Exploring the drivers of longer lifespan in subterranean species: *Astyanax mexicanus* as model species

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Background. Longevity is one of the characteristic features of cave-dwelling animals. However, not much is known about its evolution or underlying mechanisms because it is difficult to determine the age of wild animals and closely related surface species that allow direct comparison are often lacking. Here, we examined average telomere length in *Astyanax mexicanus*, a species that has both surface-dwelling and cave-adapted populations. Telomeres are non-coding DNA repeats at the end of chromosomes and their shortening is considered one of the major causes of aging. Longer telomeres and their slower shortening are positively correlated with higher longevity and survival of individuals, so we expected to observe longer telomeres and their slower shortening in cavefish, assuming that they have higher longevity.

Methods. We compared telomere length and shortening between laboratory-reared Pachón cavefish and Rio Choy surface fish of *A. mexicanus* in different tissues and at different ages.

Results. Contrary to our expectations, surface fish had longer average telomere length compared to cavefish. This allows us to hypothesize that cavefish have developed shorter and cheaper telomeres because many stressors known to affect aging are not present in the cave environment. In addition, we did not observe telomere attrition as a result of aging in adults up to 9 years old, suggesting that efficient mechanisms prevent telomere-mediated senescence in laboratory stocks of this species, at least in this time frame. This study provides the first information on telomere dynamics in *Astyanax* morphs and suggests that shorter telomeres may have evolved during adaptation to caves.

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Abstract

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Introduction

Cavefishes represent one of the most successful groups of vertebrates that colonized the subterranean world, with more than 350 species from every continent (Niemiller et al., 2019). Subterranean habitats are semi-enclosed ecosystems characterized by specific environmental features, such as the absence of light, stable microclimate, and scarcity of food resources (Culver and Pipan 2019). These features triggered the evolution of specific traits in species that settled there (Balart-García et al., 2023). Adaptive traits characteristic of subterranean species may be morphological (e.g., loss of eyes and pigmentation, elongation of appendages), physiological (e.g., lower metabolism, loss of circadian rhythm), or behavioral (e.g., increase in exploratory behavior, lower intraspecific agonistic behavior) (Christiansen 2012; Lunghi et al., in press; Mösslacher and Creuzé Des Châtelliers 1996; Poulson 1963). A recent study that compiled evidence from the available literature highlighted the higher longevity of subterranean species compared to their closely related aboveground species and hypothesized that this may be considered an additional adaptive trait to subterranean life (Lunghi and Bilandžija 2022).

A widely used method to predict the lifespan of individuals is to measure telomere length (Lulkiewicz et al., 2020). Telomeres are repeated sequences of non-coding DNA located at chromosome ends in all vertebrates and most metazoans (Gomes et al., 2010; Monaghan 2010); their function is to protect chromosomes from degradation and fusion (Armanios and Blackburn 2012). The shortening of telomeres is one of the main causes of cell senescence (Campisi and d'Adda di Fagagna 2007; Haussmann et al., 2005; Joeng et al., 2004; Takubo et al., 2010). Each time a cell divides, telomere replication is incomplete, resulting in the loss of part of the telomere sequence and the generation of two daughter cells with shorter telomeres (Campisi and d'Adda di Fagagna 2007). When telomere length is reduced to a critical length, the cell ceases replication

and enters senescence; this mechanism is the basis of aging in many organisms (Blasco 2007; Hemann et al., 2001). On the other hand, several stressors (e.g., oxidative stress, competition) can increase telomere shortening and drive cells into senescence earlier than is expected for their biological age (Boonekamp et al., 2013; Chatelain et al., 2020; Epel et al., 2004). Telomere shortening is sometimes counteracted by telomerase activity, which can maintain and extend telomere length, helping to slow overall organismal aging (Blasco 2007; McLennan et al., 2018). In this study, we compared the average telomere length (hereafter TL) between closely related cave and surface fish populations, assuming that longer telomeres positively correlate with longer lifespan (Anchelin et al., 2011; Joeng et al., 2004; Monaghan and Haussmann 2006). Given the higher longevity of belowground species compared to their aboveground relatives (Lunghi and Bilandžija 2022), we expect a longer TL in the former. We tested our hypotheses using the Mexican tetra *Astyanax mexicanus*, a model species often used in evolutionary studies (Jeffery 2020). Aside from being easily propagated in the laboratory (Elipot et al., 2014), this species is an important model because it has two ecomorphs, one fully adapted to living in caves (hereafter CF) and the other living in surface waters (hereafter SF) (Fig. 1A). The advantage of using *A. mexicanus* in evolutionary studies is that comparisons between conspecifics allow us to avoid potential biases due to the intrinsic life history differences that characterize different species, regardless of how closely related they are (Ficetola et al., 2018; Michaux et al., 2005; Verberk et al., 2008). With this study, we aim to determine if TL is one of the possible factors causing the divergence in lifespan between *Astyanax* CF and SF. The sparse information we have on the age distribution of wild *Astyanax mexicanus* comes from only a few populations (three caves and two surface rivers). In their study, Simon et al. (2017) estimated fish ages from scales and determined an age range of 2-6 years in surface populations and 2-8 years in cave

