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The cecal appendix is correlated with greater maximal longevity in mammals

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Abstract

The cecal appendix had been considered as a useless vestige since Darwin's work, but recent research questioned this idea demonstrating that the cecal appendix appeared among the mammals at least 80 million years ago and has made multiple and independent appearances without any obvious correlation with diet, social life, ecology, or size of the cecum. However, functions and probable selective advantage conferred by this anatomical structure still remain enigmatic. We found, through analyses of data on 258 mammalian species, that cecal appendix presence is correlated with increased maximal observed longevity. This is the first demonstration of a correlation between cecal appendix presence and life history. Interestingly, the classical evolutionary theory of aging that predicts an increased longevity when the extrinsic mortality is reduced has been questioned several times, but recent comparative studies asserted its validity in the taxa, which experience age-dependent and density-dependent mortality, as in mammals. Thus, the cecal appendix may contribute to the increase in longevity through a reduction of extrinsic mortality. A lower risk of fatal infectious diarrhea is one of the most plausible hypotheses that could explain it. However, several hypotheses coexist about the possible functions of the cecal appendix, and our results provide new insights about this much-disputed question. In addition, we show that the cecal appendix arose at least 16 times and was lost only once during the evolutionary history of the considered mammals, an asymmetry that supports the existence of a positive selective of this structure.

KEYWORDS
convergent evolution, diarrhea, extrinsic mortality, gastrointestinal anatomy, lifespan, phylogenetic generalized least squares, senescence, vermiform appendix

1 | INTRODUCTION

The cecal appendix is a narrow, cylindrical pouch connected to the end of the cecum in the gastrointestinal tract and is composed of an organized lymphoid tissue that achieves its greatest development shortly after birth and then regresses with age (Dasso et al., 2000). Inflammation of this structure frequently occurs in humans, and as the risk of unfavorable evolution of an acute complicated appendicitis

Michel Laurin and Eric Ogier-Denis contributed equally to this work (alphabetic order).

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could be lethal (Pattison, 1936), its current treatment is radical and consists of the surgical removal of the appendix. Appendectomy is a very common surgery as about one person out of 10 is concerned in developed countries (Anderson et al., 2012; Lee et al., 2010). Recently, a conservative approach to treat uncomplicated appendicitis by antibiotics alone without appendectomy has been evaluated in several randomized control trials (Flum et al., 2020; Podda et al., 2019) showing a high early efficiency but exposing about 40% of the patients to a recurrence of the disease (Salminen et al., 2018).

In 1871, Charles Darwin characterized the cecal appendix in humans as a rudimentary and useless vestige left by a decreased cecal size that occurred because of changed diet or habits in our distant ancestors (Darwin, 1871). However, recent studies have refuted this hypothesis by demonstrating that the cecal appendix appeared among mammals at least 80 million years ago and has made multiple independent appearances (between 6 and up to 41 times, depending on the study and analysis) without any obvious correlation with diet, social life, ecology, or size of the cecum (Smith et al., 2017; Smith, Fisher, et al., 2009; Smith, Parker, et al., 2013). All of these data suggest a positive selective value associated with the cecal appendix, although its precise function is still debated (Girard-Madoux et al., 2018).

The classical evolutionary theory of aging formulated by George Williams in 1957 predicts that when the risk of mortality is reduced, senescence is delayed, and longevity is increased (Williams, 1957). Although questioned several times (Abrams, 1993; Wensink et al., 2017), this theory is supported by observations on mammals and in four species of the annual teleost *Nothobranchius* (Shattuck & Williams, 2010; Tozzini et al., 2013) and experiments in captive fruitflies (Stearns et al., 2000). Hence, we hypothesize that if protective functions conferred by the cecal appendix reduce extrinsic mortality, such as a lower infectious mortality from diarrhea through its shape serving as a bacterial sanctuary (Laurin et al., 2011), or increased immune barrier or function through the high density of lymphoid follicles contained in the cecal appendix (Bollinger et al., 2007; Gebbers & Laissue, 2004), mammalian taxa possessing this anatomical structure should display greater longevity than their close relatives of similar body size.

Below, we test 15 evolutionary models to assess the potential impact of appendix presence on longevity, controlling for body size effect and the statistical nonindependence reflecting the presence of a phylogenetic signal. We also test the hypothesis that the appendix has a selective value by looking at the asymmetry between gains and losses of this structure in a phylogeny of 258 mammalian species.

2 | METHODS

2.1 | Compilation of data

From the open-access database published by Smith et al. (2017), we extracted the information on the body mass and the presence or absence of a cecal appendix in the database. Among the taxa in this database for which presence or absence of the cecal appendix was a missing data item, a review of publications more recent than Smith et al. (2017) was conducted in order to update these data as much as possible. The updated cecal appendix criterion was defined exactly as in that paper, morphologically, as a close-ended projection that is clearly differentiated from the cecum or the colon by a change in diameter whatever the density of lymphoid follicles in this structure. The cecal appendix was considered absent only if an anterior and posterior picture of the cecum of an anatomical dissection were provided and if these did not reveal any visible cecal appendix, or if the publication explicitly described the absence of a cecal appendix after anatomical dissection. A single picture or drawing without a visible cecal appendix was not sufficient to score appendix as absent in that taxon. Finally, we modified the scores of only two species for the cecal appendix. *Lemur catta* that was previously considered of unknown condition was reclassified as having a cecal appendix based on data from a recent study (McGrosky et al., 2019). *Rattus norvergicus* was previously considered to be polymorphic for this character. Following an extensive review of the literature on this well-studied species, all reports suggest that cecal appendix of this taxon is in fact absent (Langer, 2002; Lingohr et al., 2014).

