Why are telomeres the length that they are? Insight from a phylogenetic comparative analysis

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Running title: Phylogenetic comparison of vertebrate telomeres

Data Accessibility Statement

A fully reproducible workflow of the data analyses, including R scripts and additional supporting material, is available in the following repositories: Github https://dylan-padilla.github.io/telomere_evo/, a Dryad link will be available upon acceptance.

Conflict of interest

The authors declare no conflict of interest.

Author Contributions

Derek M. Benson conceptualized the study, collected and curated the data, assisted with the methodology and data analysis, and wrote the manuscript. Dylan J. Padilla-Perez was responsible for the majority of the methodology and data analysis, created the figures, and assisted in writing the manuscript. Dale F. DeNardo oversaw the project and contributed to conceptualization and writing.

Abstract

Telomeres are short repeating nucleotide sequences at the ends of chromosomes that shorten with every cellular replication. Despite the importance of keeping telomere length within a critical homeostatic range, adult telomere length can differ by two orders of magnitude across vertebrate species. Why telomere length varies so widely remains unknown, though popular hypotheses suggest that body size, lifespan, and endothermy are key variables that have coevolved with telomere length. To test the relationship among telomere length, telomerase activity (which extends telomeres), and these variables, we modeled the evolution of telomere length across 123 vertebrate species. We failed to find an influence of body mass, though lifespan was negatively correlated with telomere length. Furthermore, we found an effect of thermal biology, with endotherms having on average shorter telomeres than ectotherms. The presence of telomerase activity was positively correlated with telomere length across the 58 species where data for both existed. Taken together, our findings suggest that thermal biology and lifespan may have influenced the evolution of telomere length across vertebrates and indicate that telomerase activity and telomere length may have coevolved.

Keywords: aging, cellular senescence, molecular evolution, oxidative stress.

16 **Introduction**

17 Telomeres are DNA sequences that are critical for protecting the ends of chromosomes. Accordingly,
18 all vertebrate chromosomes end in telomeres. Yet, despite the crucial role of telomeres and their
19 ubiquity across vertebrates, the existing literature on absolute telomere length has not previously been
20 comparatively analyzed to better understand the variation in telomere biology across major vertebrate
21 clades.

22 Structurally, vertebrate telomeres are comprised of repeating TTAGGG nucleotide sequences
23 and a protein complex known as shelterin (Rhodes and Giraldo, 1995; De Lange, 2005). The 3' end of
24 telomeres ends in a G-strand overhand, which often forms a T-loop structure by invading and binding
25 to double-stranded telomeric DNA (Cong et al., 2002; Haussmann and Marchetto, 2010). The
26 functional limitations of DNA polymerase and the creation of Okazaki fragments lead to the
27 progressive loss of telomeric DNA with each cellular replication (Gilson and Géli, 2007). Many
28 species, including humans, experience replicative aging where cells enter senescence and stop
29 replicating when telomere length hits a critical minimum (Saretzki and Von Zglinicki, 2002; Shay
30 and Wright, 2005). In addition to the impacts of cellular replication, oxidative stress can also
31 cause significant telomere shortening (Von Zglinicki, 2002). The telomeric 5'-GGG-3' sequence
32 is particularly susceptible to damage by reactive oxygen species and conversion of one or more
33 gaunines into 8-oxoG (Kawanishi and Oikawa, 2004). Furthermore, the DNA damage response is
34 repressed at telomeric DNA, and telomeric DNA within the single-stranded T-loop is not protected
35 by hydrogen bonding and, due to the absence of a template strand, not able to be repaired by
36 base excision repair (Ahmed and Lingner, 2018). In contrast to the cellular senescence associated
37 with short telomeres, long telomeres are associated with increased cancer risk in some species, so it is
38 crucial for an organism to maintain its telomere lengths within a critical range (Rode et al., 2016;
39 Aviv et al., 2017; McNally et al., 2019).

To counter the progressive shortening of telomeres over time, organisms can prevent telomere shortening through antioxidant production and, in some cases, elongate telomeres via telomerase activity and alternative telomere lengthening (Neumann et al. 2013; Armstrong and Boonekamp 2023). Telomerase is a ribonucleoprotein reverse transcriptase comprised of catalytic (TERT) and RNA (TERC) components (Cong et al., 2002). Telomerase extends the 5' strand of telomeric DNA, after which the 3' strand is filled in by DNA polymerase $\alpha - primase$ (Bonetti et al., 2014). Despite its obvious benefits, in humans and many endotherms telomerase activity is suppressed in most somatic tissues outside of embryonic stages (Wright et al., 1996). It has been hypothesized that this inactivity of telomerase is an anti-cancer protection mechanism, as telomerase activity enables continuous cellular replication and is almost always necessary for cellular immortalization (Shay and Wright, 2011). In fact, telomerase activity is present in 85-90% of all human tumors (Shay and Bacchetti, 1997).

Since their discovery, telomeres, telomere length, and telomerase activity, collectively referred to as "telomere dynamics," have been studied extensively for their potential involvement in both aging and cancer (Aubert and Lansdorp, 2008; Xu et al., 2013). Additionally, many studies have investigated telomere dynamics in non-human vertebrates, revealing that telomere length, replicative senescence, and repressed telomerase activity are not conserved traits but rather vary among species (Gomes et al., 2010). In fact, telomere length ranges across species from one to hundreds of kb (Rocco et al., 2001; Zhdanova et al., 2005). Why telomere length varies so widely across vertebrates remains to be understood, though popular hypotheses suggest that body size, lifespan, and endothermy are key variables that have coevolved with telomere length (Heidinger et al., 2012; Olsson et al., 2018; Pepke and Eisenberg, 2022). Larger body size increases the number of cells an organism has which increases the number of potentially cancerous cells, increased lifespan enables more mutations to accumulate over time, and endothermy increases the rate of metabolism and cellular turnover, thus

64 increasing the mutation rate of cells (Peto et al., 1975; Seluanov et al., 2008; Hayes, 2010).

65 While the importance of telomere dynamics has resulted in a considerable number of
66 publications regarding the subject across numerous species, no literature has taken a
67 comprehensive, wide-scale comparative approach to review and analyze the relationships among
68 telomere dynamics, body size, lifespan, and endothermy across major vertebrate clades. The goals
69 of this review are to 1. Determine whether lifespan, body mass, and endothermy have affected the
70 evolution of telomere length; 2. Elucidate whether telomere length is differentially affected by these
71 variables among major vertebrate clades; and 3. Investigate whether telomere length and
72 telomerase have coevolved.

73 **Materials and Methods**

74 ***Literature search and data collection***

75 To search for existing literature on telomere length and telomerase activity, key terms including
76 "telomere length", "telomerase activity", and specific species or taxonomic groups were input into
77 both Google Scholar and Web of Science (Figure S1). We collected data from studies utilizing
78 fluorescent in-situ hybridization (FISH), telomere amount and length assay (TALA), and telomere
79 restriction fragment analysis (TRF), but we did not include data from studies using quantitative
80 polymerase chain reaction (qPCR) as telomere length in these studies is measured as a ratio
81 rather than in kb (see supporting information for more detail). When available and for consistency,
82 we utilized average adult telomere length as reported in previous analyses that had collected data across
83 multiple studies (see, Gomes et al., 2011; Criscuolo et al., 2021; Pepke and Eisenberg, 2022). For
84 species not included in these analyses, we used the average telomere length value reported in
85 individual studies. For studies that did not report an average telomere length, we calculated average
86 telomere length as the mean telomere length across all individuals in a study. We did not include
87 studies or datapoints on telomere length in extremely young or old individuals (see the supporting

88 information for more information on the collection of data). Additionally, as previous studies have
89 shown no evidence for sex-based differences in adult telomere length across vertebrates, we included
90 data for both sexes in our analyses (Remot et al., 2020). Telomerase activity in adult somatic tissue
91 (hereafter referred to as telomerase activity) was recorded as a binary variable, being either absent or
92 present in the somatic tissue of the study species. Telomerase activity data from gonadal tissue were not
93 utilized as many species exhibit telomerase activity in gonadal tissue but not in other somatic tissues
94 (Wright et al., 1996; Sun et al., 2021). For studies that reported telomere length but not telomerase
95 activity, we recorded telomerase activity as not available (n/a) for the species. As telomere length
96 data from Seluanov et al. (2007) was found to be highly correlated with but longer than that of
97 overlapping species analyzed by Gomes et al. (2011), we applied the same correction factor
98 (0.6617 x TL – 2.4454 kb) that was utilized by Pepke and Eisenberg (2022). Once telomere length
99 and telomerase activity data were collected, we obtained species-specific maximum lifespan in
100 years (hereafter referred to as lifespan) and adult mass in grams primarily from the AnAge
101 database https://genomics.senescence.info/species/index.html. For species that did not have an
102 entry in the AnAge database, information on adult mass and lifespan was collected from various
103 literature sources (see supplemental table 1 for more information). If no data on lifespan or adult
104 mass could be found, the species was not used for any analysis. Additionally, as domestication
105 may have influenced the evolution of telomere length, animals with a long history of domestication
106 were not included in our analyses. We utilized telomere dynamics data for 123 vertebrate species
107 across 42 orders and 88 families.
108

109 **Figure 1.**

110 ***Vertebrate phylogeny***

111 To build a vertebrate phylogeny for this review, we used the Build a Timetree function available at

112 The TimeTree of Life database https://timetree.org/, which outputs a timetree of taxa of interest
113 extracted from the global timetree connecting species and publication-specific timetrees in the
114 database. For species that did not have phylogenetic data readily available, we utilized closely related
115 species or congeners when constructing the phylogeny (see the supporting information for more
116 detail). Although the placement of taxa in our species-level tree of vertebrates was generally well
117 supported, phylogenetic comparative methods appear to be robust to perturbations such as tree
118 misspecification or soft polytomies (Stone, 2011).