populations. This allows us to hypothesize that cave ecomorphs may have a higher life expectancy. In addition, according to anecdotal observation in the laboratory, reared surface fish show signs of aging, such as a hunched back, sunken skin, and tattered fins, whereas this is not the case in cave-dwelling individuals of similar age (Riddle et al., 2018). Given this, we expect at least one of the following scenarios: *i*) TL is positively correlated with lifespan of individuals (Joeng et al., 2004), thus we expect longer TL in CF; *ii*) TL shortening is significantly amplified by various stressors individuals are exposed to (Von Zglinicki, 2002), and aboveground environments may provide more stressful conditions compared to belowground environments, increasing telomere attrition rates (Chin et al., 2018; Chin et al., 2020). Therefore, we expect lower TL attrition in CF.

Materials & Methods

Fish samples

We performed three different experiments to identify possible divergence in TL between *Astyanax* CF and SF. The samples used for our experiments were unrelated, so we provide a separate dataset for each of them (Table S1-3). All fish used in our experiments came from the Jeffery lab; the stock of SF originated from the Rio Choy river, while the stock of CF from Pachón cave. Fish employed in our experiment came from laboratory stocks and were kept in running water in not-enriched 40 liters aquariums since the age of one. Eight to fifteen individuals per aquarium are cultured at 23–25 °C under a 14–10 h light–dark photoperiod and fed daily with tetra flakes occasionally supplemented by living invertebrates (Jeffery, 2020). This study was performed in accordance with University of Maryland Animal Care and Use Committee (IACUC #R-NOV- 18–59) Project 1241065-1. Although the employment of lab-

reared fish in experiments may require a careful interpretation of the results (i.e. conditions in laboratory environments differ from those occurring at natural sites), this is the easiest and most precise way to obtain exact information on fish age. Furthermore, rearing in the laboratory conditions controls for the effects of ecology and is ideal to uncover genetic differences between the two morphs.

Caudal fin experiment

We collected fin clips (a few millimeters from the ventral lobe of the caudal fin) from 43 fish (23 SF and 20 CF) and tested the potential divergence in TL (both length and shortening) between the two ecomorphs. This first experiment aims to evaluate the use of fin tissue to study TL in *Astyanax* fish. This sampling method is simple, fast, and does not require sacrificing the fish, allowing for sustainable use of laboratory and wild animals. The dataset can be found in Table S1.

Fish organ experiment

In this experiment we wanted to assess the potential variability of telomere length and attrition between different fish organs from both surface and subterranean environments. We also aim to assess the potential correlation between the TL of caudal fin and different organs, in order to evaluate the use of caudal fin TL as a proxy for the TL of the entire organism. Twenty-two fish (12 SF and 10 CF) were euthanized in a solution of Tricaine methanesulfonate (> 2%) (Sigma Aldrich, USA, cat #: A5040), and the following organs were macrodissected: Caudal fin, bladder, skin, brain, gills, heart, liver, muscles, and gonads. Our aim was to collect fish of

various ages, no other criteria prior to euthanizing the fish was used. The dataset can be found in Table S2.

Gamete experiment

In this experiment we aim to investigate a possible correlation between the TL of the adult caudal fin and the gametes of the fish to determine if telomere attrition occurs after fertilization and during development. We obtained both fin clips and gametes (eggs or sperm) from 42 reproductive fish (16 SF and 26 CF). Spawning was induced by gradually changing the water temperature of the fish system (from 22°C to 24°C to 26°C to 24°C to 22°C) for four consecutive days, and gametes were collected on the second and third nights. Fish were placed upside down in a carved sponge soaked in water. Release of gametes was induced by massaging the abdomen. Eggs were collected with a stainless spoon, and sperm were collected with microcapillary tubes (Hirschmann, Germany, cat #: 9000105). A clip of the caudal fin was also collected before release. The dataset is provided as Table S3.

