous traits to discrete traits using arbitrary thresholds to run an analysis of transition probabilities (Figs 2a, 4a). I'm not an expert in this area, but it looks to me like BayesTraits has lots of methods for analyzing continuous traits but none of them involve just making the traits binary. This would seem to both throw away valuable information and lead to likely misclassification. Is this a common practice in the field? If so, please provide some citations justifying this approach, which is not at all intuitive.

Also, what does it accomplish that the PGLS doesn't? If body size and cooperative breeding are correlated, doesn't it necessitate that these transition probabilities have to be different? It feels unnecessary to me, in addition to being problematic.

Response: We now strongly agree with your point. We did not have the justification to convert the traits into binomial data, so all results and discussions related to BayesTraits were removed from the manuscript.

Minor comments:

Line 29: Remove the words “other than parents”

Response: The text noted was removed or changed based on comments from other reviewers.

Lines 95-97: Haven't read Arnold and Owens (1998) but why would decreased mortality lead to reduced opportunity to establish new breeding territories? And what does this have to do with clutch size or cooperative breeding?

Response: Our understanding is that Arnold and Owens (1998) considered that population saturation was related to the cost of producing offspring of the same cohort. Explanations for these hypotheses are added in L217-219, as follows, “According to the life history hypothesis, in an overcrowded breeding environment, resulting from a low annual mortality and low population turnover, natural selection may favor smaller clutch sizes.”

Lines 134-149: I appreciate the authors' forthrightness in discussing this piece of ambiguous data and its effects on their results. Could the authors extend this to see if changing the status of *N. niger* would also affect the results of the PGLS? I suspect not, but it would be nice to see that confirmed.

Response: We are honored that you have appreciated our attempts to increase the reliability of our analysis. The data from the public database you suggested included *N. niger*, so we carried out the similar approaches using phylogenetic GLMMs. In both analyses, the correlation between body size and social system was marginal, and the interpretation of this result troubled us. However, having presented our analyzes, we carefully discussed the relationship between body size, social systems and predation pressure. [L149-157]

Lines 265-277, Table 1: Should clutch size be included as a covariate in the PGLS analysis of egg size and social status? Given the predicted trade-off controlling for clutch size may help isolate possible relationships with egg size.

Response: First we here report that we have changed our PGLS analysis to MCMC GLMM with phylogeny information. Also, as you pointed out, we have incorporated the egg size (or vice versa) in the clutch size

analysis, into the model. However, our analysis did not detect the predicted strong egg size-clutch size trade-off. This result is similar in another paper we are submitting in parallel.

Lines 286-288: The PPA is neat but if the authors have a hypothesis about diet and trophic state shouldn't the $\delta^{15}\text{N}$ stuff be in the PGLS as well? I can't follow why the authors only bring up this data here, especially since they discuss diet in lines 124-129.

Response: While the point is well taken, we analyzed and discussed this variable in isolation to simplify the statistical model. More specifically, it was excluded from the model when the variable was reduced to make the model converge. This was done after PGLS confirmed that no relationship between $\delta^{15}\text{N}$ and the social system could be detected.

Line 360: Confusing wording here. Should this say "> 5"?

Response: Thanks for pointing this out to us. This was our mistake.

Once again, we sincerely thank you for providing so many valuable comments that have greatly improved our manuscript. We also apologize for the delay in responding to your feedback.

Note that our dataset and the R script for the analysis can be accessed via the URL (<http://datadryad.org/share/1VPwkhgeABi57IRJr7yaAS8NGbe5VL2PsVDjggOE1Y>).

Reviewer #2 (Remarks to the Author):

With great interest have I read the manuscript by Satoh et al. on the evolution of co-operative breeding in cichlids. I found it to be an enjoyable read, the questions are interesting, and the analyses and results make a lot of sense to me. There is quite some speculation throughout, but it is clearly communicated as being speculative, and the arguments are well sourced from the literature. I should note however, that I am not familiar enough with the ecology of these species to really critically evaluate some of the arguments made. I think there are some parts of the manuscript that can be more clear, especially the path analysis, which I'll explain below.

There's not much information presented about the path analysis. PPA, as proposed by Von Hardenberg &

Gonzalez-Voyer, is intended to confirmatory; i.e. comparing the evidence between causal hypotheses. But it is unclear what the hypotheses are. The authors mention that they compared 109 models (which is a lot! more than the number of species), but there is no information on why/how these models were chosen. Can the authors please clarify their reasoning here, and also include analytical details, such as what model of evolution was assumed, and what estimation method for the binary pglms was used.

The interpretation of the model with most support was also not very clear to me. The authors argue that the causal model supports the role of predation, but this variable is not in the model. I'm not sure if the "no effect" arrows are really helping the interpretation here, these paths aren't present at all in the final model, correct?

Response: First and foremost, we would like to extend my sincere apologies for the delay in resubmitting our revised manuscript. We are also glad that you have given positive feedback on our efforts. We made significant changes to refine all analyses based on reviewers' comments. We hereby apologize for our great misunderstanding and impropriety in the operation of the PPA. To ensure proper model selection, we have greatly reduced the number of paths and potential models. We also clearly presented what hypotheses are supported by each latent model [L263-270; L471-476; Supplementary S8].

Table 1 can do with a more detailed legend. What are the superscript numbers on the lambda parameters? What is the C.I. column? Can the degrees of freedom be added?

We changed our PGLS to BPMM. This was to address the adverse effects of averaging for species with small sample sizes.

Figure 1: Perhaps I missed it, but it is never spelled out what the numbers and arrows in this figure refer to. I assume the independent transitions to CB?

Response: Thanks for pointing this out to our figure. Arrows were removed due to changes in the method of ancestral trait restoration.

38: PPA does not reconstruct ancestral states or provide any temporal information, so it cannot reveal that cooperative breeding evolved first in smaller species. One can think of path analysis models are trying to describe what would happen in the case of an intervention. That is, if e.g. a species would become smaller, it would be more likely to become a cooperative breeding. If the authors want to make claims about what evolved first and in what species, they need to use evidence from other analyses.

Response: We apologize for our lack of understanding of the PPA. As noted in my earlier comment, we have changed our analysis per your suggestion.

156: Variations should be variation.

Response: We have changed it [L135].

174: I think “preys” should be “preyed”?

Response: This sentence has been deleted based on your other points.

231: Can the authors double-check that it is indeed a log10 BF of 40, and not just the BF? That would make the alternative hypothesis 10 duodecillion times more likely than the null, which seems rather extreme.

266: Since the authors are using Bayes Factors here, they can interpret that BF to mean strong to very strong evidence in favour of the null.

360: Double check if this is indeed supposed to say less than 5.

421: This section would be easier to read if it is specified earlier that it is all about BayesTraits Discrete (BayesTriats has more models). Also, please clarify the priors used.

428: <2 includes <0 , it's more clear to give intervals $[0, 2]$, $[2, 5]$, $[5, \text{Inf}]$.

Response: Thanks for pointing this out to us. We are writing to inform you that this analysis has been removed as noted by the reviewer#1.

238: “life history hypothesis” is such a generic term, that it would be helpful what is meant by that sensu ref [10].

Response: We have changed it [L217].

289: Cite Von Hardenberg and Gonzalez-Voyer 2013, Evolution 67(2): 378-87

Response: We have changed it [L265].

294: This needs more explanation. Predation is not in the path model, so this interpretation isn't straightforward.

Response: We have added some sentence, as follows; The most optimal PPA model showed that body size directly and significantly influenced social system, clutch size, and feeding ecology; cooperative breeding also resulted in a smaller clutch size (Fig. 5). Although we were unable to directly measure potential predation pressure occurring in the species in this study, body size can be seen as a life history trait that can determine predation pressure. Therefore, these results indicate that each relationship agrees with the following two hypotheses: 1) predation risk against species with smaller body size promotes the evolution of cooperative breeding, and 2) cooperative breeding restricts larger clutch sizes. Moreover, although body size influenced the feeding ecology (or trophic level) of each species, we could not find a significant relationship between social system and feeding ecology, as suggested by Dey et al. (2017)8. We believe that the described influence of diet on the evolution of cooperative breeding can be explained by fish eaters (or piscivorous fishes) that are larger than non-fish eaters [L271-283].

349: This reference to table 1 is not correct.