119 ***Statistical Analysis***

120 To better understand the evolution of telomere length, we estimated its ancestral state across
121 vertebrates. We used the function fastAnc in the "phytools" package of R v. 4.2.2 (Revell, 2022),
122 which performs a reasonably fast estimation of the most likely ancestral states for a continuous
123 trait by taking advantage of the fact that the state computed for the root node of the tree during
124 Felsenstein (1985) contrasts algorithm is also the most likely estimate of the root node. Thus, the
125 function re-roots the tree at all internal nodes and computes the contrasts state at the root each
126 time. The function also computes variances or 95% confidence intervals on the estimates.

127 Subsequently, we used Phylogenetic Path Analysis (van der Bijl, 2018) to test our
128 hypotheses on the relationships among body mass, thermal biology, lifespan, and telomere length
129 across species. Phylogenetic path analysis uses Phylogenetic Generalized Least-squares (PGLS)
130 to perform confirmatory path analysis based on the directed separation method–"d-separation"–as
131 described in Shipley (2013). We examined the explanatory power of 8 competing models involving
132 different relationships among variables, which enabled us to identify the most plausible hypothesis
133 given the available data, and thus infer the relative importance of mechanisms described by optimality
134 models. The most likely model suggested by path analysis was then refitted assuming that the
135 species trait values evolved via a Brownian motion model, an Ornstein–Uhlenbeck motion model, and

136 a Pagel's lambda model using the R package 'ape' v. 5.6.2. To evaluate the models' goodness of fit,
137 we used information-theoretic criteria such as AICc. We ranked the candidate models accordingly
138 and selected the most likely one for inferences (lowest value of AICc). To investigate the effects of
139 sample size on the exploratory power of our models, we conducted a sensitivity analysis with 1000
140 simulations across the entire dataset using the package "sensiPhy" v.0.8.5 (Paterno et al., 2018).
141 The samp_phylm function uses a jackknifing method to randomly remove species in order to assess
142 the effects of sample size and species sampling on parameter estimates (Paterno et al., 2018).

143 We also estimated the degree of phylogenetic signal in our data based on the most likely
144 model. The "ape" package of R enabled us to modify the correlation structure of the residuals in the
145 function gls. Thus, we assigned different fixed values to the parameter λ, ranging from zero (no
146 phylogenetic signal, equivalent to a "star" phylogeny) to one (consistent with Brownian motion).
147 Intermediate values of λ imply that the data support a model that is somewhere between a "star"
148 phylogeny and Brownian motion. λ represents the amount of phylogenetic "signal" in the data,
149 conditional on the tree. In a regression context, we used maximum likelihood to simultaneously fit
150 the regression model and estimate λ. Because confidence intervals for λ were provided by the models,
151 we tested the hypotheses that $\lambda = 0$ (i.e., independence) and $\lambda = 1$ (Brownian Motion).

152 Lastly, we investigated whether telomerase activity changes as a function of telomere length.
153 To do that, we modeled the correlated evolution of telomere length and telomerase activity under
154 the threshold model (Felsenstein, 2012). This model creates a natural framework by which one can
155 evaluate the evolutionary correlation between a continuous variable (e.g., telomere length) and a
156 discrete variable (e.g., telomerase activity). In R, the correlational threshold model can be performed
157 through the function threshBayes available in the package "phytools" (Revell, 2022). It also enabled
158 us to generate a three-panel plot showing the likelihood profile, the mean acceptance rates (using a
159 default sliding window), and a profile plot for the correlation coefficient (Figure S2). In addition, we

used the density function to later plot a posterior density of the correlation coefficient and added a 95% high probability density interval (HPD) around the value of *r*. We did this by extracting a post-burn-in sample of values of *r* (assuming a 20% burn-in) and using the function HPD interval from the package "coda". To produce good visualization of our results and ensure that they are reproducible, we carried out all the analyses in the free software for statistical computing R v.4.2.2 (R Core Team, 2022).

Results

Our results indicated no effect of body mass on telomere length. However, the evolution of telomere length across vertebrates appeared to be influenced by both the thermal biology and lifespan of species (Figure S3-4). The information-theoretic approach revealed that a model including the main effects of these variables was strongly supported (Table 1). Generally, telomere length decreased with lifespan (Figure 2). Although the rate at which telomere length decreased (i.e., the slope) seems to be more pronounced in endotherms compared to ectotherms, the difference is statistically negligible. However, on average, endotherms had shorter telomeres than did ectotherms (Figure 2). Finally, our analyses indicate that there may have been many transitions in telomere length across vertebrates (Figure 1).

Figure 1

Table 1.

Figure 2.

As the scatter in the data indicates substantial noise, we examined the impact that different sample sizes could have on our results. Randomly removing 10, 20, 30, 40, and 50% of species in the analyses reduces the likelihood of finding a significant effect of lifespan on telomere length by 0.2, 1.2, 3.3, 10.4, and 19.6%, respectively (Figure S5).

Figure 3.

Based on a likelihood ratio test (X2), models with λ fixed at 0 and 1 were significantly different from one that had λ fixed at 0.5. Thus, we rejected the hypotheses of independent residuals and Brownian motion. The most likely model suggested an intermediate degree of phylogenetic signal in the data.

Finally, the threshold model of correlated evolution indicated that the evolution of telomere length is correlated with the presence of telomerase activity. According to the posterior probability distribution of the correlation coefficient ($r = 0.62$), the presence of telomerase activity significantly increased with telomere length. This conclusion was based on the 95% high probability density (HPD = 0.35 − 0.87) interval around r, which did not include 0 (Figure 3 and S2).

Discussion

Across vertebrates, we found evidence that lifespan is negatively correlated with telomere length and that thermal biology is correlated with telomere length, with endotherms having on average shorter telomeres than ectotherms. However, we failed to find evidence that body mass or the interactions among endothermy, body mass, and lifespan are correlated with telomere length across major vertebrate clades. To our knowledge, ours is the first study to test whether these variables are correlated with telomere length across vertebrate clades, though previous studies have investigated within clades (Gomes et al., 2011; Criscuolo et al., 2021; Pepke and Eisenberg, 2022).

The reason that telomere length is correlated with thermal biology remains unclear, though several hypotheses have been raised. One possible explanation is that high stable body temperatures increase the risk of malignant tumors (Olsson et al. 2018). While plausible, many ectotherms prefer and maintain body temperatures above that of endotherms, and previous research has failed to find support for this idea (Stevenson 1985; Olsson et al. 2018). Alternatively, it may be that the difference in metabolic rate between endotherms and ectotherms has significantly affected the evolution of telomere length, as metabolic rate has been shown to be positively correlated with DNA damage (Moretton and Loizou 2020). Regardless of the reason behind the relationship between thermal biology and telomere length, it is important to consider the disparity in sample size between endotherms and ectotherms in our analyses. Vertebrate ectotherms are understudied in terms of telomere biology when compared to endotherms, and information on major clades including Anurans and Agnatha is nearly or completely absent. Given the importance of these groups for understanding vertebrate evolution, it is likely that quantifying telomere length in species from these clades will further elucidate how thermal biology is related

227 to telomere length. Nevertheless, our results indicate that thermal biology may have played a
228 significant role in the evolution of telomere length.

229 In accordance with previous research in mammals, we found evidence that telomere length
230 is negatively correlated with lifespan (Pepke and Eisenberg 2021). Regardless, conclusions on this
231 relationship remain divided, with studies both supporting and refuting the idea that a correlation
232 exists (Gomes et al. 2011; Tricola et al., 2018; Criscuolo et al., 2021; Pepke et al., 2023b). This
233 discrepancy, and our results, may in part be explained by the use of either early-life (within the
234 first few weeks of life) or adult telomere length. We utilized adult telomere length for several
235 reasons: Early-life telomere length changes rapidly for many though not all species (Watson et al.,
236 2015; Pepke et al., 2023a). Thus, early-life telomere length may not matter as much as preserved
237 telomere length. Secondly, early-life telomere length fails to account for telomere length
238 modulation later in life. Several long-lived species have been shown to increase telomere length in
239 adulthood (Haussmann et al., 2003). Furthermore, telomere rate of change has been repeatedly
240 shown to be correlated with lifespan (Tricola et al., 2018; Criscuolo et al., 2021; Whittemore et al.,
241 2019). While adult telomere length does not measure this, adult telomere length is affected by
242 shortening and lengthening and may serve as a more effective measurement of telomere length for
243 a species. However, among adults, age can vary considerably in longer lived species and such
244 variation in age among adults could confound results.

245 In contrast to lifespan and thermal biology, we failed to find a significant relationship
246 between telomere length and body mass. Previous research on birds and mammals has yielded
247 contrasting results, and the relationship between body mass and telomere length remains unclear
248 (Gomes et al. 2011; Criscuolo et al. 2021; Pepke and Eisenberg 2021). As we included fishes and
249 ectothermic diapsids, our results are not directly comparable, though they suggest no relationship
250 between body mass and telomere length. Additionally, we cannot rule out the possibility that body

mass does not differentially affect telomere length across clades. As our results indicate that thermal biology is related to telomere length, it may be that the selective pressure of body mass on telomere length is only significant in endotherms. Regardless, given ours and previous findings, it seems likely that the relationship between telomere length and body mass is not robust to varying modelling strategies, and that sample size and unequal representation among taxa may complicate the evaluation of this relationship.

