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1 **Environment and sex control lifespan and telomere length**
2 **in wild-derived African killifish**

3
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19

20 **ABSTRACT**

21 Telomere length is correlated positively with longevity at the individual level, but negatively
22 when compared across species. Here, we tested the association between lifespan and telomere
23 length in African annual killifish. We analyzed telomere length in 18 *Nothobranchius* strains
24 derived from diverse habitats and measured the laboratory lifespan of 14 strains of *N. furzeri*
25 and *N. kadleci*. We found that males had shorter telomeres than females. The longest telomeres
26 were recorded in strains derived from dry region where male lifespans were shortest. At the
27 individual level, we detected a weak negative association between rapid juvenile growth and
28 shorter telomeres in early adulthood. Overall, average telomere length was a good descriptor of
29 telomere length distribution. However, within-individual telomere length spread was not related
30 to any pattern. This substantial variation in telomere length between strains from different
31 environments provides killifish as powerful tool to understand the evolutionarily adaptive value
32 of telomere length.

33

34 INTRODUCTION

35 Telomeres comprise the ends of all eukaryotic chromosomes and are composed of repetitive
36 DNA sequences and specialized proteins (de Lange 2005). Telomeres protect chromosome ends
37 from illegitimate DNA repair and, through the action of telomerase, from DNA replication-
38 related chromosome shortening (Olovnikov 1973, Greider and Blackburn 1989, Forsyth,
39 Wright et al. 2002). Telomere attrition results in the loss of its protective function and cell
40 proliferation arrest. Critically short telomeres trigger DNA damage responses and genomic
41 instability, a hallmark of aging (López-Otín, Blasco et al. 2013). Apart from the “end-
42 replication problem”, several other factors have been linked to telomere attrition. These include
43 exposure to oxidative damage (Opresko, Fan et al. 2005, Glade and Meguid 2015, Ahmed and
44 Lingner 2018), inflammation (Zhang, Rane et al. 2016), and environmental stressors (Epel,
45 Blackburn et al. 2004, Chatelain, Drobniak et al. 2020). Cells with short telomeres may undergo
46 apoptosis and, if compensatory cell proliferation is no longer available, short telomeres result
47 in cell senescence and inflammation, ultimately disrupting tissue homeostasis (Roos and Kaina
48 2006, Armanios, Alder et al. 2009, Carneiro, Henriques et al. 2016, El Mai, Marzullo et al.
49 2020). Impaired tissue homeostasis is at the core of ageing-associated degeneration.

50 Although not completely understood, telomere shortening in animals, particularly in
51 humans, is a strong promoter of aging-associated degenerative phenotypes (Armanios, Alder et
52 al. 2009, Moskalev, Shaposhnikov et al. 2013). Short telomeres are closely associated with age
53 pathologies such as diabetes mellitus, cardiovascular disease, atherosclerosis, dementia and
54 various cancers (Kota, Bharath et al. 2015, Sethi, Bhat et al. 2016, Aviv, Anderson et al. 2017,
55 Martinez and Blasco 2018). Loss-of-function mutations in genes related to telomere
56 maintenance are responsible for several human syndromes, such as dyskeratosis congenita,
57 aplastic anemia and idiopathic pulmonary fibrosis (Vulliamy, Marrone et al. 2004, Yamaguchi,

58 Calado et al. 2005, Tsakiri, Cronkhite et al. 2007). Similarly, studies using animal models have
59 revealed that mutations in telomerase give rise to age-associated deficiencies and reduction of
60 lifespan (Rudolph, Chang et al. 1999, Henriques, Carneiro et al. 2013, Harel and Brunet 2015,
61 Lex, Gil et al. 2020).

62 Previous studies have highlighted an association between telomere length and lifespan
63 (Fick, Fick et al. 2012, Heidinger, Blount et al. 2012, Barrett, Burke et al. 2013, Ibanez-Alamo,
64 Pineda-Pampliega et al. 2018). Correlative analyses suggest that, within the same species,
65 individuals with shorter telomeres are prone to disease and die earlier (Salomons, Mulder et al.
66 2009, Fairlie, Holland et al. 2016, Wilbourn, Moatt et al. 2018), but it remains unclear whether
67 telomere length is an indicator of individual condition (Glei, Goldman et al. 2016). While the
68 protective functions of telomeres are conserved across eukaryotes, telomere shortening with
69 age is not observed in all species (Monaghan and Ozanne 2018, Burraco, Comas et al. 2020).
70 Unlike humans, some animals do not repress telomerase in somatic cells and maintain long
71 telomeres throughout their lives (Sherr and DePinho 2000, Forsyth, Wright et al. 2002). Across
72 species, long telomeres do not correlate with longer lifespans. In fact, longer-lived mammals
73 possess shorter telomeres than short-lived mammals, such as mice and rats (Gomes, Ryder et
74 al. 2011). Instead, the rate of telomere attrition, rather than telomere length per se, appears to
75 correlate with species-specific lifespans (Hausmann, Winkler et al. 2003, Epel, Merkin et al.
76 2008, Boonekamp, Mulder et al. 2014, Dantzer and Fletcher 2015, Whittemore, Vera et al.
77 2019).

78 In humans, women typically have longer telomeres, experience lower rates of telomere
79 attrition and live longer (Gardner, Bann et al. 2014, Lapham, Kvale et al. 2015, Aubert,
80 Baerlocher et al. 2012). While not universal across species, sex differences in telomere length
81 have been reported in most vertebrate taxa studied (reviewed in Barret and Richardson 2011).

82 However, many of these studies report relatively weak effects that fail to be reproduced. It is
83 also not consistent which sex possesses longer telomeres and it is even less clear whether sex-
84 specific telomere length correlates with lifespan or individual condition (Barrett and
85 Richardson 2011, Wilbourn, Moatt et al. 2018, Bauch, Gatt et al. 2020). Hormonal profiles and
86 reproductive stress have been suggested as the causes of differential telomere shortening, but
87 the mechanisms underlying sex differences in telomere length remain largely unexplored
88 (Hartmann, Reichwald et al. 2009, Barrett and Richardson 2011, Graf, Hartmann et al. 2013,
89 Bauch, Gatt et al. 2020).

90 We performed a comparative experimental study to examine the association between
91 telomere length and lifespan in African annual killifishes from the genus *Nothobranchius*. This
92 group of small and short-lived fishes, and the Turquoise killifish (*Nothobranchius furzeri*) in
93 particular, has recently been established as a model for aging studies. These fish combine the
94 advantages of available resources (reviewed in Cellerino, Valenzano et al., 2016; Hu and Brunet
95 2018), cheap and convenient husbandry (Polačik, Blažek et al. 2016) and, importantly, links
96 between laboratory studies and natural populations (Reichard and Polačik 2019). We used a set
97 of strains derived from natural populations, which have evolved along a cline of aridity in their
98 natural habitat (Tozzini, Dorn et al. 2013, Blažek, Polačik et al. 2017), to test how variation in
99 lifespan among strains relates to telomere length. As an extension of the association of longer
100 telomeres with shorter-lived species (Gomes, Ryder et al. 2011), we predicted that telomere
101 length would be negatively associated with lifespan across strains.

102 Within populations, male killifish are shorter lived than females (Reichard, Polačik et al.
103 2014, Reichard, Blažek et al. 2020), leading to a prediction that females may possess longer
104 telomeres than males. Telomerase expression can be detected in *Nothobranchius* throughout
105 their lives (Hartmann, Reichwald et al. 2009), but telomeres do shorten with age. Hartmann,

106 Reichwald et al. (2009) reported that in a longer-lived strain (MZM 0304), old adults (26 weeks
107 old) had 8% and 27% shorter telomeres in muscle and skin tissues, respectively, compared to
108 young adults (5 weeks old). In contrast, telomeres do not appear to shorten between the ages of
109 5 and 13 weeks in the short-lived GRZ strain (Hartmann, Reichwald et al. 2009).