A previous study (Smith et al., 2017) collected data on appendix presence that pertained to given species (which they called "observed appendix") and used generalizations about higher taxa to infer it in some species ("inferred presence"). Given the fast evolutionary rate of the appendix, we prefer to rely only on accounts that bear on the species that we included in our study, so we used only the "observed appendix" from that study (Smith et al., 2017). In addition, species for which the presence of the cecal appendix was considered as polymorphic in previous studies (Smith et al., 2017; Smith, Parker, et al., 2013) were described as present by some authors and absent by others. These discrepancies had been interpreted as genuine biological variability by Smith, Parker, et al. (2013) and Smith et al. (2017), but we prefer, for the main analysis of the study, to consider that this represents uncertainty about the morphology, and we excluded these data because we fear that they are of low quality. To our knowledge, a proper treatment of polymorphism in analysis of variance does not exist. Nevertheless, results including the polymorphic species treated as having a cecal appendix are also reported in Supplementary Material.

The maximum longevity observed for all the mammalian species analyzed was retrieved from two databases available online: on the one hand the database Animal Diversity Web (https://animaldiversity.org/) and on the other hand the database AnAge (http://genomics.senescence.info/species/). When these two databases provided different maximum observed longevities within the same species, the longest longevity was kept. From the 317 species explored, maximal longevity was missing simultaneously in these two databases for 59 species (19%). The short dataset included the 258 species for which body mass, longevity, presence, or absence of a cecal appendix and phylogenetic history were successfully compiled. The long dataset concerned all the species for which presence or absence of a cecal appendix was compiled but with missing data regarding body mass and/or longevity. This dataset was useful to infer the evolutionary history of the cecal appendix.

The mean longevity of each species was not collected because of its very low availability and reliability in the literature. Thus, we decided to focus our study on maximal longevity. Of course, maximal values depend on sample size, but there is no reason to suspect that this introduced a bias in our database.

2.2 | Timetrees

Because of the uncertainty of the current knowledge about the phylogenetic tree of mammals, we downloaded two sets of 100 Bayesian time-trees (one of node-dated trees and another one of tip-dated trees) from Upham et al. (2019). These were sampled from the "completed" phylogenetic trees, filtering to retain only the mammalian species for which data on appendix presence or absence were available in their database. All the analyses of our work have been performed on the two sets of 100 trees to incorporate the phylogenetic uncertainty. The use of "completed" trees may add some uncertainty (Rabosky, 2015), but the practice of using such trees (including supertrees, which are also loosely tied to phylogenetic data) is well-established in comparative biology, as shown by the 2026 Google Scholar citations (as of April 22, 2021) of one of the classical comparative studies based on a supertree (Bininda-Emonds et al., 2007). Thus, to address this concern, we report (in Supplementary Material) the results on our analysis performed on two sets of 100 Bayesian time-trees (node-dated and tip-dated) that were sampled from "DNA-only" phylogenetic trees (Upham et al., 2019). Note that our approach to incorporate phylogenetic uncertainty through a population of completely resolved trees differs from that of recent studies of the evolution of the cecal appendix (Smith et al., 2017; Smith, Parker, et al.. 2013), which instead used incompletely resolved trees. This had the disadvantage that polytomies create character optimization problems (Maddison, 1989).

2.3 | Longevity analysis

As closely related taxa share a part of their evolutionary history, they may resemble each other more than expected by chance alone. Thus, we used the Phylogenetic Generalized Least Squares regression to test the relationship between the longevity, the mass, and the presence of cecal appendix while taking into account the fact that character data about species are not independent observations (i.e., not independent and identically distributed). The set of models all have the longevity expressed in year, which is log (ln)-transformed as the dependent variable, and they differ by the independent variable(s), their interaction, and the error structure: (1) *M* is the log (ln) of the mass in grams; (2) *A* is the absence/presence of the cecal appendix; (3) *M_A* corresponds to the model with both variables as predictors without interaction (two intercepts and one slope); (4) *M_MA* the effect of the mass can be different given the absence or presence of the cecal appendix (i.e., one intercept and two slopes); (5) *A_MA* both variables included with interaction (i.e., two intercepts and two slopes). The R language formulae are *M*: longevity ~mass, *A*: longevity ~appendix, *M_A*: longevity ~mass + appendix, *M_MA*: longevity ~mass + mass: appendix, *A_MA*: longevity ~mass * appendix. Models vary also by the covariation expected between observations to take into account their phylogenetic relationships. The phylogenetic signal, if any, was integrated into the error term of these models through three types of structures: (1) the identity matrix: the observations are considered independent (*no phylogeny*); the phylogenetic covariance between related taxa follows (2) a Brownian model whose intensity is estimated by Pagel's lambda (Pagel, 1999); and (3) the Ornstein–Uhlenbeck model modeling the attraction (i.e., alpha) towards an optimal value (Felsenstein, 1985; Martins & Hansen, 1997).

The Akaike information criterion (AIC) of each model was calculated, then the AIC weights were obtained (Akaike, 1974). The AIC weight allows an intuitive assessment of the relative support for each model; they can interpret as the probabilities that each model is the best, among those considered (Wagenmakers & Farrell, 2004). We use the usual calculation that consists of the relative likelihood of one model *i* over all *K* models for a given dataset. Values are scaled between 0 and 1. The best model has the highest value. The larger the weight difference between the best model and others is, the higher is the confidence in that this model is better than the others. To perform these analyses, we used various R packages: ape (Paradis & Schliep, 2019), phytools (Revell, 2011), and nlme (Pinheiro et al., 2020).

For each dataset, 100 trees were used to take into account topological and branch lengths uncertainties. As a consequence, a distribution of 100 results is obtained for each model. Wilcoxon tests have been performed on AIC weights for each pair of models to check if the differences are significant. We used the pairwise.wilcoxon.test of the R package *stats* because variances of samples vary greatly, and we used the Holm method to adjust *p* values for multiple comparisons to avoid false positives and false negatives (Holm, 1979).

