Defying expectations: sex-biased telomere dynamics and extended lifespan in the tropical bat species, *Molossus molossus*.

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Abstract

Telomeres are key biomarkers of cellular ageing, yet their dynamics remain poorly studied in tropical and short-lived bat species. Here, we present the first investigation of telomere length across age in *Molossus molossus*, a tropical bat historically categorised as the shortest-lived bat on record. Through a multi-year mark-recapture study in Gamboa, Panama, we sampled 492 individuals (n = 317 females, 175 males) and documented a female *M. molossus* surviving to at least 13 years of age, more than doubling the previously reported maximum lifespan of 5.6 years. Across the population, relative telomere length (rTL) showed no overall decline with age. However, sex-specific models revealed clear divergence: males tended to have longer rTL than females but showed significant age-related shortening, whereas females exhibited stable rTL across the observed age range. The pattern of telomere stability in females aligns closely with results observed in long-lived bats of the *Myotis* genus while male-specific attrition suggests differential physiological and life-history costs between the sexes. Overall, the findings here

challenge previous assumptions about the lifespan and ageing biology of *M. molossus*, demonstrate that telomere maintenance is not limited to temperate bats and reinforce the value of long-term field studies for understanding ageing processes in the wild.

Keywords: telomeres, chiroptera, longevity, short-lived, mark-recapture

Introduction

Vertebrate lifespan shows extraordinary variation, from species such as pygmy goby (*Eviota sigillata*) living ~8 weeks (Depczynski and Bellwood, 2005) to the Greenland shark (*Somniosus microcephalus*) living up to 500 years (Nielsen et al., 2016). Body size reflects much of this range, with large animals typically living longer than smaller animals due to lower extrinsic mortality and slower metabolic turnover (Speakman, 2005). Within mammals, in particular, there is a log-linear relationship between body mass and maximum lifespan, where larger species tend to live longer than smaller species (Austad, 2010). However, bats (order Chiroptera) defy this relationship, displaying exceptional longevity and are the longest-lived mammals relative to their body size (Austad, 2010, Foley et al., 2020). Many bat species can live considerably longer than terrestrial nonflying mammals of a similar size (Healy et al., 2014, Seim et al., 2013, Wilkinson and Adams, 2019). Indeed, the oldest bat record is an individual (*Myotis brandtii*) which was re-captured at >41 years old, living more than eight times longer than predicted for its size, weighing just ~7g (Foley et al., 2018, Podlutsky et al., 2005). This exceptional lifespan has positioned bats as valuable models for ageing research (Cooper et al., 2024, Teeling et al., 2018). One promising approach for investigating ageing in wild mammals is telomere dynamics, a widely used biomarker of biological ageing across taxa (Lopez-Otin et al., 2023).

Telomeres are short repetitive nucleotide sequences which protect the ends of linear chromosomes and prevent genomic instability (Blackburn, 1991). In most organisms, telomeres shorten with each round of cell division due to incomplete end replication during cell division, known as the “end replication problem” (Levy et al., 1992, Watson, 1972). Telomere length can also shorten when exposed to sources of cellular damage, such as reactive oxygen species (Armstrong and Boonekamp, 2023, Reichert and Stier, 2017, von Zglinicki, 2002). When

telomere length becomes critically short, replicative cell senescence is triggered, halting cell division (Harley et al., 1990, Jones-Weinert et al., 2024). In some species, however, the enzyme telomerase restores telomere length, counteracting this shortening, although in most mammals telomerase activity is downregulated in somatic tissues after development. This is thought to have evolved as a tumour-suppressive mechanism, reducing the risk of malignant transformation at the cost of progressive cellular senescence (Gomes et al., 2011, Shay and Wright, 2019). However, studies focusing on long-lived bat species, particularly within the *Myotis* genus, have revealed unique telomere dynamics.

Unlike most mammals, where telomeres shorten with age, in the genus of bats with the longest maximum lifespan, *Myotis,* there is no correlation between telomere length and chronological age (Foley et al., 2018, Foley et al., 2020, Ineson et al., 2020). This occurs without detectable telomerase activity in somatic tissues, suggesting alternative mechanisms for preserving telomere integrity (Foley et al., 2018, Foley et al., 2020). However, little is known about telomere dynamics across bats. Despite more than 1400 bat species recorded globally, nearly all studies to date are based on longer-lived temperate insectivorous bats (Foley et al., 2018, Foley et al., 2020, Ineson et al., 2020, Power et al., 2022, Power et al., 2023). A recent study in the tropical bat *Phyllostomus hastatus* provided the first evidence of age-related telomere shortening in a non-temperate species, based on cross-sectional sampling and methylation-derived age estimates (Rayner et al., 2025). However, *P. hastatus* is a relatively long-lived tropical bat (females can live up to 22 years; Wilkinson and Adams, 2019) and therefore a gap remains in research concerning telomere dynamics in short-lived bat species, with no known studies to date. Investigating such species could clarify whether short-lived bats follow more conventional mammalian telomere ageing patterns, and whether telomere maintenance is restricted to certain long-lived temperate lineages (e.g., *Myotis*).