110 We took advantage of the diversity of killifish strains derived from different wild
111 populations and data on their natural habitats. We measured the lifespan of nine *N. furzeri*
112 strains and five strains of the closely related *N. kadleci* (Bartáková, Reichard et al. 2015) in
113 laboratory conditions. We estimated telomere length in 12 of these strains at the time of sexual
114 maturity (4 weeks) and linked them to juvenile growth. Using Telomere Restriction Fragment
115 (TRF) by Southern blot analysis, we tested the statistical distribution of telomere length within
116 individual samples to understand how variability in telomere length, rather than its mean, is
117 associated with juvenile growth and lifespan estimates. Finally, we report telomere lengths for
118 another seven strains of five other *Nothobranchius* species with various lifespans.

119 RESULTS

120 Lifespan: males from dry-region strains have shorter lifespans

121 We used strictly standardized conditions to estimate the lifespan of 14 strains from the *N.*
122 *furzeri-N. kadleci* species complex. Across strains, median lifespan varied almost twofold, from
123 151 days (inbred GRZ strain) to 276 days (Table 1). Overall, males were shorter lived than
124 females (Mixed effects Cox model controlling for population identity nested within species
125 identity: $z = 5.39$, $p < 0.001$). This pattern was especially consistent in *N. furzeri*. Sex-specific
126 lifespan estimates for each strain are detailed in Table 1.

127 The *N. furzeri-kadleci* complex is composed of locally constrained populations living in
128 small ephemeral pools which are genetically distinct from adjacent populations (Bartáková,
129 Reichard et al. 2013). All strains used in our analyses were derived from natural populations,
130 for which we knew the exact location of their original pool. We characterized the environmental
131 conditions in which these natural populations lived using long-term data on precipitation
132 (www.worldclim.org). The wild populations are distributed along a clear cline, where
133 increasing altitude and decreasing latitude are associated with lower precipitation (Figure 1).
134 Our comprehensive set of wild-derived strains confirmed previous conclusions using contrasts
135 between pairs of strains (Tozzini, Dorn et al., 2013, Blažek, Poláček et al., 2017) that median
136 lifespan correlates positively with local precipitation ($t_{12} = 2.64$, $P = 0.021$) for data on both
137 sexes pooled). Interestingly, for sex-specific lifespan estimates (which were not tested in
138 previous studies), this relationship holds in males (LM: $t_{12} = 3.19$, $p = 0.008$) but not females
139 ($t_{13} = 0.32$, $p = 0.752$) (Figure 2). Our results indicate that males, but not females, from arid
140 regions have shorter median lifespans than those from humid regions, suggesting that perhaps
141 strains from arid regions would exhibit shorter telomeres.

142

143 **Average telomere length is a good descriptor of telomere length distribution**

144 Telomere length was estimated using Telomere Restriction Fragment (TRF) by Southern blot
145 analysis on samples from 11 wild derived populations (10 fish per population, with equal sex
146 ratio). The final sample size ($n = 92$, Table 1) was slightly reduced due to lower quality of
147 genomic DNA. All fish examined were young adults of the same age (28 days old), except for
148 a single population (p222; 69 days old). All tissue samples comprised small biopsies from the
149 caudal fin.

150 We first explored the relationships between various descriptors of telomere length
151 distributions. Using a biplot based on a Principal Component Analysis (PCA) of the data
152 distribution parameters (Figure 3), we found that disparate measures of telomere length (mean,
153 median, 5-95% quantiles) were all highly positively correlated (Supplementary Table 1). The
154 measures of telomere length variability within individual fish (inter-quartile range and
155 coefficient of variation) were not strongly related to the estimates of telomere length (Figure 3,
156 Supplementary Table 1). Based on this information, we used the mean as the measure of
157 telomere length and the coefficient of variation (CV) as a measure of intra-individual telomere
158 length variability in further analyses. These two measures were not correlated (Pearson, $r = -$
159 0.01 , $n = 92$, $p = 0.940$).

160

161 **Telomere length is shorter in males and in faster growing individuals**

162 The exploratory analysis revealed a difference in mean telomere length, from 5.29 (s.e. 0.42)
163 to 9.92 (s.e. 0.42) kbp across various strains (Figure 4, Supplementary Figure 1, Supplementary
164 Table 2). When comparing telomere length between males and females, we accounted for inter-
165 strain variation using strain ID as a random effect.

166 We first tested how parameters of telomere length vary in relation to sex and juvenile
167 growth (measured as individual body size at age of 28 days). Our set of candidate models
168 contained the two explanatory variables and their interaction, along with a null model (Table
169 2). Candidate models were fitted to the data and compared using the Akaike Information
170 Criterion corrected for small sample size (AICc). The best model contained only sex (Table 2a)
171 and demonstrated that males had shorter telomeres than females (Linear Mixed Model with
172 strain ID modelled as a random factor: $F_{1,80.4} = 23.13$, $P < 0.001$). Across strains, mean telomere
173 length was 7.37 kbp (s.e. 0.45) in females and 6.18 (s.e. 0.24) in males. Strain identity explained
174 a large part of variation in all models; the values of $R^2_{\text{GLMM conditional}}$ were approximately 6 times
175 larger than $R^2_{\text{GLMM marginal}}$ (Table 2).

176 We note that the second best model (model M2 in Table 2a, $\Delta\text{AIC} = 1.99$) indicated that
177 rapid juvenile growth (i.e. large size at the age of 28 days) was associated with relatively shorter
178 telomere length ($F_{1,88.9} = 4.47$, $P = 0.037$) but the trend became weaker and statistically non-
179 significant after removal of the strain with older (and larger) fish (p222: 69 old vs. 28 days old
180 fish) ($F_{1,75.7} = 3.52$, $P = 0.064$; Supplementary Table 3).

181 Intra-individual variation in telomere length was not related to sex, juvenile growth or
182 their interaction (Table 2b).

183

184 **Telomere length tends to be shorter in strains from more humid environments**

185 We then tested whether total annual precipitation in the original habitat, which is associated
186 with (male) lifespan, also correlated with telomere length. The best fitting model (Table 3a)
187 included sex differences in telomere length ($F_{1,80.5} = 23.08$, $P < 0.001$) and a trend towards
188 negative association with precipitation totals (Figure 5; $F_{1,9.0} = 4.11$, $P = 0.072$), suggesting that
189 killifish strains from regions with higher precipitation tend to possess shorter telomeres.

190 Telomere length variability (CV) was neither related to precipitation nor to sex (Table
191 3b), although it varied considerably across strains (Figure 4b).

192

193 **Telomere length is not associated with median lifespan**

194 Given that the sexes differed in their lifespans, we used sex-specific lifespan estimates to test
195 the association between telomere length and population-level longevity (Figure 6). The best
196 model (Table 4a) confirmed sex differences in telomere length ($F_{1,8071} = 18.57$, $P < 0.001$), but
197 did not demonstrate a link between mean telomere length and median lifespan estimate ($F_{1,88.7}$
198 $= 2.39$, $P = 0.126$). Therefore, consistent with previous studies at the inter-specific level,
199 telomere length does not appear to predict longevity among different killifish strains.

200 For telomere length variability (CV), none of the candidate models was better than a null
201 model (Table 4b).

202

203 **Telomere length in other *Nothobranchius* species**

204 Finally, we expanded telomere length analysis to three other *Nothobranchius* species from
205 Mozambique (*N. orthonotus*, *N. pienaari*, *N. rachovii*) that widely coexist with *N. furzeri-*
206 *kadleci* species complex (Reichard, Janáč et al. 2017). We also included two species from
207 Tanzania (*N. rubripinnis*, *N. eggersi*), where a more humid climate is linked to a longer duration
208 of wet habitat phase (Reichard 2015).