2.4 | Gains and losses of the cecal appendix analysis

The number of gains and losses on the two sets of 100 phylogenetic trees was assessed using the parsimony optimization of the R package *phangorn* (Schliep, 2011). We could not use maximum likelihood for this because our data obviously depart from the required assumption that the probability of change is proportional to the branch lengths as shown by the fact that several changes occur in *Euarchontoglires* and none in *Laurasiatheria*, two very large, speciose clades. This fact about the evolutionary mode of this character is unsurprising given that Goloboff et al. (2019) already showed that most morphological datasets used by systematists do not meet the assumptions of the available evolutionary models for phenotypic characters, but this is a particularly extreme example, as shown below. To resolve optimization ambiguities, we applied the accelerated transformation (ACCTRAN) algorithm in order to privilege losses over parallel gains, as our working hypothesis is that appearances are more frequent than losses; this approach is thus conservative. A binomial test assessing the probability of the null hypothesis

under which gains and losses are equally probable is used to test the hypothesis that the appendix lacks a function (and hence, confers no selective advantage).

Another binomial test is performed to test the hypothesis that the appendix (considered as a binary, presence/absence character) evolved according to a homogeneous evolutionary model in placental mammals. Our test takes into consideration the sampled phylogenetic diversity of *Euarchontoglires*, where 19 transitions occur in the presence/absence of the appendix after ACCTRAN optimization and that of *Laurasiatheria*, its sister group, where no change occurs; note that we italicize all taxon names, in conformity with recommendation 6.1.A of the PhyloCode (De Queiroz & Cantino, 2020). Our test then assesses the probability of the null hypothesis (homogeneous evolutionary model) that such an extreme distribution in the number of transitions in two sister taxa can be generated randomly.

2.5 | Data availability

The database (Dataset S1), the code used for the statistical analysis (Script.R), nexus files containing the 100 trees node-dated (Completed: TreesNodeCompleted.nex, DNA-only: TreesNodeDNAonly.nex), and 100 trees tips-dated (Completed: TreesTipsCompleted.nex, DNA-only: TreesTipsDNAonly.nex) visible with Mesquite software (www.mesquiteproject.org) are available in the Supplementary Material.

3 | RESULTS

We compiled presence or absence of a cecal appendix and phylogenetic history in 317 taxa (long dataset; see Section 2 for the differences between short and long datasets) typically ranked as species and representing all main mammalian clade (including 43 with and 274 without cecal appendix). For regression analyses, we retained 258 terminal taxa (short dataset) for which body mass and longevity were also documented (including 39 with and 219 without cecal appendix).

3.1 | Impact of the presence of a cecal appendix on the longevity of mammals

Of the 15 tested models, the best one was achieved by the model considering mass and presence or absence of the cecal appendix as explicative variables for longevity variability between species (M_A model) with phylogenetic covariance under the Brownian motion on tip-dated trees with a median weights of AIC (wAIC) of 0.49 and a median absolute deviation of 0.092 (Table 1). This median value was significantly higher than the median wAIC of all other models (Table S1) and especially higher than the median wAIC of the second-best model, which was the model considering only the mass as explicative variable for longevity variability between species (M model) under the same conditions (p value of both models being equally good = 1.19E-15). Focusing only on node-dated trees with Brownian motion, the M_A model was still the best model (Table 1), significantly superior to the M model (p value = 1.19E-15) (Table S1), which was still the second-best model. Whatever the phylogenetic tree considered among the 100 tip-dated trees and the 100 node-dated trees, in Brownian motion condition, the M_A model was always the best model of the five models with the highest wAIC (Table S2). All the models that consider the phylogenetic covariance under the OU process always fit very poorly whatever the phylogenetic trees set considered, with a very low median wAIC ranging between 3.0E-28 ± 3.8E-28 and 3.5E-21 ± 5.1E-21 (Table 1).

As expected, an overall negative allometric relationship between the log-longevity and log-mass of species was observed in our data (Figure 1), meaning that longevity increases with but slower than body size.

For the best model of the longevity (M_A model on tip-dated trees with Brownian motion) and among the 100 tip-dated trees, the median intercept was significantly higher with than without cecal appendix, indicating the positive association between the presence of the cecal appendix and longevity (Table 2). The model that included only the presence of appendix (A model) was poorly supported. This shows that its effect was only detectable with the analysis of covariance when the effect of mass was taken into account. Models with interactions (M_MA and A_MA) are not better suited to our data, meaning that the additional variance explained in is not sufficient to justify the addition of parameters, compared with the best (M_A) model.

3.2 | Evolutionary history of the cecal appendix

On all 100 tip-dated trees and the 100 node-dated trees corresponding to the two sets of trees used for the regressions abovementioned, 16 non-ambiguous gains for the short dataset and 17 for the long dataset of the cecal appendix were identified versus one non-ambiguous loss for both datasets (Table 3).

This loss concerns *Hapalemur griseus* (Figure 2) and was revealed mainly on the updated data of its closest neighbor *L. catta* in which the presence of a cecal appendix has been recently reported (McGrosky et al., 2019).

Ambiguity in optimization with parsimony was resolved by using the ACCTRAN method, which privileges losses over parallel gains. Given that our hypothesis assumes that gains in this character are more frequent than losses, this is the worst-case scenario for our hypothesis. This analysis reported a minimum of 18 gains and a maximum of four losses for the short dataset (Table 3). A binomial test assessing the probability of the null hypothesis under which gains and losses are equally probable was performed. This test yielded a p value of 2.17E-03, highlighting a significant asymmetry between gains and losses of the cecal appendix in favor of the gains. While this asymmetry had already been found before (Smith et al., 2017; Smith, Parker, et al., 2013), the new analysis we carried out with our

TABLE 1 Summary of the best models (according to Akaike information criterion weights) explaining the longevity as a function of cecal appendix and body size

	Phylogenetic signal	1. M model	2. A model	3. M_A model	4. M_MA model	5. A_MA model
No phylogeny		3.1E-28 (2.3E-28)	3.1E-54 (2.3E-54)	5.1E-25 (3.7E-25)	1.5E-25 (1.1E-25)	6.3E-26 (4.6E-26)
Tip-dated trees	*BM*	0.19 (0.061)	6.5E-10 (7.2E-10)	**0.49 (0.092)**	0.11 (0.021)	0.04 (0.0084)
	OU	3.0E-28 (3.8E-28)	3.5E-21 (5.1E-21)	3.2E-25 (3.4E-25)	9.1E-26 (8.3E-26)	3.8E-26 (3.4E-26)
Node-dated trees	*BM*	0.039 (0.042)	8.7E-11 (1.1E-10)	0.096 (0.1)	0.021 (0.022)	0.0082 (0.0083)
	OU	7.3E-28 (9.6E-28)	3.3E-21 (4.9E-21)	3.6E-25 (3.7E-25)	7.3E-26 (7.1E-26)	3.7E-26 (3.2E–26)