Molossus molossus, commonly known as Pallas's mastiff bat or the velvety free-tailed bat, offers the ideal short-lived contrast. *M. molossus* has been categorised as the shortest-lived bat species on record, with a maximum recorded lifespan of about 5.6 years in the wild and median survival for wild females estimated to be approximately 1.8 years (Gager et al., 2016a). *M. molossus* is among the most widespread and abundant species in the Neotropics, with a distribution ranging

from southern Mexico through Central and South America and the Caribbean (Loureiro et al., 2021, Reid, 2006, Simmons, 2005). In Panama, stable roosting groups dominated by adult females have been well studied (Dechmann et al., 2010, Gager et al., 2016a). These groups are typically joined by one or two adult males with frequent replacement of harem males. *M. molossus* is strictly tropical and does not hibernate, however they reduce heart rate, along with metabolic rate by >70% while maintaining body temperature around 32 °C, similar to the daytime torpor employed by temperate zone bats (O'Mara et al., 2017, Wojciechowski et al., 2007). Such a trait may reduce oxidative damage and influence telomere dynamics.

Here, we provide the first assessment of telomere length dynamics in *Molossus molossus* leveraging over 15 years of mark-recapture data from wild populations in Panama. Utilizing population-level qPCR estimates, we investigated age-related telomere changes in this tropical insectivorous bat. Given its previously reported short lifespan, we hypothesised that telomere length would decline with advancing age, in this putative short-lived bat.

Materials and Methods

Study population

This study focuses on a long-term mark-recapture programme for *Molossus molossus* in Gamboa (N 09,07; W 079,41) Panama, initiated in 2008 (Dechmann et al., 2010, Gager et al., 2016a). Gamboa is located near the banks of the Panama Canal and the Chagres River, surrounded by Soberanía National Park, and the study area is covered by semi-deciduous tropical lowland rainforest (Leigh, 1999, Windsor, 1990). Between 2008 and 2014, 14 social groups were captured on 81 occasions with approximately 500 individuals PIT-tagged, producing the first survival and longevity estimates for this species (maximum lifespan 5.6 years; Gager et al. 2016). Smaller yearly mark-recapture catches have continued since 2014, up until the start of this study.

Individuals for the present study were caught and sampled over the course of four years (2021 – 2024). Sampling was carried out consistently over two-three weeks in October/ November of

each year, where the majority of female bats have finished lactating. In addition to these annual samplings, a subset of individuals (n = 109) were also opportunistically sampled during the summer months (May - August) in 2022 and 2023. All capture and handling of bats was carried out with appropriate permission from the Autoridad Nacional del Ambiente in Panama and with approval from the Institutional Animal Care and Use Committee of the Smithsonian Tropical Research Institute.

Capture and sampling

In total, 30 sampling events were conducted across 14 different roost sites in Gamboa, (n = 583 capture events). Roosts were selected based on prior knowledge of *Molossus* bat presence and through systematic searches in human-made structures, typically crevices on the exterior of buildings. Bats were caught using mist nets placed at roost entrances, shortly after sunset. Upon capture, all bats were placed in soft cloth bags prior to processing. Bats were sexed, weighed (to an accuracy of ± 0.1g using a precision weight balance), and forearm length was measured (to an accuracy of ± 0.1mm using Vernier callipers). Newly caught individuals were marked with a subcutaneous Passive Integrated Transponder (PIT) tag (2.12x11mm, 0.1g, Trovan® & Biomark™), containing a unique 10-digit code which can be read using passive Radio-Frequency Identification (RFID) readers and facilitate the identification of individuals at each subsequent recapture. Wing punches were taken from all individuals at every capture using a sterile 3mm biopsy punch and immediately flash frozen in the field in liquid nitrogen (LN_2). Blood samples were also collected for each bat, by piercing the cephalic vein along the forearm with a sterile needle (27 gauge), creating a drop of blood on the patagium surface from which a volume of <100 μL was pipetted into a cryotube (2 ml, Nalgene labware). Haemostatic gel was applied on the vein to prevent further bleeding and all bats were checked before release to ensure bleeding had completely stopped (Huang et al., 2016). Blood samples were also immediately flash frozen in LN_2. All bats were released in the same area in which they were captured. As *M. molossus* bats are unable to take flight from low levels, all bats were released onto nearby trees where they could climb to an appropriate height before taking flight. Bats were monitored post-release to ensure they successfully took flight.