209 Telomere length among these species was comparable (range 3.6-7.3 kbp, Supplementary
210 Figure 2) but generally lower than the *N. furzeri-N. kadleci* group. Estimated telomere length
211 was generally longer for the relatively shorter-lived *N. orthonotus* (strain MZCS 002 Limpopo:
212 mean (s.e.) = 5.63 (0.29), n=4; MZCS 528 Beira: 6.03 (0.35), n=2) compared to *N. pienaari*

213 (MZCS 505 Limpopo: 4.29 (0.25), n=4; MZCS 514 Beira: 5.86 (0.29), n=3) and *N. rachovii*
214 (MZCS 096 Beira: 3.65, n=1). Tanzanian *Nothobranchius* species had telomere lengths
215 comparable to Mozambican species (*N. rubripinnis* T33: 5.60 (0.25), n=4; *N. eggersi* T52: 5.45
216 (0.35), n=2). Finally, we also tested telomere length in a highly inbred GRZ strain of *N. furzeri*
217 that was bred under different laboratory conditions from the other fish and, therefore, was not
218 included in the overall analysis. Mean telomere length in the GRZ fish (7.33 (0.29), n=3)
219 corresponded to the predicted values based on their origin in a relatively dry region.

220

221 **DISCUSSION**

222 Telomeres and telomerase prevent the continuous erosion of chromosome-ends caused by
223 lifelong cell division. In several species, including humans, telomeres shorten with age and their
224 decline is considered a hallmark of ageing (López-Otín, Blasco et al. 2013). Telomere structure
225 and function is conserved across eukaryotes and their exhaustion leads to multiple effects both
226 at the cellular and systemic level, causing disruption of organ homeostasis and disease
227 (Armanios, Alder et al. 2009, Carneiro, Henriques et al. 2016). Traditionally, telomere length
228 has been considered indicative of “biological age” and a useful proxy to predict individual
229 health status and longevity. However, evidence from several non-human vertebrates now
230 contradicts this assumption as simplistic.

231 In our large sample of wild-derived annual killifish strains obtained from a gradient of
232 environmental conditions and natural lifespans, we found that telomere length varied across
233 strains and tended to be shorter in populations that came from more humid regions where
234 populations naturally live longer. The natural habitats of annual killifish are seasonal pools in
235 the African savannah. They are inundated during the rainy season, fish hatch, grow rapidly and
236 attain sexual maturity within a few weeks (Vrτίlek, Žák et al. 2018a). The pools desiccate during

237 the dry season and killifish populations persist as dormant eggs in the pool sediment. The wet
238 phase may vary from a few weeks to several months (Vrtílek, Žák et al. 2018b) depending on
239 the amount of precipitation in the area (Blažek, Polačik et al. 2017). Habitat duration, which
240 varies considerably across the precipitation gradient of killifish distribution, determines
241 maximum lifespans. Male lifespans in our laboratory assays were strongly associated with
242 annual precipitation levels in their area of their origin, though female lifespans were not.
243 Consistent with previous observations across species (Gomes, Ryder et al. 2011), killifish from
244 more humid regions (and hence with longer lifespans, both predicted and observed) had shorter
245 telomeres than those from arid regions. This complements an analogous trend found across
246 mammalian species (Gomes, Ryder et al. 2011) and demonstrates that telomere length
247 differences among populations reflect patterns found among species.

248 Alternatively, telomere length differences across strains may simply have been fixed
249 through genetic drift. Natural *Nothobranchius* populations are genetically distinct units
250 (Bartáková, Reichard et al. 2015) but are more fragmented and have smaller effective
251 population sizes in the dry part of their range (Willemsen, Cui et al. 2020). Killifish populations
252 from dry regions experience increased mutation load, leading to a reduction in their lifespans
253 (Cui, Medeiros et al. 2019, Willemsen, Cui et al. 2020). However, our data demonstrate that
254 dry-region populations tend to have longer telomeres, including the highly inbred GRZ
255 population with apparently the strongest mutation load. This trend is further strengthened by
256 the telomere length of other three *Nothobranchius* species from Mozambique (*N. orthonotus*,
257 *N. pienaari*, *N. rachovii*), widely coexisting with the *N. furzeri-kadleci* species complex and
258 two species from a more humid region in coastal Tanzania (*N. eggersi*, *N. rubripinnis*).
259 Telomere length was comparable in all these longer-lived species (Blažek, Polačik et al. 2017,
260 Wildekamp 2004) but generally shorter than in the *N. furzeri-N. kadleci* complex, suggesting

261 that genetic drift is unlikely a driver of such consistent population-specific differences in
262 telomere length.

263 Sex differences in telomere length were consistent across strains (Supplementary Table
264 2), with male telomeres 22% shorter on average. Remarkably, males also had consistently
265 shorter lifespans than females in all nine *N. furzeri* (but only in 2 of 5 *N. kadleci*) populations.
266 All our experimental fish were housed in social tanks. This pattern of sex differences in lifespan
267 corroborates conclusions from a separate study (Reichard, Blažek et al. 2020) where *N. furzeri*
268 (but not *N. kadleci*) males in social tanks suffered higher baseline mortality than females.
269 Higher male mortality was not reflected in sex differences in any measure of functional aging
270 (oxidative stress, kidney and liver histopathology, accumulation of lipofuscin) (Reichard,
271 Blažek et al. 2020). Individual variation in telomere length distribution did not differ between
272 the sexes, discounting the possibility that females possess mechanisms for countering cells with
273 short telomeres. However, it is plausible that males are more susceptible to telomere erosion
274 due to higher hormonal and environmental stress. *Nothobranchius* fish display remarkable
275 sexual dimorphism and dichromatism. Males are larger and more brightly colored than females
276 and continuously compete for mating opportunities (Cellerino, Valenzano et al., 2016, Reichard
277 and Polačik 2019). Male reproductive strategy (higher growth, investment in bright coloration
278 and aggressive interactions) therefore implicates a higher metabolic cost and more rapid growth
279 than the female reproductive strategy (Bonduriansky, Maklakov et al. 2008). This may underlie
280 the faster decline in telomere length in males, which we recorded as a shorter telomere length
281 in young adult males.

282 Even within the sexes, rapid juvenile growth tended to be associated with relatively
283 shorter telomere length. Our sampling at the age of 28 days encompassed the period of most
284 rapid growth rate in *Nothobranchius* lifespan, with fish growing from an initial body length of
285 5 mm to 50 mm (Blažek, Polačik et al. 2013, Vrtílek, Žák et al. 2018a). This raises a plausible

286 hypothesis that growth rate, primarily in early life, at least partly affects the kinetics of telomere
287 erosion and overall telomere length. We now plan a follow-up study with repeated longitudinal
288 sampling of the same individuals to test this hypothesis.

289 Studies on telomere length in the field of ecology and evolution typically use real-time
290 PCR assays to determine Telomere-to-Single Copy Gene (T/S) ratio which provides an indirect
291 estimate of mean telomere length (Lai, Wright et al. 2018). However, the detrimental effects of
292 telomere shortening are expected to arise from a frequently dividing subpopulation of cells
293 within a particular tissue, which may not be well represented in the T/S ratio. We estimated
294 telomere length using the robust TRF method that yields a direct distribution of telomere
295 fragments in the sample. Importantly, we confirmed that estimates of mean values are a useful
296 proxy for telomere length distribution as they closely correlated with other parameters of
297 telomere length distribution, including a set of 5-95% quantiles. Thus, mean telomere length
298 represents a suitable estimate of telomere kinetics in adult *Nothobranchius* killifishes.
299 Additionally, the measure of telomere length variability within an individual, the coefficient of
300 variation, was not associated with any pattern in our data (between sexes, among strains, or in
301 juvenile growth). This suggests that the dispersion in telomere length distribution is a strain-
302 specific character controlled by regulatory pathways other than those used in determining
303 telomere length. As telomeres shorten, wider telomere distributions will give rise to more
304 critically short telomeres than narrow ones, thus providing an independent parameter to control
305 telomere decline with age.

306 Overall, our results demonstrate a clear variation in telomere length between sexes and
307 environments among wild-derived strains of *Nothobranchius* fishes and highlight their
308 suitability for understanding the adaptive value of telomere length across individuals,
309 populations, and species.