Response: We have removed table 1. Thank you.

356: I have an inkling, but can the authors please clarify their reasoning behind removing ovary mass from the body size measure? E.g. predators don't care about this distinction, and body size is used a lot in this paper in relation to predation.

Response: We have added some explain, as follows; This is because female body weight was greatly affected by changes in ovary weight due to the breeding cycle. [L349-350]

367: What about tissue shrinkage? Would the eggs kept in formalin not be measured to be smaller?

Response: All samples were once fixed in formalin. However, since some samples did not have a storage period recorded, we were not able to take into account the effect of the period of time they were stored.

We did not observe any extreme shrinkage of the eggs, and we consider those effects to be small. We provided a more adequate description of these methods [L347-362].

396: It is phytools, not phytool.

Response: we have changed it. Thank you.

440: I assume this refers to Pagel's lambda model? Why was this model of evolution chosen, were others considered? Was the same model of evolution assumed for the path analysis?

Response: We have changed our PGLS to BPMM. PPA continues to use Pagel's lambda model.

460: It's probably the CICc that was used, which is the CIC with correction for small sample sizes.

396: It is phytools, not phytool.

Response: We have changed it, as follows; The C-statistic Information Criterion corrected for small sample size (CICc) was calculated for model selection. [L476-477]

Once again, thank you for providing so many helpful comments to improve our manuscript. We also apologize for the delay in responding to your comments.

Note that our dataset and the R script for the analysis can be accessed via the URL (<http://datadryad.org/share/1VPwkhgeABi57IRJr7yaAS8NGbe5VL2PsVDjgqOE1Y>).

Reviewer #3 (Remarks to the Author):

In this study, Satoh and co-workers examine the evolution of cooperative breeding in cichlid fishes from Lake Tanganyika. This is an exciting topic, as the lamprologine cichlids from Lake Tanganyika are a textbook example for cooperative breeding in fish. Through character state reconstructions, the authors confirm the previous finding that cooperative breeding has evolved several times in the Lamprologini. They provide a large (but far from complete) dataset on cooperative breeding in Lamprologini and estimates for two life history traits, and it is obvious that they have spent quite some effort into collecting these data in the field. Unfortunately, the manuscript does not do justice to the interesting research question of broad relevance and the efforts in the field by the authors as well as their expertise on the cichlids. The analyses performed are standard at best, and much of the manuscript is more like story-telling and full of speculation, with many of the statements not being supported by data or analyses (which would nevertheless be possible).

Response: First and foremost, we would like to extend my sincere apologies for the delay in resubmitting our revised manuscript. In addition, thank you for providing so many critical and constructive comments. In order to properly address your suggestion, we have conducted additional field sampling and collected data from database. We strongly hope that our new manuscript will satisfy you.

One problem that I have is that the authors use only a subset of species (see below) and that they pick out a few traits and/or phenotypic axes. All lamprologine cichlids, also the non-cooperative breeders, perform brood care, but I do not see the diversity of "breeding types" reflected in their analyses, potentially leading to circularities. For example, the authors focus on shell dwellers but do not take into account other types of "protection against predators", such as digging sand tunnels, living below rocks or in caves, or breeding in crevices.

Response: We obtained breeding habitat data from publicly available databases and analyzed the relationship between habitat characteristics and social systems, extending our analysis beyond the previously examined shell-brooding species. Our analyses revealed a noteworthy relationship between habitat type and social structure, which we present and discuss in the revised manuscript.

Since shell dwellers have the property of being small (otherwise they would not fit into *Neothauma* shells), body size might simply be a confounding factor in their analyses. It would be important to see what would happen to a pPGLS when also other breeding types are included. Similarly, while the authors speculate about such confounding factors (see e.g. L298FF), they do not formally test for it. While I fully understand their focus on cooperative versus non-cooperative breeders, I do not see how some of the predictions can be applied to this strict distinction. For example, why should cooperative breeders have stronger predation than non-cooperative ones, especially at their juvenile age (e.g. LINE 178FF)? And if large predators are included in the noncooperative group, and clutch size is correlated to body size, then of course noncooperative ones have larger clutch sizes, as a confounding effect of body size.

Response: We here first report that we have changed PGLS to BPMM. This is to address the adverse effects of averaging that you pointed out. In these analyses, body size was controlled as a fixed effect along with clutch size. Similarly, the correlation between social system and clutch size was detected in the PPA after accounting for body size.

According to Ronco et al. (their reference 16), there are 100 species of lamprologine cichlids in Lake Tanganyika. In the present study, they analyzed 74 of those (for cooperative versus non-cooperative breeders) and much less than that for most of the other comparisons. At the same time, the authors show that their analyses are highly sensitive to the character state of single species (notably, they present the case of *N. niger*). In the light of the latter observation, I am wondering how credible their results are on the

whole. What if some of the *Telmatochromis* species are cooperative? Wouldn't that increase the probability for "just" 3 origins of cooperative breeding? In any case, it would be important to know the rationale behind their selection of subsets of species for the respective analyses. Perhaps the authors could also comment on the riverine lamprologines that they kept in their analyses (Fig. 1), any suggestion/prediction on those?

Response: We strongly agree with your concerns. We have attempted to scrutinize the existing previous studies to see if we can increase our knowledge of the social system. However, while we did achieve some success, we were unable to significantly increase our sample size. Instead, we varied the method of ancestral trait reconstruction with evolutionary simulation to identify a range of the number of evolutionary transition that could be obtained with the existing data set. We also carefully noted the uncertainty of inferences due to data limitations and the need for additional research [L113-131].

I also have an issue with the sample sizes used for some of the analyses, in particular the female body size analyses (And why only females??? There is only one species with a substantial male dimorphism, for all the other species an analysis of males would work well!). Judging from the methods section (L368), 155 females have been examined for 39 species. According to table S1, only 1-8 individuals per species (typically less than 4) were measured, which, in my opinion, is not sufficient to measure intra- and inter-specific variation (and way below current standards in the field). At minimum, these measurements should be presented, for example, in a figure with box-blots per species. The statement "female body size was smaller in cooperative breeders than in noncooperative breeders" (L164F) is problematic - what if you repeat this analysis without the lepidiolamprologine clade (large predators that might strongly influence all analyses that include female body size)? If the authors are interested in what small species do (see statement in L167, then they should only analyze small species that suffer from predation). In general, I find it problematic that the data of this study have not been made available to the reviewers, and I strongly suggest to the authors to make their primary data (and not just tables derived from analyzing them, such as Table 1) available publicly before submitting their work.

Response: We strongly agree with your point and have implemented multiple approaches to address the issue. The first step was to collect breeding males in the lake, and 351 breeding males were added to the data set. This is because it was not possible to collect the necessary male individual data from previous studies and databases. We also changed our PGLS to BPMM. Because the effect of species is controlled as a random effect in BPMM, individual data can be used to test the relationship between traits. While we were not able to completely solve the problem of small sample sizes for some of the species you point out, we recognize that we were able to partially eliminate the detrimental effects of averaging for species with small sample sizes. All figures have been modified to make the scope of the data set more recognizable.

We also apologize that our earlier manuscripts did not publish the required datasets. We have published the dataset and R code used in our analysis and modified it so that the analysis can be reproduced.

Another aspect that is entirely missing from this manuscript is that the other half of the cichlid species in Lake Tanganyika are mouthbrooders, which is not even mentioned in the manuscript. While I do not ask the authors to include those in their study, it should be at least discussed what can be expected in those (in the light of predation pressure, clutch size, etc.).

Response: We have added a discussion on mouth-brooding cichlids. Thanks for the suggestion [290-295].

I have not checked it for each and every analysis that the authors have performed, but by default, all analyses presented should be phylogenetically corrected (and not all are). Everything else does not make sense in this case. In general, the material and methods section is rather vague, program versions are often not provided, and links to the scripts and datasets used are entirely lacking, making it impossible for this reviewer to judge the validity of their analyses. Any revised version should contain a link to datasets and scripts used, etc.

Response: We apologize for any lack of clarity in our original description of the methodology. In response, we have revised the Methods section and have made the R code and datasets used in our analysis publicly available. While these data will be released upon completion of the peer-review process, they are currently accessible to reviewers via the following URL:

<http://datadryad.org/share/1VPwkfhgeABi57IRJr7yaAS8NGbe5VL2PsVDjggOE1Y>.