Age determination

The age of the bat was calculated based on the first time the bat was caught and PIT tagged. Captures were timed to occur around the time juveniles were fledged but still present in the maternal roost. If a bat was caught and tagged as a juvenile for the first time, its age was recorded as 0 and it was considered known aged. If a bat was caught for the first time as an adult, then it was assigned the age of “1+” indicating they were at least one year old at time of capture. If re-captured a year later this individual was recorded as “2+” and so forth. Juvenile bats were differentiated from adults based on the degree of ossification of the epiphyseal cartilage in the metacarpal-phalangeal joints (Kunz and Anthony, 1982) as well as by darker pelage colour than adults.

Species identification

While this study was designed to focus on *Molossus molossus*, the cryptic species *(Molossus coibensis*) was identified at a subset of roosts. Morphological classification was performed using forearm length, body mass, and pelage colour (Gager et al., 2016b). To validate the field morphological assignments, we confirmed the species identity of a subset of individuals (n = 72) using molecular phylogenetics. Total RNA was extracted from whole blood using an RNAzol BD kit, following the manufacturer’s instructions with minor modifications. The full RNA extraction from bat blood protocol is described in detail by Huang et al., (2016). Quantity and quality of RNA were assessed using a Bioanalyzer 2100 (Agilent Technologies). All samples that met the criteria of having >2 µg of total RNA and an RNA Integrity Number score >8.0 were chosen for RNA-Seq Illumina library preparation. The generated RNAseq data were quality trimmed using *Trim Galore!* v0.6.4 (Martin, 2011), then mapped on the *M. molossus* genome from Jebb et al. (2020) with STAR software v2.7.10b_alpha using default parameters (Dobin et al., 2013). Genomic variants were extracted from expressed regions using *bcftools* “mpileup” and “call” functions (Danecek et al., 2021), and filtered for a minimum quality of 20 and coverage of 10. The BRCA1 sequence for each individual was reconstructed by applying the filtered variants to the longest annotated isoform of the *M. molossus* reference, BRCA1_x2, using *bcftools* consensus. A *Myotis myotis* BRCA1 sequence was used as an outgroup (Jebb et al., 2020). Multiple sequence alignment was performed using the MUSCLEv3 program (Edgar,

2004) with default parameters. A maximum likelihood phylogeny was constructed using IQ-tree (Trifinopoulos et al., 2016), with the substitution model GTR+G+I determined by the software based on the lowest AICc (Corrected Akaike Information Criterion) score. Branch support was assessed using two statistical tests available in IQ-tree: SH-like aLRT (approximate Likelihood Ratio Test, 1,000 replicates) and UfBoot (ultrafast bootstrap, 1,000 replicates).

DNA extractions and qPCR assay

DNA was extracted from wing biopsies using the membrane filter-based extraction kit - Promega Wizard SV DNA Extraction Kit (catalogue no. A2371, Promega Corporation, Madison, WI). This 96 well extraction protocol was partially automated using the Hamilton Star Deck liquid Handling Robot but otherwise followed the manufacturer's instructions. DNA concentration and purity was quantified using a Nano Drop 8000 Spectrophotometer (ThermoScientific). Wing tissue has been shown to be a suitable proxy for global tissue telomere dynamics in bats (Power et al., 2021). Relative telomere length (rTL) was measured by real-time quantitative PCR (qPCR) as the concentration of telomeric DNA relative to a single copy gene (SCG) that is constant in number and optimised for use in bats. qPCR amplifications were carried out using the Applied Biosciences Quantstudio 7 Flex Real-Time PCR. qPCR assay followed a modified version of the Cawthon (2002) protocol adapted for bats as described in Foley et al. (2018). Samples were run on either 96-well or 384-well plates, with six technical replicates. A "golden sample" calibrator prepared from pooled *M. molossus* wing tissue was run in triplicate on each plate to account for among-plate variation as well as a negative control to detect contamination.

Raw data was exported from the qPCR machine and the software package LinRegPCR (Ruijter et al., 2009) was used to calculate baseline correction, per well amplification efficiencies and Cq values (the number of cycles the qPCR amplification curve crosses a set fluorescence threshold). Data were normalised using the 'golden sample' as reference across both telomere and SCG reactions. The average Cq was calculated across the six replicates for each sample for both amplicons. rTL was calculated for each sample, following Pfaffl (2001). Co-efficient of variation (CoV) thresholds were applied: 5% for telomere reactions and 2.5% for single-copy gene (SCG) reactions (Foley et al., 2018, Foley et al., 2020).