310 MATERIALS AND METHODS

311 Fish origin and environmental data

312 Study strains were descendants of wild populations imported from Mozambique and the
313 adjacent area of Zimbabwe. In this region, a clear gradient of aridity is generated by a decrease
314 in total precipitation with increasing distance from the coast, increasing altitude and decreasing
315 latitude associated with lower precipitation (Figure 1; Blažek, Polačik et al. 2017). Fish from
316 wild populations were imported between 2009 and 2012 during dedicated expeditions and wild-
317 derived strains were maintained in the Institute of Vertebrate Biology (IVB) in Brno, Czech
318 Republic according to published protocol (Polačik, Blažek et al. 2016). One strain (GRZ) was
319 imported in 1969 from Zimbabwe and another strain (MZM-0410, coded as pMZM in our
320 dataset) was imported in 2004. The origin of each study strain is illustrated in Figure 1 and GPS
321 coordinates are provided in Table 1. Other strains of Mozambican (*N. orthonotus*, *N. pienaari*,
322 *N. rachovii*) and Tanzanian species (*N. eggersi* and *N. rubripinnis*) originate from our own
323 imports between 2008 and 2017 (Supplementary Table 4). Housing conditions were the same
324 for all species.

325 Data on precipitation (represented by annual rainfall totals) and aridity were downloaded
326 from www.worldclim.org. Aridity was measured as aridity index (total precipitation/model-
327 estimated evapotranspiration). Given that precipitation and aridity were highly collinear
328 (Pearson $r = 0.989$, $n = 12$, $P < 0.001$), we only present analyses using total precipitation.

329

330 Fish housing and lifespan estimates

331 Fish were kept at the IVB facility under standard conditions and care was taken to minimize
332 inbreeding (i.e. a large number of fish were sources of offspring for the next generation, though
333 no specific breeding design was followed).

334 For the estimates of lifespan, eggs of 14 strains were hatched simultaneously. The eggs
335 were kept in diapause at 19°C. Two weeks prior to hatching, the eggs were placed in 28°C to
336 initiate release from Diapause II and development to prehatching stage. In August 2016, the
337 incubation substrate (peat) was watered with dechlorinated tap water (conductivity
338 600microS/cm, temperature 16°C) in a 2L tank. Fish hatched within 1 day and were
339 immediately fed on freshly hatched *Artemia* nauplii. After 5 days, fish were moved to larger
340 tanks (40 L) and their density was standardized across strains. At 3 weeks, fish were weaned
341 from *Artemia* nauplii to frozen chironomid larvae, a standard diet of captive *Nothobranchius*
342 (Polačik, Blažek et al. 2016).

343 At 6 weeks, fish density was standardized to 50 fish per strain, though some strains had
344 a slightly lower density (Table 1). All tanks were monitored daily and any dead fish was
345 recorded and removed. Daily censuses continued until all fish were dead.

346 Median lifespan was calculated for each strain (and each sex within a strain) using the
347 *survfit* function in the *survival* package. 95% confidence intervals for median lifespan estimates
348 were calculated using a log-log function.

349

350 **Telomere length estimates**

351 Telomere length was estimated through Telomere Restriction Fragment (TRF) analysis
352 followed by Southern blotting. Fish from 12 strains (all strains used in lifespan estimate except
353 for GRZ, p108, and p109) were hatched on 6 May 2019, following the same protocol as for

354 lifespan estimates. When all fish were sexually mature, at the age of 28 days, a biopsy from the
355 caudal fin was taken from 5 male and 5 female fish from each population and flash frozen in
356 liquid nitrogen. Samples of other species were handled in the same way, except they were stored
357 in 100% ethanol instead of being flash frozen. Due to logistic reasons, one population (*N.*
358 *furzeri*, MZCS 222) was hatched earlier (26 March 2019) and was older (69 days) at the time
359 of sampling, although it was raised following identical methods and kept in the same room. To
360 account for the potential effect of age on telomere length, we re-ran statistical analyses with
361 this population excluded from the dataset. This population had no effect on the qualitative
362 interpretation and, in the main text, we present the final analysis with this population included.
363 The exception is the test of relationship between body length at sexual maturity (28 days) and
364 telomere length, where we present both the full and truncated datasets as this is where the
365 removal of MZCS 222 strain affected the strength of statistical association.

366 Genomic DNA was extracted using a lysis buffer (Thermo Scientific #K0512)
367 supplemented with 1 mg/ml Proteinase K (0.5 mg/ml final concentration, Sigma, MO, USA)
368 and RNase A (1:100 dilution, Sigma, MO, USA). Samples were incubated at 50°C for 18 h in
369 a thermomixer. Purification of genomic DNA was carried out using equilibrated phenol-
370 chloroform (Sigma, MO, USA) and chloroform-isoamyl alcohol extraction (Sigma, MO, USA).
371 Following quantification and normalization, genomic DNA was digested with RsaI and HinfI
372 enzymes (NEB, MA, USA) for 12 h at 37°C. Digested samples were electrophoresed on a 20
373 cm 0.6% agarose gel, in 0.5% TBE buffer, at 4°C for 17 h at 110 constant voltage. Southern
374 blotting was performed as previously described (Kimura, Stone et al. 2010) with minor changes;
375 a telomere probe, (CCCTAA)₄, labelled with [α -³²P]-dCTP through the Prime-it II random
376 primer labelling kit (Stratagene) was used. Raw data from Southern Blots are presented as
377 Supplementary Figure 1.

378

379 **Statistical analysis**

380 We first extracted pre-selected parameters of the telomere distribution and analyzed them using
381 Principal Component Analysis (PCA) to test their covariance. We then selected two parameters
382 (mean telomere length and coefficient of variation) representing two uncorrelated descriptors
383 of individual-fish telomere populations for further analyses.

384 To test the effects of predictors on telomere length and intra-individual variability, we
385 used an information theoretic approach (Burnham and Anderson 2002). A set of biologically
386 plausible models was constructed, with various level of complexity, and including a null
387 (intercept-only) model. All models included strain identity as a random effect to control for
388 non-independence of telomere length across individuals from the same population. We then
389 compared the fit of the models using the Akaike Information Criterion corrected for small
390 sample size (AICc). The best model minimizes AICc value. Model within $\Delta AICc$ between 2
391 and 5 are considered to have a comparable power.

392 Analyses were performed in *lmer* and *MuMIn* packages in the R 3.5.2 environment.
393 Before applying statistical models, data exploration was undertaken as recommended by Zuur
394 and Ieno et al. (2016).

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399 with relevant guidelines and regulations. Collection of wild strains complied with legal
400 regulations of Mozambique (collection licenses: DPPM/053/7.10/08, 175/154/
401 IIP/2009/DARPE, DPPM/083/7.10/10, DPPM/330/7.10/10, DPPM/069/7.10/11,
402 DPPM/088/7.10/12). Experimental work was approved by the Ethical Committee of the
403 Institute of Vertebrate Biology (No. 163-12) and by Ministry of Agriculture (CZ 62760203) in
404 accordance with legal regulations of the Czech Republic. We thank R. Spence for valuable
405 comments and English corrections. All primary data are available at Figshare repository (doi:
406 10.6084/m9.figshare.12888566).

407

408 **Author contributions**

409 MR and MGF conceived and designed the study. RB and MP collected data on lifespan. MV
410 and MR raised fish for telomere length estimates. KG, TF performed genomic DNA extractions,
411 TRF and Southern hybridizations. KG, TF and MGF analyzed and interpreted telomere length.
412 MR and MV conducted statistical analyses. MR, KG and MGF drafted the manuscript. All
413 authors significantly contributed to the final text.