Throughout, the authors compare their results to previous studies in birds, which I find problematic, in particular, since birds have way smaller clutch sizes than most of the lamprologine cichlids; if such a comparison is intended, then I feel that the authors would need to perform similar measurements as have been done in birds (e.g. egg sizes; L268).

Response: We continue to consider the comparisons between birds and cichlids to be conceptually valid. However, we appreciate your observation that the previous version of our manuscript lacked certain

methodological details and supporting arguments. In response, we have substantially revised the manuscript to address these points, and have now included additional relevant data—such as egg size—to enable a more thorough and informed discussion.

I also have a problem with the title of this study: Where do the authors show convergent evolution of life history traits in their study? In general, it is a matter of debate if they show “convergence” in cooperative breeding (that would basically mean that the behavior is the same in all lineages it evolved), so it would be safer to say “Repeated evolution of cooperative breeding in Tanganyikan cichlid fishes”.

Response: We have changed our title. Thank you for your suggestion.

Finally, I feel that for the non-cichlid experts, it would be good to provide a bit more background on the study system in general and the Lamprologini in particular, and to define what are “tribes”.

Response: We have added an explanation [L76].

OTHER COMMENTS:

LINE28FF: This definition for cooperative breeding is somewhat different from the one provided in the beginning of the Introduction (LINE47FF) in that, in the abstract, individuals other than the parents “raise” offspring that is not their own, whereas in the Introduction they provide care to them, i.e., they “contribute to raising” them. To me, these are two different scenarios, please clarify and provide a consistent definition throughout.

Response: Thank you for sharing your uncomfortable point with us. We have changed the text to a more general definition of cooperative breeding system [L31].

LINE36: Should be “from the field” or from “field observations”.

Response: We have changed it to “field observations” [L38-39].

LINE65: If you use "significant", then I'd expect a P-value to back this up.

Response: We have changed "significant" to "great" [L67].

LINE105: Reference format is different to the other references.

Response: We have changed it.

LINE120: The phylogenetic hypotheses used is not for the "whole tribe" but for the entire radiation.

Response: We have changed this sentence, as follows; Previous studies^{8,27} suggest the convergent evolution of cooperative breeding in Tanganyikan cichlids, but the number of evolutionary events remains unclear. An accurate evolutionary history of this social system can be inferred using the most recent phylogenetic tree based on full-genome information of the cichlid fishes endemic to Lake Tanganyika¹⁵. [L101-105]

LINES134F: This sentence is somewhat awkward ("multiple occurrences of evolution"). I guess it should read "...of multiple gains and losses of cooperative breeding", but then I think that "multiple" is a bit of an over-exaggeration.

Response: We have changed it to "repeated". Moreover, we have tried to improve these sentences [L113-114].

LINE137: Should be "insufficiently".

Response: We have changed it. Thank you [L116].

LINE139F: Maybe better "in which" instead of "when".

Response: We have changed it [L115].

LINE167FF: Why "or" here? What about *N. multifasciatus*? This species is highly social (perhaps the most social of all lamprologines according to some authors) AND lives in shells.

Response: We apologize for the inappropriate manner in which we expressed here. We have changed "or" to "and" [L193].

LINE176: Remove "-" before "mm".

Response: Thanks for pointing that out. This sentence has been removed in present manuscript.

LINE198: What is a "simple" substrate? Is rock more "simple" than gravel or sand?

Response: We have changed it [L177].

LINE203: You mean "maintenance by parents"?

Response: We have changed It to "group members" [L184].

LINE210: Should be "temporalis".

Response: Sorry for our mistakes. We have changed it [190].

LINE216: I do not get the logic here: First, what is the difference between "small species" (and please define this) versus "lineages with small body size" (please define)? And how can you know in which environment they evolved? This cannot be answered by the type of character state reconstructions done by the authors, and I doubt that it is possible with the available data to reconstruct how large the species was, in which cooperative breeding evolved some millions of years ago, and in which environment it lived. All this is pure speculation.

Response: We removed this sentence as it was overly elaborated in the context of our analysis. We apologize for the carelessness of our previous discussion and sincerely appreciate your comments.

LINE220: Remove "a".

Response: Thanks for pointing that out. This sentence has been removed as we have added our analysis of males.

LINE252: According to this logic, shell dwellers should have an even lower predation pressure and, hence, a higher survival rate, because juveniles are protected in the shell; similarly, tunnel diggers should have a higher survival rate. All these are testable predictions. And what about mouthbrooders???

Response: Our revised analysis—while not without limitations—yielded results that align with your observation. Accordingly, we have added a sentence to reflect this point [L195-205]. Additional text has

also been included to address the topic of mouth-brooding [L290-295].

LINE280: Please say "two life history traits".

Response: Thanks for pointing that out. We have changed it [L254].

L298FF: "We believe...". Please provide a formal test for the influence of larger fish eaters on these results.

Response: Dey et al. (2017) appeared to suggest that species with a higher reliance on carnivory tend to have lower population densities and are less likely to evolve cooperative breeding. In light of these hypotheses, we considered it more appropriate to use stable isotope ratios in our analysis rather than relying solely on categorical classifications of species as piscivorous. Indeed, even among species generally regarded as fish-eaters, there are interspecific differences in the degree of reliance on piscivory. From this perspective, we determined that stable isotope analysis would provide a more accurate and nuanced approach. We apologize for the lack of clarity in previous drafts and have accordingly revised this section to better reflect our rationale [L265-271; L282-284].

L319: What is novel to this study then? It has been shown before that cooperative breeding evolved more than once and that cooperative breeding is associate to life history traits.

Response: We have changed it [L297-303].

L327: "Neothauma" should be in italics.

Response: We have changed it [L317].

L336: I do not see the point of overruling what is known from the literature. If it is known that *N. multifasciatus* also breeds on rocks, then I do not see the point of making them "obligate shell brooders" just for this study. Please repeat the analyses with defining them as facultative.

Response: We apologize for the inadequacy of our previous description. In this context, we focused on their life history of these species. Specifically, *Neolamprologus multifasciatus* is described as a facultative shell-brooder because, in certain populations, it utilizes not only shells but also fine rock crevices. This suggests that the breeding habitat of this species exists within a mixed environment of gravel and coiled shells, in contrast to species that inhabit exclusively rocky environments. Moreover, in the population we sampled, *N. multifasciatus* is characterized as an obligate shell-brooder, as it consistently uses shells as breeding nests throughout its life. To illustrate these ideas we have changed the text [L322-327].

LINE 376F: What about catfishes?

Response: As for catfish, we could not target them for our study because they hunt at night. We have, however, added text to the method section to explain the limitations of this study [L392-394].

LINE379: These coordinates are very vague.

Response: We have changed it [L387].

LINE388FF: I do not get this section. First of all, the phylogeny of Ronco et al. includes all cichlids from Lake Tanganyika (not just the lamprologines). Second, the phylogeny of Ronco et al. is available as tree file, so what have the authors "reconstructed" with RAxML? Which data have they used to do so? Finally, the phylogeny of Ronco et al. is time-calibrated, so what's the logic of dating it with BEAST2?

Response: Sorry. This was our mistake. Thank you for your comment. We have changed it [L398-401].

L406FF: All these analyses should be corrected for phylogeny, please redo.

Response: Thank you for your comments. We have changed it [398-420].

Thank you very much for your many thoughtful comments. We sincerely hope that the revised manuscript meets your expectations.

Response to Reviewer#1

General comment 1

I am reviewing the manuscript “Repeated evolution of cooperative breeding and life history traits in Tanganyikan cichlid fishes” for the second time. I apologize for the delay in completing the review. I initially had significant comments about both the analyses, writing, and interpretation. I now feel that the paper is much improved, but I still have comments about the interpretations regarding predation as well as interpretations regarding constraint. I also point out some grammatical issues that the authors can start to work on cleaning up as this gets closer to acceptance.

Response

We sincerely thank the reviewer for taking the time to provide a second evaluation of our manuscript. We greatly appreciate the thoughtful feedback and constructive suggestions. We are pleased to hear that the reviewer finds the manuscript much improved, and we carefully have revised the text to address the remaining concerns regarding interpretations of predation and ecological constraints. In addition, we thoroughly have reviewed the manuscript for grammatical issues to further improve clarity and readability.