Average reaction efficiencies (mean ± SE) across the 12 qPCR plates were 1.984 ± 0.012 for the SCG reaction and 1.931 ± 0.026 for the telomere reaction. Intra-plate repeatability (intraclass correlation coefficient, ICC) calculated with the rptR package (Stoffel et al., 2017) was 0.971 ± 0.002 (95 % CI [0.967–0.974], n = 3528 technical replicates, $P < 0.001$) for SCG and 0.957 ± 0.003 (95 % CI [0.952–0.962], n = 3528 technical replicates, $P < 0.001$) for telomere. (1,000 parametric bootstraps; 1,000 permutations, rptR). Inter-plate consistency was calculated on the subset of samples analysed in triplicate on two separate plates. Plate-to-plate effects accounted for only ≈ 6 % of the telomere Cq variance (ICC_plate = 0.067 ± 0.070) and ≈ 16 % of the SCG variance (ICC_plate = 0.157 ± 0.111). However, differences among samples remained the dominant source of variation (ICC_sample = 0.823 ± 0.065 for telomere; 0.652 ± 0.091 for SCG; all $P < 0.001$).

Statistical analysis

All statistical analyses were carried out in R v.3.6.3 (R Core Team, 2021). As residual diagnostics indicated deviations from normality and heteroscedasticity, a Box-Cox transformation (Box and Cox, 1964) was applied to the response variable to improve model fit. rTL measures were subsequently transformed to z-scores to facilitate comparability across studies (Verhulst, 2020). Linear mixed effect models were constructed using the R packages MASS (Venables, 2002) and lme4 (Bates et al., 2015) to investigate relationships between rTL, age and sex. Models included random effects for individual ID, qPCR assay plate, site, and sampling year to control for repeated measures and non-independence in the data. The best-fitting model was selected based on Akaike's Information Criterion corrected for small sample sizes (AICc), and residuals were examined to ensure model assumptions were met. We also tested for random effects of capture month, but its inclusion did not improve model fit or affect fixed effect estimates and was therefore excluded from the final models. To test for sex-specific telomere dynamics, a sex × age interaction term was included. Separate age-only models were finally fitted to male and female subsets to examine within-sex age effects.

In our dataset, females were more numerous than males due to the harem based structure of most *Molossus* colonies. To account for the resulting imbalance in sex and age distributions, we

applied an iterative subsampling approach to test the robustness of sex differences in rTL when age distributions were equalized (Rayner et al., 2025). For each of 10,000 iterations, males were randomly matched to females of the same exact age, and a simple linear model was used to test the effect of sex on rTL. A Wilcoxon rank-sum test was used in each iteration to confirm matched age distributions between sexes. The proportion of iterations where the sex effect was statistically significant ($p < 0.05$) was recorded. Additionally, the distribution of sex effect estimates across all iterations was plotted to assess the consistency and direction of any observed sex bias.

The *M. molossus* dataset contains many individuals which were first captured as adults and thus have an approximate age e.g. '1+'. As such the data was analysed two ways: using individuals of known age (n = 151) and in a minimum age based analysis (n = 466) where the 'x+' ages were converted to whole ages and treated as the minimum age of the bat, to maximise usable data.

Results

Capture and species identification

Over the course of this study, 583 captures (428 individuals) of the genus *Molossus* were recorded across 14 different roosts sites in Gamboa, Panama from 2021 to 2024. Species identification was carried out on all individuals based on morphological characteristics and, on a subset (n = 72) using BRCA1 gene transcripts derived from Illumina blood-transcriptome reads. 492 bats were morphologically identified as *M. molossus* and 91 as *M. coibensis.* All genetically identified individuals matched their infield, morphological classification, confirming the reliability of morphological criteria used in the larger dataset. A morphological comparison between species shows clear species groupings based on forearm length and body mass (Fig. S1). *M. coibensis* individuals were generally smaller in forearm length but heavier in body mass relative to *M. molossus*. Phylogenetic reconstruction based on BRCA1 sequences revealed distinct species-level clustering consistent with field identifications for *M. molossus* and *M. coibensis* (Fig. S1). This analysis confirmed the placement of each specimen within the expected *M. molossus* or *M. coibensis* clades. Of the 492 *M. molossus* captures, 367 unique individuals

were identified, of which 317 were females and 175 males. Among these, 163 were of known age (tagged as juveniles), while the remaining 329 were assigned a minimum estimated age. A total of 82 individuals were recaptured at least once, giving a recapture rate of 22.3%. Capture and recapture data for *M. molossus* are summarised in Table S1.

Molossus molossus **- New longevity record**

A significant finding of this study was the identification of an individual that was at least 13 years old at her last capture in 2024, representing the oldest known *M. molossus* recorded to date (Fig.1). Bat 0006C8684C was originally caught from the same roost and tagged as an adult in 2012. Previously, the maximum reported lifespan for the species was 5.6 years and this new estimate has more than doubled the recorded maximum longevity for this species. This new age record also changes the longevity quotient of *M. molossus* from 0.99 to 2.37 (Fig.1).