DNA extraction and Real time (q)PCR

All the sampled tissues and gametes were stored in 500 µl of lysis buffer (Zymo Quick-DNA Miniprep). Samples were homogenized in Bead Ruptor 4 (Omni International) with ceramic beats. DNA was extracted following the Zymo Quick-DNA Miniprep protocol. DNA concentration was measured with fluorometer DS-11 FX (DeNovix, USA) using the DeNovix dsDNA Broad Range Kit (cat #: KIT-DSDNA-BROAD-2). DNA concentration was adjusted to 15 ng/µl for all samples to improve qPCR readability and comparability of the results. This concentration was chosen after a series of qPCR runs with different concentrations (9, 0.9, 0.09,

0.009) aiming to: i) identify the lowest DNA concentration that guarantee high PRC efficiency (see below), while ii) having the peak of DNA amplification on average < 15 Ct. This allowed to use tissues from which low DNA concentration were obtained, and to avoid confounding amplification factors of TEL primer, as it naturally shows PCR amplifications > 30 Ct. After testing DNA dilution on tissues from five different individuals, only the concentration of 9 ng/µl produced on average 15 Ct (see Table S4). Therefore, we arbitrary chose to set the DNA concentration to 15 ng/µl (50% more) to ensure qPCR results for TEL primers below Ct 15.We run the real time PCR on BIO RAD CFX96. We used the following primers for telomere length (Vasilishina et al., 2019) TEL; forward (F) 5'-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3', reverse (R) 5-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3' and designed our own for a gene used as reference (OCA2; forward (F) CAAGAACACTCTGGAGATGGAG, reverse (R) ACGCAGCTCGTCAAAGTT). Gene *oca2* (ENSAMXG00000012753) was chosen as a reference following recommendations of Vasilishina et al. (2019) because it is a single-copy gene and allows to estimate the relative average telomere length. Primers were designed in the second exon and the amplicon was 109 bp in length. We checked target specificity using primerBLAST tool (NCBI) and by melt curve analysis. We first performed the serial dilution test using at least three DNA concentrations and six of primers, to assess the primer concentration with the highest efficiency (i.e., between 95% and 105%; Sveca et al., 2015): for TEL we chose 10 µM/µl (slope -3.4, r^2 0.99, efficiency 96.36%) and for OCA2 1 µM/µl (slope -3.21, r^2 0.99, efficiency 104.73%). Each 10 µl reaction consisted of 5 µl of ITAQ Universal SYBR Green Supermix (Biorad, USA, cat #: 172-5122) + 2 µl of RNA/DNA free water + 1 µl of primer F and 1 µl of primer R (working concentration of TEL or OCA2) + 1 µl of DNA. Each sample was

assayed in triplicates. We ran samples in 96-well plate and each plate had six negative controls (the DNA was replaced by RNA/DNA free water), three for each primer. We set the qPCR with the following cycles: 30 sec at 95 °C, 40 cycles of 95 °C for 5 s, 60 °C for 30 sec (data collection) followed by a dissociation stage. We checked the reliability between different runs using a randomly chosen 8 samples from 3 individuals and running three different qPCR plates placing the samples in different order; standard deviation of Ct was < 0.15, therefore we considered results from different runs comparable.

Data analysis

We used the built-in software (Bio-Rad CFX Maestro 2.2) to extrapolate the Ct values for TEL and OCA2 from each sample. qPCR plates were considered contaminated and consequently discarded if Ct of negative controls were > 30 for TEL and ≠ 0 for OCA2 (see Fig. S1 for an example of qPCR results). We assessed the presence of potential pipetting errors by comparing the Ct values of each triplets: those showing a difference in Ct ≥ 0.5 were discarded and the DNA from the same sample was reanalyzed in a new qPCR run. The average Ct of the three replicates for both TEL and OCA2 was used for subsequent calculations. We calculated the TEL/OCA2 ratio applying the following formula $2^{-(\mathrm{TELCt}-\mathrm{OCA2Ct})}$, to further standardize qPCR results allowing comparisons between different samples and machine runs (Vasilishina et al., 2019). The results of this formula were log-transformed (hereafter, $\log_{Ct}$) to improve normal distribution.

We performed subsequent analyses and prepared the figures in R environment using packages *lmer4*, *lmerTest* and *visreg* (Breheny and Burchett 2017; Douglas et al., 2015; Kuznetsova et al., 2016; R Development Core Team 2021).

200

201 *Experiment with caudal fins*.

202 We performed Analysis of Variance (ANOVA) in which we used the relative telomere length

203 (log_{Ct}) as dependent variable, while the fish age and fish ecomorph (SF vs CF) as independent

204 variables.