Despite the inconsistencies among clades in terms of variation in telomere length, our analyses indicate that long telomeres are correlated with the presence of adult telomerase activity. These traits may have coevolved to reduce cancer risk, as previous literature suggests that selection favors short telomeres and the repression of telomerase as a mechanism to prevent cancer (Risques and Promislow, 2018). However, while cancer is detrimental to organismal health, the vast majority of wild animals do not live to the age where cancer becomes a significant risk factor (Rozhok and DeGregori, 2016). Thus, other variables such as pace of life and reproductive investment, rather than cancer risk, may have exerted stronger selective pressures on the evolution of telomere length and telomerase activity. Additionally, given that our and previous analyses are strongly mammal and bird biased, it is possible that the correlations between telomerase activity, long telomeres, and cancer risk are not reflective of vertebrates in general (Gomes et al., 2011; Pepke and Eisenberg, 2022). For example, the limited data in fishes demonstrate that most species in our analysis display telomerase activity with telomeres <15 kb in length, a phenomenon rarely seen among mammals (Zhdanova et al., 2005; Elmore et al., 2008; Gomes et al., 2011).

Making comparisons across and among subclades of a larger tree can prove valuable, particularly where large timescales are involved and thus where evolutionary patterns and processes are unlikely to be constant. However, there are important considerations when choosing which clades to study, including sample size and whether there is sufficient within-clade diversity

275 for statistical analysis (Baker et al., 2021). Given our findings and the indications that thermal
276 biology may play a role in shaping the evolution of telomere length as well as the current
277 literature's bias towards mammals and birds, future studies need to include a wider range of
278 vertebrates, preferably with a similar degree of representation across the major taxa, to gain a
279 more thorough understanding of (1) how telomere length varies within and among major clades,
280 and (2) how the effects of variables such as lifespan and body mass on telomere length may differ
281 among clades.

282 Our data may suffer from both an extremely variable number of species per clade and a
283 large variation in telomere size among species, preventing us from making a general conclusion
284 about the evolution of telomere size across vertebrates. While sample size may have influenced
285 our results, our findings appear to be fairly robust given that the random removal of up to 30% of
286 the species in our study results in < 5% decrease in the chance of finding a significant negative
287 correlation between lifespan and telomere length. In support of the idea that sample size may be
288 impacting our findings, previous studies have found differing results depending on the clade and
289 range of taxa used when investigating the relationship between telomere length and lifespan
290 (Seluanov et al., 2007; Gomes et al., 2011; Criscuolo et al., 2021; Pepke and Eisenberg, 2022). For
291 instance, work in rodents found no evidence that lifespan was correlated with telomere length,
292 while several analyses that include a wider range of mammals have concluded that telomere length
293 is strongly correlated with lifespan (Seluanov et al., 2007; Gomes et al., 2011; Pepke and
294 Eisenberg, 2022).

295 While our greater overall sample size may explain the differences between our results and
296 those of previous studies, many variables, such as diet and habitat, may have also significantly
297 influenced the evolution of telomere length. While diet has been shown to significantly affect
298 telomere length in mice and humans, it is not known whether carnivory or herbivory differentially

impact telomere length in mammals (Paul, 2011; Gokarn et al., 2018). Furthermore, research on a wide range of neotropical bat species found that frugivorous, omnivorous, and carnivorous species differ in oxidative stress and antioxidant capacity (Schneeberger et al., 2014). Given that telomere length is associated with oxidative stress, it may be that diet has had a significant impact on the evolution of telomere length (Von Zglinicki, 2002). In addition, habitat and environment have been shown to impact telomere length. For example, moose (*Alces alces*) populations in boreal regions have significantly longer telomeres than do those in sarmatic mixed forest and montane regions (Fohringer et al., 2022). Furthermore, tropical and temperate birds have differentially evolved responses to oxidative damage, and telomere length in young birds of the genus *Saxicola* differs between species in tropical and temperate regions (Jimenez et al., 2013; Apfelbeck et al., 2019). In addition to needing greater and more balanced phylogenic coverage, future studies investigating the evolution of telomere length should also investigate a wider range of life history and ecological traits.

In conclusion, despite the possible complications including a mammal-biased sample size, our study provides evidence that telomerase activity and telomere length in adult life have coevolved, with longer telomeres being associated with the presence of telomerase activity. Additionally, we show that lifespan is negatively correlated with telomere length and that thermal biology is related to telomere length, with endotherms having shorter telomeres than ectotherms. Finally, we failed to find any relationship between body mass and telomere length. Future studies should incorporate a wider range of non-mammalian species in their analyses to further our understanding of the variables affecting telomere length while also attempting to account for other potentially confounding variables such as unbalanced sample sizes, chromosome size, diet, habitat, pace of life, and additional life history traits.

Acknowledgements

We thank the anonymous reviewers for feedback on earlier drafts.

References

Ahmed, W., & Lingner, J. (2018). Impact of oxidative stress on telomere biology. *Differentiation*, *99*, 21–27. https://doi.org/10.1016/j.diff.2017.12.002

Apfelbeck, B., Haussmann, M. F., Boner, W., Flinks, H., Griffiths, K., Illera, J. C., Mortega, K. G., Sisson, Z., Smiddy, P., & Helm, B. (2019). Divergent patterns of telomere shortening in tropical compared to temperate stonechats. *Ecology and Evolution*, *9*(1), 511–521. https://doi.org/10.1002/ece3.4769

Armstrong, E., & Boonekamp, J. (2023). Does oxidative stress shorten telomeres *in vivo*? A meta-analysis. *Ageing Research Reviews*, *85*, 101854. https://doi.org/10.1016/j.arr.2023.101854

Aviv, A., Anderson, J. J., & Shay, J. W. (2017). Mutations, cancer and the telomere length paradox. *Trends in Cancer*, *3*(4), 253–258. https://doi.org/10.1016/j.trecan.2017.02.005

Aubert, G., & Lansdorp, P. M. (2008). Telomeres and aging. *Physiological Reviews*, *88*(2), 557–579. https://doi.org/10.1152/physrev.00026.2007

Baker, J., Meade, A., Pagel, M., & Venditti, C. (2021). Nothing wrong with the analysis of clades in comparative evolutionary studies: A reply to Poe et al. *Systematic Biology*, *70*(1), 197–201. https://doi.org/10.1093/sysbio/syaa067

Bonetti, D., Martina, M., Falcettoni, M., & Longhese, M. P. (2014). Telomere-end processing: Mechanisms and regulation. *Chromosoma*, *123*(1), 57–66. https://doi.org/10.1007/s00412-013-0440-y

Cong, Y.-S., Wright, W. E., & Shay, J. W. (2002). Human telomerase and its regulation. *Microbiology and Molecular Biology Reviews*, *66*(3), 407–425. https://doi.org/10.1128/mmbr.66.3.407-425.2002

Criscuolo, F., Dobson, F. S., & Schull, Q. (2021). The influence of phylogeny and life history on telomere lengths and telomere rate of change among bird species: A meta-analysis. *Ecology and Evolution*, *11*(19), 12908–12922. https://doi.org/10.1002/ece3.7931

Elmore, L. W., Norris, M. W., Sircar, S., Bright, A. T., McChesney, P. A., Winn, R. N., & Holt, S. E. (2008). Upregulation of telomerase function during tissue regeneration. *Experimental Biology and Medicine (Maywood, N.J.)*, *233*(8), 958–967. https://doi.org/10.3181/0712-RM-345

Felsenstein, J. (2012). A comparative method for both discrete and continuous characters using the threshold model. *The American Naturalist*, *179*(2), 145–156. https://doi.org/10.1086/663681

Fohringer, C., Hoelzl, F., Allen, A. M., Cayol, C., Ericsson, G., Spong, G., Smith, S., & Singh, N. J. (2022). Large mammal telomere length variation across ecoregions. *BMC Ecology and Evolution*, *22*(1), 105. https://doi.org/10.1186/s12862-022-02050-5

Gilson, E., & Géli, V. (2007). How telomeres are replicated. *Nature Reviews Molecular Cell Biology*, *8*(10), Article 10. https://doi.org/10.1038/nrm2259

Gokarn, R., Solon-Biet, S., Youngson, N. A., Wahl, D., Cogger, V. C., McMahon, A. C., Cooney, G. J., Ballard, J. W. O., Raubenheimer, D., Morris, M. J., Simpson, S. J., & Le Couteur, D. G. (2018). The relationship between dietary macronutrients and hepatic telomere length in aging mice. *The Journals of Gerontology: Series A*, *73*(4), 446–449. https://doi.org/10.1093/gerona/glx186

Gomes, N. M. V., Ryder, O. A., Houck, M. L., Charter, S. J., Walker, W., Forsyth, N. R., Austad, S. N., Venditti, C., Pagel, M., Shay, J. W., & Wright, W. E. (2011). Comparative biology of mammalian telomeres: Hypotheses on ancestral states and the roles of telomeres in

longevity determination. *Aging Cell*, *10*(5), 761–768. https://doi.org/10.1111/j.1474-9726.2011.00718.x