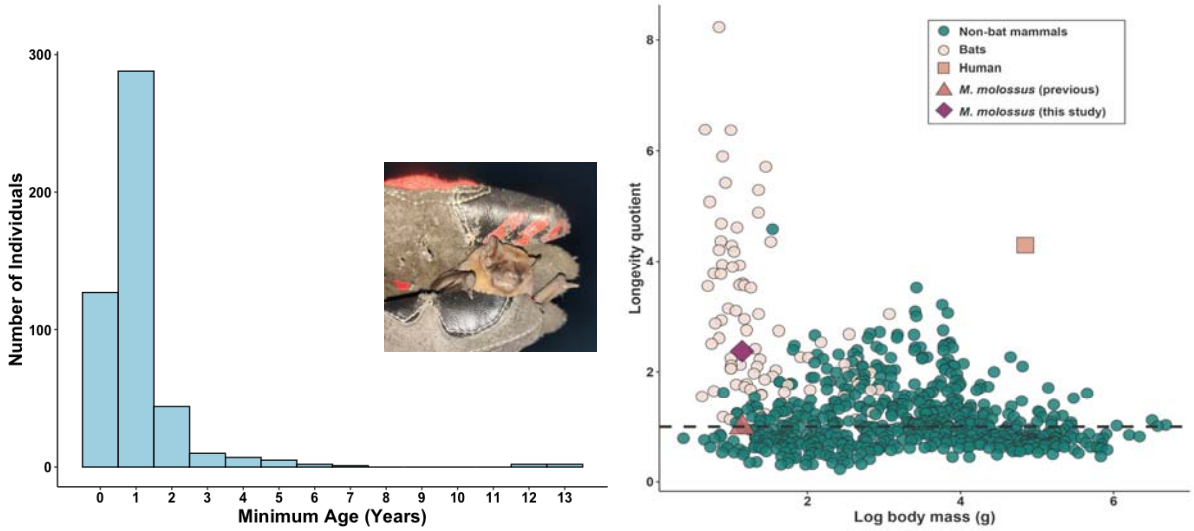

Figure 1: Minimum age distribution and updated longevity quotient for *Molossus molossus*. (a) Distribution of minimum age (in years) for *M. molossus* individuals captured between 2021 - 2024. The inset photograph shows Bat 0006C8684C, the oldest known *M. molossus* to date. (b) Relationship between longevity quotient (LQ = observed/expected longevity) and log body mass across 829 mammalian species. Each point represents a different species, with non-bat mammals shown as teal circles, bats as pale pink circles, and humans indicated by a light orange square. The previously reported *M. molossus* LQ (Gager et al., 2016) is shown as a red triangle, while the new LQ based on the longevity record discovered in this study is indicated by a purple

diamond. The dashed horizontal line represents an LQ of 1, indicating expected longevity based on body mass. Species above this line live longer than predicted for their size. Modified from Foley et al., (2018).

Telomere length and age in *M. molossus*

Accounting for samples which failed DNA extractions and/or qPCR assay, a total of 466 rTL measurements were calculated for *M. molossus*, with an age range of 0 to 13+ years. Linear mixed-effects models initially indicated a significant negative relationship between age and rTL for *M. molossus* (p = 0.016, Table. S2), suggesting telomere shortening with age (Fig. S2). However, this effect was strongly influenced by a single individual, the oldest bat in the dataset, which had three of the highest age data points. When this individual was removed from the analysis, the relationship between age and rTL was no longer significant (p = 0.188, Table. 1, Fig. 2a). The variance explained by individual identity also decreased, suggesting that much of the detectable age effect was driven by this single bat. A separate analysis including only individuals of known age (n = 151) did not detect a significant relationship between age and rTL (p = 0.822, Table. 1, Fig. 2b). The lack of significance across these models suggests that telomere shortening with age may be weak or highly variable in *M. molossus*, and that larger datasets with more older individuals may be needed to confirm this pattern.