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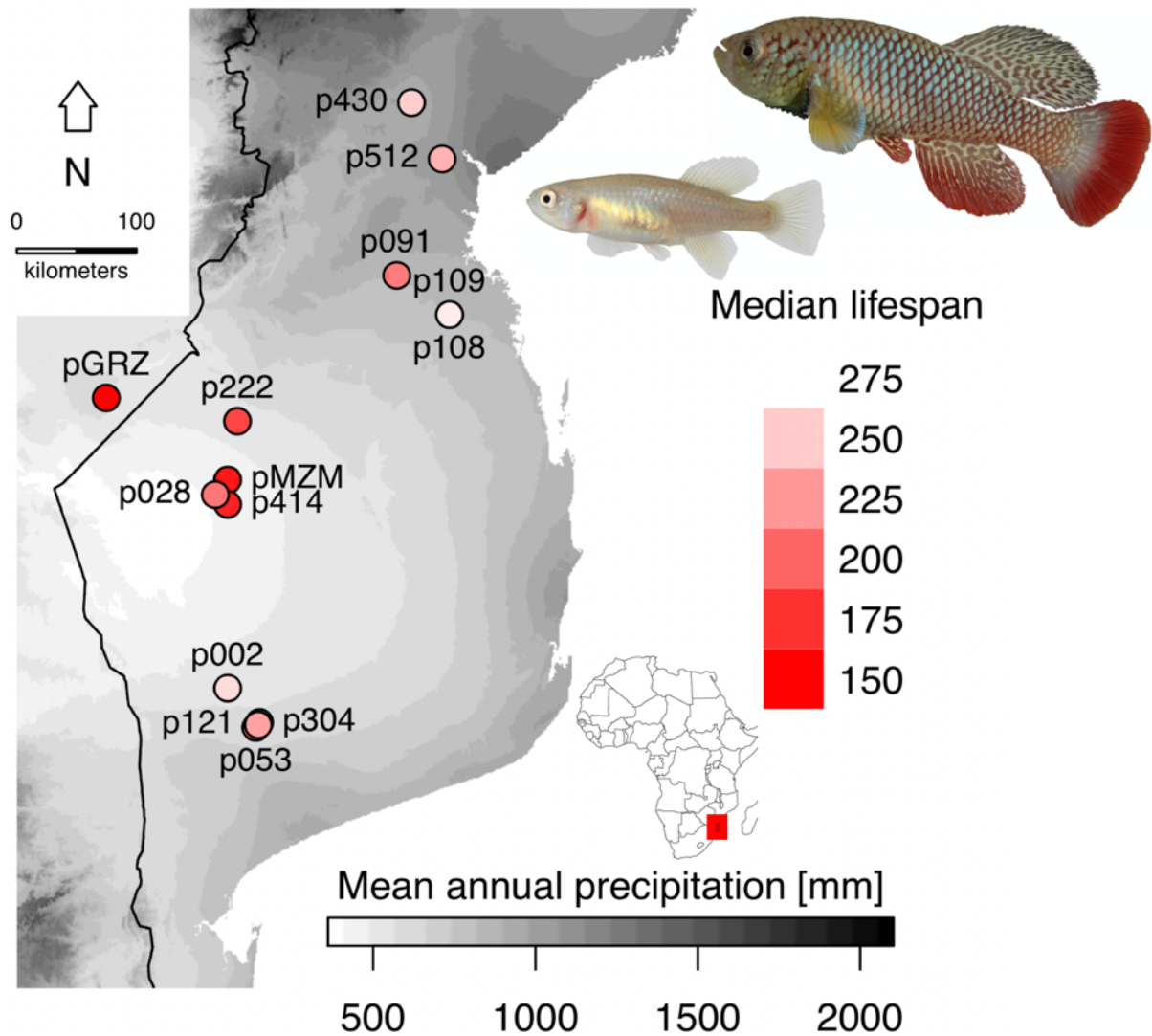
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624

625 **FIGURES**

626 Fig. 1. Geographic origin of study strains along the cline of precipitation, with male (larger)
627 and female (smaller) *Nothobranchius furzeri* inset. Median lifespan (sexes pooled) is
628 visualized by intensity of coloration for each original strain location (red: shortest lifespan;
629 white: longest lifespan). Sex differences in fish body size are proportional.

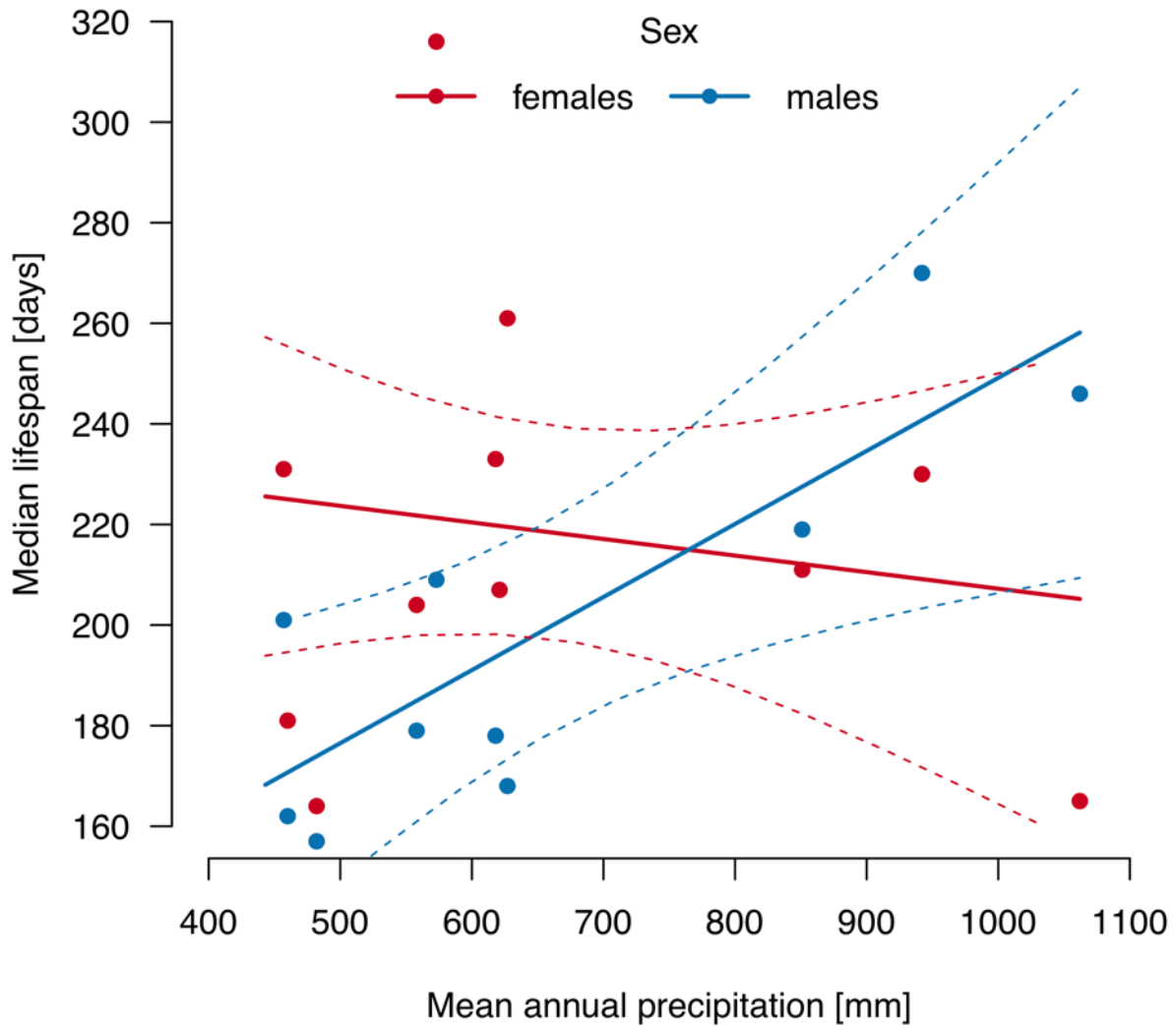


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632 Fig. 2. Relationship between median lifespan of males (blue) and females (red) and mean
633 annual precipitation in their region of origin. Lines were fitted using linear regression.