Gomes, N. M. V., Shay, J. W., & Wright, W. E. (2010). Telomere biology in Metazoa. *FEBS Letters*, *584*(17), 3741–3751. https://doi.org/10.1016/j.febslet.2010.07.031

Haussmann, M. F., & Marchetto, N. M. (2010). Telomeres: Linking stress and survival, ecology and evolution. *Current Zoology*, *56*(6), 714–727. https://doi.org/10.1093/czoolo/56.6.714

Haussmann, M. F., Winkler, D. W., O'Reilly, K. M., Huntington, C. E., Nisbet, I. C. T., & Vleck, C. M. (2003). Telomeres shorten more slowly in long-lived birds and mammals than in short–lived ones. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1522), 1387–1392. https://doi.org/10.1098/rspb.2003.2385

Hayes, J. P. (2010). Metabolic rates, genetic constraints, and the evolution of endothermy. *Journal of Evolutionary Biology*, *23*(9), 1868–1877. https://doi.org/10.1111/j.1420-9101.2010.02053.x

Heidinger, B. J., Blount, J. D., Boner, W., Griffiths, K., Metcalfe, N. B., & Monaghan, P. (2012). Telomere length in early life predicts lifespan. *Proceedings of the National Academy of Sciences*, *109*(5), 1743–1748. https://doi.org/10.1073/pnas.1113306109

Jimenez, A. G., Harper, J. M., Queenborough, S. A., & Williams, J. B. (2013). Linkages between the life-history evolution of tropical and temperate birds and the resistance of cultured skin fibroblasts to oxidative and non-oxidative chemical injury. *Journal of Experimental Biology*, *216*(8), 1373–1380. https://doi.org/10.1242/jeb.079889

Kärkkäinen, T., Briga, M., Laaksonen, T., & Stier, A. (2022). Within-individual repeatability in telomere length: A meta-analysis in nonmammalian vertebrates. *Molecular Ecology*, *31*(23), 6339–6359. https://doi.org/10.1111/mec.16155

Kawanishi, S., & Oikawa, S. (2004). Mechanism of telomere shortening by oxidative stress. *Annals of the New York Academy of Sciences*, *1019*(1), 278–284. https://doi.org/10.1196/annals.1297.047

Lai, T.-P., Wright, W. E., & Shay, J. W. (2018). Comparison of telomere length measurement methods. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1741), 20160451. https://doi.org/10.1098/rstb.2016.0451

Lange, T. de. (2005). Shelterin: The protein complex that shapes and safeguards human telomeres. *Genes & Development*, *19*(18), 2100–2110. https://doi.org/10.1101/gad.1346005

McNally, E. J., Luncsford, P. J., & Armanios, M. (2019). Long telomeres and cancer risk: The price of cellular immortality. *The Journal of Clinical Investigation*, *129*(9), 3474–3481. https://doi.org/10.1172/JCI120851

Moretton, A., & Loizou, J. I. (2020). Interplay between Cellular Metabolism and the DNA Damage Response in Cancer. *Cancers*, *12*(8), Article 8. https://doi.org/10.3390/cancers12082051

Neumann, A. A., Watson, C. M., Noble, J. R., Pickett, H. A., Tam, P. P. L., & Reddel, R. R. (2013). Alternative lengthening of telomeres in normal mammalian somatic cells. *Genes & Development*, *27*(1), 18–23. https://doi.org/10.1101/gad.205062.112

Olsson, M., Wapstra, E., & Friesen, C. (2018). Ectothermic telomeres: It's time they came in from the cold. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1741), 20160449. https://doi.org/10.1098/rstb.2016.0449

Paterno, G. B., Penone, C., & Werner, G. D. A. (2018). sensiPhy: An r-package for sensitivity analysis in phylogenetic comparative methods. *Methods in Ecology and Evolution*, *9*(6), 1461–1467. https://doi.org/10.1111/2041-210X.12990

Paul, L. (2011). Diet, nutrition and telomere length. *The Journal of Nutritional Biochemistry*, *22*(10), 895–901. https://doi.org/10.1016/j.jnutbio.2010.12.001

Pepke, M. L., & Eisenberg, D. T. A. (2022). On the comparative biology of mammalian telomeres: Telomere length co-evolves with body mass, lifespan and cancer risk. *Molecular Ecology*, *31*(23), 6286–6296. https://doi.org/10.1111/mec.15870

Pepke, M. L., Kvalnes, T., Wright, J., Araya-Ajoy, Y. G., Ranke, P. S., Boner, W., Monaghan, P., Sæther, B.-E., Jensen, H., & Ringsby, T. H. (2023b). Longitudinal telomere dynamics within natural lifespans of a wild bird. *Scientific Reports*, *13*(1), Article 1. https://doi.org/10.1038/s41598-023-31435-9

Pepke, M. L., Ringsby, T. H., & Eisenberg, D. T. A. (2023a). The evolution of early-life telomere length, pace-of-life and telomere-chromosome length dynamics in birds. *Molecular Ecology*, *32*(11), 2898–2912. https://doi.org/10.1111/mec.16907

Peto, R., Roe, F. J., Lee, P. N., Levy, L., & Clack, J. (1975). Cancer and ageing in mice and men. *British Journal of Cancer*, *32*(4), Article 4. https://doi.org/10.1038/bjc.1975.242

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing. https://www.R-project.org

Revell, L. J., & Harmon, L. J. (2022). Phylogenetic comparative methods in R. Princeton University Press.

Rhodes, D., & Giraldo, R. (1995). Telomere structure and function. *Current Opinion in Structural Biology*, *5*(3), 311–322. https://doi.org/10.1016/0959-440X(95)80092-1

Risques, R. A., & Promislow, D. E. L. (2018). All's well that ends well: Why large species have short telomeres. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1741), 20160448. https://doi.org/10.1098/rstb.2016.0448

Rocco, L., Costagliola, D., & Stingo, V. (2001). (TTAGGG)n telomeric sequence in selachian chromosomes. *Heredity*, *87*(5), Article 5. https://doi.org/10.1046/j.1365-2540.2001.00945.x

Rode, L., Nordestgaard, B. G., & Bojesen, S. E. (2016). Long telomeres and cancer risk among 95 568 individuals from the general population. *International Journal of Epidemiology*, *45*(5), 1634–1643. https://doi.org/10.1093/ije/dyw179

Rozhok, A. I., & DeGregori, J. (2016). The evolution of lifespan and age-dependent cancer risk. *Trends in Cancer*, *2*(10), 552–560. https://doi.org/10.1016/j.trecan.2016.09.004

Saretzki, G., & Von Zglinicki, T. (2002). Replicative aging, telomeres, and oxidative stress. *Annals of the New York Academy of Sciences*, *959*(1), 24–29. https://doi.org/10.1111/j.1749-6632.2002.tb02079.x

Schneeberger, K., Czirják, G. Á., & Voigt, C. C. (2014). Frugivory is associated with low measures of plasma oxidative stress and high antioxidant concentration in free-ranging bats. *Die Naturwissenschaften*, *101*(4), 285–290. https://doi.org/10.1007/s00114-014-1155-5

Seluanov, A., Chen, Z., Hine, C., Sasahara, T. H. C., Ribeiro, A. A. C. M., Catania, K. C., Presgraves, D. C., & Gorbunova, V. (2007). Telomerase activity coevolves with body mass not lifespan. *Aging Cell*, *6*(1), 45–52. https://doi.org/10.1111/j.1474-9726.2006.00262.x

Seluanov, A., Hine, C., Bozzella, M., Hall, A., Sasahara, T. H. C., Ribeiro, A. A. C. M., Catania, K. C., Presgraves, D. C., & Gorbunova, V. (2008). Distinct tumor suppressor mechanisms evolve in rodent species that differ in size and lifespan. *Aging Cell*, *7*(6), 813–823. https://doi.org/10.1111/j.1474-9726.2008.00431.x

Shay, J. W., & S. A. Bacchetti. (1997). A survey of telomerase activity in human cancer. *European Journal of Cancer*, *33*, 787–791. https://doi.org/10.1016/S0959-8049(97)00062-2

Shay, J. W., & Wright, W. E. (2005). Senescence and immortalization: Role of telomeres and telomerase. *Carcinogenesis*, *26*(5), 867–874. https://doi.org/10.1093/carcin/bgh296

Shay, J. W., & Wright, W. E. (2011). Role of telomeres and telomerase in cancer. *Seminars in Cancer Biology*, *21*(6), 349–353. https://doi.org/10.1016/j.semcancer.2011.10.001

Shipley, B. (2013). The AIC model selection method applied to path analytic models compared using a d-separation test. *Ecology*, *94*(3), 560–564. https://doi.org/10.1890/12-0976.1

Stone, E. A. (2011). Why the phylogenetic regression appears robust to tree misspecification. *Systematic Biology*, *60*(3), 245–260. https://doi.org/10.1093/sysbio/syq098

Sun, J., Liu, W., Guo, Y., Zhang, H., Jiang, D., Luo, Y., Liu, R., & Chen, C. (2021). Characterization of tree shrew telomeres and telomerase. *Journal of Genetics and Genomics*, *48*(7), 631–639. https://doi.org/10.1016/j.jgg.2021.06.004

Tricola, G. M., Simons, M. J. P., Atema, E., Boughton, R. K., Brown, J. L., Dearborn, D. C., Divoky, G., Eimes, J. A., Huntington, C. E., Kitaysky, A. S., Juola, F. A., Lank, D. B., Litwa, H. P., Mulder, E. G. A., Nisbet, I. C. T., Okanoya, K., Safran, R. J., Schoech, S. J., Schreiber, E. A., … Haussmann, M. F. (2018). The rate of telomere loss is related to maximum lifespan in birds. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1741), 20160445. https://doi.org/10.1098/rstb.2016.0445