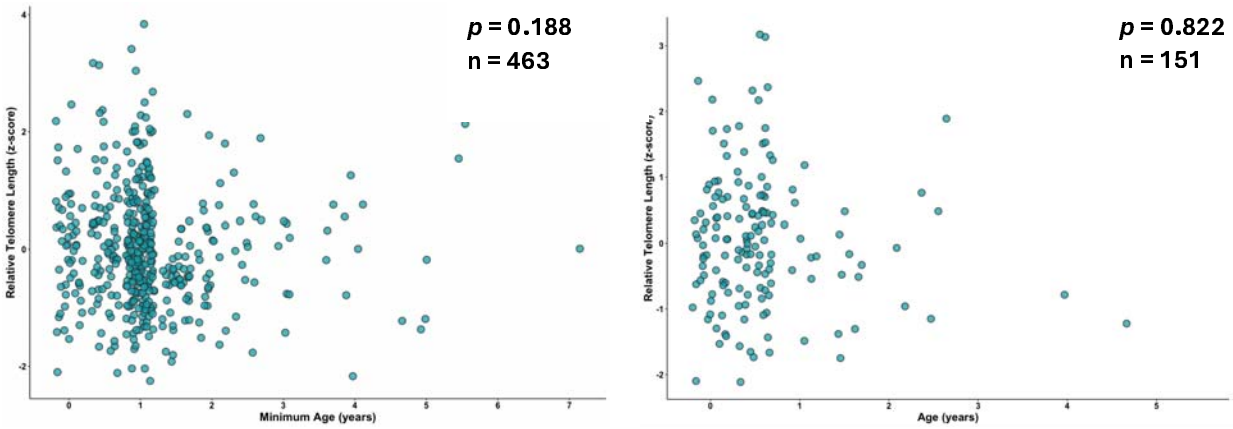

Figure 2: Relationship between age (years) and relative telomere length (rTL) in *Molossus molossus.* (a) Full dataset excluding the oldest individual (0006C8684C; n = 463). No significant

relationship between age and rTL (p = 0.188). **(b)** Known aged individuals only (n = 151), also showing no significant relationship between age and rTL (p = 0.822).

Table 1: Results of Linear Mixed-Effects Models examining the relationship between age and relative telomere length in *M. molossus.* Results shown for two models: the full model excluding the oldest bat and a separate model including only individuals of known age. Each model includes random intercepts for Individual identity, qPCR plate, capture Site and Year.

Excluding old bat (n = 463)				
Fixed Effects				
(Intercept)	0.134	0.267	0.503	0.637
Age	-0.062	0.048	-1.323	0.188
Random Effects	**Variance**			
Individual	0.060			
qPCR Plate	0.090			
Site	0.034			
Year	0.207			
Residual	0.648			
Known Age (n = 151)				
Fixed Effects				
(Intercept)	0.090	0.265	0.343	0.744
Age	-0.021	0.094	-0.226	0.822
Random Effects	**Variance**			
qPCR Plate	0.173			
Site	0.084			
Year	0.121			
Residual	0.760			

Telomere length and sex in *M. molossus*

A linear mixed-effects model examining the relationship between sex and rTL, including age as a fixed effect and individual, qPCR plate, site, and year as random effects, indicated that male *M. molossus* had slightly longer telomeres than females (p = 0.024, Table. 2, Fig. 3a). A sex × age interaction was not significant (p = 0.155) although sex-specific models revealed contrasting telomere-age dynamics: males showed significant telomere shortening with age (p = 0.023),

whereas no such pattern was detected in females ($p = 0.697$, Fig. 3b). To test the robustness of the observed sex difference, age-matched subsampling was performed. In 10,000 iterations, 73.8% showed a significant sex effect ($p < 0.05$), with a consistent positive estimate favouring males (mean estimate ≈ 0.23), and matched age distributions across all subsamples (Fig. S3). These results suggest that the observed male-biased telomere shortening is not simply a byproduct of age imbalance, but likely reflects an underlying biological difference between the sexes in *M. molossus*. Random effects showed a strong year component, particularly in females, suggesting annual variation in rTL. By contrast, the larger estimate of individual variance in the male-only model is likely an artefact of sparse recaptures, as most males were only sampled once.