General comment 2

1. I initially commented that I was not convinced of a relationship between predation risk and cooperative breeding. In this revision, the authors still claim to be testing a relationship between predation risk and cooperative breeding using body size as a proxy. I still don’t find this compelling. The authors should feel free to speculate that predation might be the mechanism by which body size and social status are related, but they should not state so directly that they are testing predictions about this (e.g. lines 90-93). Body size is related to so much more than predation risk.

Response

We apologize that our previous revised manuscript did not fully address your concerns. In the revised version, we have clarified that while we touched upon the predation pressure hypothesis, our analysis was based on the premise that body size influences predation pressure, as follows: *Second, we tested the relationships among social system, body size, and habitat type as ecological factors that potentially drive the evolution of cooperative breeding among cichlids*^{23–25}. *In particular, we focused on the relationship between the social system and body size, based on the premise that smaller body size may increase predation risk.* [L92-96]

We now also explicitly stated that body size affects not only predation pressure but various ecological factors, and discussed the need for further research in the future, as follows:

The present analysis was predicated on the assumption that body size is strongly correlated with predation pressure. Indeed, consistent with patterns observed in many fish species, stomach content analyses indicated that smaller fish in Lake Tanganyika are subjected to disproportionately higher predation pressure than their larger counterparts. It should be noted, however, that body size is influenced by a multitude of ecological factors beyond predation⁴¹. What this study demonstrates is a phylogenetically controlled correlation between social system and body size—an association between social system and predation pressure that warrants further rigorous investigation. [Line 208-215]

General comment 3

2. I'm a bit confused about the use of the word "constraint" throughout this manuscript. The authors seem to be suggesting that predation is an "ecological constraint" on social evolution – but at the same time they are arguing that predation drives social evolution. I'd suggest they stop using that word; there's no evidence here for anything constraining social evolution, just that there may be certain ecological factors that select for cooperative breeding behavior.

Response

We have removed the term "ecological constraints" from the manuscript. As you pointed out, in many cases the word 'constraints' could be replaced with "factors." [e.g., Line 65; 300; 309]

General comment 4

3. I'm finding the 15N analysis and Figure 5 a bit confusing and more importantly pretty tangential to the exciting questions and analyses in the paper. I guess I can live with it, but the authors could consider simplifying and shortening this paper a bit by just removing the whole analysis. In my opinion, it doesn't contribute anything.

Response

We consider the hypothesis proposed by Dey et al. 2018 to be highly important. Therefore, while recognizing that the discussion becomes more verbose by including it, we wished to retain these analyses. However, we carefully edited this part and hope that is now more straightforward to follow. Furthermore, the analytical model has been refined following the suggestions of Reviewer #2. The relevant sections read now as follows:

The 16 candidate models incorporated causal hypotheses reflecting four primary predictions, grounded in the results of the BPMMs and previous studies: (Hypothesis A) body size influences the evolution of social systems and clutch size; (Hypothesis B) feeding ecology affects the evolution of social systems; (Hypothesis C) body size drives feeding ecology; and (Hypothesis D) social system drive the clutch size. [Line 273-278]

Sixteen candidate models, including a null model, were constructed from combinations of four hypotheses (Hypothesis A: cooperative breeding evolved in species with smaller body size due to predation pressure; Hypothesis B: feeding ecology influences the evolution of cooperative breeding; Hypothesis C: body size affects feeding ecology; Hypothesis D: cooperative breeding reduces clutch size; see Supplementary S8). For this analysis, we employed Pagel's lambda model to estimate associations between continuous variables and the logistic MPLE method for those involving binary variables. [Line 492-499]

Minor Comments:

Lines 57-58: It feels a bit obvious that “evolutionary processes” mediate the development of cooperative breeding systems. Could you elaborate on a specific type of evolutionary process that these citations have revealed to be involved in this evolutionary innovation?

Response

Thank you for your suggestion. Since what we wanted to examine was an understanding of ecological or social factors driving cooperative breeding, we have revised the text as follows:

Recent advances in comparative methods have spurred discussions on the ecological and social factors that promote the evolution of cooperative breeding systems⁶⁻⁹. [Line 61-63]

Lines 193-205: See major comment 2 above. This is also a weird argument to make, that ecological factors are restricting the evolution of cooperative breeding. If the authors are arguing that shell-brooding is another solution to the problem of high predation, then a more straightforward explanation is that here, just like in large species, there is little selection for cooperative breeding. The words “constraint” and “restrict” imply that the trait would be beneficial but there is some other factor, genetic, developmental, or environmental, that prevents the trait from evolving. I don't see any need to invoke that concept here (or anywhere).

Response

We removed the term “ecological factors” from the entire manuscript and replaced it with the word “factors.” When revising the manuscript, we additionally deleted the paragraph containing the sentence you pointed out.

We still believe, as you stated, that shell brooding possesses a function to avoid strong predation pressure, though analyses demonstrating this have not been conducted yet. Furthermore, in our previous analysis of body size and social systems, we had implemented BPMM with body size as the response variable; however, this was removed following Reviewer #2's comment and replaced to PGLM, where social system fitted as response term. Therefore, we have determined that we currently lack the basis to develop these arguments.

Figure 1: Can the authors increase the contrast on the scales for the quantitative traits on the phylogeny? For total length especially, all the little green boxes look the same color to me – so I can't use that to evaluate the key result that body size is phylogenetically correlated to social system.

Response

Thank you for your suggestion.

We have improved the intensity of the color coding for the trait data in Figure 1. Additionally, we determined that the background color was making it difficult to judge the intensity of colors, so we have changed it.

Grammatical Comments

Line 61: Remove the “However” at the start of the sentence.

Line 65: Should say “...single breeding event”.

Lines 75-78: Rewrite as “Lamprologine chichlids, most of which are endemic to Lake Tanganyika, have diverse social and mating systems ranging from biparental care, polyandry, polygyny, polygynandry, and highly complex cooperative breeding systems.”

Line 81: Remove the space after species.

Lines 81-84: Awkward. Rewrite as “The combination of a well-resolved phylogenetic tree, their monophyletic origins, and their diverse ecological and social systems make lamprologines a suitable model system for comparative analyses of social evolution.”

Lines 109-110: Change to “... although the loss of cooperative breeding was not found.”

Line 114: Remove “the depiction of the social system in”

Line 116: change “the cooperative breeder” to “a cooperative breeder”

Line 117: remove “the”

Line 167: What is the “362” doing here?

Line 231: Remove space before citation 45.

Response

Thank you very much for taking the time to check the manuscript for grammatical shortcomings. We have made all the suggested changes according to your suggestions. We also checked the entire manuscript for similar errors, such as extra spaces after citations.

We would like to once again express our sincere gratitude for your thoughtful comments on our manuscript.

Response to Reviewer #2

I would like to start my review by expressing my dissatisfaction with the response letter. Many of my comments simply have not received any response at all, making it unclear if or how these have been addressed in the revision. I notice several comments from Reviewer 1 have also gone without response. The responses which are present are often terse and give little information on the changes made or the intent behind them. The authors have also re-ordered my comments, which is a minor point but adds to the confusion as it seems at least one of my comments has disappeared altogether:

Response

We would like to sincerely apologize for that several of the referees' previous comments were not adequately incorporated into the earlier revised manuscript. It appears that in the revision process, including data processing, interpreting the reviewers' intentions, and acquiring additional data, some of the referee's comments have inadvertently been deleted or their order was re-arranged. We very much appreciate the effort and care the referees has devoted to reviewing our work, and we have taken particular care in the present revision to ensure that each of their comments is addressed thoroughly and transparently. We sincerely hope that the revisions made in the current version will meet the referee's expectations.

General Comment 2

L362: Is it not possible to estimate egg volume? E.g. one can use the volume of an ellipse: $V = (\pi * L * W * H) / 6$, and plug in the values that already have been measured. It seems to me that it is the volume of the egg (or the log volume) that matters.

Response

I apologize for overlooking this suggestion in the first round of reviews.

We have now changed the analysis from being based on egg size to being based on egg volume. Since it was difficult to measure the thickness of the eggs, the volume was calculated using the major and minor axes with the following formula: $\text{egg volume} = \frac{4}{3} \pi \times (\text{long radius}) \times ((\text{short radius}))^2$. The relevant changes are in lines 373-380.