205

206 *Experiment with fish organs.*

207 We built a Generalized Linear Mixed Models (GLMM) in which we used the log_{Ct} of the organs

208 as the dependent variable, while the type of organ, ecomorph, and fish age were added as

209 independent variables; we added the interaction between organ and ecomorph as further

210 independent variable to assess the potential variability for each organ between the two

211 ecomorphs. The identity of the individuals was used as a random factor because we obtained

212 multiple organs from each individual. Likelihood ratio test was used to evaluate the significance

213 of the variables in the GLMM. We evaluated the potential correlation of TL between the organs

214 for each ecomorph.

215

216 *Experiment with gametes.*

217 We built a GLMM in which we used the log_{Ct} of the two different tissues as the dependent

218 variable, while sample type (caudal fin vs. gametes) and fish age served as independent

219 variables; the interaction between ecomorph and tissue was added as further independent

220 variable. The identity of individuals was used as a random factor since two different samples of

221 each individual were analyzed. In this analysis, we added fish sex (male vs. female) as an

222 additional random factor. The likelihood ratio test was used to evaluate the significance of the

variables in the GLMM. Finally, we evaluated the potential correlation of TL between caudal fin and gametes for each ecomorph.

Results

Experiment with caudal fins.

The average telomere length (TL) was significantly correlated with the fish ecomorph (df = 1, F = 10.479, P = 0.002), while no significant correlation with fish age (df = 1, F = 0.062, P = 0.774) was observed; CF had shorter TL compared to SF (Fig. 1B).

Experiment with fish organs.

We successfully extracted DNA from 165 organs: bladder (18), brain (22), caudal fin (22), gills (22), gonads (14), heart (21), liver (16), muscle (15), skin (15) (Table S1). Overall, TL was significantly correlated with ecomorph ($F_{1, 18.24}$ = 10.82, P = 0.004) and organ type ($F_{8, 129.20}$ = 4.96, P < 0.001), while no significant effect was detected for age ($F_{1, 23.61}$ = 1.2, P = 0.284) and for the interaction between organ and ecomorph ($F_{8, 130.18}$ = 0.38, P = 0.93). The SF showed longer TL than CF, while among organs the bladder and skin had the shortest TL (Fig. 1C). We found six significant correlations of TL in different tissues for SF, while only one for CF (Table 2). In SF, the gill had the highest number of significant correlations with other tissues (4), followed by the caudal fin, brain, and heart (2 each), and muscle (1). In contrast, in CF, only the correlation between brain and liver was significant (Table 1).

Experiment with gametes.

246 The ecomorph significantly correlated with TL ($F_{1, 38.16} = 7.26$, $P = 0.01$), while no significant
247 effect was observed for fish age ($F_{1, 38.36} = 0.06$, $P = 0.806$) and tissue type ($F_{1, 40} = 2.28$, $P =$
248 0.139). The interaction between ecomorph and tissue type was marginally not significant ($F_{1, 40} =$
249 3.63, $P = 0.064$). The TL was generally longer in SF compared to CF, while TL of gametes were
250 relatively shorter in SF (Fig. 1D). Caudal fin TL was significantly correlated with gametes TL in
251 SF ($R = 0.6$, $P = 0.013$), whereas no significant correlation was found between TL of these two
252 tissues in CF ($R = 0.36$, $P = 0.068$).
253
254 ## Discussion

255 In this study, we observed a striking divergence in TL between the Pachón CF and Rio Choy SF
256 (Figs. 1B-D). In general, CF had shorter TL, which contradicts our original hypothesis in which
257 we predicted that CF should have longer TL than SF, as this trait may be correlated with higher
258 longevity of the cave species (Joeng et al., 2004; Lunghi and Bilandžija 2022). A longer TL
259 could enable *A. mexicanus* SF to survive better in a more stressful environment (Epel et al.,
260 2004). Compared to subterranean animals, individuals from surface rivers are exposed to a
261 variety of biotic and abiotic stressors, including oxidative stress (i.e., faster metabolism), UV
262 radiation, greater environmental fluctuations (e.g., microclimate), and predation risk (Culver and
263 Pipan 2019). All of these stressors contribute to the acceleration of telomere shortening (von
264 Zglinicki 2002). Therefore, TL in *A. mexicanus* may be considered a proxy for somatic
265 redundancy rather than a biomarker for aging (Boonekamp et al., 2013; Sauer et al., 2021).
266 Furthermore, cave animals live in environments where they must cope with prolonged periods of
267 starvation and hypoxia (Bizjak Mali et al., 2013; Lipovšek et al., 2019; van der Weele and
268 Jeffery 2022) both of which have been shown to increase TL (Iglesias et al., 2019; Wang et al.,
269 2018). To conserve energy, subterranean organisms also tend to reduce metabolism, slow

