C. R. Palevol 12 (2013) 339–354

Contents lists available at ScienceDirect

Comptes Rendus Palevol

www.sciencedirect.com

General palaeontology, systematics and evolution (Evolutionary processes, microevolution)

Multiple independent appearances of the cecal appendix in mammalian evolution and an investigation of related ecological and anatomical factors

Multiples apparitions indépendantes de l'appendice du cæcum dans l'évolution des mammifères et une étude des facteurs écologiques et anatomiques pertinents

Heather F. Smith[a,b,*], William Parker[c], Sanet H. Kotzé[d], Michel Laurin[e]

[a] *Department of Anatomy, Arizona College of Osteopathic Medicine, Midwestern University, Glendale, AZ 85308, USA*
[b] *School of Human Evolution and Social Change, Arizona State University, Tempe, AZ 85287, USA*
[c] *Department of Surgery, Duke University Medical Center, Durham, NC 27710, USA*
[d] *Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, University of Stellenbosch, Tygerberg, South Africa*
[e] *UMR 7027, CNRS/MNHN/UPMC, centre de recherches sur la paléobiodiversité et les paléoenvironnements, Muséum national d'histoire naturelle, 75005 Paris, France*

ARTICLE INFO

Article history:
Received 7 November 2012
Accepted after revision 8 December 2012
Available online 7 February 2013

Presented by Philippe Taquet

Keywords:
Cecal appendix
Mammalian phylogeny
Gastrointestinal anatomy

Mots clés :
Appendice du cæcum
Phylogénie des mammifères
Anatomie gastro-intestinale

ABSTRACT

Although the cecal appendix has been widely viewed as a vestige with no known function or a remnant of a formerly utilized digestive organ, the evolutionary history of this anatomical structure is currently unresolved. A database was compiled for 361 mammalian species, and appendix characters were mapped onto a consensus phylogeny along with other gastrointestinal and behavioral characters. No correlation was found between appearance of an appendix and evolutionary changes in diet, fermentation strategy, coprophagia, social group size, activity pattern, cecal shape, or colonic separation mechanism. Appendix presence and size are positively correlated with cecum and colon size, even though this relationship rests largely on the larger size of cecum and colon in taxa that have an appendix. The appendix has evolved minimally 32 times, but was lost fewer than seven times, indicating that it either has a positive fitness value or is closely associated with another character that does. These results, together with immunological and medical evidence, refute some of Darwin's hypotheses and suggest that the appendix is adaptive but has not evolved as a response to any particular dietary or social factor evaluated here.

RÉSUMÉ

Même si l'appendice du cæcum a été considéré comme un vestige sans fonction connue ou comme un reste d'organe digestif autrefois fonctionnel, son histoire évolutive demeure énigmatique. L'évolution de l'appendice, d'autres caractères du tractus gastro-intestinal, ainsi que de caractères comportementaux est étudiée à l'aide d'une nouvelle banque de données de 361 espèces de mammifères. Aucune corrélation n'est détectée entre l'apparition de l'appendice et des changements de régime alimentaire, de la stratégie de fermentation, de la coprophagie, de la taille du groupe social, du patron d'activité, de la forme du cæcum, ou du mécanisme de séparation du côlon. La présence et la taille de l'appendice sont

---

* Corresponding author. Department of Anatomy, Arizona College of Osteopathic Medicine, Midwestern University, Glendale, AZ 85308, USA.
E-mail addresses: hsmith@midwestern.edu, heather.f.smith@asu.edu (H.F. Smith).

1631-0683/$ – see front matter © 2012 Académie des sciences. Publié par Elsevier Masson SAS. Tous droits réservés.
http://dx.doi.org/10.1016/j.crpv.2012.12.001

positivement corrélées à la taille du côlon et du cæcum, même si cette relation semble provenir surtout de la plus grande taille du côlon et du cæcum chez les espèces pourvues d'un appendice. L'appendice est apparu au moins 32 fois, mais il a été perdu moins de sept fois, ce qui indique, soit une valeur sélective positive, soit une association étroite avec un caractère possédant une telle valeur sélective. Ces résultats, avec des données immunologiques et médicales, réfutent certaines hypothèses de Darwin et suggèrent que l'appendice est adaptatif, mais qu'il n'a pas évolué en réponse aux régimes alimentaires ou facteurs sociaux considérés dans cette étude.

1. Introduction

The cecal appendix has long been an anatomical structure of intrigue, with historically uncertain function and a well-earned reputation for inflammation that results in frequent surgical removal (MacFayden et al., 2000). Its interest to early anatomists is apparent in the numerous original publications on the subject (Arvy, 1972; Bockman, 1983; Bürgi, 1905; Cave, 1936; Eggeling, 1920; Gluckmann, 1939, 1947a, b; Jacobshagen, 1922; Keith, 1912; Kostanecki, 1913, 1926; Muthmann, 1913). Recent studies have revealed that the appendix has undergone a complicated evolution, which has not been easy to reconstruct (Laurin et al., 2011; Smith et al., 2009). It projects from the cecum, the most proximal section of the large intestine, which typically functions as a fermentation chamber involved in cellulose breakdown; as such, the appendix could be inferred to be affected by diet, cellulose content in the diet, cecal size and shape, and/or other anatomical characteristics of the gastrointestinal system.

The lack of evident function of this organ in humans was discussed by Charles Darwin in his famous *On the Descent of Man...* (Darwin, 1871). Darwin observed that humans, along with other apes, uniformly possess a cecal appendix, but that its size and structure render it incapable of participating to any notable degree in digestion, despite its location along the intestinal tube. Darwin posited that in extant hominoids (great apes), who are for the most part frugivorous, the shift from a predominantly folivorous ancestor to a descendent with diet requiring less fermentation led to a reduction in cecal size, which was in turn associated with the appearance of the appendix. During Darwin's time, the presence of a cecal appendix had not been documented in many nonhuman taxa, so the idea that this structure was a uniquely hominoid trait was entirely reasonable. Thus, for many decades, the idea that the cecal appendix was a synapomorphy of the Hominoidea predominated (Clark, 1971; Groves, 1986; Hill, 1972; Napier and Napier, 1985; Scott, 1980).