General Comment 3

As just one example of a terse response, I had asked: "440: I assume this refers to Pagel's lambda model? Why was this model of evolution chosen, were others considered? Was the same model of evolution assumed for the path analysis?" To which the authors respond: "We have changed our PGLS to BPMM. PPA continues to use Pagel's lambda model." So this doesn't

answer why this model of evolution was chosen, or whether alternatives were considered. It also ignores what the assumed model of evolution of the BPMM is. I am not helped by the fact that the marked up manuscript does not have all changes marked. Overall, the response letter is really minimally useful to me, and makes my job as a reviewer more difficult than necessary.

I would kindly ask the authors to take care when writing their response letter to 1) reproduce my comments verbatim and in the same order, and 2) clearly respond to each point individually. When changes have been made, clearly indicate where I can find those, and what motivated the changes. If a comment is no longer relevant, or if the authors disagree with comment, please also indicate this clearly.

Response

Since the evolution of cooperative breeding has occurred independently multiple times, phylogenetic covariation may be small. Therefore, we employed the Pagel-Lambda model, which corrects for phylogenetic effects. For binary data, logistic MPLE was used. This was stated in the paper, as follows:

PGLM: The model was constructed with the social system (cooperative or non-cooperative breeding) as the response variable and body size (mean log10-transformed BW for females) and breeding habitat (with crevices/caves, facultative shell-brooding, obligatory shell-brooding, rocky area, or sandy/muddy area) as explanatory variables, applying a logistic regression framework (logistic MPLE method). A separate PGLM was fitted for the body size of mature males in the same manner. [Line 446-451]

PPA: For this analysis, we employed Pagel's lambda model to estimate associations between continuous variables and the logistic MPLE method for those involving binary variables. [Line 497-499]

A Brownian motion model was used in BPMM. During the revision process, we changed the used R package from MCMCGLMM to brms, still the evolutionary model remained the Brownian motion model. The reason for using the Brownian motion model was that when using these packages, it was not possible to incorporate the estimation of the lambda value into the analysis. In the paper, we have stated as follows:

The correlation matrix of phylogenetic distance, assuming a Brownian motion model, was

incorporated as a group random effect. [Line 478-480]

Once again, we sincerely apologize for the mentioned omissions. We sincerely hope that the new manuscript satisfactorily answers all questions.

Specific comments

Comment 1

Overall, I don't find the revised version of the manuscript more convincing than before. In particular, the new ancestral state reconstructions suggest many more independent transitions than before, but the authors are not very self-critical of this result (also see below).

Response

We added a more self-critical discussion regarding phylogenetic analysis from several perspectives. One point is phylogenetic uncertainty: here, while confirming the phylogenetic tree estimates from Ronco et al. 2021, we discussed the uncertainty in the number of evolutionary events, as follows:

Moreover, several inferred independent transitions in ancestral state reconstructions rely on the presence of a single cooperative species (N. meeli, N. niger, N. buescheri, N. bifasciatus, and N. obscurus) (Figure 1). As the results of ancestral state reconstruction were based on a published phylogenetic tree¹⁵, it was not possible to fully incorporate phylogenetic uncertainty in our analyses. Examination of the tree¹⁵ indicates that the clade comprising N. prochilus and N. obscurus is sister to the clade containing N. bifasciatus; however, this relationship is supported only weakly (bootstrap = 64%)¹⁵, leaving open the possibility that either the N. prochilus–obscurus clade or N. bifasciatus may instead form a sister group with N. buescheri. Under such a topology, the inferred number of evolutionary transitions to cooperative breeding would be reduced by one, and a subsequent loss of cooperative breeding could be detected. Regarding N. niger, uncertainty lies not only in the characterization of its social system but also in its placement in the phylogenetic tree¹⁵, both of which may influence the inferred frequency of cooperative breeding evolution. This differs for N. meeli, which represents an additional, independent evolutionary origin of a cooperative social system. Here, no other cooperative breeding species are present in the Lepidiolamprologus clade; the effects of phylogenetic uncertainty are hence expected to be minimal. [Line126-142]

Furthermore, as the referee pointed out, we mention the uncertainty in our conclusions that accompanies the updating of knowledge about cichlid life histories, as follows:

In addition to these interspecific variations in the reproductive skew in cooperatively breeding cichlids, a comprehensive comparative analysis of the prevalence of cooperative breeders, the modes of helping behavior (e.g., direct and indirect care), and their impacts on and responses from offspring would be essential³⁰. Taken together, future revisions to behavioral descriptions of social structures, including the discovery of new cooperatively breeding cichlids, and phylogenetic analyses may therefore necessitate corresponding updates to the conclusions drawn in this study. [Line148-154]

Comment 2

Secondly, they now present phylogenetic regressions of a range of variables (including breeding mode) on body mass, clutch size and egg size. However, their conclusions are all about breeding mode, which was not used as an independent variable. While in a simple $Y \sim X$ regression the direction of the regression can be swapped more or less indiscriminately, this is not the case for multiple regression, nor is it the case for phylogenetically informed regression. I therefore believe these models to be inappropriate for the goal the authors have in mind.

Response

We removed the BPMM with body size as the response variable and replaced it with a PGLM in which the social system is the response variable and body size the explanatory variable. As the referee correctly noted, in the previous draft we had constructed a model that differed slightly from our original hypothesis because the model with social system as the response variable failed to converge [former manuscript, Lines 455–457]. This had forced us to treat body size as the response variable instead. Upon further examination, we identified that the lack of convergence arose because, although the response variable was specified at the species level, we had incorporated individual-level variation as an explanatory variable. We had done so under the assumption that intra-species replication would be advantageous. In the present revision, following the referees' advice, we have restructured the analysis so that the social system is treated as the response variable, with body size averaged per species and included as the explanatory variable in PGLM. The following sections have been modified:

. A phylogenetic generalized linear model (PGLM) with a binomial error structure, based on field data from these 39 cichlid taxa, revealed that the social system was significantly affected by the body size of mature females ($z = -2.48$, $p = 0.013$) (Fig. 2a; Table 1), with cooperative breeders exhibiting smaller body sizes than non-cooperative breeders (Fig. 3a). Furthermore, in terms of habitat effects, the intercept was significantly positive only for the category "crevices and caves,"

indicating that habitat type modulates the relationship between social system and body size (Fig. 2a; Fig. 3a; Table 1). Similar results were obtained from an analysis using the body size of male breeders (Fig. 2b and Fig. 3b; Table 1). [Line 164-172]

To assess the reliability of our analyses based on field-obtained data, we performed PGLMs using body size data from 61 taxa available on FishBase (<https://fishbase.org/>) and their phylogenetic tree. Also, this analysis revealed a significant association between social system and body size, as well as a significant effect of certain habitat types on the intercept (Supplementary S4). The relationship between body size and social system remained robust when *N. niger* was classified as a non-cooperative breeder (Supplementary S5) or excluded from the analysis altogether (Supplementary S6). These findings suggest that cooperative breeding evolved more readily in smaller species that might be more vulnerable to predation. [Line 173-181].

We examined the relationships between social system and body size, egg number, or egg size. Because the statistical analyses incorporated either 39 or 61 taxa, we pruned the MCMC tree from Ronco et al. (2021)¹⁵ to retain only the relevant taxa. When a species was represented by multiple terminal tips, the dataset corresponding to the tip geographically closest to the species' natural distribution was used in the phylogenetic tree for this life-history trait analysis.

First, we used phylogenetic generalized linear models (PGLMs; R package *phylolm* (ver 2.6.5)⁶⁵) to test the association between social system and body size across 39 taxa. Our analysis is grounded in the hypothesis that cooperative breeding is driven by predation pressure, under the assumption that body size and predation pressure are strongly correlated. Before fitting PGLMs, log-transformed body weights were averaged by taxa, separated by sex. The model was constructed with the social system (cooperative or non-cooperative breeding) as the response variable and body size (mean log₁₀-transformed BW for females) and breeding habitat (with crevices/caves, facultative shell-brooding, obligatory shell-brooding, rocky area, or sandy/muddy area) as explanatory variables, applying a logistic regression framework (logistic MPLE method). A separate PGLM was fitted for the body size of mature males in the same manner.

