1. BASIC REPORTING

This study is investigating the effects of maternal separation on early-life telomere length and telomere dynamics in kittens, which has not been investigated so far. It is therefore filling a gap in the literature. However, a lack of context in the abstract and the introduction, together with a not sufficiently detailed state-of-the-art and a too few number of studies cited, are masking the importance of this study. I provide detailed suggestions along with references (with 10 examples, but the authors are of course free to extend this suggestion and to choose other papers than those I suggested) that should be included to improve the background and context of this study.
In addition, my ability to assess the strength and validity of the findings at that stage is hampered by a lack of various key information in the methods. I provide some suggestions and have several questions that should help the authors advancing this issue. I hope the authors will find my comments helpful. I remain available for further exchanges or review of this article, would it be needed.
With best regards,
Mathilde Tissier

Clear, unambiguous, professional English language used throughout.
Yes

Intro & background to show context.

1. Abstract: although your objective and results are clear, I think that the abstract is lacking context. You should add 1-2 lines of background before your objective and 1-2 lines after your results to replace your study into a broader context, so the reader might understand rapidly the extent of your work. In addition, you should state on which cell you measured telomere length (L21): white blood cells? For the results, it seems to me that your sentences L25-29 are redundant with what you said L24-26. I would keep only the sentence L25-29 that seem more adapted for an abstract. This would provide space for a sentence replacing your results into a broader context.

2. Introduction: I find the introduction well written. Perhaps consider starting with a broader context, not directly and solely on telomeres. For instance, you could broaden your sentence L33-34 by stating that "Studies in multiple species have found that adverse early life experiences can result in modifications of adults' behavioral, biochemical or molecular phenotypes. For instance, studies have pinpointed that telomeres shortening is increased in individuals facing harsh early life conditions". In addition, you do not mention the potential relationship nor the existing knowledge/literature on the link between maternal effects, maternal separation and telomere length/telomere dynamics in other species, which is at the core of your study. You should include a paragraph on this (L42-43, between the state-of-the art on early life experiences and your specific hypothesis on the effect of maternal separation on kittens' telomere length).

Below, I suggest some minor edits regarding the introduction.

Minor comments on the introduction
L35-37- consider revising. Here is a suggestion: Cells have a limited reproductive capacity, referred as the "Hayflick Limit"[5]. Telomeres shorten during mitosis and once they reach a threshold, the cell stops reproducing and enters a state of senescence or dies [6].

L38-39 – Telomeres are also

L41 – you mention health and well-being but you only cited studies referring to lifespan and mortality. Consider replacing well-being by "life-history" and to include studies connecting telomeres to health to support your statement.

L43-53 – references on other taxa are critically lacking to support your hypothesis and predictions in this section (see my suggestions).

L47 – are subsequently hand-reared by humans

L48-49 – no, you cannot say that this is the first study on telomeres in kittens; see Brümmendorf, T. H., Mak, J., Sabo, K. M., Baerlocher, G. M., Dietz, K., Abkowitz, J. L., & Lansdorp, P. M. (2002). Longitudinal studies of telomere length in feline blood cells: implications for hematopoietic stem cell turnover in vivo. *Experimental hematology*, *30*(10), 1147-1152 > this is just one example.
You should rephrase this sentence, and make it more specific to the exact objective of your study, which is investigating the effects of maternal separation on early-life telomere length and telomere dynamics in kittens, which has not been investigated so far.

L49 – telomere length

L54 – Please include a prediction on the dynamics of telomeres, in addition to your predictions on telomere length (since you mention telomere shortening in your abstract and introduction + results)

<u>Literature well referenced & relevant.</u>
The literature on telomeres is extremely vast and I don't expect you to cite all the articles published, but your state-of-the art should be a bit more referenced, including key papers, especially for the relationships between telomere length and life-history traits and health, on how telomere shortening in early-life may have long-term consequences or on the relationship between maternal effects and telomeres. You only have 22 references, which is very few, and offers room to cite more papers in your introduction.

Some examples (among which reviews or meta-analyses):

a) *Relationship between telomeres and health or life-history traits*

Monaghan P, Haussmann MF. 2006 Do telomere dynamics link lifestyle and lifespan? *Trends Ecol. Evol.* 21, 47–53. (doi:10.1016/j.tree.2005.11.007)

Bize P, Criscuolo F, Metcalfe NB, Nasir L, Monaghan P. 2009 Telomere dynamics rather than age predict life expectancy in the wild. *Proc. Biol. Sci.* 276, 1679–83. (doi:10.1098/rspb.2008.1817)

Seeker, L. A., Ilska, J. J., Psifidi, A., Wilbourn, R. V., Underwood, S. L., Fairlie, J., ... & Whitelaw, B. (2018). Bovine telomere dynamics and the association between telomere length and productive lifespan. *Scientific reports*, *8*(1), 1-12.

b) Relationship between early life experiences, stress and telomeres

Price, L. H., Kao, H. T., Burgers, D. E., Carpenter, L. L., & Tyrka, A. R. (2013). Telomeres and early-life stress: an overview. *Biological psychiatry*, *73*(1), 15-23.