Note: Values are medians of AIC weights for each combination of dependent variables and error structure, followed in parenthesis by median absolute deviation. The best model is in bold type. All models all have the longevity as dependent variable, and they differ by the independent variable(s), their interaction, and the error structure. *M* is the log of the mass, *A* is the absence/presence of the appendix, *M_A* corresponds to the model with both variables as predictors without interaction between them, *M_MA* assumes that the effect of the mass can be different given the absence or presence of the appendix (i.e., one intercept and two slopes), and *A_MA* has both variables included with interactions (i.e., two intercepts and two slopes). Models vary also by the covariation expected between observations to take into account their phylogenetic relationships. Three models of error structure are included: a null model with observations considered independent and identically distributed (no phylogeny), Brownian motion (BM), and Ornstein–Uhlenbeck (OU). Two sets of 100 phylogenies are used, the one with branch lengths based on tip dating (tip-dated trees) and the other with branch lengths based on nodes (node-dated trees).

Abbreviation: AIC, Akaike information criterion.

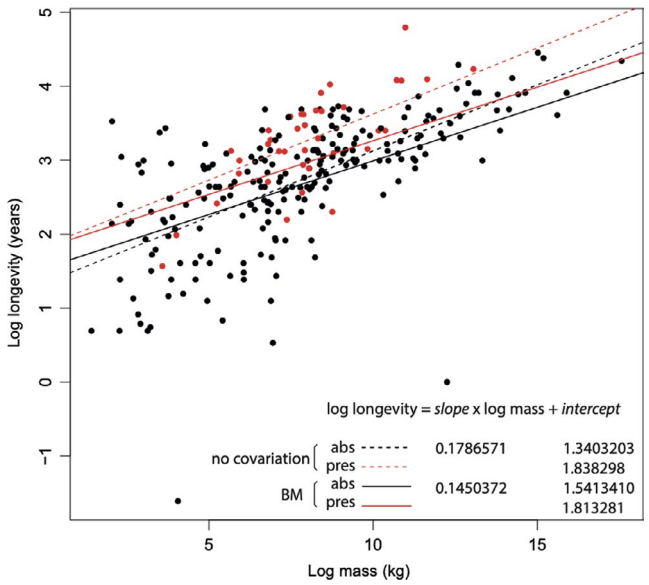


FIGURE 1 Plot of size against longevity with regression lines of the models mass + cecal appendix (M_A model). Colors correspond to absence (black) of presence (red) of appendix. Dotted lines correspond to taxa considered independent and identically distributed (no phylogeny) and full lines to Brownian motion + Pagel's lambda error structure (with phylogeny)

updated data and trees confirms previous results and describes the first non-ambiguous loss of the cecal appendix in mammals.

The null hypothesis that the cecal appendix evolved according to a homogeneous evolutionary model in placental mammals, tested through the comparison of two sister groups, *Euarchontonglires* (Figure 2) and *Laurasiatheria* (Figure 3), is rejected because its probability is extremely small (1.345E-06). The cecal appendix also appeared among *Monotremes* (which document one of the oldest appearances of this structure), *Marsupials*, *Afrotheria*, and *Afrosoricida* (Figure 4).

3.3 | Potential impact of tree completion and polymorphism on model selection

All results presented above rely on "completed" trees that excluded species polymorphic for the presence of a cecal appendix. We also conducted all the analyses on "DNA-only" trees excluding polymorphic species (Tables S3–S7) and on "completed" trees including polymorphic species, treated as if they had an appendix (Tables S8–S12). The order of the Supplementary Tables follows the same order as the tables of the main analysis of the manuscript. The results of all these analyses (presented in the Supplementary Material) are similar to the main analyses commented here and support the same conclusions. Using "DNA-Only" trees, the M_A model on tip-dated trees under the Brownian motion had the highest median wAIC of 0.56 (Table S3). This median value was significantly higher than the median wAIC of all other models (Table S6) and especially higher than the median wAIC of the second-best model (M_MA model under the same condition) (p value =3.96E-15). For this best model of longevity, the median intercept was significantly higher with than without cecal appendix, indicating the positive impact of the presence of the cecal appendix on longevity (Table S4). With "Completed" trees including polymorphic species, the M_A model on tip-dated trees under the Brownian motion still has the highest median wAIC of 0.42 (Table S8). This median value was significantly higher than the

TABLE 2 Summary of the fitted coefficients and their respective *p* values for the best model: longevity ~mass + cecal appendix (M_A model) with the error structure following a Brownian motion with Pagel's lambda

	Intercept appendix absent	*p* value	Intercept appendix present	*p* value	Slope (mass)	*p* value	Lambda
Tip-dated trees	1.5 (0.027)	0.0038 (0.0015)	1.78 (0.0067)	0.011 (0.0018)	0.14 (0.0024)	1.0E-15 (1.1E-15)	0.85 (0.014)
Node-dated trees	1.5 (0.022)	0.00052 (0.00036)	1.78 (0.0064)	0.01 (0.0015)	0.15 (0.0026)	5.8E-16 (6.3E-16)	0.81 (0.021)

Note: Values are medians computed from the set of 100 trees; values in brackets are median absolute deviation.