growth, and limit reproduction (Bulog et al., 2000; Howarth and Moldovan 2018; Poulson 1963). These activities usually have a positive effect on telomere length, as they are associated with a lower frequency of cell division and lower oxidative stress (Eisenberg 2011; Sudyka 2019; von Zglinicki 2002) resulting in reduction of telomere attrition. Therefore, the relief from some sources of stress present on the surface and the reduction of potential body damage in cave species, for example through negative inter- and intraspecific interactions (Plath and Schlupp 2008; Rétaux and Elipot 2013), may have contributed to relaxed selection on TL maintenance in CF. Considering that the subterranean form of *A. mexicanus* is descended from its surface ancestors (Gross 2012) and that telomere length is a highly heritable trait (Boonekamp et al., 2021), we can hypothesize that CF TL shortened during colonization of caves. So, can we consider the shortening of telomere length as an adaptation to subterranean habitats? According to the "thrifty telomere" theory (Eisenberg 2011), having longer telomeres is costly, and it does not always pay off. For example, in nutrient-poor environments such as caves, organisms have fewer resources to devote to their biological activities (Culver and Pipan 2019) so cheaper and shorter telomeres could be beneficial and selected for. Overall, environmental characteristics of caves may have indirectly or directly contributed to the shorter telomeres in CF. However, another possibility is that telomere length is controlled by pleiotropic effects of a gene selected for a different phenotypic trait (Pathak et al., 2021) as is the case in the evolution of eye degeneration and albinism in *Astyanax* CF (Bilandžija et al., 2018; Bilandžija et al., 2013; Krishnan et al., 2022; O'Gorman et al., 2021; Yamamoto et al., 2009). In the future, investigation of these hypotheses will allow to assess whether the shorter telomere length observed in the subterranean morph can be considered an adaptive trait for cave life.

Our results revealed no significant effects of aging on telomere shortening in adult *A. mexicanus*, suggesting that telomere maintenance mechanisms may be very efficient in this species throughout fish life (Blasco 2007). Lost telomere sequences are mainly replaced by telomerase activity (Campisi and d'Adda di Fagagna 2007; Gomes et al., 2010; Xie et al., 2008). Several studies conducted on different fish species, reported that despite the high telomerase activity in different organs, there was a significant decrease in TL with the age of the fish, which is a clear indication of the senescence of the organism (Hartmann et al., 2009; Hatakeyama et al., 2008). In short-lived zebrafish, on the other hand, TL does not shorten until 24 months of age, implying that individuals avoid senescence for approximately four-fifths of their lives due to telomerase activity (Anchelin et al., 2011; Lau et al., 2008; Lund et al., 2009). In our model organism, *A. mexicanus*, we found no evidence of telomere attrition in adult individuals up to 9 years old, which is consistent with the estimated age of the oldest wild-caught *A. mexicanus* (Simon et al., 2017). Therefore, senescence in this species might not be correlated with TL shortening, but is regulated by other mechanisms (Gruber et al., 2014). However, we cannot rule out the possibility that *A. mexicanus* does not age significantly within the age range examined in our study (1.5 - 9 years) and that telomere attrition may occur in older fish like some other signs of senescence (Anchelin et al., 2011; Riddle et al., 2018).

Our study yielded unexpected results. TL in SF increased with age. Fish from the laboratory are not exposed to most biotic and abiotic stressors that may contribute to TL shortening in the wild (Debes et al., 2016; Epel et al., 2004; Peterson et al., 2015), implying that the rate at which the species normally ages may be altered. Under the controlled conditions of the laboratory facility, both CF and SF ecomorphs can reach the age of 15 years, which is double the lifespan observed in the wild (Riddle et al., 2018; Simon et al., 2017). If increased telomerase

315 activity is the mechanism that enables SF to thrive in more stressful river environments
316 (Anchelin et al., 2011; Chin et al., 2018; Chin et al., 2020), this may explain the extension of TL
317 in the laboratory SF (Fig. 1C) where almost no stressors contribute to telomere attrition (Chin et
318 al., 2020; Monaghan 2010). This explanation is also supported by the third experiment, where
319 TL of the caudal fin resembles that of gametes (Fig. 1D), where telomeres should be longest
320 because a new individual arises from the division of these cells (Espinasa et al., 2005; Siderakis
321 and Tarsounas 2007). This is surprising because the caudal fin is expected to have a high rate of
322 telomere degradation due to frequent injury and regeneration. SF is, in fact, very aggressive
323 (Burchards et al., 1985) and the difference in TL between dominant and subordinate individuals
324 may explain the highest variance in SF fins among all other organs (Fig 1C). Although the
325 difference in TL between gametes and fin in the two ecomorphs was slightly above the
326 significance threshold ($P = 0.05$), a trend showing larger TL difference between these two tissues
327 in CF can be observed (Fig. 1D). Telomere attrition in CF may be occurring during embryonic
328 development and early adulthood, which is contrary to our second hypothesis that CF has lower
329 telomere attrition than SF. This suggests that telomere dynamics differ between the two morphs
330 and establishes *Astyanax* as a model system to understand divergent patterns of telomere
331 dynamics within a single species. Future studies aimed at assessing telomerase expression in
332 organs of wild fish of different ages may help to understand the dynamics of senescence in *A.*
333 *mexicanus*.