Chatelain M, Drobniak SM, Szulkin M. 2020 The association between stressors and telomeres in non-human vertebrates: a meta-analysis. *Ecol. Lett.* 23, 381–398. (doi:10.1111/ele.13426)

Ilska-Warner, Joanna J., et al. "The genetic architecture of bovine telomere length in early-life and association with animal fitness." *Frontiers in genetics* 10 (2019): 1048.

c) Relationship between maternal effects, maternal separation, telomeres in early life and phenotypes

Haussmann MF, Longenecker AS, Marchetto NM, Juliano SA, Bowden RM (2011) Embryonic exposure to corticosterone modifies the juvenile stress response, oxidative stress and telomere length. Proc R Soc Lond B Biol Sci 279:

Tissier, M. L., Williams, T. D., & Criscuolo, F. (2014). Maternal effects underlie ageing costs of growth in the zebra finch (Taeniopygia guttata). *PloS one*, *9*(5), e97705

Chen, X., Zeng, C., Gong, C., Zhang, L., Wan, Y., Tao, F., & Sun, Y. (2019). Associations between early life parent-child separation and shortened telomere length and psychopathological outcomes during adolescence. *Psychoneuroendocrinology*, *103*, 195-202.

BOTHA, Martmari, GRACE, Laurian, BUGARITH, Kishor, *et al.* The impact of voluntary exercise on relative telomere length in a rat model of developmental stress. *BMC research notes*, 2012, vol. 5, no 1, p. 697.

Structure conforms to PeerJ standards, discipline norm, or improved for clarity.
Ok

Figures are relevant, high quality, well labelled & described.

Raw data supplied (see PeerJ policy).
I thank you for sharing your raw data.

2. EXPERIMENTAL DESIGN

Original primary research within Scope of the journal.
Yes

Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.

Yes, although a lack of context is hampering the importance of this study. Please see my comments above.

Rigorous investigation performed to a high technical & ethical standard & Methods described with sufficient detail & information to replicate.

I have four main issues resulting in my incapacity to assess the strength and validity of the findings, mainly arising from a lack of information in the methods section. These information are also crucial to assess the repeatability of your study.

1. You did not mention how you determined age of OR kittens, for which date of birth is likely not known (you say line 62 “if known”: how did you proceed for the “not known”?). This could create a strong bias in your analyses, since date of birth is likely much more precise for MR and OR kittens. Therefore, you should address this in details in your Methods.

2. On which tissue did you measure telomere length? Did you isolate white blood cells from your blood samples? Or did you measure it on total blood? If so, please justify by witing relevant references.

3. Please provide information on whether you did the qPCR measurements in duplicates or triplicates. On one or two plates (did you use 96-plates or 384-plates)? Please also detail the composition of the Mix that you used to carry-out your qPCR, which primers and what were the cycling conditions. Please also include intra and inter-plate (if any) coefficients of variation. These are extremely important to assess the strength and the repeatability of your measurements.

4. There is no section detailing the data nor statistical analyses that you conducted. Please add one. L95-98 of the results should be included in this section but is not sufficient to assess what you did. For instance, you say that you used a generalized linear model, but I assume that you mean generalized linear mixed model, since you included a random factor? In addition, which law did you use (normal)? If so, did your residuals follow normality? I am also concerned about conducing a Linear Mixed Model on these data given the (apparent) difference in the variance and the large difference of sample size between your two groups.

General comments and questions

L70: you should indicate at which age the kittens died (to understand whether this had an influence on your experimental design and analyses or not).

L74: approximately 1 and 8 weeks > please be more specific (give the information in days±SE)

L73-82: consider providing a table of how many viable samples with extracted DNA you managed to obtain at 1 and 8 weeks, for OR and MR kittens, respectively, as it is hard to have a clear idea when reading the text.

L81-82 – that's a lot regarding your already low sample size for MR kittens (9 at both points and 6 at one point). This is raising the question on "how many samples did you have at each point at the end?'

General question: do you have any information on whether the 42 kittens made it to adulthood? If yes, you could include an analysis with a binary response (yes/no), and looked at whether telomere length or telomere dynamics influenced survival in kittens (while comparing OR and MR kittens).

3. VALIDITY OF THE FINDINGS

Unfortunately, the lack of information on the analyses conducted, the methodology (especially on the qPCR approach), the sample size of each category does not allow me to assess the strength, validity and repeatability of the findings. I could use the supplementary datasets to conduct analyses myself but my numerous questions on qPCR analyses would in any cases do not allow me to have sufficient information to assess the validity of the findings. In addition, I think that this information should be specified in the article, so that, by reading the methods, results and looking at the figure, the reader may be able to assess whether the analyses were performed correctly. You seem to have a much greater variation on your OR groups (Fig 1) than on your MR group (Figure 1). Nonetheless, I cannot assess if this is due to a difference in sample size, an unsuitable statistical/analytical approach, to a real difference in the variance between the two groups or to measurement errors.

I appreciate your supplementary sensitivity analysis but this should be developed in the methods section and not in the results.