More recently, Fisher conducted a broad survey of published descriptions of the presence or absence of cecal appendices across primate species (Fisher, 2000). She discovered that the appendix is present in many more species than is traditionally recognized. It is found, at least variably, in several species of lorises, lemurs, New World monkeys, and Old World monkeys, and is found ubiquitously in all apes (Fisher, 2000). Based on these findings, Fisher argued that the appendix should be identified and defined as more than simply a distinct narrow-lumened apex projecting off the cecum, but that related characteristics such as a concentration of lymphoid tissue and a thickening of the cecal wall should also be considered (Fisher, 2000).

Although Darwin initially posited that the appendix lacked an important biological function, the idea that the appendix must have some important function has not been without support. For example, the observation that the vermiform appendix is associated with substantial amounts of gut-associated lymphoid tissue (GALT) was made more than a century ago (Berry, 1900), and suggested that the appendix may have an immune function. A few years later, Keith argued strongly, based on phylogenetic observations and other evidence, that the vermiform appendix probably did have a function, and in fact that appendicitis was due to changes in behavior associated with industrialization rather than a "degenerate" nature of the structure (Keith, 1912). Keith's ideas were supported by numerous others in the following decades (Barker et al., 1988; Boroda, 1961; Bremner, 1964; Burkitt, 1969, 1971; Gelfand, 1956; Janssens and de Muynck, 1966; Scott, 1980; Trowell, 1960; Walker et al., 1973), culminating with the identification of the vermiform appendix as a "safe-house" for beneficial bacteria with the capacity to re-inoculate the gut following depletion of the normal flora after diarrheal illness (Bollinger et al., 2007; Laurin et al., 2011). The identification of this function was based on old observations regarding the appendices' size, shape, location, and association with GALT, in combination with more recent findings that the immune system supports microbial biofilm growth in the large intestine (Everett et al., 2004; Sonnenburg et al., 2004; Thomas and Parker, 2010), and that intestinal biofilms are most abundant in the appendix (Bollinger et al., 2007).

The idea that the appendix is a safe-house for beneficial bacteria can be demonstrated by deduction (Bollinger et al., 2007). This function is consistent with a wide range of corroborating evidence (Laurin et al., 2011; Smith et al., 2009). First, the observation that the appendix is associated with a large amount of GALT (Berry, 1900) and the discovery that the immune system supports intestinal biofilms (Everett et al., 2004; Sonnenburg et al., 2004; Thomas and Parker, 2010) are consistent with this function as a safe-house. Second, the size and anatomical location of the appendix are well suited for inoculation of the gut and for avoidance of contamination by pathogens that might infect the main fecal stream (Laurin et al., 2011). Third, the newly recognized importance of the microbiome to human health (Cho and Blaser, 2012) and the high impact of diarrheal illness in developing countries (Guerrant et al., 1990) suggest that rapid recolonization of the gut following diarrheal disease may be important for survival. Fourth, a broad consideration of symbiotic relationships in biology indicates that host support of microbial biofilms is a widespread component of host-microbial interactions. Finally, the safe-house function of the appendix is consistent with the very low rates of complications following appendectomy in post-industrial culture, where modern medicine, a general lack of malnourishment coupled with routine sanitation, and water treatment practices makes diarrheal disease of little overall concern. On the other hand, recent evidence indicates that individuals without an appendix are susceptible to recurrent overgrowth of the opportunistic pathogen, *Clostridium difficile* (Im et al., 2011). While this observation does not directly demonstrate that the vermiform appendix functions as a safe-house for bacteria, it does demonstrate some function and it is consistent with the function as a safe-house even in the presence of modern sanitation and water treatment practices.

Given the recently improved understanding of the function of the appendix in biology and in medicine, the evolution of the appendix has garnered increased attention. In a broad survey of the cecal appendix across mammalian families, Smith et al. (2009) determined that the appendix appeared (and in some cases disappeared) several times in at least three major mammalian clades: primates, glires (lagomorphs and rodents), and marsupials. Within these groups, the appendix takes on three discernable morphotypes: the classic primate "vermiform" morphology, an elongated cecum that tapers into an appendix (in some lagomorphs, marsupials, and rodents), and a small appendix-like structure in the complete absence of a cecum, as in the wombat (Smith et al., 2009). Since the latter category is of unclear ontogenetic origin, the authors acknowledged that it may derive from a cecum through a drastic size reduction and shape change.

In previous surveys of the cecal appendix at the family level, the appendix has been estimated to have appeared a minimum of two times throughout the course of mammalian evolution (Laurin et al., 2011; Smith et al., 2009); however, this taxonomic scale provides limited resolution regarding evolutionary events compared to the information that could be obtained by investigating at the species level, and the authors indicated that this number is likely an underestimate (Smith et al., 2009). Almost half of the families that contain species possessing an appendix also contain species lacking one, suggesting greater intrafamilial variation in this structure than has been typically recognized (Jones, 1929). In any case, sampling at a given taxonomic level corresponding to a Linnaean category places inappropriate constraints on comparative studies (Laurin, 2010b), and in this study, we have tried to lift this constraint to the extent possible by sampling to the lowest taxonomic level for which information was available, namely the species. Additionally, while several studies have postulated various ecological and anatomical hypotheses as to why the appendix evolved and what potential function(s), if any, it might serve, until this point no study has attempted to identify specific aspects of diet, social behavior, and digestive anatomy that correlate with the presence of an appendix across the entire mammalian diversity. In the present study, we attempt to determine whether appendix presence is correlated with other anatomical and ecological variables, and obtain a more accurate estimate of how many times it has evolved. In this manner, we probe the hypothesis that the evolution of the appendix is not associated with decreasing cecum size, but rather that it is associated with particular social or dietary factors that might increase the survival advantage of an intestinal safe-house for bacteria.