In addition to PGLMs analyses for the life history traits (39 taxa) gained from field observation, we performed comparable analyses of the evolutionary relationship between body size and social system using a larger number of species (61 taxa) in the public database FishBase. While these data were collected using non-standardized methods, they provided a broader species representation than our field collection data. We used PGLMs, fitting the pruned phylogenetic tree with 61 taxa. In these models, body size (defined as log₁₀-transformed total length in cm)

was used as the response variable, with social system and breeding habitat included as explanatory variables. Given that the FishBase body size data included *N. niger*, we conducted separate PGLMs, treating *N. niger* either as a cooperatively breeding species, an undetermined social species (i.e., excluded from the dataset), or as a non-cooperatively breeders. [Line435-462]

With respect to egg size and egg number, we continue to regard the social system as an explanatory variable, and thus the structure of that model remains unchanged.

Comment 3

From my previous review: What estimation method for the binary pglms was used in the PPA?

Response

We used the logistic MPLE method. Since this was not mentioned in the previous draft, we added the following text.

For this analysis, we employed Pagel's lambda model to estimate associations between continuous variables and the logistic MPLE method for those involving binary variables. [Line497-499]

Comment 4

From my previous review: The interpretation of the model with most support was also not very clear to me. I'm not sure if the "no effect" arrows are really helping the interpretation here, these paths aren't present at all in the final model, correct?

Response

In the PPA, we selected the model based on CIC. Therefore, the no-effect arrows indicate paths that were incorporated into some latent models but were not included in the final model. Consequently, as you pointed out, these paths do not exist at all in the final model.

Comment 5

From my previous review: Is it not possible to estimate egg volume? E.g. one can use the volume of an ellipse: $V = (\pi * L * W * H) / 6$, and plug in the values that already have been measured. It seems to me that it is the volume of the egg (or the log volume) that matters. This is more appropriate than estimate egg size as is currently done.

Response

We now individually calculated the egg volume from the major and minor axes of an egg. Therefore, in the current manuscript, all analyses concerning egg size have been changed to use egg volume (add line numbers again). We have also uploaded the egg volume data to Dryad.

Comment6

Line 69: "However, only a few studies have focused on the relationship between cooperative breeding and life history traits in vertebrates except for birds and mammals due to the limited accumulation of knowledge." This reads as saying that there have been few studies on this because there have been few studies, which is tautological.

Response

Thank you for pointing that out.

We have revised the text, as follows:

However, only a few studies have focused on the relationship between cooperative breeding and life history traits in animal taxa that are neither birds^{10,12,14} nor mammals^{9,13}. [Line73-775]

Comment 7

108: Several of these independent transitions are supported by a single species. How sensitive is this result to uncertainty in the phylogeny? My understanding is that the analyses did not consider this.

Response

Our analyses are based on the most phylogenetic tree of Lake Tanganyika cichlids, based on full genomes of all hitherto described species (Ronco et al). We were therefore unable to apply a bootstrap-generated tree. Examination of the Ronco et al. tree indicates that the clade comprising *N. prochilus* and *N. obscurus* is sister to the clade containing *N. bifasciatus*; however, this relationship is somewhat weakly supported, and we cannot exclude the possibility that this clade instead forms a sister group with the outgroup *N. buescheri*. Under such a topology, the number of inferred evolutionary origins of cooperative breeding would be reduced by one, suggesting that *N. prochilus* may represent a case of secondary loss. For other single inferred transitions, the influence of phylogenetic uncertainty is considered low. With respect to *N. meeli*, because the Lepidiolamprologus clade containing this species lacks any other cooperative breeders, the impact of phylogenetic uncertainty is also regarded as minimal.

We have not been able to statistically verify these possibilities (or using phylogenetic analysis)

in our new manuscript. However, we have instead chosen to convey the uncertainty of our conclusions to readers by incorporating statements such as the following.

Moreover, several inferred independent transitions in ancestral state reconstructions rely on the presence of a single cooperative species (N. meeli, N. niger, N. buescheri, N. bifasciatus, and N. obscurus) (Figure 1). As the results of ancestral state reconstruction were based on a published phylogenetic tree¹⁵, it was not possible to fully incorporate phylogenetic uncertainty in our analyses. Examination of the tree¹⁵ indicates that the clade comprising N. prochilus and N. obscurus is sister to the clade containing N. bifasciatus; however, this relationship is supported only weakly (bootstrap = 64%)¹⁵, leaving open the possibility that either the N. prochilus–obscurus clade or N. bifasciatus may instead form a sister group with N. buescheri. Under such a topology, the inferred number of evolutionary transitions to cooperative breeding would be reduced by one, and a subsequent loss of cooperative breeding could be detected. Regarding N. niger, uncertainty lies not only in the characterization of its social system but also in its placement in the phylogenetic tree¹⁵, both of which may influence the inferred frequency of cooperative breeding evolution. This differs for N. meeli, which represents an additional, independent evolutionary origin of a cooperative social system. Here, no other cooperative breeding species are present in the Lepidiolamprologus clade; the effects of phylogenetic uncertainty are hence expected to be minimal. [Line126-142]

Comment 8

143: While this is far from clear from the text here, my understanding is that these analyses use body size as the independent variable, not cooperative breeding. So they suggest that body size evolution is affected by CB, but I do not believe they provide evidence that CB is affected by body size, which was the stated aim of this paper.

Response

As the referee pointed out, our objective is to verify whether body size influences changes in social systems. In the previous draft, since the BPMM failed to converge, we conducted an analysis with body size as the response variable. In the current draft, by changing the BPMM to PGLM, we successfully performed an analysis with the social system as the response variable. In this analysis, individual data were averaged, so we could no longer obtain repetition; however, we believe we were able to construct a model more aligned with the intended purpose.

Accordingly, we have revised not only the main text [Line 164-181; 435-462] but also Figures 2 and 3.

On the other hand, as described in Lines 95-97, the purpose of our analysis of egg investment patterns was to verify whether changes in social systems affect these patterns. This possibility has also been suggested in previous studies of birds and fish. Therefore, regarding the analysis of the egg investment pattern, we have not updated the model structure. Furthermore, in these analyses, we continue to use BPMM, which includes individual-based intraspecific repetition.

However, following the referees' suggestion to change the egg size to egg volume, the wording on the BPMM has been updated, as follows:

Second, we performed a Bayesian phylogenetic mixed model analysis (BPMM; R package brms (ver 2.22.0)⁶⁶ with Stan (ver 2.32.2)⁶⁷) to determine the factors driving clutch size variation across 39 taxa using individual data of females (n = 155 females from 39 taxa). The model was constructed with log10-transformed clutch size as the response variable and included social system, breeding habitat, log10-transformed egg volume, and body size (log10-transformed BW) of female breeders as explanatory variables. Third, we tested whether the social system influenced egg volume. In this model, log10-transformed egg volume was set as the response variable, with social system, breeding habitat (crevices/caves as the reference, along with facultative shell-brooding, obligatory shell-brooding, rocky area, or sandy/muddy area), log10-transformed clutch size, and body size (log10-transformed BW) of female breeders as explanatory variables.

In both BPMMs, the error distribution for continuous variables followed a Gaussian distribution. A Markov chain Monte Carlo (MCMC) simulation was performed with a total of 1,000 iterations, including a burn-in of 500 iterations, a thinning interval of 1, and four chains were applied. We assessed model convergence with R-hat via the convergence diagnostic. All R-hats were kept less than 1.01. The correlation matrix of phylogenetic distance, assuming a Brownian motion model, was incorporated as a group random effect. [Line463-480]

Moreover, figures 4 and 5 have also been updated.

Comment 9

151: "As consequence of analysis using data from FishBase, we found that the marginal correlations between social system and body size (total length) among lamprologini cichlids (n = 61 species) even when N. niger as classified as a non-cooperative breeder, cooperative breeder, and undescribed species (Fig. 3c; Supplementary S4-S6)." I don't follow what this sentence is trying to say. What is meant by the marginal correlations? Marginal to what? Do

the authors mean this in a statistical sense, like after adjusting for other effects, or do mean that the effects are weak? I would recommend rephrasing. Why are there no statistics reported from the GLMM, like was done in 148 for the BPMM?