Bijl, W. van der. (2018). phylopath: Easy phylogenetic path analysis in R. *PeerJ*, *6*, e4718. https://doi.org/10.7717/peerj.4718

Watson, W., & Walker, H. J. (2004). The world's smallest vertebrate, *Schindleria brevipinguis*, a new paedomorphic species in the family Schindleriidae (Perciformes: Gobioidei). *Records of the Australian Museum*, *56*(2), 139–142. https://doi.org/10.3853/j.0067-1975.56.2004.1429

Watson, H., Bolton, M., & Monaghan, P. (2015). Variation in early-life telomere dynamics in a long-lived bird: Links to environmental conditions and survival. *Journal of Experimental Biology*, *218*(5), 668–674. https://doi.org/10.1242/jeb.104265

Whittemore, K., Vera, E., Martínez-Nevado, E., Sanpera, C., & Blasco, M. A. (2019). Telomere shortening rate predicts species life span. *Proceedings of the National Academy of Sciences*, *116*(30), 15122–15127. https://doi.org/10.1073/pnas.1902452116

Wright, W. E., Piatyszek, M. A., Rainey, W. E., Byrd, W., & Shay, J. W. (1996). Telomerase activity in human germline and embryonic tissues and cells. *Developmental Genetics*, *18*(2), 173–179. https://doi.org/10.1002/(SICI)1520-6408(1996)18:2<173::AID-DVG10>3.0.CO;2-3

Xu, L., Li, S., & Stohr, B. A. (2013). The role of telomere biology in cancer. *Annual Review of Pathology: Mechanisms of Disease*, *8*(1), 49–78. https://doi.org/10.1146/annurev-pathol-020712-164030

Zglinicki, T. von. (2002). Oxidative stress shortens telomeres. *Trends in Biochemical Sciences*, *27*(7), 339–344. https://doi.org/10.1016/S0968-0004(02)02110-2

Zhdanova, N. S., Karamisheva, T. V., Minina, J., Astakhova, N. M., Lansdorp, P., Kammori, M., Rubtsov, N. B., & Searle, J. B. (2005). Unusual distribution pattern of telomeric repeats in the shrews *Sorex araneus* and *Sorex granarius*. *Chromosome Research*, *13*(6), 617–625. https://doi.org/10.1007/s10577-005-0988-3

Table with captions

Table 1: Contrast of parameters estimated by the most likely model according to the information-theoretic criteria of selection.

	DF	F-value	p-value
(Intercept)	1	84.65	<0.001
Thermal biology	1	16.65	<0.001
Log lifespan	1	7.96	0.006
Log mass	1	1.71	0.194

Figures with captions

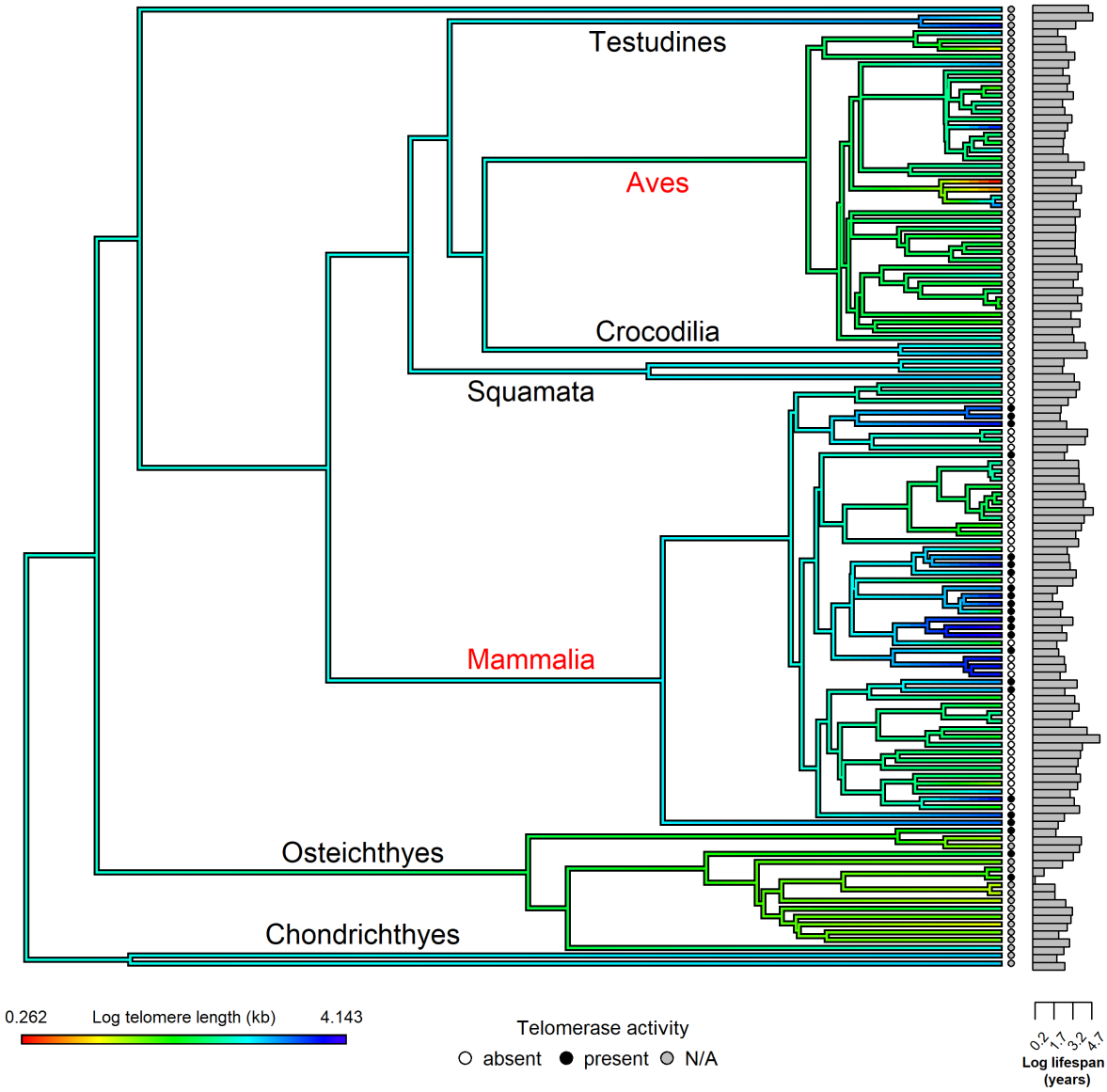

Figure 1: Telomere length, lifespan, thermal biology, and telomerase activity across 123 vertebrate species. Warmer colors represent shorter telomeres.

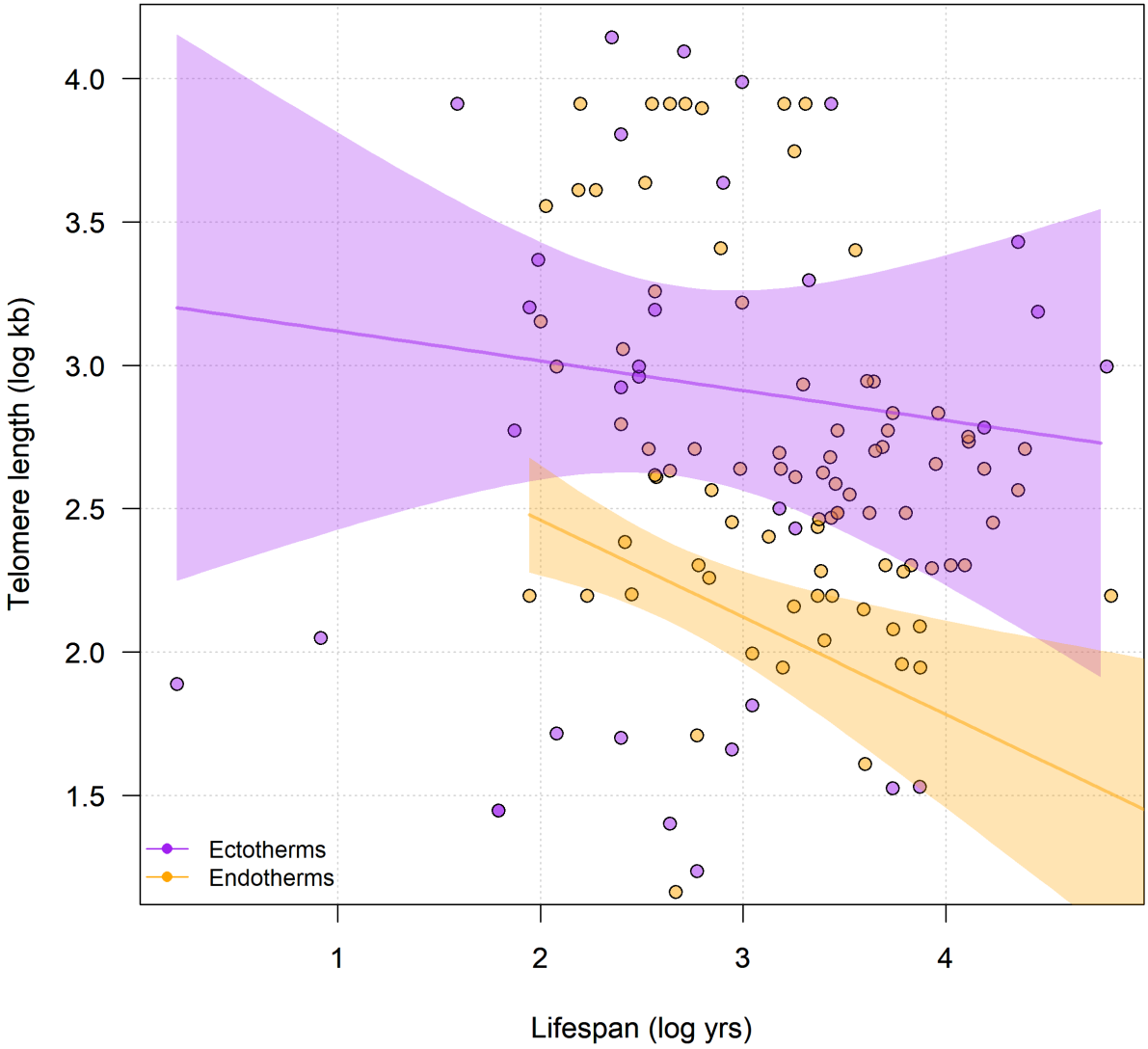

Figure 2: Effect of the thermal biology and lifespan on telomere length across vertebrates. Shading around the regression lines represents the 95% confidence intervals.