334 We observed a clear divergence of TL between the fish organs considered; in both
335 ecomorphs, the shortest TL was observed in the bladder and skin. In bony fish, the bladder is an
336 organ that serves to regulate buoyancy. It is composed mainly of collagen fibers and generally
337 lacks vessels (Pough et al., 2013). Therefore, oxidative stress and cell renewal in the bladder are

likely to be very limited (Mizushima and Komatsu 2011; von Zglinicki 2002), so reducing mechanisms that counteract telomere attrition in this organ may be beneficial to reduce maintenance costs (Eisenberg 2011). At the same time, the skin represents the protective layer that constantly defends the organism against external stressors and therefore undergoes high cell division, which accelerates the shortening of its telomeres (Buckingham and Klingelhutz 2011). On the other hand, other fish organs are involved in various biological processes such as metabolism, locomotion, processing information, etc., and constant cell turnover and production of energy are essential for maintaining the vitality of these organs (Mizushima and Komatsu 2011; Pough et al., 2013), which means that the cells composing their tissues are constantly under oxidative stress, one of the major exogenous causes of telomere shortening (Eisenberg 2011; von Zglinicki 2002). Therefore, prolonged TL in these organs may not only help maintain their vitality, but also increase overall survival and longevity of fish (Boonekamp et al., 2013; Haussmann et al., 2002; Haussmann et al., 2005).

The TL of the different organs correlated poorly with each other, showing only six significant correlations for SF and one for CF (Table 2). Of the total significant correlations, only two in SF involved the caudal fin. For these relatively small fish, fin clipping is likely a more practical and sustainable method of DNA sampling because it does not necessarily require sacrificing the fish and can be used in longitudinal studies due to the ability of *Astyanax* to regenerate fins (Stockdale et al., 2018). Our goal was to establish a harmless protocol for examining TL in *Astyanax* in order to identify potential variation in telomere attrition over the course of fish life. Unfortunately, TL of the caudal fin did not correlate significantly with other organs (especially in CF) in the populations we studied. However, repeating this experiment in

other populations may increase the possibility of developing a sustainable method for studying TL in *Astyanax* fish.

Conclusions

Our study provided the first information on average telomere length in *Astyanax mexicanus* and suggest that cavefish have shorter telomeres compared to surface form. These results apply to the Pachón cavefish and surface Rio Choy stocks cultured in the laboratory (Jeffery 2020). Studying the different cave and surface lineages, especially in the wild (Espinasa et al., 2020; Gross 2012), will be important to understand the evolution of telomere length, telomere attrition and senescence in *Astyanax* populations. The results of our analyses support the hypothesis that average telomere length, at least in the laboratory stocks of *A. mexicanus* used here, is more indicative of somatic redundancy than of the lifespan of individuals. The less stressful (fewer predators, reduced environmental fluctuations) and longevity-promoting ecological and biological conditions (lack of UV irradiation, caloric restriction, hypoxia) in subterranean habitats may have contributed to cavefish having shorter telomeres that require less energy to maintain. We observed no evidence of telomere attrition as an indicator of senescence in surface ecomorph of *A. mexicanus* until 9 years of age. These results may have been influenced by the favorable conditions in the laboratories, such as the constant environment and food supply or the lack of predation risk (Chin et al., 2018; Chin et al., 2020). Under these favorable conditions, the potential telomerase activity, particularly useful for SF to thrive under stressful conditions in the wild (i.e., counterbalancing the telomere attrition due to environmental stress; Boonekamp et al., 2013) may contribute in masking telomere attrition due to aging. Additional analyses on wild fish and other *Astyanax* populations will be important in order to confirm and interpret this result.

Identification of the mechanisms responsible for lifespan extension could lead to the development of strategies to increase human longevity. Because aging is a complex phenotype, it is important to incorporate research on nontraditional and atypical species and integrate approaches from different biological disciplines (Cohen 2018). Our study provided unexpected results and added *Astyanax* to the list of model species that can be used to deepen our understanding of telomere dynamics and senescence.