Response

We apologize for the inappropriate description in the previous analysis. In the earlier manuscript, the value slightly exceeded 0.05 based on the analysis results, leading to that description. In the new analysis, following the referees' suggestion, the response variable was changed to social system, and the analysis results were updated accordingly. Therefore, the term "marginal correlations" has been removed from the manuscript. Additionally, the reason the analysis results obtained from FishBase were not included in the main text was that this analysis is positioned as supplementary to the analysis of field data.

To make this clear, we have changed the sentence, as follows:

*To assess the reliability of our analyses based on field-obtained data, we performed PGLMs using body size data from 61 taxa available on FishBase (<https://fishbase.org/>) and their phylogenetic tree. Also, this analysis revealed a significant association between social system and body size, as well as a significant effect of certain habitat types on the intercept (Supplementary S4). The relationship between body size and social system remained robust when *N. niger* was classified as a non-cooperative breeder (Supplementary S5) or excluded from the analysis altogether (Supplementary S6). These findings suggest that cooperative breeding evolved more readily in smaller species that might be more vulnerable to predation. [Line173-181]*

Comment 10

406: Can the authors comment on what the multiple tips per species means for their analyses? How have they assigned trait data to these tips? Are any the species not monophyletic?

Response

We apologize for the confusing description in the previous manuscript. In fact, we followed the classification of Ronco et al. (2019, 2021), in which "species" included taxonomic ranks below the species level. Accordingly, we have used the term "taxa" in the revised manuscript. Some currently recognized species consist of multiple taxa, and among these, two did not form a monophyletic group. In our ancestral trait reconstructions, we assumed that all taxa within a species shared the same social system. However, in the regression analyses, the available social system data could be assigned to a single taxon, and thus no issues arose.

In PGLM and BPMM, when a species was represented by multiple terminal tips, the dataset corresponding to the tip geographically closest to the species' natural distribution was used in the phylogenetic tree for this life-history trait analysis.

These points have now been clarified, and the following sentences have been added. In addition, a correspondence table between species names and taxon names has been provided in Supplementary S1.

We conducted data collection following the taxonomic inventory of Ronco et al. (2019)¹⁶. This review suggested that single described species sometimes consist of multiple distinct sub-units (termed taxa from now on), like local varieties and undescribed species. Social system data were collected from previous studies (Supplementary S1). According to data collection, we determined that 31 taxa from 27 species were cooperative breeders, while 50 taxa from 44 species were non-cooperative breeders (Fig. 1). If a single species contained multiple taxa, we assumed they had the same social systems. The correspondence between scientific names and taxon names used in Ronco et al. (2019; 2021)^{15,16} is provided in Supplementary S1. [Line315-323]

To account for the effects of phylogeny, we included a modified, time-calibrated phylogenetic tree of the Tanganyika cichlids¹⁵ in our analyses. To that aim, we pruned the Ronco et al. (2021) tree¹⁵ using the 'droptip' function in R (ver 4.4.2)⁶⁰ in package ape (ver 5.8-1)⁶¹. Using the trimmed tree, ancestral states of social systems were assessed using phytools (ver 2.4-4)⁶². We included all 81 taxa (31 taxa from 27 cooperative breeders and 50 taxa from 44 non-cooperative breeders) in the analysis. [Line413-419]

We examined the relationships between social systems and body size, egg number, and egg size. Because the statistical analyses incorporated either 39 or 61 taxa, we pruned the MCMC tree from Ronco et al. (2021)¹⁵ to retain only the relevant taxa. When a species was represented by multiple terminal tips, the dataset corresponding to the tip geographically closest to the species' natural distribution was used in the phylogenetic tree for this life-history trait analysis. [Line435-440]

Comment 11

408: Can the authors provide appropriate references for the ARD and ER models, as well as the "equal" and "FitzJohn" models? I am not quite sure what the authors mean by the latter, do mean the prior for the state at the root, i.e. the flat vs FitzJohn priors? How do these choices

affect their estimate of the number of transitions? I don't believe it is appropriate to compare the results of models using different priors with AIC, but I could be mistaken. Can the authors provide a reference for this approach?

Response

As you rightly pointed out, we recognized that comparing different root states using AIC is inappropriate. Because our primary interest lies in the evolutionary processes of social systems, including root trait states, we therefore used only the FitzJohn estimation and compared the ARD model with the ER model—both based on the FitzJohn estimation—using AIC in current draft. Although the main results remained unchanged because best fit model in former draft was ARD model with FitzJohn, we have revised the methodological description accordingly. We sincerely appreciate your valuable feedback.

We also performed ancestral trait reconstruction using the ARD model with equal estimation to confirm, but the results were nearly identical to those from ARD with Fitzjohn (CB to nCB transition = 0 time; nCB to CB transition = 8 to 11 times (median = 9 times)). Estimates of the emergence period of social systems using LLT were also largely consistent.

We have also attached references for root estimation and the methodology of ancestral state reconstruction that we referred to.

[root estimation]

- FitzJohn, R. G. Diversitree: comparative phylogenetic analyses of diversification in R. *Methods Ecol. Evol.* 3, 1084–1092 (2012).

[methodology of ancestral state reconstruction]

- Revell, L. J. phytools 2.0: an updated R ecosystem for phylogenetic comparative methods (and other things). *PeerJ* 12, e16505 (2024).
- <https://www.rdocumentation.org/packages/phytools/versions/2.4-4/topics/fitMk>

Using the trimmed tree, ancestral states of social systems were assessed using phytools (ver 2.4-4)⁶². We included all 81 taxa (31 taxa from 27 cooperative breeders and 50 taxa from 44 non-cooperative breeders) in the analysis. We constructed two models for the probability of change between traits (All Rates Different [ARD] and Equal Rates [ER] models). In both models, the stationary probabilities of root traits were estimated using the maximum likelihood methods of

*FitzJohn (2012)⁶³. Comparisons based on AIC (Akaike Information Criterion)⁶⁴ values show that the ARD with FitzJohn root estimation provided the best fits to the data and represents the non-uniformity of evolutionary rates and heterogeneous transition probabilities between social systems (ARD, AIC = 60.088; ER, AIC = 61.426). Subsequently, based on the best model, 1000 stochastic character mappings were performed to estimate the probability of transitions between social systems⁶². Moreover, a lineage-through-time plot was created based on the simulation results to examine how the number of lineages in each social system changed over evolutionary time. As the breeding system of *N. niger* is only anecdotally described, we also performed a similar analysis, treating either as an undetermined social species (i.e., excluded from the dataset) or as a non-cooperative breeding species. [417-432]*

Comment 12

Figure 1: The figure legend leaves me with many questions. Many of the species abbreviations are not unique, which makes the figure confusing. Are these the multiple populations of the same species mentioned in the text? One tip is labeled "Neo sp.", which seems like it represents more than 1 species? Why are some species names underlined? What does it mean for a node to be "unpredictable"? It is not clear to me what panel B adds to this figure.

Response

As noted earlier, our classification of “taxa” follows Ronco et al. (2019, 2021). In the previous draft, however, some names had been independently converted to species names, which caused confusion. In the present manuscript, we have avoided such inconsistencies by adhering strictly to the taxon names of Ronco et al. (2021) in the phylogenetic tree. For clarity, readers may refer to Supplementary S1, which provides a correspondence table between species names and taxon names.

The taxa defined by Ronco et al. (2019) represent units larger than population-level differences and are considered regional species or taxonomic groups requiring future formal description. In the previous manuscript, “Neo sp.” referred to *Neolamprologus* sp. “Kombe,” which is listed as an undescribed species in Ronco et al. (2019, 2021). In the present manuscript, this taxon is designated “Neokom,” consistent with the original classification of Ronco et al. (2019, 2021).

Taxa for which we successfully collected field data are underlined. Gray circles indicate nodes at which the ancestral state of the social system could not be estimated due to missing tip data. Panel B presents a lineage-through-time (LTT) plot, generated from ancestral state reconstruction and 1,000 stochastic character mappings. To address the lack of detail in the previous draft, additional explanatory text has been included.