2. Materials and methods

2.1. Data compilation

In order to assess the phylogenetic distribution of the cecal appendix across mammals and attempt to correlate it with ecological and anatomical variables, a broad literature search of published studies on mammalian gastrointestinal anatomy was conducted. At the species level, anatomical data were compiled for appendix presence and length, cecal size and morphology, colon length, presence of colonic separation mechanisms (CSMs), and stomach histological lining (entirely glandular *vs.* some squamous epithelium) for a broad sample of 361 species representing all major mammalian clades (SOM 1, 2). For quantitative variables, numeric values were compiled from the literature. For qualitative characters, published descriptions were used. The nomenclature of species names follows the on-line edition of a standard on-line reference (Wilson and Reeder, 2005). To determine whether the presence of an appendix is associated with dietary factors, data on published dietary categories of these species were also compiled (SOM 3). Data on activity pattern (time of day that an animal is typically most active), body mass, and mean social group size for each species were taken from the PanTHERIA database (Jones et al., 2009). We also collated data on whether each species practiced coprophagia, which we define for the purposes of this study as the regular eating of any type of feces in sufficient amounts to make a significant contribution to the nutrition of the animal as reported in the literature.

Following Smith et al. (2009), we defined a cecal appendix as a narrow, close-ended extension off the apex of the cecum with a distinct change in diameter of the lumen between it and the cecum. While previous studies have also used characteristics such as a thickened wall and concentration of lymphoid tissue to define this structure (Fisher, 2000), there were insufficient published data on these characters in most species for their inclusion here.

Given that the presence and size of the cecum and appendix are thought to be related to cellulose richness in the diet (Darwin, 1871), we have scored diet type and ordered the states in a manner which roughly reflects increasing order of cellulose richness: 0, carnivory; 1, insectivory; 2, omnivory; 3, frugivory; 4, granivory; 5, gummivory; 6, folivory. To verify that our results did not depend too heavily on a detailed ordering scheme, we have also lumped the states into a binary character, in which state 0 represents a cellulose-poor diet (including carnivory, insectivory, omnivory, and frugivory) and state 1 represents a cellulose-rich diet (including folivory, granivory, and gummivory). We also examined slightly different coding schemes to assess the sensibility of our results to this factor, but these did not alter the results substantially (probabilities were about the same), so we do not report the results of these additional analyses below. The examined alternative, for the multi-state character, consisted of inverting the position of gummivory (to state 3) and frugivory (to state 5). For the binary character, we also assessed the related issue of impact of food hardness (hard, including insectivory, folivory, granivory, and gummivory; soft, including carnivory, omnivory, and frugivory), and again moving frugivory into the "hard" category. To determine whether a cecal appendix was found more frequently in combination with any particular cecal shape, a qualitative cecal morphology character was created with the following states: 0, cecum absent; 1, appendix-like; 2, spherical; 3, cylindrical; 4, tapering; 5, spiral; 6, paired.

2.2. Statistical analyses

The phylogeny follows a recent, nearly exhaustive, species-level mammalian phylogeny (Bininda-Emonds et al., 2007) for topology and branch lengths. More specifically, we have used the electronic version of the phylogeny (in NEXUS format), in the variant using the best estimates of molecular ages. We then pruned the tree to retain only the species in our sample, and added a few taxa from our sample to the tree using the literature (usually, the taxonomy in Wilson and Reeder [2005]) to determine the best topology and approximate divergence time. These added taxa are the marsupials *Chaeropus ecaudatus*, *Thylacinus cynocephalus*, and *Antechinmys laniger*, the afrotherians *Rhynchocyon cirnei* and *Elephantulus pilicaudus*, the phocid *Pagophilus groenlandicus*, and the wild horse *Equus caballus przewalskii*. The tree includes 361 terminal taxa representing all main mammalian clades. There are 19 polytomies, each with three to 13 daughter branches. Given that comparative tests require fully dichotomous trees or perform better with such trees, for the analyses using pairwise comparisons (described in more detail below), we used a set of 10 randomly resolved trees produced by Mesquite (Maddison and Maddison, 2011) by the addition of zero-length branches, and averaged the results (probabilities) over the 10 trees. In any case, when the average probability suggests significance, most tests using the individual random resolutions and the test using the tree with polytomies suggest significance, and conversely, when the average probability is over 0.05, in most cases, most other tests gave congruent results. This procedure is not problematic because pairwise comparisons are a non-parametric test that does not use branch lengths (thus, adding length to the randomly added branches would not have altered the results). We used the "most pairs" selection algorithm, which maximizes the number of compared pairs.

To compare the relative size of various parts of the digestive system, we divided linear measurements by the cubic root of body mass estimates that we collected from the literature. The adequacy of this transformation was checked through linear regressions in the Microsoft Excel analytical tools. The data matrix is available in Mesquite (Maddison and Maddison, 2011) NEXUS format (SOM 2).

To select appropriate analytical methods for our data, we performed several exploratory analyses. We determined if there was a phylogenetic signal in the data by comparing the tree length using squared-change parsimony, character by character, to a population of random trees. Because of the presence of polytomies in our master tree, we used the random taxon reshuffling procedure (Laurin, 2004). This procedure has the advantage, over the equiprobable tree algorithm, of retaining the same tree resolution and branch length distribution. The latter is required to deal with continuous characters, if squared-change parsimony is used (Maddison, 1991), because the squared length depends on branch lengths as well as on topology.