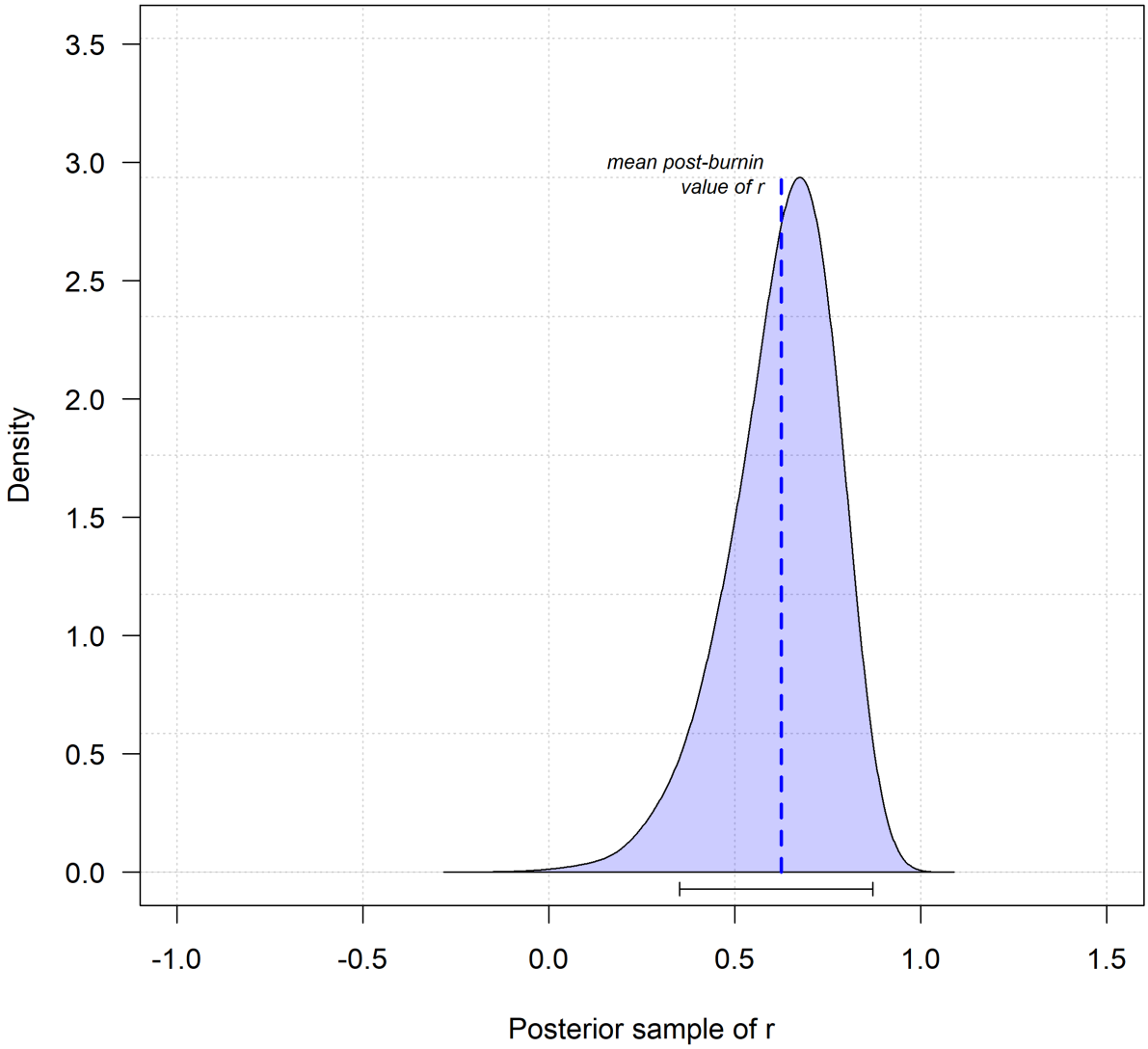

Figure 3: Posterior density of the correlation between telomere length and telomerase activity in vertebrates. The dashed line represents the estimated correlation coefficient (r = 0.62), representing the correlation between telomere length and the presence or absence of telomerase activity. Additionally, the 95% high probability density (HPD = 0.35 − 0.87) is represented by the solid black bar below the distribution.

Supplementary Material

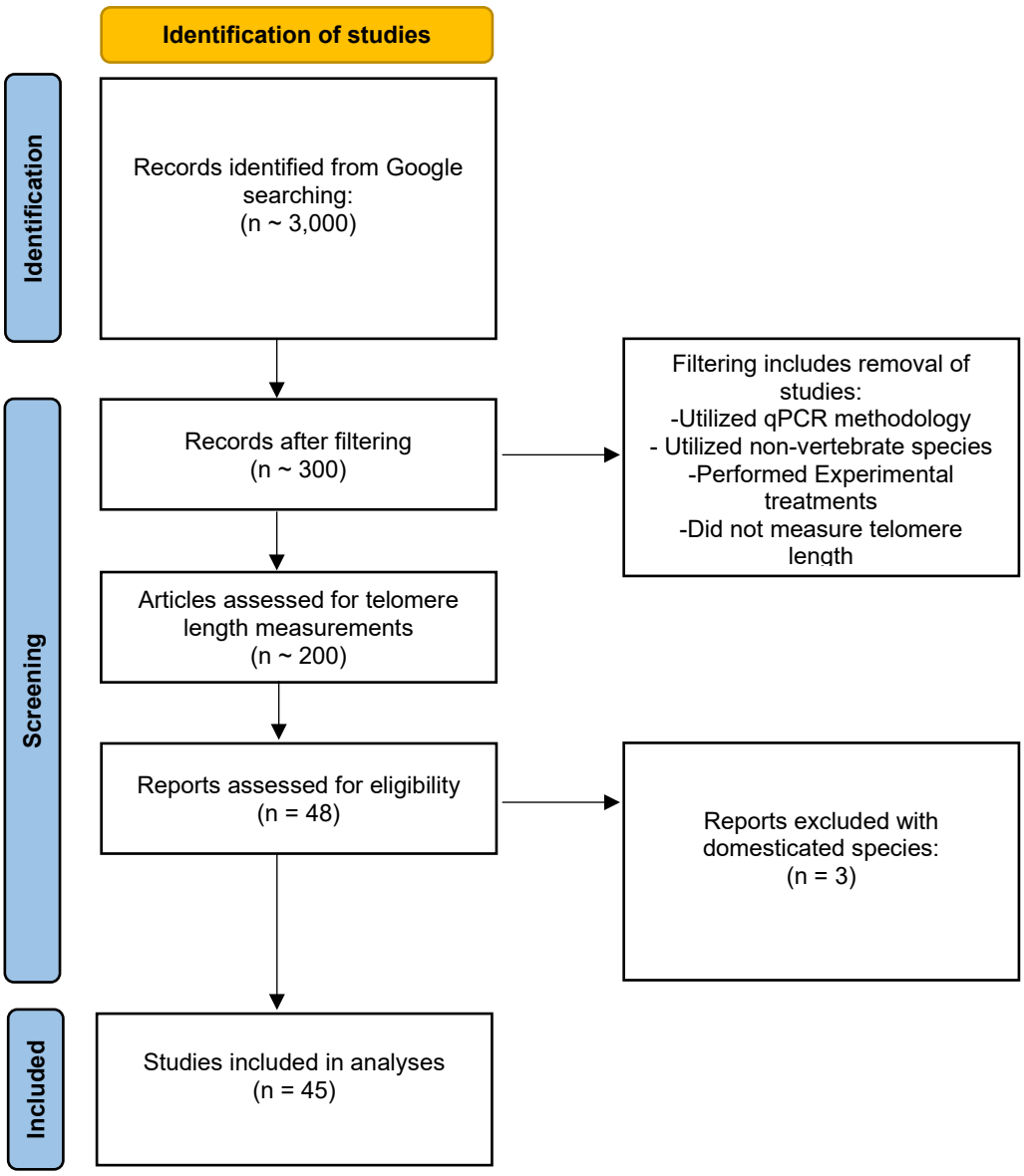

Figure S1. PRISMA flow diagram depicting the data collection for this study.