Based on the referees' feedback above, we have changed the figure caption, as follows:

Figure 1. Phylogenetic distribution of cooperative breeding and diversity of life history traits among lamprologine cichlids. (a) Blue and red circles indicate cooperative (n =31 taxa from n = 27 species) and non-cooperative breeders (n =50 taxa from 44 species), respectively. Variation in clutch size, egg size, body weight, total length, and habitat is indicated by color intensity. Pie charts represent the likelihood of the social system as estimated by ancestral state reconstruction. Tips with unidentified social systems due to missing life-history data are depicted as circles with diagonal lines. Nodes of tips for which the ancestral state of the social system could not be estimated because of missing tip data are indicated by gray circles. Underlined taxon names denote taxa for which life-history trait data were obtained through standardized field sampling. The scale bar corresponds to one million years. (b) A lineage-through-time (LTT) plot visualizing the relationship between time (million years) and the cumulative number of taxa with non-cooperative (blue) and cooperative breeding (red). Each thin line represents a single LTT plot for 1/1000 stochastic character maps; the thicker central lines represent the average. The Bayesian MCMC tree was produced by Ronco et al. 2021.

Comment 13

Figure 2: The length of the plotted tails of these distributions are quite arbitrary. Can the authors please add the limits of the credible interval? The x-axis reads "Z-score", but it seems this is not true for the binary predictors?

Response

As the referee correctly noted, the confidence intervals were difficult to discern; therefore, we have revised the figure and added shading to improve clarity (We kindly ask you to refer to Figure 4). In addition, we have prepared a table to present the confidence intervals explicitly. Since the Z-score is calculated as the coefficient divided by its standard error, we understand that it can be obtained even for discrete lightweight data. We have also added Table 1 and Table 2 to make it easier for readers to review the statistical information.

We would like to confirm whether we have understood the referees' point correctly here. If not, we would greatly appreciate any further clarification.

Comment14

Figure S8: Why are there multiple models for some combinations of hypotheses (e.g. APA, PPP, PAP) but not others (e.g. APP)? Is AAA the same as the null model?

Response

The inclusion or exclusion of a path from Body size to Feeding—which had not been incorporated into any initial hypothesis—resulted in multiple models arising from certain combinations of hypotheses. To address this, we added the corresponding hypothesis (i.e., Feeding is determined by Body size), thereby increasing the total number of latent models to 16. The AAA hypothesis represents the null model.

Accordingly, we have revised the text, supplementary S8, and S9, as follows:

The most optimal PPA model showed that body size directly and significantly influenced social system, clutch size, and feeding ecology; cooperative breeding also resulted in a smaller clutch size (Fig. 6). Although we were unable to directly measure potential predation pressure occurring in the species in this study, body size can be seen as a life history trait that can be linked to predation pressure. Therefore, these results indicate that each relationship agrees with the following three hypotheses: Hypothesis A) predation risk of species with smaller body size promotes the evolution of cooperative breeding, and Hypothesis D) cooperative breeding restricts larger clutch sizes. Although body size influenced the feeding ecology (or trophic level) of each species (Hypothesis C), we could not find a significant relationship between social system and feeding ecology, as suggested by Dey et al. (2017)8. Instead, the described influence of diet on the evolution of cooperative breeding can be explained by the fact that piscivorous fishes are larger than non-piscivorous fishes. [Line 279-291]

Sixteen candidate models, including a null model, were constructed from combinations of four hypotheses (Hypothesis A: cooperative breeding evolved in species with smaller body size due to predation pressure; Hypothesis B: feeding ecology influences the evolution of cooperative breeding; Hypothesis C: body size affects feeding ecology; Hypothesis D: cooperative breeding reduces clutch size; see Supplementary S8). For this analysis, we employed Pagel's lambda model to estimate associations between continuous variables and the logistic MPLE method for those involving binary variables. [Line 492-499]

Despite our initial oversight of some of the referee's suggestions, we are sincerely grateful for their guidance, which has greatly helped us to improve our manuscript.

Point-by-point rebuttal letter

I was not asked to provide my own review of the paper. However, several points do crop up, which relate to some of R2's comments, which I would like the authors to consider in their future work. And a couple of points that can be easily fixed in the present work.

Response to Refree#4: We sincerely thank Reviewer #4 for carefully reviewing our manuscript and for taking the time to examine our previous response letter in detail. We also appreciate the reviewer's positive evaluation of our work, as well as the constructive and insightful advice provided for our future research.

Modelling issues:

Comment 1: Report the phylogenetic signal in your traits.

Response to Comment 1: Thank you for your omments. We have calculated the phylogenetic signal and added it to the manuscript and the supplementary table S8. The relevant changes are as follows:

Additionally, although not reported in the results section, we calculated the phylogenetic signals for each trait (male and female body weight, total length, clutch size, and egg size) using the phytools package (ver 2.4-4)⁶⁵. Significant phylogenetic signals were detected for female body size, total length, and clutch size. Detailed statistical results are provided in Supplementary table S8. [L433-437]

Comment 2: When using multiple regression, interpreting the intercept and other parameter estimates can be difficult. In some of your models, you have one fixed effect with five levels (breeding habitat) and another that is continuous (log body size). The intercept here is the mean value of Y for the first level of breeding habitat when log body size = 0. The other coefficients for breeding habitat are then differences from this baseline. Are these contrasts biologically meaningful? I guess that the reference level was chosen appropriately. An alternative would be to interact the fixed effects.

Response to Comment 2: We recognize the habitats as control factors affecting transitions in social systems. We also considered testing for two-way interaction, but as you later pointed out, we did not perform this modeling due to our limited sample size.

Comment 3: In phylogenetic multiple regressions, only phylogenetic signal in the

response is considered. This means that swapping which trait is the response (eg. between your PGLS vs BPMM models) changes the interpretation. This is true for the path analysis as well. Using multi-response BPMMs can help avoid this. The paper cited below provides an excellent description of the issues and the solutions to the problem. Unfortunately such models are data hungry.

Response to Comment 3: We have acknowledged your comment as advice for future research. Thank you very much. The analyses you suggest are currently not feasible due to limitations in sample size. We believe the approach we can take to address these issues is to increase the number of comparable species through field observation, but we are currently in the process of planning the relevant field observation at the deep water of the Lake.

Reproducibility issues:

Comment 4: Unfortunately, the Dryad link didn't work so I couldn't check the code, underlying data and whether or not the authors uploaded a README file.

Response to Comment 4: We apologize for our shortcomings. We were operating Dryad incorrectly. Currently, Dryad data is publicly available, and you can check the README file and datasets.

Comment 5: The data in Table S1 seems incomplete, as this only includes breeding system information.

Response to Comment 5: We have added information on breeding habitats. Thank you for your suggestion.

Comment 6: Line 357, life history data in Table 1... but Table 1 is a results table?

Response to Comment 6: This was a quote that should have been deleted during the revision. We have removed this citation for Table 1.

Comment 7: In general, comparative studies struggle with reproducibility, especially when data are compiled from existing sources because any subjective decisions regarding, eg. intraspecific variation or sex differences in trait values, are not made plain. Having a data extraction file detailing where exactly the value comes from and any subjective

decisions would be wonderful.

Response to Comment 7: For clarity, we here mention that the body size and egg data used in our analyses were not extracted from existing literature, but were obtained directly from our own field sampling. Therefore, no subjective decisions regarding data extraction from multiple sources were involved for these traits. All measurements were taken using standardized procedures and restricted to breeding individuals (females bearing mature eggs and males confirmed through behavioral observations), which are explicitly stated in the Methods. For traits compiled from external sources (total length from FishBase), we now explicitly state that FishBase provides a single species-level value without sex differentiation, and that this value was used consistently across species, as follows:

We also collected body size data of lamprologines endemic to Lake Tanganyika from the FishBase. Data of maximum total length (in cm) were obtained from 66 lamprologines. Of these taxa, 61 taxa were matched to social system data ($n = 22$ cooperative breeders and $n = 39$ non-cooperative breeders). Note that FishBase provides a single species-level value of maximum total length without sex differentiation. [L349-353]

In addition, we have revised Supplementary Table S1 to provide clearer documentation of habitat classification and social system assignments, allowing readers to verify all trait information.

Referencing issues:

Comment 8: Lines 55 to 57 in the main text - good to cite Boomsma's monogamy hypothesis here and other classical theory.

Response to Comment 8: Thank you for your comment. We have cited classical theories and their review papers, including Boomsma's paper, here.

Comment 9: There are quite a few comparative studies now from other clades which have tried to tease apart direct and indirect fitness benefits which could be cited.

Response to Comment 9: We have added references regarding studies of direct/indirect benefits of the cooperative breeding system here.