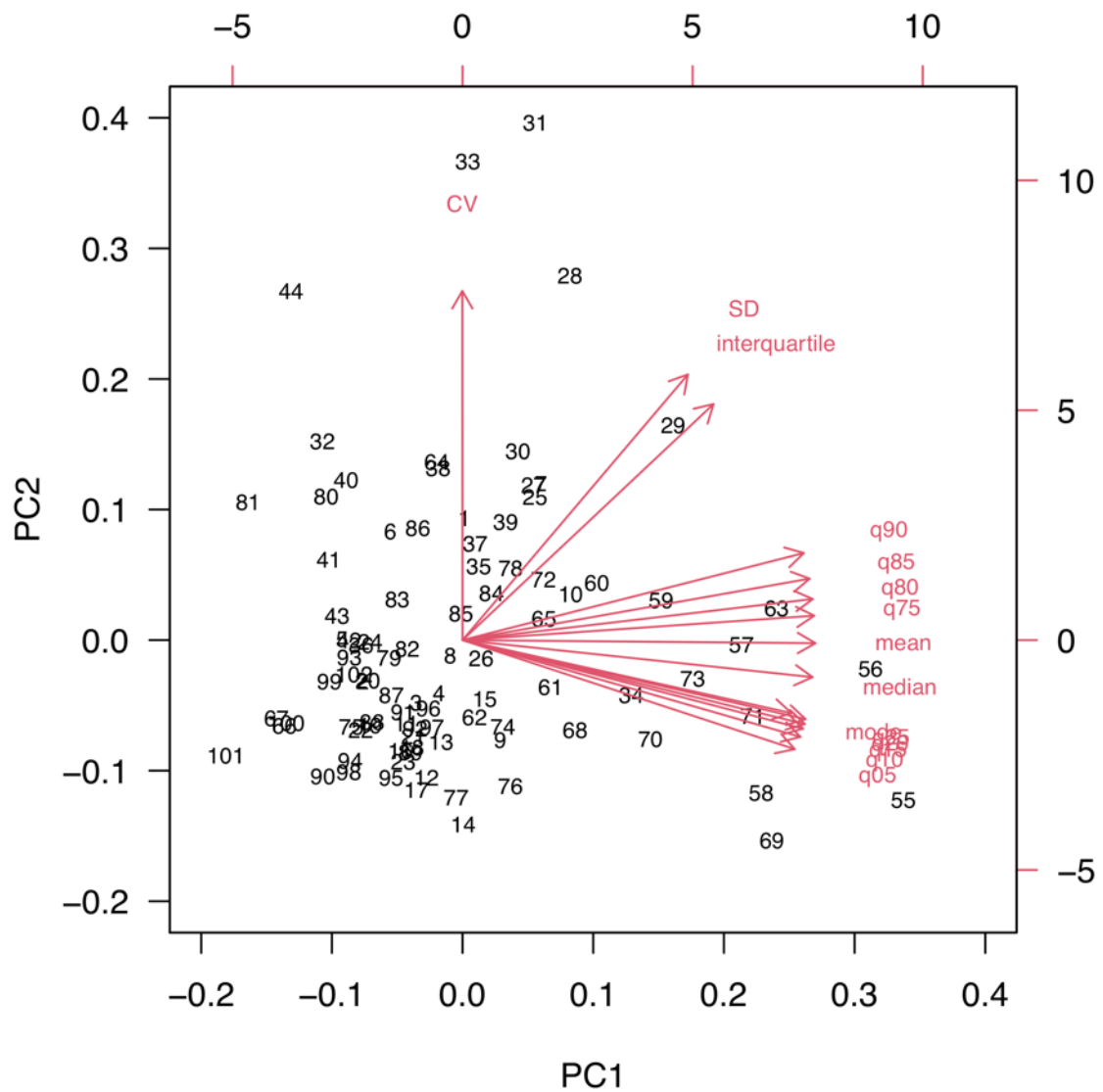
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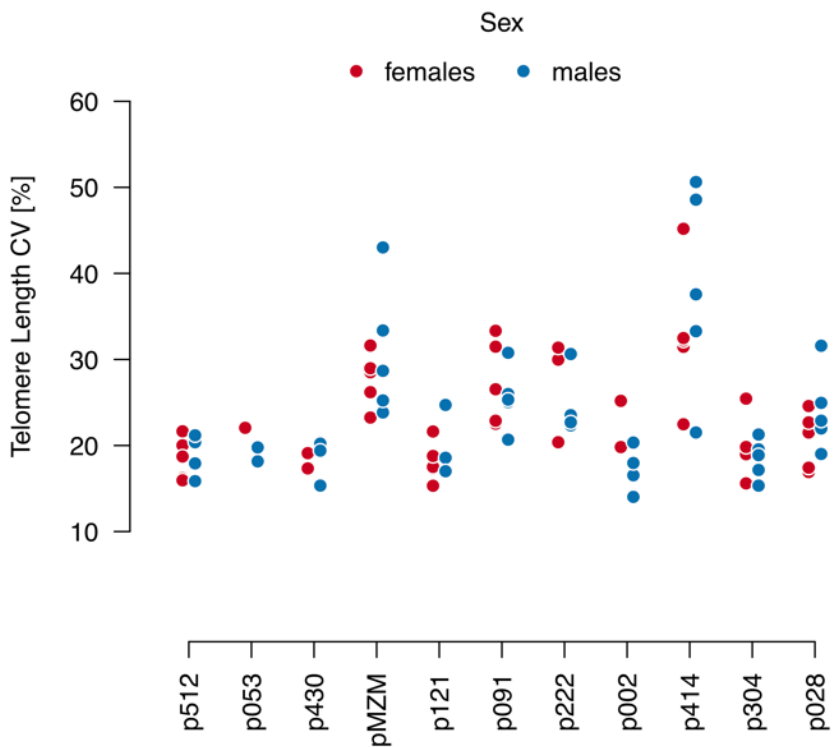
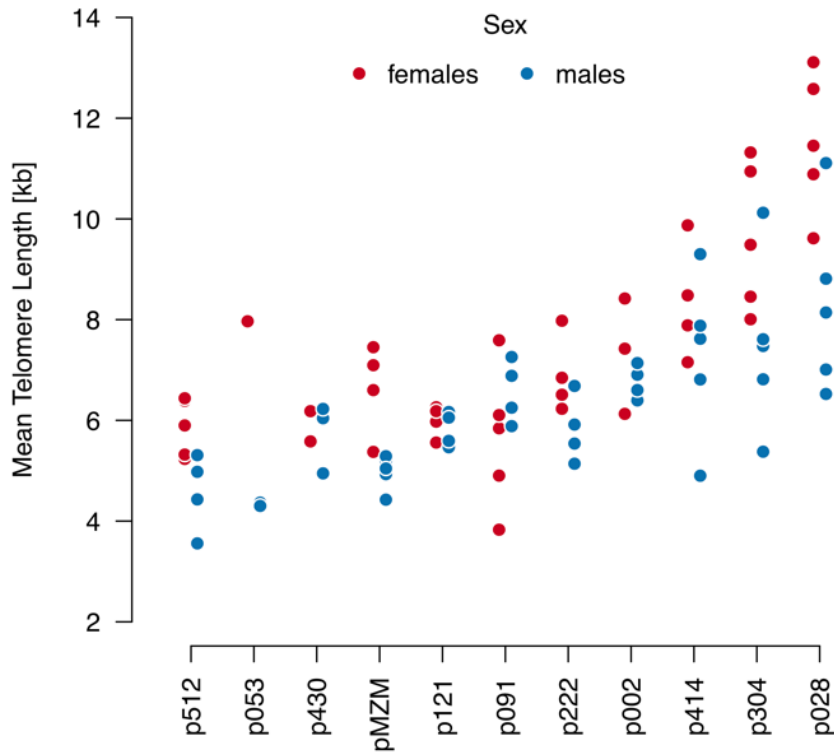
637 Fig. 3. Biplot visualization of telomere length distribution parameters, based on Principal
638 Component analysis. Vectors represent estimated parameters; the numbers denote sample ID.