TABLE 3 Number of gains and losses of the cecal appendix inferred by parsimony

	Gains	Losses	*p* value
Short MPR	16	1	1.37E-04
Short ACCTRAN	18	4	2.17E-03
Long MPR	17	1	7.25E-05
Long ACCTRAN	20	5	2.04E-03

Note: Most parsimonious reconstruction (MPR) can produce ambiguous optimization, which is not counted in the corresponding lines of the table. ACCTRAN corresponds to counts for which ambiguities are resolved by favoring losses, which results in more identified gains and losses. We used the 200 trees from Upham et al. (2019), and we filtered taxa present in the data used to produce the models (short, 258 taxa) and those for which data on appendix were available (long, 317 taxa). In each of these categories, all reconstructions have the same numbers of the various types of transitions.

median wAIC of all other models (Table S11) and especially higher than the median wAIC of the second-best model (M_MA model under the same condition) (*p* value =5.93E-16). For the best model of longevity (M_A model on tip-dated trees with Brownian motion), the median intercept was significantly higher with than without cecal appendix, indicating the positive impact of the presence of the cecal appendix on longevity (Table S9).

4 | DISCUSSION

The significant association between cecal appendix presence and increased maximal longevity suggests a reduction in mortality in mammals possessing a cecal appendix, while the evolutive asymmetry between gain and loss supports a positive selection of this anatomical structure.

The existence of a confounding bias that could explain the association found between the cecal appendix and longevity cannot be formally excluded in this comparative study. However, no correlation between a life history trait and the cecal appendix presence or size was found previously in comparative studies of mammals, which militates against the possible implication of such a confounding factor (Smith et al., 2017; Smith, Parker, et al., 2013). The only previous significant association found with the cecal appendix was related to the morphology of the cecum and to cecal apex thickness, suggesting the co-evolution of these two anatomical structures to form a "cecoappendicular complex" (Smith et al., 2017). This did not support Darwin's hypothesis of a cecal atrophy being responsible for the appearance of the cecal appendix. Here, we found the first positive correlation with a life history trait, namely, a greater longevity among mammalian taxa possessing a cecal appendix. In line with the updated scientific data, a reduction of extrinsic mortality though cecal appendix presence is the most plausible explanation. This suggestion relies on the classical theory of aging of Williams (Williams, 1957), which hypothesizes that when the extrinsic mortality is high, few individuals attain an old age, and thus, natural selection has

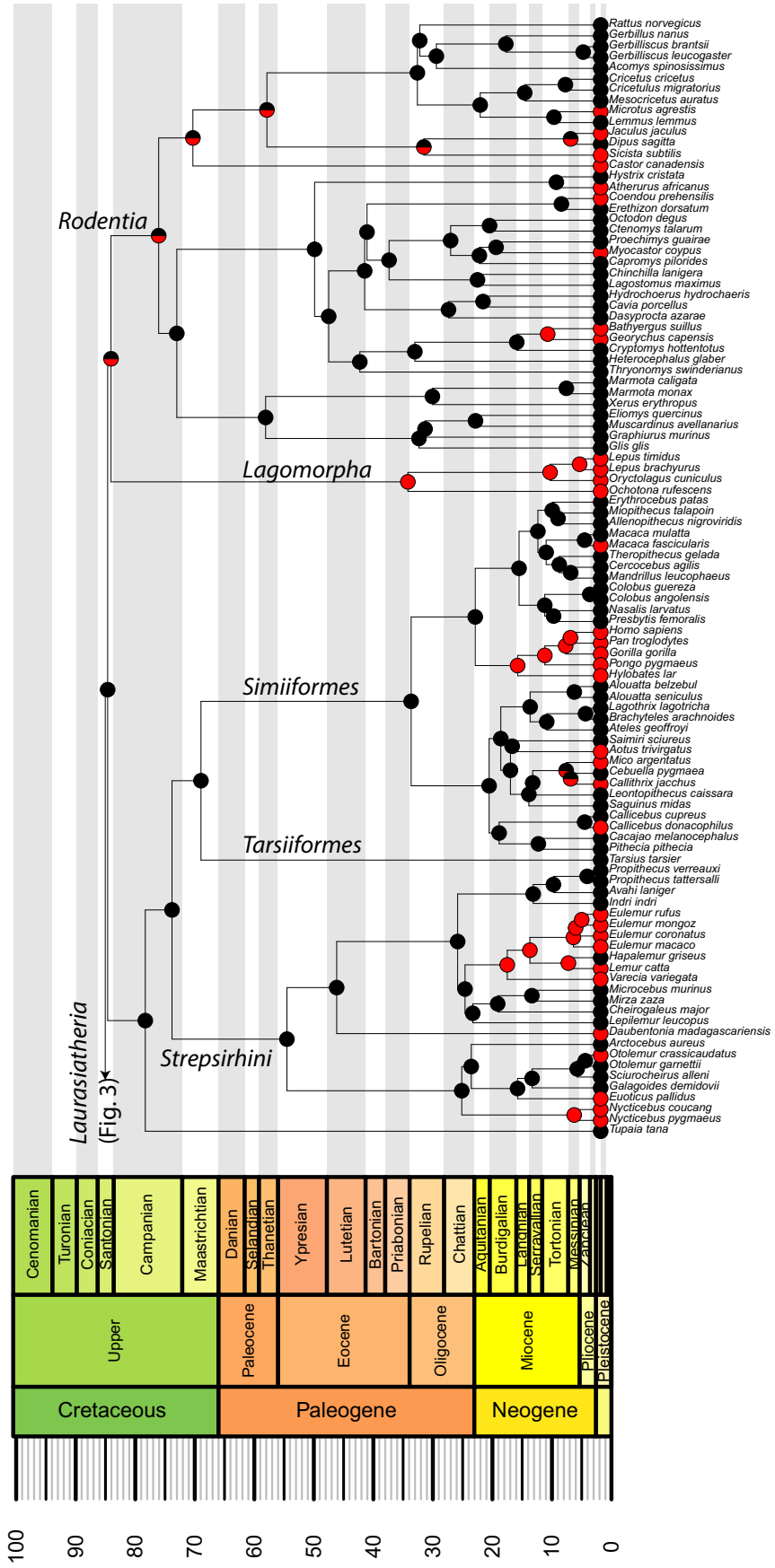


FIGURE 2 Phylogeny of *Euarchontoglires* (tree *n* 100 tips-dated) with MPR of cecal appendix presence. MPR, most parsimonious reconstruction