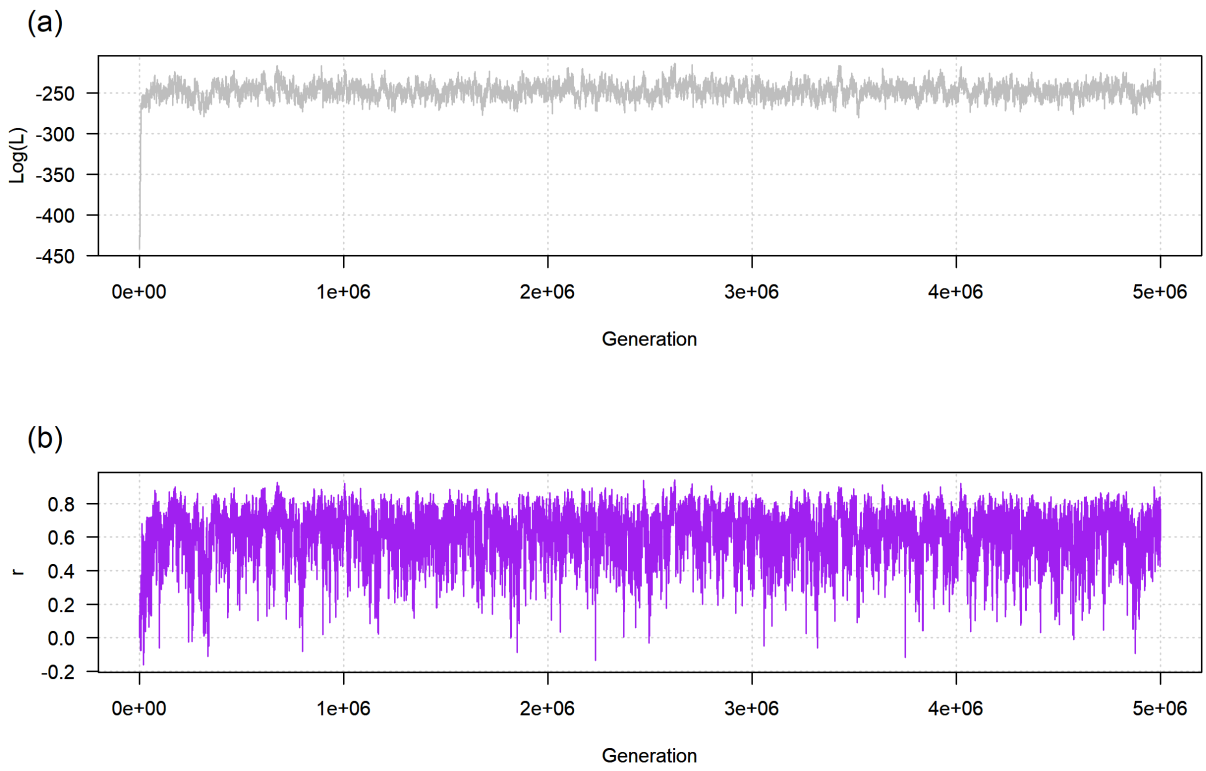

Figure S2: Profile plots from a Bayesian MCMC analysis of the threshold model for telomere length and telomerase activity in vertebrates. (a) The likelihood profile and (b) Profile of the correlation coefficient, r.

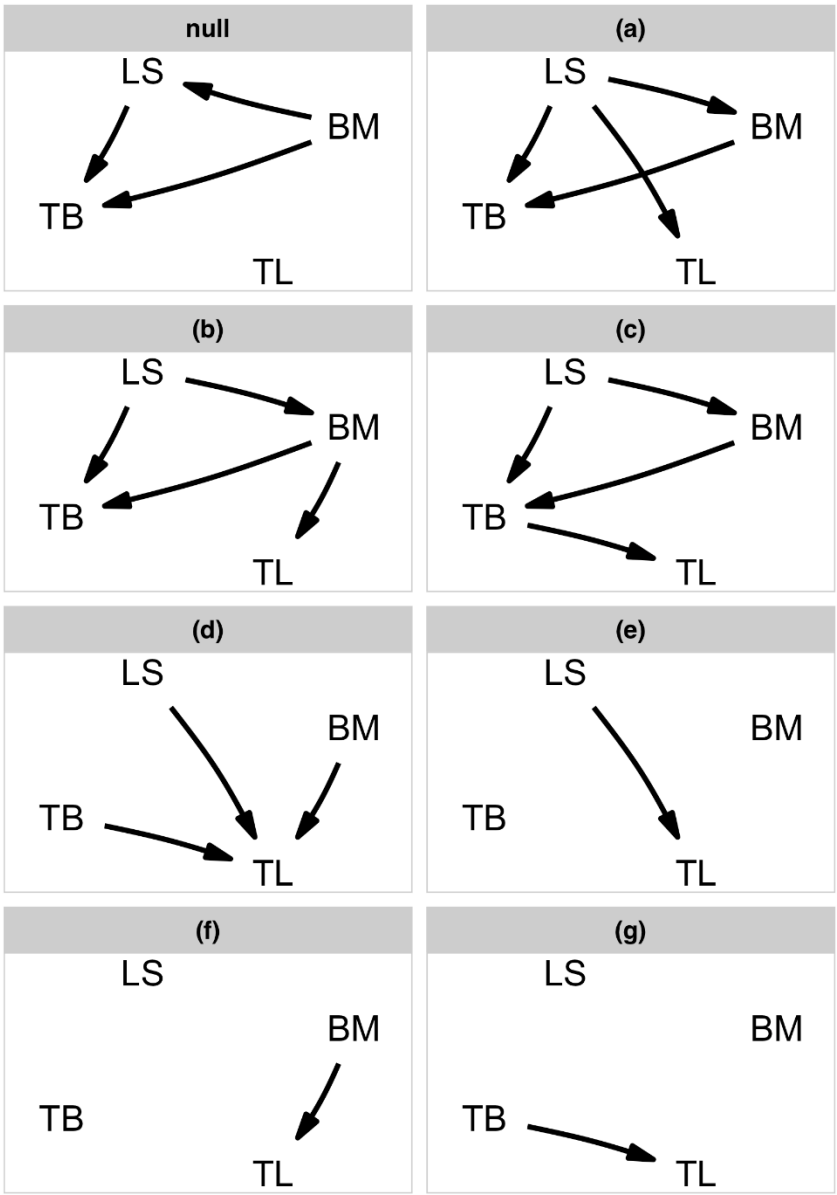

Figure S3: Path models depicting relationships among telomere length and predictor variables derived from optimality models (see text for details). Abbreviation in the path diagrams are as follows: TL, telomere length; BM, body mass; LS, lifespan; TB, thermal biology.

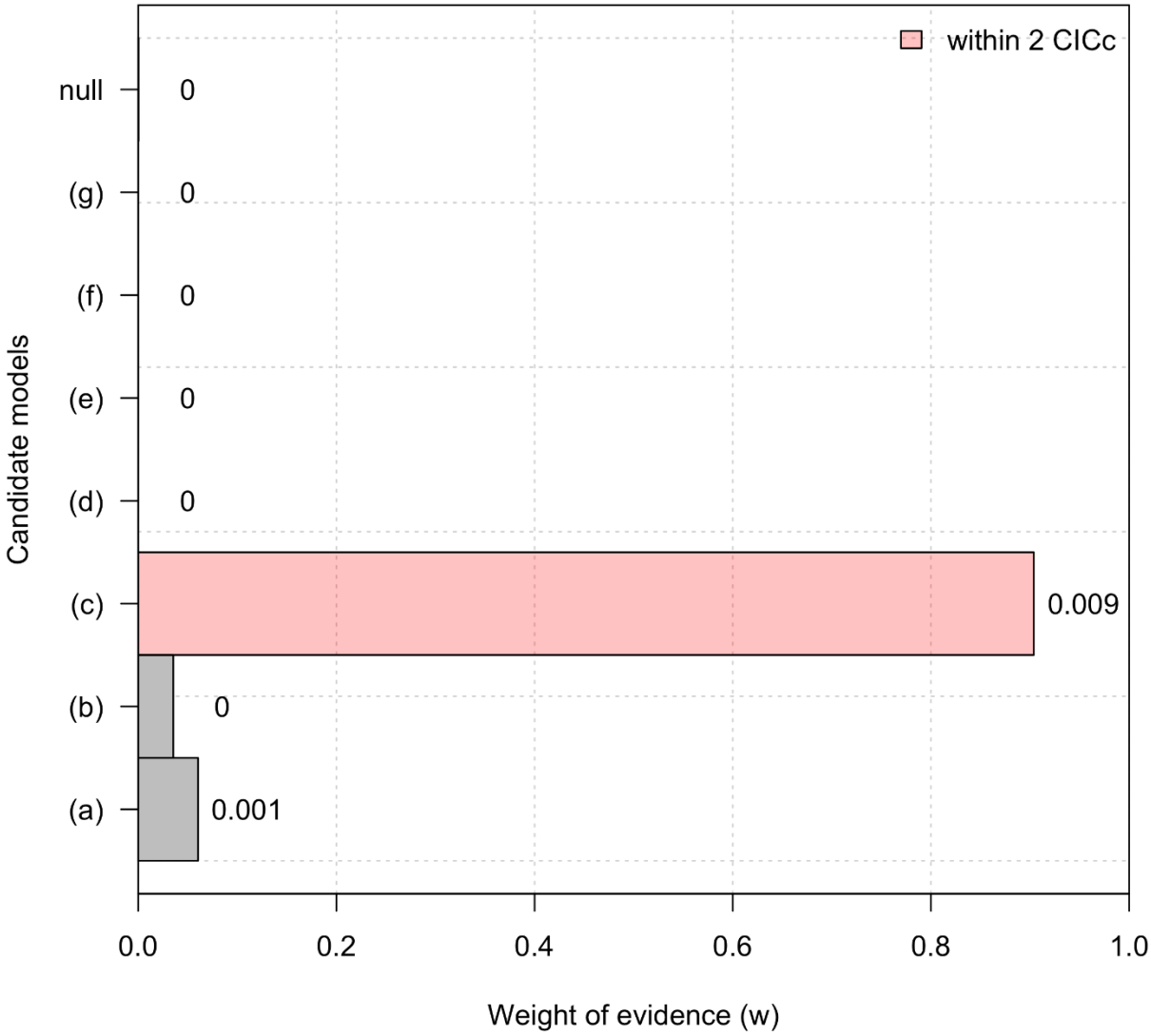

Figure S4: Relative importance of the causal models describing the evolution of telomere length across vertebrates.