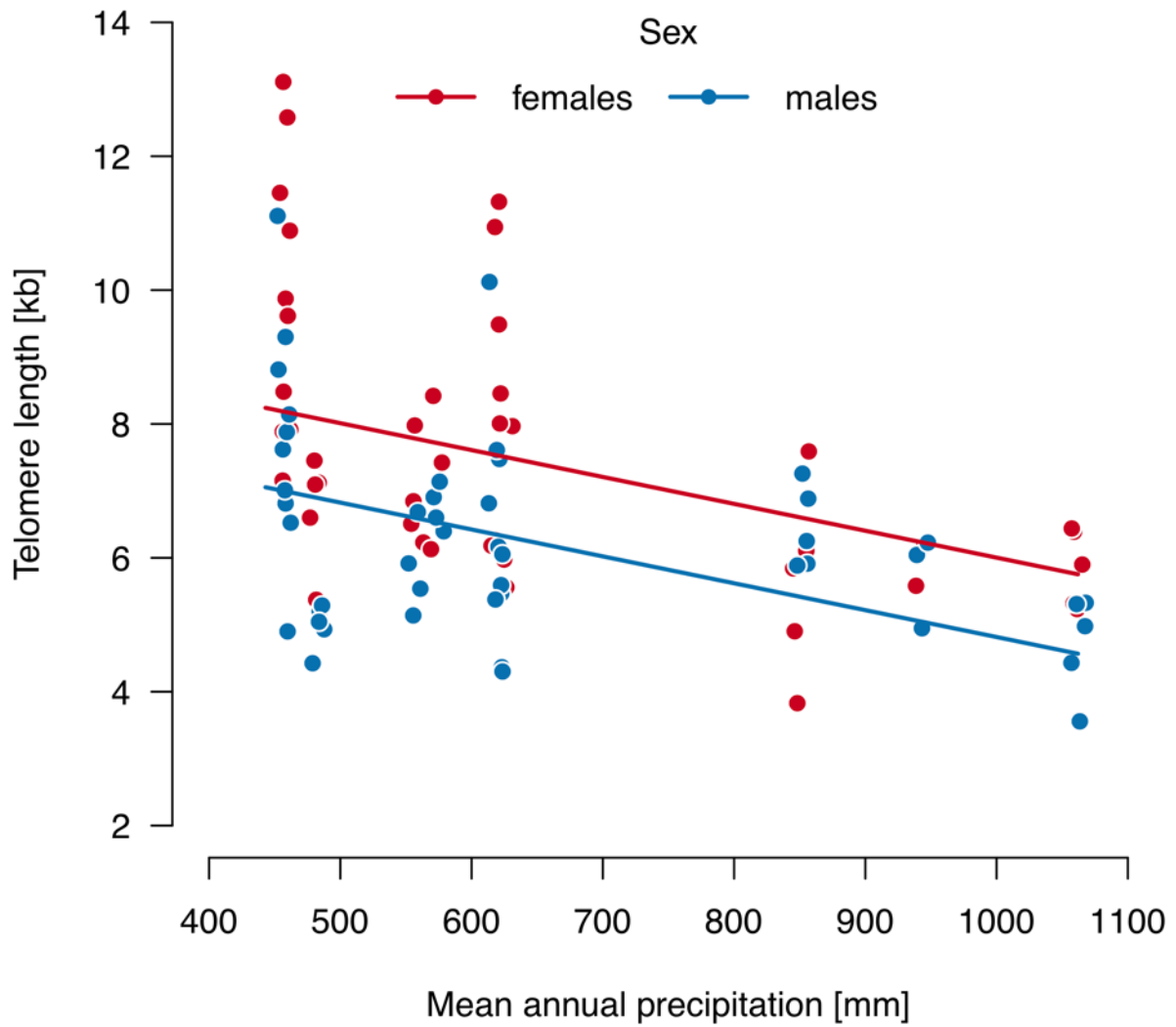
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641 Fig. 4. Among strains variability in (A) mean telomere length and (B) intra-individual
642 variation in telomere length, estimated as Coefficient of Variation (CV). Strains are ranked
643 from shortest to longest telomere length.



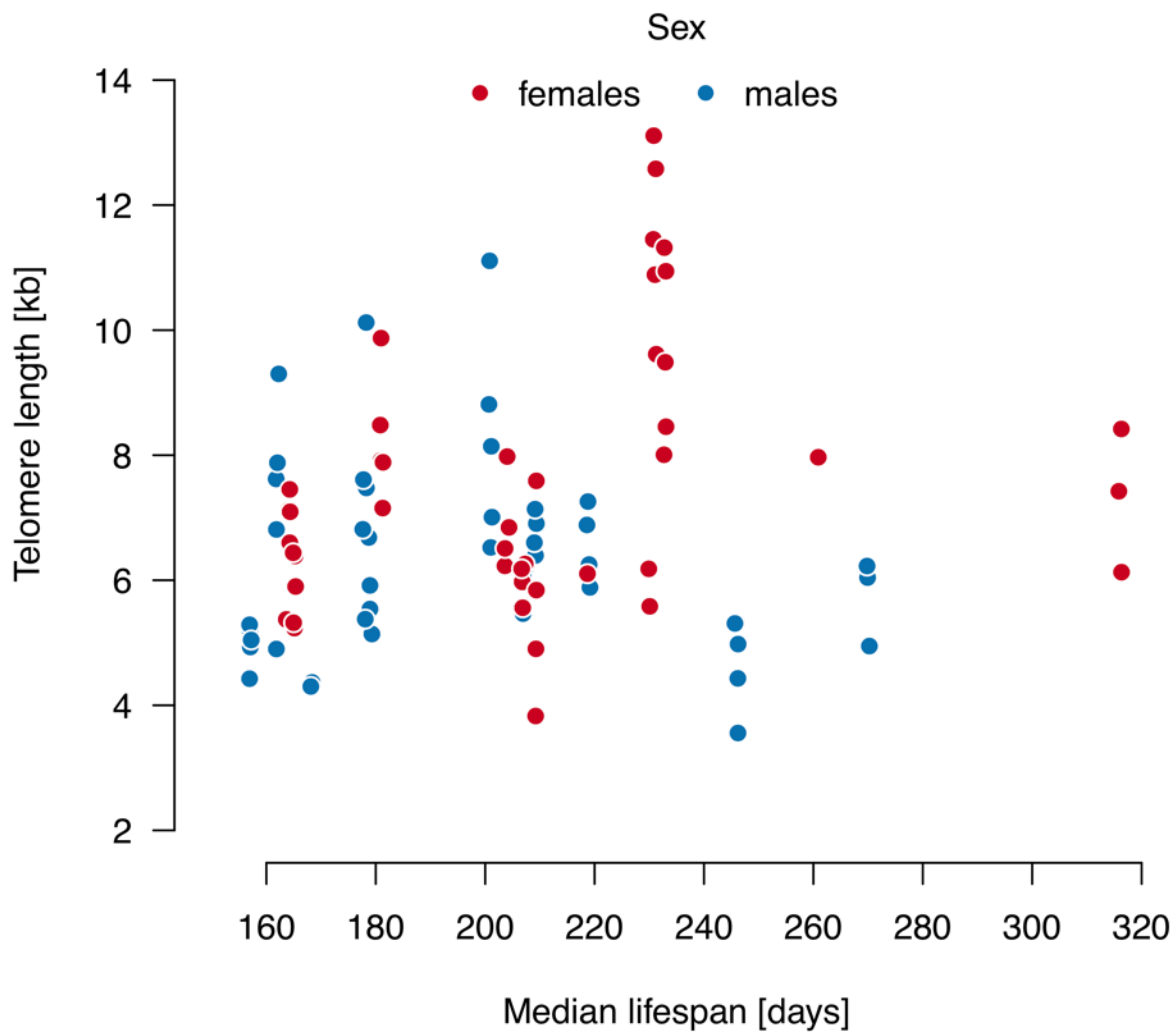
646 Fig. 5. Relationship between mean telomere length and mean annual precipitation in male
647 (blue) and female (red) killifish. Solid lines represent relationship fitted with a mixed model
648 including sex and precipitation as fixed factors and strain ID as a random factor.