For continuous data, we had initially planned to use phylogenetic independent contrasts or PIC, for short (Felsenstein, 1985) to assess character correlation. However, diagnostic checks using the four tests available in the Phenotypic Diversity Analysis Program (PDAP: PDTREE) module of Mesquite (Midford et al., 2008) revealed very highly significant artifact that various data and branch length transformations failed to adequately remove. These artifacts include the four classical ones that Mesquite allows testing for, namely the following four regressions: 1, absolute value of the standardized contrasts vs. their expected standard deviation; 2, absolute value of standardized contrasts vs. nodal value; 3, absolute value of the standardized contrasts vs. node height; and 4, estimated nodal value vs. node height. In an ultrametric tree (as is the case here because only extant and very recently extinct taxa are represented), significant relationships in any of these four characters implies that the characters did not evolve according to Brownian motion over the reference tree, which may mean that the characters followed another evolutionary model, or that the topology and/or branch lengths are wrong. In any case, this means that PIC would yield unreliable results. Because of this, and because we have both discrete and continuous data, we decided to use the pairwise comparison test (Maddison, 2000; Read and Nee, 1995), which effectively evaluates the association between changes in two variables. All tests of correlations reported in this manuscript were made using pairwise comparisons. This test does not require the assumption that characters evolved according to a Brownian motion model (contrary to PIC), and can handle discrete as well as continuous variables, polymorphism, and even polytomies (at the cost of reduced power). More sophisticated methods are either designed for continuous data only, such as Phylogenetic Generalized Least Squares (PGLS), or disallow polymorphism (more than one state being present in a given taxon, which is abundant in our data), such as the Mesquite implementation of Pagel's (Pagel, 1994) correlation method.

To test the hypothesis that gains and losses of the appendix are equally frequent, we used a binomial distribution to assess the probability of this pattern, assuming that gains are as probable as losses. Binomial probabilities were calculated using GraphPad Software (http://www.graphpad.com/quickcalcs/binomial2.cfm).

To compare the evolutionary rates of the appendix in various clades, we have simply divided the number of inferred transitions (using parsimony) in each clade by that clade's sampled phylogenetic diversity index (Faith, 1992), which was calculated using the Stratigraphic Tools of Mesquite (Josse et al., 2006). To assess the statistical significance of the extreme difference in evolutionary rates between Euarchontoglires (fast evolution of the appendix) and its sister-group Laurasiatheria (no evolution of the appendix), we did a binomial test to assess the probability that all 33 changes observed in the smallest clade that includes Euarchontoglires and Laurasiatheria occur in Euarchontoglires, under the null hypothesis that chances are equally likely to occur in lineages of either clade. The null hypothesis is that the proportion of changes reflects the relative sampled phylogenetic diversity of both clades. The sampled phylogenetic diversity of Euarchontoglires is 4762.9 million years (Ma), and that of Laurasiatheria is 3220.1 Ma; thus, each of the 33 events had a probability of 0.592 of occurring within Euarchontoglires.

The use of parsimony both for phylogenetic signal detection and for inferring character history is justified largely by pragmatic considerations. We are unaware of software implementing maximal likelihood or Bayesian methods with the diversity of data that we have (continuous characters, multi-state discrete characters, both ordered and unordered, binary data, missing data, with a tree incorporating polytomies). In any case, parsimony has the advantage of having been used for such analyses for decades, so its properties are well understood. Bayesian methods are advantageous especially when there is a population of trees in which node frequency reflects support, but this is not the case here (in our randomized trees, node frequencies are essentially random).

Given the number of statistical tests performed below, we corrected for multiple testing using the False Discovery Rate, a procedure that is simple to use, statistically valid, and more powerful than conventional Bonferroni corrections (Benjamini and Hochberg, 1995; Curran-Everett, 2000).

3. Results

3.1. Appendix presence

Of the 361 mammalian species sampled here, 50 were found to possess a cecal appendix (SOM 2). As previously noted by Smith et al. (2009), several different morphotypes of the appendix have been described in mammals (Fig. 1). An appendix was found to be present in the Metatheria and Euarchontoglires, but not Laurasiatheria.

3.2. Phylogenetic signal

Most characters display a strong phylogenetic signal (Table 1), which confirms that phylogeny-informed analyses (such as pairwise comparisons) are required to assess correlation between characters. This result also indicates that character history can be reliably inferred from optimizations (Cubo et al., 2002; Laurin, 2004).

3.3. Correlation between the evolution of the appendix and the evolution of other characters

Darwin's suggestion that the hominoid appendix is a vestige of a larger cecum was derived in part from the assertion that the appearance of the appendix occurs concomitantly with decreases in cecum size and a shift from folivory to frugivory resulting in decreased dietary cellulose consumption. Parsimony optimization (Swofford and Maddison, 1987), a procedure that minimizes the changes on a tree to account for character state distribution, indeed confirms that the appearance of the appendix in hominoids is associated with a decrease in cecum size (Figs. 2–6) in these mostly frugivorous taxa. However, this may be a coincidence because no statistical test was performed to assess the probability that this association is random, and this is typically very difficult to do on singular events (as is the case here; there is a single appearance of the appendix in hominoids). Furthermore, our tests fail to support the general application of this model to the evolution of the appendix in mammals, since the changes in the state of the appendix were not associated with changes in the state of cellulose richness of diet (Table 2). However, changes in the character state of the cecum size were also not correlated with changes in cellulose digestion (Table 2), a counter-intuitive result that might possibly change with a denser taxonomic sampling.