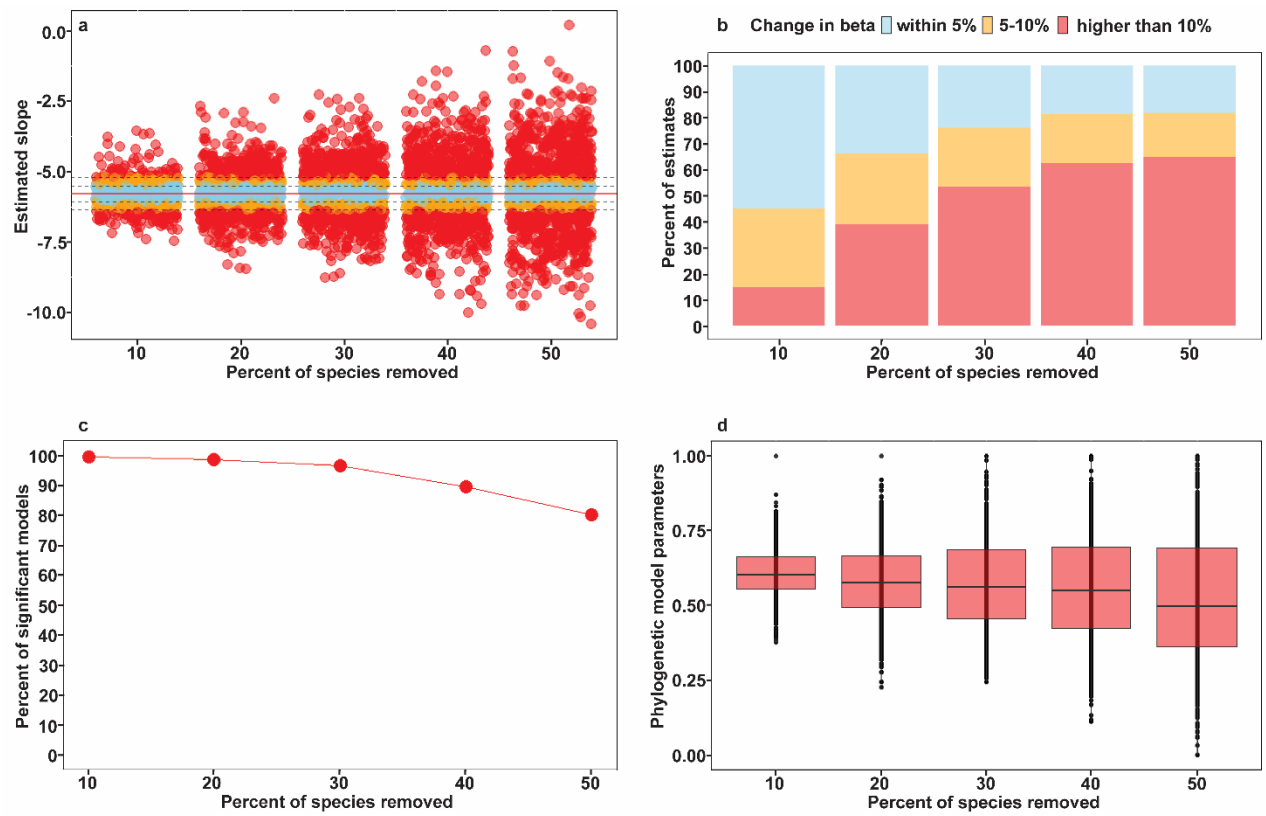

Figure S5: Sensitivity analysis investigating the effect of sample size on the regression slope comparing telomere length and lifespan across all species. A. The change in estimated slope across differing proportions of random species removed. Each point represents a simulation with blue, orange, and red representing lower than 5%, 5-10%, and greater than 10% changes in slope. B. The percent of estimated slopes across the proportions of species removed. Blue, orange, and red represent lower than 5%, 5-10% and greater than 10% change in slope. C. The percent of models finding a significant correlation between telomere length and lifespan as species are removed. D. The percent of significant slopes as species are removed.

Supporting information

Addressing potential confounding variables

Methodology

We included methodology as a covariate in our main model. We utilized data on 6 species using fluorescent in-situ hybridization (FISH), telomere amount and length assay (TALA) on 4 species, and telomere restriction fragment analysis (TRF) was used for the remaining 132 species. As methodology did not have a significant effect on any of our models, data from all methodologies listed above was included in our analyses.

Tissue type

To assess the effect of tissue on telomere length measurements, we included tissue type as the predictor variable and telomere length as the response variable. We found no significant effect of tissue type on telomere length ($t_{123} < 0.92$, $p > 0.36$ for all tissues). As such, we included data from all tissue types in our analyses involving telomere length, lifespan, body mass, and thermal biology. Additionally, because measuring telomere length and telomerase activity in different tissues could lead to erroneous conclusions, we included only studies which measured both variables from the same tissue type when analyzing the correlated evolution of telomere length and telomerase activity. These results did not differ significantly from analyses including telomerase activity and telomere length measurements from different somatic tissues within the same organism.

Standardization of telomere length measurements between studies

When possible, we analyzed data that was analyzed in previous meta-analyses, reviews and studies that have collected data on telomere length from multiple sources (Gomes et al. 2011, Criscuolo et al. 2021, Pepke and Eisenberg 2022). This covers the vast majority of mammal and bird species. We applied the same correction factor to data from Seluanov et al. (2007) that precious analyses used (Pepke and Eisenberg 2022). As data on telomere length in fishes and non-avian archelisaurs and a handful of birds and mammals has not previously been analyzed in a comparative study, we were unable to apply a correction factor. However, given that telomere length measurements not including qPCR are highly repeatable, and that our study provides valuable insight, we included this data in our analyses (Lai et al. 2018, Kärkkäinen et al. 2022).

Domestication

Given that previous literature has demonstrated that the relationship between lifespan and telomere length is significantly impacted by the inclusion or exclusion of domesticated species, we did not include domesticated mammals in our analyses. The species excluded were the chicken (*Gallus gallus)*, pig (*Sus scrofa*), cow (*Bos taurus*), dog (*Canis lupus familiaris*), horse

(*Equus caballus*), house mouse (*Mus musculus*), Norway rat (*Rattus norvegicus*), cat (*Felis catus*), sheep (*Ovis aries*), dromedary camel (*Camelus dromedarius),* and goat (*Capra hircus*).

The calculation of telomere length and average adult telomere length

Telomere rate of change differs drastically among species, and telomere attrition and lengthening occurs at different times in different species (Whittemore et al., 2019). Given this, standardizing adult telomere length among species is a difficult task, and a general approximation based on several individuals across a range of ages within a species may be the best indicator of adult telomere length. We excluded studies with measurements from extremely young or old individuals. For the majority of birds and mammals, we utilized the average adult telomere length values reported in previous analyses (Gomes et al. 2011, Criscuolo et al. 2021, Pepke and Eisenberg 2022). For species not included in these analyses, we used the reported average telomere length when available. For studies that did not report this, we calculated the average telomere length by adding up the measured telomere length for each individual and dividing by the number of individuals.

Alterations to phylogeny

Species that lacked easily-accessible phylogenetic information were replaced with closely related species and when possible congeners in order to increase our sample size. The following alterations were made:

Torpedo marmorata replaced with *Tetronarce macneilli*
Urolophus paucimaculatus replaced with *Urogymnus asperrimus*
Mustelus asterias replaced with *Mustelus antarcticus*
Ailurus fulgens replaced with *Ailurus*
Giraffa camelopardalis replaced with *Giraffa reticula*
Saimiri sciureus replaced with *Saimiri boliviensis*
Fregata minor replaced with *Fregata*
Larus crassirostris replaced with *Larus marinus*
Trachemys scripta replaced with *Trachemys dorbigni*
Larus fuscus replaced with *Larus cachinnans*
Phalacrocorax aristotelis replaced with *Phalacrocoracidae*
Corvus monedula replaced with *Corvus brachyrhynchos*
Erythrura gouldiae replaced with *Erythrura trichroa*
Elephantulus rufescens replaced with *Elephantulus brachyrhynchus*
Antigone antigone formerly *Grus antigone*
Calidris pugnax formerly *Philomachus pugnax*
Thalassarche melanophrys replaced with *Thalassarche chrysostoma*