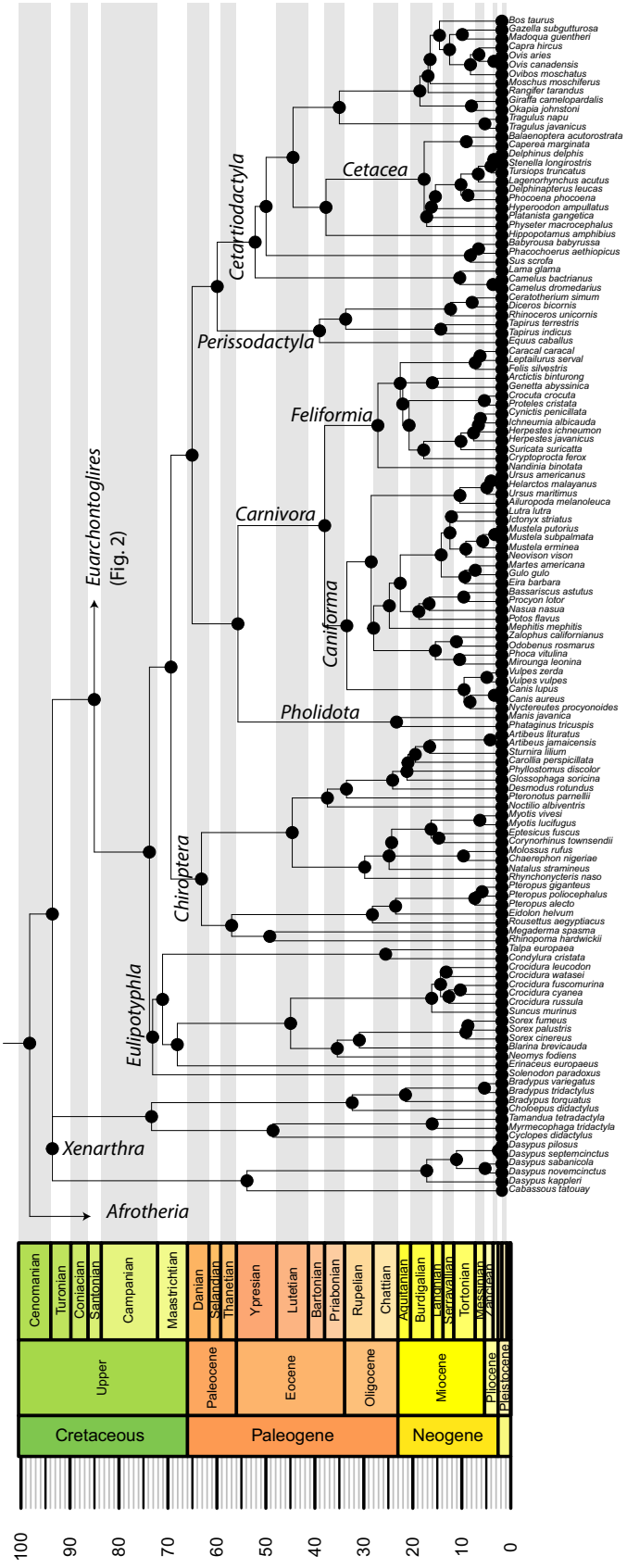


FIGURE 3 Phylogeny of *Laurasiatheria* (tree *n* 100 tips-dated) with MPR of cecal appendix presence. MPR, most parsimonious reconstruction

little opportunity to favor alleles delaying senescence and increasing longevity. This relationship between reduction in extrinsic mortality and increased longevity has been observed in wild (Shattuck & Williams, 2010; Tozzini et al., 2013) and captive animals (Stearns et al., 2000). However, this historical theory of aging has been challenged several times through mathematical approach of senescence (Abrams, 1993; Moorad et al., 2019; Wensink et al., 2017), by animal observations (Reznick et al., 2004) and by theoretical considerations (Day & Abrams, 2020; Gaillard & Lemaitre, 2017; Kozlowski et al., 2020). We propose an application of the theory of aging to our observed increased maximal longevity in mammals with a cecal appendix (Figure 5). This evolutive sequence suggests that the cecal appendix has a function.

Our study improves our understanding of the evolutionary history of the cecal appendix. Previous studies had identified between six (Smith, Fisher, et al., 2009) and up to 41 appearances of the appendix (Smith et al., 2017), but this high number (41) resulted partly from the inclusion of several polytomies, which create character optimization problems (Maddison, 1989). To a lesser extent, the lower number of included species (317 species in the long dataset, compared with 533 in Smith et al. (2017), other topological changes, and rescoring of a few taxa (we mentioned two such cases) explain these differences. The numbers that we report here should thus be more reliable and more conservative than those reported in previous studies.

The highly significant heterogeneity in the evolutionary rate of the cecal appendix among mammal clades is both extreme and intriguing (Figures 2 and 3). They are well documented in our database to the extent that they are based on the scores of about 200 taxa, which represent two very large mammalian clades (*Laurasiatheria* and *Euarchontonglires*), which form *Boreoeutheria* and account for more than 95% of the biodiversity of placental mammals (4641 species, according to http://www.onezoom.org/life.html, consulted on April 22, 2021). This well-corroborated evolutionary pattern suggests that the classical Markov evolutionary model for discrete characters (Lewis, 2001) would not suitable to study the evolution of the cecal appendix, as is the case for many phenotypic characters (Goloboff et al., 2019), though more complex models might possibly be appropriate (Dang & Golding, 2016).