Parsimony optimization of appendix presence and cecum presence and size (Figs. 2–6) refutes the suggestion that appendix appearance is linked to a decrease in cecum size within mammals. Thus, the ancestral mammal and monotreme are reconstructed as having had a small cecum (Fig. 2), and there is no reason to believe that a large cecum was present in any older ancestor of these taxa, yet, monotremes have an appendix. The same applies to the marsupial *Caenolestes fuliginosus*. The other marsupials with an appendix (*Phascolarctos cinereus, Vombatus usrinus, Lasiorhinus krefftii*, and *Phalanger carmelitae*) are in a part of the tree in which the optimization is ambiguous (Fig. 2), but in which the cecum may be small or mid-sized. In Euarchontoglires (and specifically within primates and glires) we find an appendix often associated with a large cecum (Figs. 5 and 6). A large cecum is clearly primitive for that clade, but only a small proportion of primates (and no glires) with a reduced cecum have an appendix. This last pattern, the only one consistent with Darwin's hypothesis, occurs only in the strepsirhine *Daubentonia madagascariensis*, in hominoids, and in some cercopithecids (Fig. 5). In the latter, an appendix is present in *Macaca nigra*, and the appendix is polymorphic (not being present in all individuals) in several other cercopithecids (*Procolobus verus, Pliocolobus badius, Colobus polykomos, Cercopithecus mitis, C. mona, Chlorocebus aethiops*, and *Papio hamadryas*). Among eutherians, the only other example consistent with Darwin's hypothesis is provided by the sirenian *Trichechus manatus*, in which appendix appearance is associated with a reduction in size of the cecum, from mid-sized to small (Fig. 3). However, a cecum reduction occurs in other clades that lack an appendix (Fig. 5), such as the strepsirhine *Cheirogaleus major*, the cebids *Alouatta* and *Ateles*, and several cercopithecids (*Nasalis larvatus, Presbytis femoralis, Colobus angolensis, Allenopithecus nigroviridis, Miopithecus talapoin, Erythrocebus patas, Macaca mulatta, Cercocebus agilis, Mandrillus leucophaeus*, and *Theropithecus gelada)*. Thus, among mammals, only *Trichechus manatus* and some catarrhine primates (especially hominoids) exhibit the

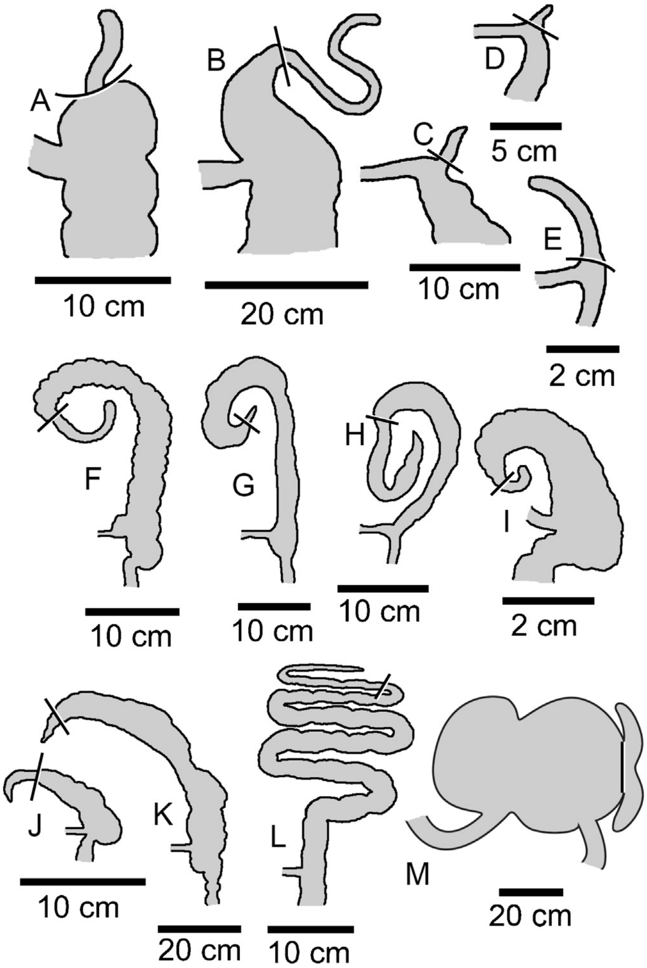

Fig. 1. The morphology of the cecal appendix in a variety of mammals. The black line indicates the approximate boundary of the appendix. (A) Human, *Homo sapiens*; (B) Orangutan, *Pongo pygmaeus*; (C) Southern hairy-nosed wombat, *Lasiorhinus latofrons*; (D) Echidna, *Tachyglossus aculeatus*; (E) Platypus, *Ornithorhynchus anatinus*; (F) Rabbit, *Oryctolagus cuniculus*; (G) Ground cuscus, *Phalanger gymnotis*; (H) Common brush-tail possum, *Trichosurus vulpecula*; (I) Cape dune mole-rat, *Bathyergus suillus*; (J) Brush-tailed porcupine, *Atherurus africanus*; (K) Beaver, *Castor canadensis*; (L) Koala, *Phascolarctos cinereus*; (M) Florida manatee, *Trichechus manatus*. (A, B, D, E, F, G, J, K, L) redrawn from Stevens and Hume (1995); (C) redrawn from Barboza and Hume (1992); (M) redrawn from Snipes (1984); (I) drawn based on samples from Sanet Kotzé. In the present study, cecal shape was defined as: 0, cecum absent; 1, appendix-like (as in C, D, E); 2, spherical (as in A, B, J); 3, cylindrical (as in I, K, M); 4, tapering (as in F, G, H, L); 5, spiral (not pictured); 6, paired (not pictured). Figure drawn by Brent Adrian.