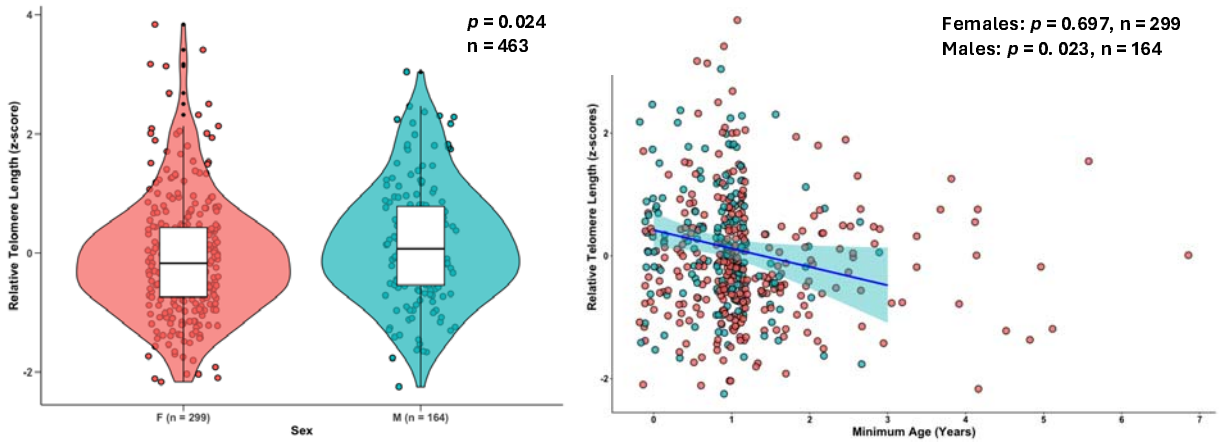

Figure 3: Sex specific patterns of relative telomere length (rTL) in *Molossus molossus*. (a) Violin plot showing the distribution of rTL by sex. Females (F) are shown in red, while males (M) are shown in blue. The boxplot within each violin represents the median and interquartile range. Males have significantly longer telomeres than females ($p = 0.024$). **(b)** Sex specific telomere-age patterns in *M. molossus*. Scatterplot of rTL (z-scores) against minimum age for females (red, n = 299) and male (blue, n = 164). Blue line represents sex-specific model fit with 95% confidence interval. In males, rTL declines with age ($p = 0.023$) whereas no age effect is detected in females ($p = 0.697$). The oldest *M. molossus* was removed to avoid skewing results.

Table 2: Results of linear mixed-effects models testing age and sex effects on relative telomere length (z-scores) in *Molossus molossus*. Results shown for three models: the full model including an age-sex interaction term, and separate models fitted to females and males only. Age represents the minimum known age in years. Each model includes random intercepts for Individual identity, qPCR plate, capture Site and Year. Significant values in bold $p < 0.05$.

Age × Sex Interaction (n = 463) **Fixed Effects**	**Estimate**	**Std Error**	***t* value**	***p*-value**
(Intercept)	0.027	0.267	0.102	0.922
Age	–0.022	0.051	–0.435	0.664
Sex (Male)	0.341	0.149	2.288	**0.024**
Age × Sex (Male)	–0.191	0.134	–1.423	0.155
Random Effects	**Variance**			
Individual	0.058			
qPCR Plate	0.086			
Site	0.037			
Year	0.202			
Residual	0.640			

Females only (n = 299) **Fixed Effects**	**Estimate**	**Std Error**	***t* value**	***p*-value**
(Intercept)	0.043	0.280	0.152	0.885
Age	–0.020	0.050	–0.391	0.697
Random Effects	**Variance**			
Individual	0.018			
qPCR Plate	0.092			
Site	0.052			
Year	0.219			
Residual	0.660			

Males only (n = 164) **Fixed Effects**	**Estimate**	**Std Error**	***t* value**	***p*-value**
(Intercept)	0.431	0.248	1.738	0.131
Age	–0.305	0.133	–2.293	**0.023**
Random Effects	**Variance**			
Individual	0.257			

Males only (n = 164) **Fixed Effects**	**Estimate**	**Std Error**	***t* value**	***p*-value**
qPCR Plate	0.058			
Year	0.149			
Residual	0.524			

Discussion

This study represents the first investigation into telomere dynamics in *M. molossus*, a tropical species of bat historically categorised as the shortest-lived bat on record (Tacutu et al., 2018). Through a multi-year mark-recapture study in Gamboa, Panama, we report a new longevity record for *M. molossus*, with an individual female surviving to at least 13 years, significantly exceeding the previously reported maximum lifespan of 5.6 years (Gager et al., 2016). This earlier estimate placed *M. molossus* as a stark outlier compared to temperate-zone bats such as *M. brandtii* (41 years, LQ 8.23), *M. myotis* (37 years, LQ 5.71) and *R. ferrumequinum* (30 years, LQ 4.98) (Gaisler, 2003, Podlutsky et al., 2005, Ransome, 1995). This apparent short lifespan was hypothesised to result from its energetically demanding niche as a non-hibernating, socially foraging insectivorous bat (Gager et al., 2016). However, our findings suggest that the reported short lifespan of this bat likely stemmed from historical under-sampling rather than true biological limits to its longevity. Like many bats, *M. molossus* is challenging to monitor due to its high mobility, skewed population age structure, and the dispersal of juveniles, which limit the chance of recapturing individuals. Long-term mark-recapture studies are logistically difficult to maintain, especially in tropical systems, yet they remain the only reliable method for empirically determining true lifespan in wild bats. The findings here challenge existing assumptions regarding *M. molossus* lifespan and underscore the value of long-term field studies in generating reliable life-history data.

Telomere length typically shortens with age in vertebrates, including humans (Müezzinler et al., 2013), chimpanzees (Tackney et al., 2014), reptiles (Ujvari and Madsen, 2009), sheep (Froy et al., 2021) and fish (McLennan et al., 2016). However, exceptions exist, particularly in long-lived bat species. Species of the *Myotis* genus have shown no correlation between rTL and age despite the absence of detectable telomerase activity in somatic cells (Foley et al., 2018, Foley et al.,

2020). In *M. molossus*, we initially found no significant age-related decline in rTL across the full dataset, after excluding the single oldest individual which was disproportionately influencing model outputs. Similarly, a separate analysis of individuals of known age revealed no relationship between age and rTL, suggesting that this tropical bat may also exhibit some degree of telomere maintenance with age.