The biological significance of this pattern is difficult to decipher. It has been previously shown that this evolutionary heterogeneity does not seem to be caused by ecological factors (Smith et al.,

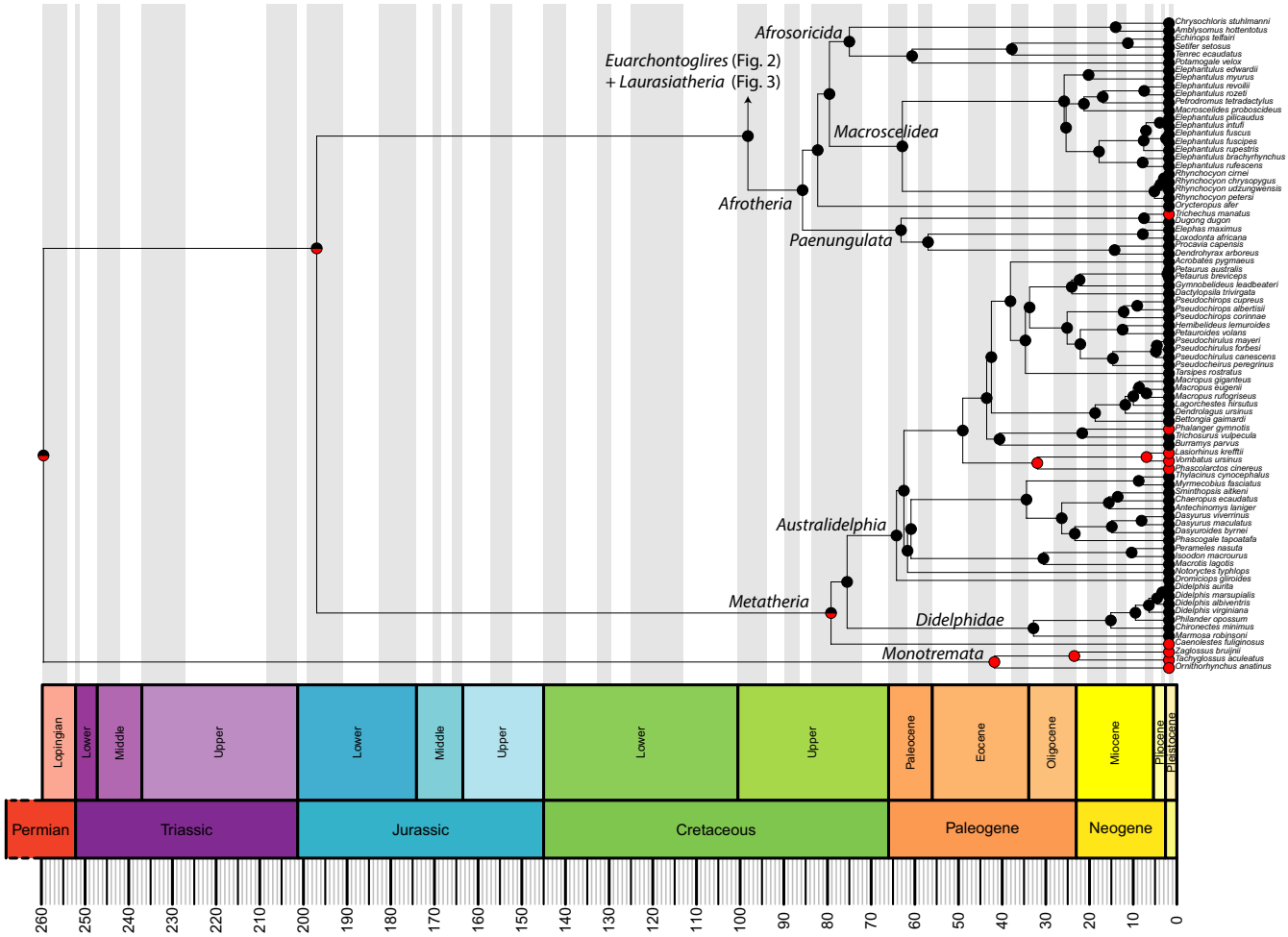


FIGURE 4 Phylogeny of *Monotreme*, *Marsupials*, and *Afrotheria* (tree *n* 100 tips-dated) with MPR of cecal appendix presence. MPR, most parsimonious reconstruction

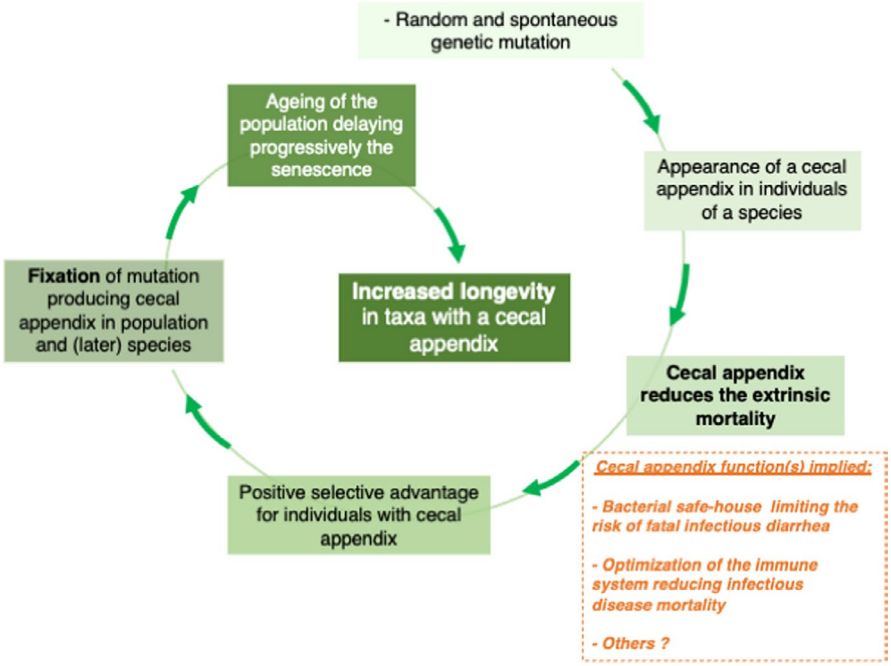


FIGURE 5 Chart of the consequences of the spontaneous appearance of the cecal appendix within a species

2017). Perhaps, among *Laurasiatheria*, the function performed by the cecal appendix may be assumed by different structures, or extrinsic mortality may differ in those clades, thus decreasing the potential benefits of a cecal appendix. But this question currently remains unanswered.