Fig. 1. Morphologie de l'appendice du cæcum chez les mammifères. La ligne noire indique la limite approximative de l'appendice. (A) Humain, *Homo sapiens* ; (B) Orang-outan, *Pongo pygmaeus* ; (C) Wombat à nez poilu du sud, *Lasiorhinus latofrons* ; (D) Echidné, *Tachyglossus aculeatus* ; (E) Ornithorhynque, *Ornithorhynchus anatinus* ; (F) Lapin, *Oryctolagus cuniculus* ; (G) Phalanger, *Phalanger gymnotis* ; (H) Phalanger-renard, *Trichosurus vulpecula* ; (I) Rat-taupe du Cap, *Bathyergus suillus* ; (J) Athérure africain, *Atherurus africanus* ; (K) Castor, *Castor canadensis* ; (L) Koala, *Phascolarctos cinereus* ; (M) Lamantin des Caraïbes, *Trichechus manatus*. (A, B, D, E, F, G, J, K, L) redessinés d'après Stevens et Hume (1995) ; (C) redessinés d'après Barboza et Hume (1999) ; (M) redessinés d'après Snipes (1984) ; (I) dessin fondé sur des spécimens de Sanet Kotzé. Dans l'étude présente, la forme du cæcum est définie comme : 0, cæcum absent ; 1, similaire à un appendice (comme dans C, D, E) ; 2, sphérique (comme dans A, B, J) ; 3, cylindrique (comme dans I, K, M) ; 4, fuselé (comme dans F, G, H, L) ; 5, en spirale (pas illustré) ; pair (pas illustré). Figure dessinée par Brent Adrian.

predicted combination of an appendix accompanying a small cecum.

A more convincing demonstration that Darwin's hypothesis on the evolution of the cecal appendix is not generally applicable outside of hominoids comes from the strong positive correlation that we have found between changes in the relative lengths of the appendix, cecum, and colon (Table 2). When the appendix is absent, its length is considered to be 0, so this character also captures information about the presence or absence of the appendix, and this correlation is no longer significant if 0-values are excluded, reflecting the small sample size ($n = 9$) of the other taxa for which comparisons can be made. Darwin's hypothesis predicts a reverse relationship between sizes of the appendix and cecum, but instead, we find a fairly strong positive relationship ($P = 0.005$).

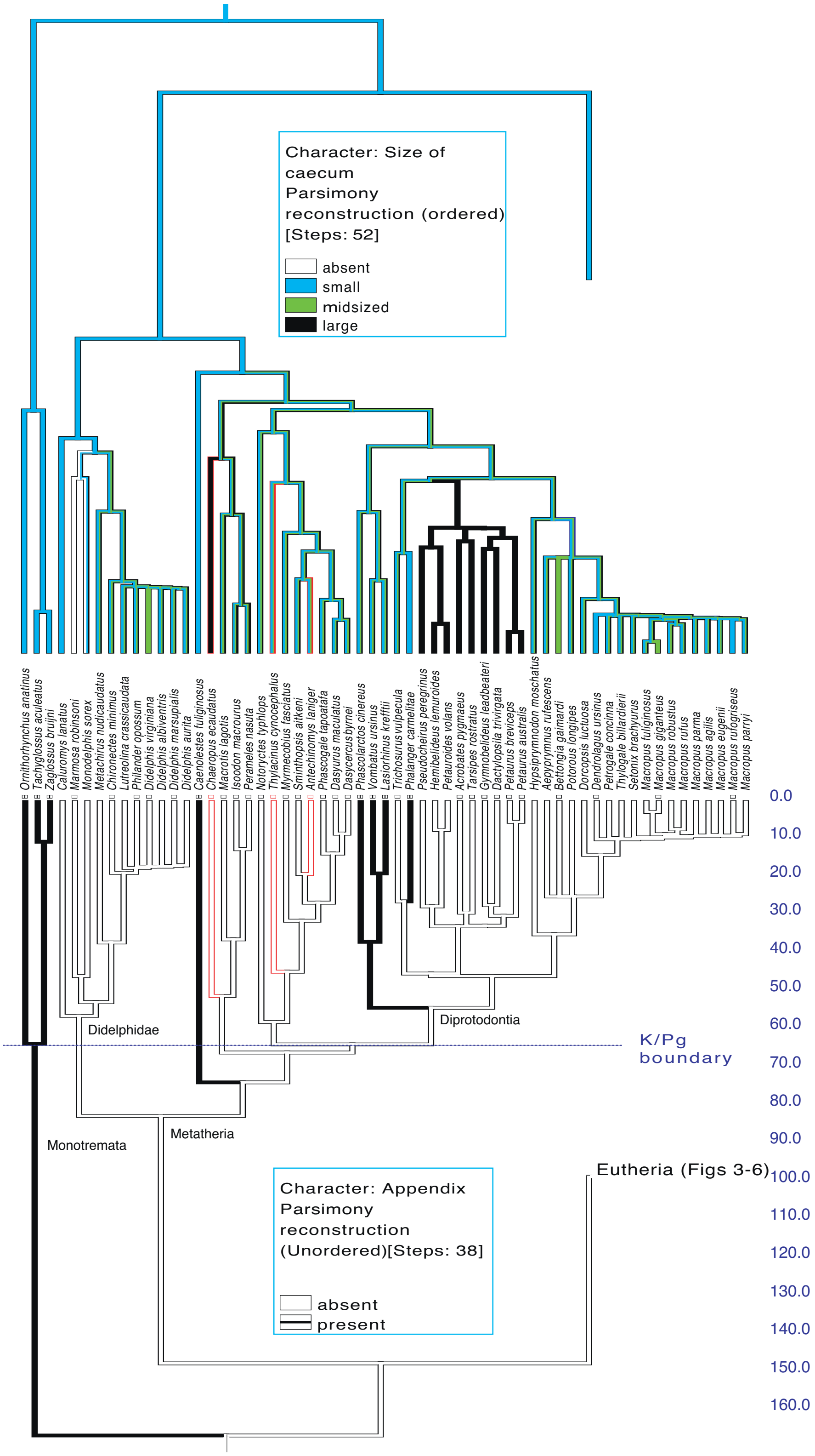

Fig. 2. Evolution of the appendix and cecum in monotremes and marsupials.