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Table 1(on next page)

Correlations among the fish organs used in the *Fish organs experiment.*

The correlation between organs in surface Rio Choy fish is shown above, and for Pachón cavefish below.

Table 1. Correlations among the fish organs used in the *Fish organs experiment*. The correlation between organs in surface Rio Choy fish (SF) is shown above, and for Pachón cavefish (CF) below.

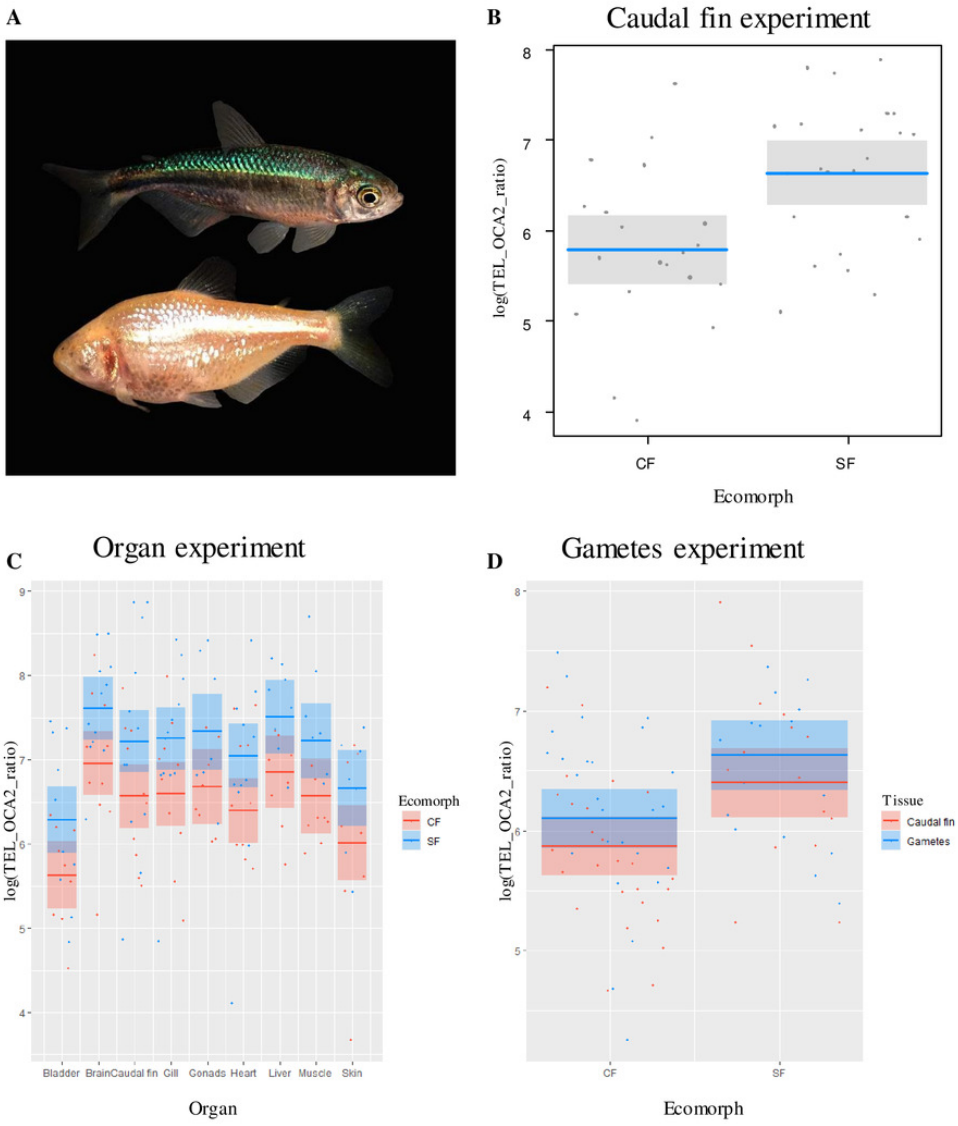
Surface fish	Brain	Caudal fin	Gill	Gonads	Heart	Liver	Muscle	Skin
Bladder	$P = 0.317$ $R = -0.38$	$P = 0.895$ $R = 0.05$	$\mathbf{P = 0.029}$ $R = -0.72$	$P = 0.904$ $R = 0.07$	$P = 0.508$ $R = -0.28$	$P = 0.860$ $R = -0.09$	$P = 0.78$ $R = 0.15$	$P = 0.905$ $R = -0.06$
Brain		$\mathbf{P = 0.01}$ $R = 0.71$	$\mathbf{P = 0.049}$ $R = 0.58$	$P = 0.871$ $R = -0.08$	$P = 0.02$ $R = 0.69$	$P = 0.324$ $R = -0.40$	$P = 0.89$ $R = 0.06$	$P = 0.168$ $R = 0.58$
Caudal fin			$P = 0.303$ $R = 0.32$	$P = 0.23$ $R = -0.52$	$\mathbf{P = 0.007}$ $R = 0.76$	$P = 0.62$ $R = 0.21$	$P = 0.485$ $R = 0.32$	$P = 0.232$ $R = 0.52$
Gill				$P = 0.314$ $R = 0.45$	$\mathbf{P = 0.027}$ $R = 0.66$	$P = 0.758$ $R = -0.13$	$\mathbf{P = 0.02}$ $R = -0.83$	$P = 0.334$ $R = 0.43$
Gonads					$P = 0.281$ $R = -0.53$	$P = 0.844$ $R = -0.09$	$P = 0.775$ $R = -0.15$	$P = 0.953$ $R = 0.05$
Heart						$P = 0.74$ $R = -0.15$	$P = 0.346$ $R = -0.47$	$P = 0.781$ $R = -0.15$
Liver							$P = 0.259$ $R = 0.49$	$P = 0.464$ $R = -0.54$
Muscle								$P = 0.089$ $R = -0.91$