Prevention of the severity of infectious diarrhea is one of the suspected functions, which would act as a bacterial "safe-house" (Bollinger et al., 2007; Laurin et al., 2011). Infectious diarrhea constitutes a major cause of extrinsic mortality among mammals. In humans, it is currently the second worldwide leading cause of death before the age of 5 years according to the World Health Organization (Bryce et al., 2005). This mortality is clearly age dependent in humans, given that it affects mostly neonates and elderly individuals (Khalil et al., 2018). This high mortality caused by infectious diarrhea has been also observed among other mammalian taxa (Virtala et al., 1996; Watson et al., 2017). A study dedicated to the exploration of diarrhea among 18 colonies of nonhuman primates reported a particularly high annual incidence of diarrhea of 10.6% with a global annual mortality related to an episode of diarrhea of 1.2% (Hird et al., 1984). Its shape and its secreted biofilm (Bollinger et al., 2007) make the cecal appendix a bacterial sanctuary that facilitates the colonic recolonization by the nonpathogen and symbiotic gut microbiota. *Clostridium difficile*-colitis illustrates perfectly this hypothetical function. This disease caused by the overgrowth of *C. difficile*, which temporarily displaces part of the colonic microbiota, which becomes toxicogenic and harmful, has been described in several mammalian taxa such as horses (Diab et al., 2013), elephants (Bojesen et al., 2006), or humans (Im et al., 2011). Interestingly, in humans, a significant increased risk of recurrence of this infectious colitis has been observed in patients that have undergone appendectomy (Im et al., 2011). To sum up, the greater longevity in taxa possessing a cecal appendix may result from decrease in extrinsic mortality, resulting from a lower risk of fatal infectious diarrhea. Interestingly, the cecal appendicular microbiota analysis from the primate *Daubentonia madagascariensis* revealed a different composition than the cecal or colonic microbiota characterized by a lower alpha-diversity associated with an enrichment of beneficial bacteria and a greater evenness (Greene & McKenney, 2018).

Prevention of infectious diarrhea through a microbiota "safe-house" is the most frequently mentioned function in the literature, but other hypotheses should be considered, such as an immune function. Indeed, we considered only an anatomical definition of the cecal appendix, but in many cases, the cecal appendix is also the site of a high density of gut-associated lymphoid tissue (GALT). This association suggests that the cecal appendix may be the site of an immune education that could optimize the immune response against pathogens, leading to a lower extrinsic mortality due to infectious diseases. However, few data in the literature allow testing this hypothesis because information about the presence or not of high lymphoid tissue concentration at the cecal appendix site in each taxon is often missing. In our database, this important information was available in only for 21 out of 39 nominal species that we consider to have a cecal appendix, and 95% (20/21) of these species were found to have a high concentration of lymphoid tissue in the appendix. This information is particularly difficult to obtain with certitude in the literature because GALT appears early in ontogeny and involutes with aging, as it was reported in humans (Gebbers & Laissue, 2004) and rabbits (Dasso et al., 2000). Thus, analyses of the cecum at different ontogenetic stages as well as a precise definition of the lymphoid tissue concentration should be better documented for future comparative studies, particularly in the absence of a lymphoid concentration in the cecum of an old mammal specimen. Although

some species without cecal appendix present a high concentration of lymphoid tissue in the cecum (Malla, 2003), the reported significant positive correlation between high concentration of lymphoid tissue and the presence of a cecal appendix suggests a functional relationship between these two elements (Smith, Parker, et al., 2013). Bacterial translocations occur within these cecal lymphoid tissues and participate to the immune system education and tolerance by controlled antigenic presentation (Gebbers & Laissue, 2004). Thus, the narrow shape of the cecal appendix could improve this microbiota antigenic exposure by trapping specific bacteria. This hypothesis on the function of the cecal appendix is compatible with a decrease in infectious mortality.

Other functions are suspected. For example, in human patients followed for a chronic inflammation of the colon and rectum in the context of ulcerative colitis, appendectomy is suspected to increase the risk of colorectal cancer (Harnoy et al., 2016; Stellingwerf et al., 2019). This observation may indicate that the cecal appendix could also play an antitumor function, although the fundamental mechanism involved is unknown. To summarize, various possible functions of the cecal appendix are currently investigated. The coexistence of several functions reducing extrinsic mortality may also reinforce the positive selective advantage conferred by the cecal appendix that we observed among mammals.

Finally, would the appendectomy so frequently performed in humans be harmful to longevity? This question has never been directly studied and would constitute an interesting topics for future research. Our conclusion, using comparative data, is that there is an increased longevity within taxa possessing a cecal appendix linked to a delayed senescence by a prolonged decrease in extrinsic mortality. If the main function of the cecal appendix is to act as a bacterial reservoir limiting the risk of fatal infectious diarrhea, many arguments that suggest that appendectomy should have no impact on human longevity. These include improvement of water quality and of medicine, in particular by antibiotic therapy. In addition, appendectomy most often occurs after the first years of life, when the risk of fatal infectious diarrhea is the highest. If a main function of the cecal appendix is the optimization of antigenic exposure via the lymphoid tissue, appendicitis may be an excellent enhancer of immune function, and as the appendectomy often occurs after this intense inflammation, it should be of no consequence. All this argumentation is based on the uncertain functions of the cecal appendix, and our results do not provide any decisive arguments for fearing a decreased longevity after appendectomy at the individual level in humans. But in other mammalian taxa, the possible negative selective value caused by complications linked to the cecal appendix, such as its inflammation for example, should be more than compensated by the positive selective value conferred by the function of this structure. This would explain, on the one hand, the evolutionary asymmetry of this structure largely favorable to the gain and, on the other hand, the benefit in terms of extended longevity.

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CONFLICT OF INTEREST

The authors declare no competing interests.

AUTHOR CONTRIBUTIONS

MKC, ML, and EO-D performed the conceptualization. JB, ML, and EO-D performed the methodology. MKC performed the data collection. JB performed the formal analysis. MKC did the writing—original draft. JB, ML, and EO-D did the writing—review and editing. ML and EO-D did the supervision.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the Supplementary Material of this article.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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