Fig. 2. Évolution de l'appendice et du cæcum chez les monotrèmes et les marsupiaux.

Table 1
Phylogenetic signal in the analyzed characters. The probability that the co-variation between character and phylogeny is random was assessed by comparing the number of steps (for discrete characters) or squared length (for continuous characters) over the reference tree to a population of 1000 random trees produced by randomly reshuffling the terminal taxa (Laurin, 2004). Corrections for multiple testing are done through the False Discovery Rate (Benjamini and Hochberg, 1995; Curran-Everett, 2000). Sample size is the number of taxa in which the character is scored.

Tableau 1
Signal phylogénétique dans les caractères analysés. La probabilité que la covariation entre le caractère et la phylogénie soit aléatoire est établie en comparant le nombre de pas (pour des caractères discrets) ou la longueur carrée (pour les caractères continus), sur l'arbre de référence à une population de 1000 arbres produits par réallocation aléatoire des taxons terminaux (Laurin, 2004). Des corrections pour tests multiples ont été faites par la méthode du « False Discovery Rate » (Benjamini et Hochbert, 1995 ; Curran-Everett, 2000). L'effectif de l'échantillon est le nombre de taxons pour lequel le caractère pertinent a été codé.

Character name	Type	Prob.	Prob. rank	FDR thr.	Sample size	Sig.
Cellulose richness in diet	Binary	< 0.001	5.5	0.009	275	Yes
Cellulose richness in diet	Multi-state, ordered	< 0.001	5.5	0.009	275	Yes
Coprophagia	Binary	< 0.001	5.5	0.009	78	Yes
Activity pattern	Multi-state, ordered	< 0.001	5.5	0.009	262	Yes
Group mean size	Continuous	0.223	17	0.029	154	No
Concentration of lymphoid tissue in cecum/appendix	Binary	1.000	26.5	0.046	25	No
Colonic separation mechanism	Binary	1.000	26.5	0.046	37	No
Stomach type (glandular or squamous)	Binary	< 0.001	5.5	0.009	140	Yes
Appendix presence	Binary	< 0.001	5.5	0.009	224	Yes
Appendix length (cm)/cubic root of body mass (g)	Continuous	0.008	7	0.025	185	Yes
Cecal morphology (shape)	Multi-state, ordered	< 0.001	5.5	0.009	194	Yes
Cecal haustrations	Binary	< 0.001	5.5	0.009	150	Yes
Cecum presence and size	Multi-state, ordered	< 0.001	5.5	0.009	145	Yes
Cecum length (cm)/cubic root of body mass (g)	Continuous	0.102	15.5	0.027	144	No
Colon length (cm)/cubic root of body mass (g)	Continuous	0.010	13	0.022	106	Yes

FDR thr.: False Discovery Rate threshold; Prob.: probability; Sig.: statistical significance.

Table 2
Correlation between characters assessed through pairwise comparisons in Mesquite. The probability represents the average probability over 10 random resolutions of the reference tree, which is slightly modified from Bininda-Emonds et al. (2007, 2008) by the addition of a few taxa. Corrections for multiple testing are done through the False Discovery Rate (Benjamini and Hochberg, 1995; Curran-Everett, 2000). When more than one test gave the same probability, the average rank was attributed to these tests. For some tests, polarity is undetermined because their number of positive and negative pairs was equal. All tests are based on comparisons of 180 pairs of terminal taxa, although not all pairs were informative due to missing data (Table 1) or lack of change in at least one character.

Tableau 2
Corrélation entre caractères, déterminée par le test de comparaisons de paires dans Mesquite. La probabilité représente la moyenne des probabilités de dix résolutions aléatoires de l'arbre de référence, qui est légèrement modifié de Bininda-Emonds et al. (2007, 2008) par l'ajout de quelques taxons. Des corrections pour tests multiples ont été faites par la méthode du « False Discovery Rate » (Benjamini et Hochbert, 1995 ; Curran-Everett, 2000). Quand plus d'un test a donné la même probabilité, le rang moyen a été utilisé pour ces tests. Pour certains tests, la polarité n'est pas déterminée car le nombre de paires positives et négatives était égal. Tous les tests sont fondés sur la comparaison de 180 paires de taxons terminaux, même si certaines paires ne sont pas informatives car il y a des données manquantes (Tableau 1) ou au moins un des caractères est invariable dans la paire.

Independent character	Dependent character	Polarity/Probability	Prob. rank	FDR thr.	Sig.
Cellulose richness (binary)	Cecum length/cubic root of body mass	Positive/0.102	15.5	0.027	No
Cellulose richness (binary)	Appendix length/cubic root of body mass	Negative/0.229	18	0.031	No
Cellulose richness (multi-state, ordered)	Cecum length/cubic root of body mass	Positive/0.413	20	0.034	No
Cellulose richness (multi-state, ordered)	Appendix length/cubic root of body mass	Negative/0.400	19	0.033	No
Coprophagia	Appendix presence	Undetermined/1.000	26.5	0.046	No
Stomach type (glandular or squamous)	Appendix presence	Positive/0.500	21	0.036	No
Activity pattern	Appendix presence	Positive/0.650	23	0.040	No
Concentration of lymphoid tissue in cecum/appendix	Appendix presence	Undetermined/1.000	26.5	0.046	No
Colonic separation mechanism	Appendix presence	Undetermined/1.000	26.5	0.046	No
Cecal haustrations	Appendix presence	Undetermined/1.000	26.5	0.046	No
Cecal morphology (shape)	Appendix presence	Negative/0.631	22	0.038	No
Appendix length/cubic root of body mass	Cecum length/cubic root of body mass	Positive/0.005	11	0.019	Yes
Appendix length/cubic root of body mass	Colon length/cubic root of body mass	Positive/0.017	14	0.024	Yes
Cecum length/cubic root of body mass	Colon length/cubic root of body mass	Positive/< 0.001	5.5	0.009	Yes

FDR thr.: False Discovery Rate threshold; Prob.: Probability; Sig.: statistical significance; –: non-significant; *: significant.