However, sex-specific models revealed a clear divergence in telomere dynamics. Males in this study tended to have longer average rTL than females and showed a significant decline in rTL with age, while females showed no evidence of age-related telomere shortening. This finding remained consistent even after stringent age-matched subsampling, indicating a true biological difference in telomere dynamics between sexes in this dataset. Although the sex × age interaction term was not statistically significant when both sexes were analysed together, likely due to limited sample size and male age range, the consistent contrast between male and female trajectories in separate models supports the presence of sex-specific telomere dynamics in this species. Many bat telomere studies sample predominantly females, largely because maternity roosts are more accessible and stable across years, allowing for consistent longitudinal sampling. Our female-only results are therefore directly comparable to much of the existing literature. The stability of rTL with age in female *M. molossus* parallels patterns reported in long-lived *Myotis* species (Foley et al., 2018, Foley et al., 2020), though other species such as *R. ferrumequinum* show measurable age-related declines (Power et al., 2023). Sex-specific differences in telomere dynamics have recently been reported in another tropical bat species, *Phyllostomus hastatus* (Rayner et al., 2025). However, in contrast to our results, female telomere length declined with age while males showed no evidence of age-related telomere shortening.

Several factors could account for the observed sex specific telomere dynamics in *M. molossus*. Males may experience greater oxidative load. Differences in reproductive effort, hormone profiles, and energy expenditure are widely linked to variation in telomere attrition across taxa (Barrett and Richardson, 2011, Olsson et al., 2011). The harem social structure, with frequent replacement of harem males, in *M. molossus* may impose greater physiological costs on males than females (Gager et al., 2016a, McCracken and Gustin, 1991). This likely elevates reproductive competition, social dominance behaviours, or increased energetic expenditure by

males to maintain access to females, all of which can elevate oxidative stress and contribute to telomere attrition. Finally, sexual size dimorphism, males being larger, can increase cumulative cell divisions during growth, a factor associated with shorter telomeres or faster loss (Barrett and Richardson, 2011, Tombak et al., 2024).

One possible explanation for the telomere preservation observed in females may be linked to the unusual activity patterns of *M. molossus*. Individuals concentrate foraging into very brief bouts of about 30 - 60 minutes, typically after sunset and before sunrise (Dechmann et al., 2009, Dechmann et al., 2010). This contrasts sharply with most insectivorous bats, which forage for several hours per night and sometimes in multiple peaks (Davidson Watts and Jones, 2006, Harding et al., 2022, Jones and Morton, 1992). Outside of these short foraging windows, *M. molossus* spends the majority of the day and night roosting, with telemetry studies recording mean flight activity of only ~37 minutes per 24 hours (Dechmann et al., 2011). This compressed foraging schedule is combined with exceptionally low daily energy expenditure and frequent use of shallow torpor-like states, even at relatively high ambient temperatures (Dechmann et al., 2011, O'Mara et al., 2017). Such declines in heart rate and metabolic rate are expected to reduce oxidative damage, and studies show that torpor and hibernation can promote telomere maintenance or elongation in mammals, including bats (Turbill et al., 2013, Power et al., 2023). While these processes likely benefit both sexes, the additional energetic costs of reproductive competition and social dominance may offset these benefits in males, resulting in detectable attrition, whereas females maintain rTL across adulthood.

Interpretation of these results must account for the modest recapture rate (27%), which could reflect both true mortality, emigration and in some cases roost desertion. Inaccessible roosting areas, predation, and human disturbance (e.g. roost sealing or destruction) were observed and likely contributed to missing individuals. Low recapture of older males, due to the social structure of *M. molossus* colonies, in particular limits our ability to detect late-life attrition in that sex. Additionally, selective disappearance of individuals with shorter telomeres could bias apparent age trends upward (Salmón et al., 2017). Furthermore, although rTL did not decline with age across the dataset, the shorter telomeres in the single oldest individual raise the

possibility of attrition at advanced ages, underscoring the importance of continued sampling of older age classes.

This study challenges previous assumptions about the lifespan of *M. molossus*, demonstrating that individuals can live over twice as long as previously recorded. Telomere analyses revealed striking sex differences with females showing no evidence of age-related telomere shortening, whereas males exhibited significant attrition. The stability of female telomeres aligns with patterns in long-lived *Myotis* bats, suggesting that telomere maintenance may have evolved independently in multiple lineages, while the male trajectory highlights how sex-specific ecology and life history can shape ageing within a species. These findings emphasise that bats do not conform to a single telomere-ageing pattern but instead exhibit diverse strategies, likely linked to their unique ecologies and life histories. Ultimately, this research underscores the need for rigorous, long-term mark-recapture field studies to accurately characterise the ageing patterns of wild animals and highlights *M. molossus* as a valuable system for investigating how telomere dynamics, sex differences and lifespan interact in bats.

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