649

650

651 Fig. 6. Relationship between telomere length and sex-specific strain-level lifespan estimates.



652

653

Table 1. List of all strains from the *N. furzeri-N. kadleci* species complex used in the study. Populations are ordered according to median lifespan. Precipitation represents mean annual precipitation total, aridity is the index of aridity calculated from precipitation-evaporation differentials (both derived from CGIAR Consortium for Spatial Information: www.cgiar-csi.org). Sample sizes for telomere length study (telomeres) and longevity estimates (N fish) are shown. Median lifespan was calculated using the *surv* function in the *survival* package.

Pop	Clade	Collection Year	Precip	Aridity	Lat	Long	Telomeres (Nmale,fem)	Median lifespan (95% CI)	N fish	Median lifespan (95% CI) Females	Median lifespan (95% CI) Males
pGRZ	Chefu	1969	588	0.3264	-21.68	31.73	(3)	151 (136-174)	29	155 (131-178)	148 (111-NA)
pMZM	Chefu	2004	482	0.2657	-22.35	32.72	5, 5	162 (140-203)	33	164 (136-220)	157 (124-203)
p414	Chefu	2012	460	0.2523	-22.55	32.72	5, 5	168 (162-176)	47	181 (165-183)	162 (136-168)
p222	Chefu	2011	558	0.3082	-21.87	32.8	4, 4	185 (137-203)	45	204 (119-218)	179 (126-195)
p028	Chefu	2013	457	0.251	-22.47	32.62	5, 5	209 (177-233)	45	231 (182-264)	201 (154-229)
p024	Chefu	2010	443	0.2435	-22.5	32.55	5, 5	215 (142-246)	43	228 (112-264)	209 (118-246)
p304	Limpopo	2013	618	0.3395	-24.33	32.98	5, 5	215 (180-240)	35	233 (200-276)	178 (137-237)
p121	Limpopo	2011	621	0.3426	-24.35	32.97	4, 5	230 (206-265)	49	274 (2014301)	207 (174-221)
p002	Limpopo	2012	573	0.3203	-24.05	32.72	4, 3	258 (218-287)	46	316 (240-350)	209 (183-260)
p091	<i>N. kadleci</i>	2010	851	0.5114	-20.68	34.1	5, 5	212 (179-226)	49	209 (122-224)	219 (173-250)
p512	<i>N. kadleci</i>	2012	1065	0.6390	-19.73	34.47	5, 5	238 (165-257)	43	165 (83-249)	246 (198-292)
p430	<i>N. kadleci</i>	2011	939	0.5051	-19.27	34.22	3, 2	252 (229-275)	48	230 (183-271)	270 (231-270)
p109	<i>N. kadleci</i>	2010	847	0.5357	-21.00	34.53	No	266 (211-287)	45	289 (265-298)	211 (175-266)
p108	<i>N. kadleci</i>	2008	847	0.5357	-21.00	34.53	No	276 (177-288)	41	288 (233-301)	186 (105-284)

Table 2. List of candidate models to test the relationship of mean telomere length (a) and its intra-individual variation (b) with sex and juvenile growth. Model structure (fixed effects and their interaction, and random effect of strain ID), degrees of freedom (d.f.), Akaike Information Criterion corrected for small sample size (AICc), difference between the best fitting model and candidate model ($\Delta AICc$) indicating strength of support for a particular model, amount of variability explained by fixed effects (R^2_{marginal}) and by combination of fixed and random effects ($R^2_{\text{conditional}}$).

Model		d.f.	AICc	$\Delta AICc$ s	R2 marginal	R2 conditional
(a) Mean telomere length						
M0	(1 pop)	3	343.6612	17.4639	0,000	0.5235916
M1	sex + (1 pop)	4	326.1973	0	0.09654603	0.6228926
M2	sex + body + (1 pop)	5	328.1906	1.9933	0.1331258	0.6611852
M3	sex + body + sex:body + (1 pop)	6	333.9235	7.7262	0.1331096	0.6589753
M4	body + (1 pop)	4	334.4206	8.2233	0.1355438	0.6662607
(b) Coefficient of variation						
M0	(1 pop)	3	-255.5706	0	0	0.5459538
M1	sex + (1 pop)	4	-246.4165	9,1541	0.00171725	0.5455763
M2	sex + body + (1 pop)	5	-235.5418	20,0288	0.02346989	0.5503774
M3	sex + body + sex:body + (1 pop)	6	-223.6337	31,9369	0.02662207	0.5501118
M4	body + (1 pop)	4	-244.7485	10,8221	0.02161716	0.5520438

Table 3. List of candidate models to test the relationship of mean telomere length (a) and its intra-individual variation (b) with precipitation. Model structure (fixed effects and their interaction, and random effect of strain ID), degrees of freedom (d.f.), Akaike Information Criterion corrected for small sample size (AICc), difference between the best fitting model and candidate model (ΔAICc) indicating strength of support for a particular model, amount of variability explained by fixed effects (R^2_{marginal}) and by combination of fixed and random effects ($R^2_{\text{conditional}}$).

Model		d.f.	AICc	ΔAICc	R2 marginal	R2 conditional
(a) Mean telomere length						
M0	(1 pop)	3	343.6612	-8.341	0	0.5235916
M1	precip + (1 pop)	4	352.6750	-17.3548	0.1741896	0.5407045
M2	precip + sex + (1 pop)	5	335.3202	0	0.2649685	0.6376086
M3	precip + sex + precip:sex + (1 pop)	6	344.3068	-8.9866	0.2844145	0.6560993
(b) Coefficient of variation						
M0	(1 pop)	3	-255.5706	0	0	0.5459538
M1	precip + (1 pop)	4	-238.9254	16.6452	0.1275769	0.5670824
M2	precip + sex + (1 pop)	5	-229.7255	25.8451	0.1286001	0.5665069
M3	precip + sex + precip:sex + (1 pop)	6	-211.1557	44.4149	0.1348708	0.5715629

Table 4. List of candidate models to test the relationship of mean telomere length (a) and its intra-individual variation (b) with median sex-specific strain-level lifespan estimates. Model structure (fixed effects and their interaction, and random effect of strain ID), degrees of freedom (d.f.), Akaike Information Criterion corrected for small sample size (AICc), difference between the best fitting model and candidate model (ΔAICc) indicating strength of support for a particular model, amount of variability explained by fixed effects (R^2_{marginal}) and by combination of fixed and random effects ($R^2_{\text{conditional}}$).

Model		d.f.	AICc	ΔAICc	R2 marginal	R2 conditional
(a) Mean telomere length						
M0	(1 pop)	3	343.6612	8.7919	0	0.5235916
M1	lifespan + (1 pop)	4	348.6886	13.8193	0.0540819	0.5801477
M2	lifespan + sex + (1 pop)	5	334.8693	0	0.1116633	0.6378792
M3	lifespan + sex + lifespan:sex + (1 pop)	6	343.9329	9.0636	0.1139724	0.6578448
(b) Coefficient of variation						
M0	(1 pop)	3	-255.5706	0	0	0.5459538
M1	lifespan + (1 pop)	4	-238.2521	17.3185	0.0009664	0.5333689
M2	lifespan + sex + (1 pop)	5	-229.0534	26.5172	0.0019424	0.5387066
M3	lifespan + sex + lifespan:sex + (1 pop)	6	-216.2014	39.3692	0.0307574	0.5483350