Cavefish	Brain	Caudal fin	Gill	Gonads	Heart	Liver	Muscle	Skin
Bladder	$P = 0.594$ $R = 0.21$	$P = 0.097$ $R = 0.59$	$P = 0.891$ $R = -0.05$	$P = 0.341$ $R = -0.47$	$P = 0.793$ $R = 0.1$	$P = 0.288$ $R = 0.47$	$P = 0.632$ $R = 0.22$	$P = 0.988$ $R = -0.01$
Brain		$P = 0.301$ $R = 0.36$	$P = 0.124$ $R = 0.52$	$P = 0.404$ $R = 0.38$	$P = 0.84$ $R = 0.07$	$\mathbf{P = 0.017}$ $R = 0.8$	$P = 0.224$ $R = 0.48$	$P = 0.062$ $R = 0.68$
Caudal fin			$P = 0.302$ $R = 0.36$	$P = 0.719$ $R = 0.17$	$P = 0.604$ $R = 0.19$	$P = 0.26$ $R = 0.45$	$P = 0.723$ $R = 0.15$	$P = 0.987$ $R = -0.01$
Gill				$P = 0.329$ $R = 0.43$	$P = 0.397$ $R = -0.30$	$P = 0.613$ $R = 0.21$	$P = 0.558$ $R = 0.24$	$P = 0.22$ $R = 0.49$
Gonads					$P = 0.675$ $R = 0.19$	$P = 0.215$ $R = 0.54$	$P = 0.257$ $R = 0.50$	$P = 0.917$ $R = -0.05$
Heart						$P = 0.452$ $R = 0.31$	$P = 0.89$ $R = 0.06$	$P = 0.747$ $R = 0.14$
Liver							$P = 0.114$ $R = 0.6$	$P = 0.782$ $R = 0.15$
Muscle								$P = 0.766$ $R = -0.16$

Figure 1

Average telomere length in *Astyanax mexicanus* Pachón cavefish and Rio Choy surface fish.

A) The two *Astyanax mexicanus* ecomorphs. On top the surface form (SF) showing normal eyes and pigmentation. On the bottom the cave form (CF) characterized by the lack of eyes and pigmentation. Source: https://commons.wikimedia.org/wiki/File:Astyanax_mexicanus_(cavefish)_(27589386037).jpg . B) Partial regression plots showing the log-transformed relative telomere length (TEL-OCA2 ratio) for the caudal fin of the two studied populations of *A. mexicanus*: Rio Choy river SF and cave Pachón CF. C) Partial regression plots showing the log-transformed relative telomere length (TEL-OCA2 ratio) for the studied organs in the two *A. mexicanus* ecomorphs. D) Partial regression plots showing the log-transformed relative telomere length (TEL-OCA2 ratio) for both gametes and caudal fin in the two *A. mexicanus* ecomorphs. Horizontal line represents mean values, while shaded box are 95% CI.


A
B
Caudal fin experiment
log(TEL_OCA2_ratio)
Ecomorph
CF
SF
C
Organ experiment
Bladder
Brain
Caudal fin
Gill
Gonads
Heart
Liver
Muscle
Skin
Organ
Ecomorph
D
Gametes experiment
Tissue
Caudal fin
Gametes