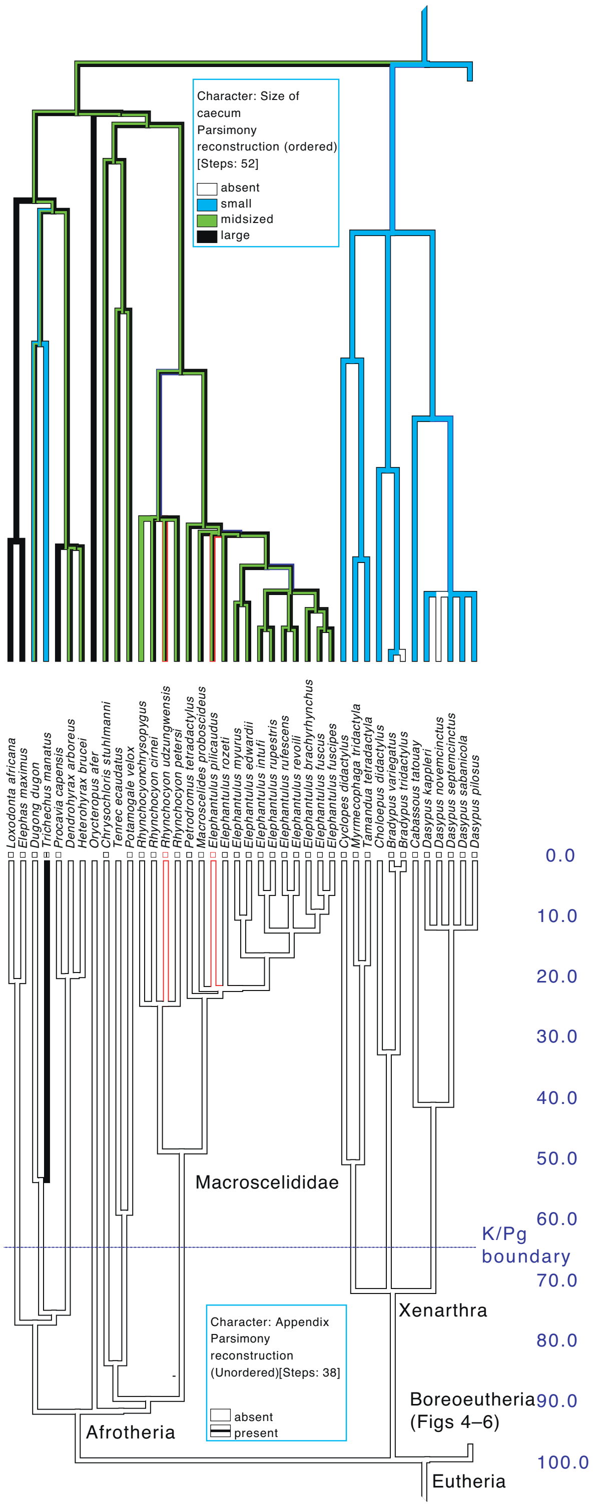

Fig. 3. Evolution of the appendix and cecum in Afrotheria and Xenarthra.
Fig. 3. Évolution de l'appendice et du cæcum dans Afrotheria et Xenarthra.

3.4. *Association between various characters and the evolution of the cecal appendix*

To evaluate other potential relationships that might provide insight into the evolution of the morphology of the proximal colon, we tested the relationship between appendix and colon size, and between cecum and colon size. Here our results show that all three parts of the digestive system that we studied are positively correlated with each other, and that the correlation between cecum and colon length is the strongest ($P < 0.001$), whereas the correlation between appendix and colon is the weakest, although still clearly significant ($P = 0.017$). This cannot be an indirect correlation reflecting body size because we had divided all length measurements by the cubic root of body mass, and linear regressions indicated that this effectively removed the size effect (which was present in the raw measurements). However, these correlations hold partly because most species without an appendix appear to have a smaller colon and cecum than species with an appendix, so this correlation reflects both presence and size of the appendix. Because of sample size, we are not currently able to determine if this correlation holds within species with an appendix, but this will be tackled in a subsequent study with a greater sample size.

The pairwise comparison test revealed no statistically significant correlations between changes in the appendix and changes in colonic separation mechanisms, cellulose in the diet, stomach wall histological composition, or cecal haustrations during the course of evolution. However, the relationship with CSM is less certain, because this character contains quite a bit of missing data. Alternate schemes of binary cellulose richness against group mean size and appendix, colon, and cecum length divided by cubic root of body mass, also resulted in statistically non-significant results. At least one of our multi-state coding schemes had granivory ranking as lower-cellulose than frugivory, but this also yielded non-significant results.

Additionally, appendices were not found to occur significantly more or less frequently in any particular character state of cecal morphology. Rather, the cecal appendix was observed to occur in at least one species in five of the seven categorical cecal shape states. Further, more highly social (those living in larger groups) and/or diurnal animals do not appear to be more likely to have an appendix than species living in smaller groups, or nocturnal or cathemeral/crepuscular species. A post hoc analysis including only taxonomic groups for which some species possess an appendix, the Euarchontoglires and Metatheria, also yielded no significant correlations ($P = 0.25–1.0$), suggesting that the appendix-less Laurasiatherians are not obscuring an otherwise observable pattern.

3.5. *Rate of evolution of the cecal appendix*

Across the entire mammalian phylogeny, the appendix was found to have undergone 38 evolutionary events, including 32 to 38 gains, and a maximum of six losses. The Chi^2 test shows that there are significantly more gains than expected by chance alone if gains were equally probable as

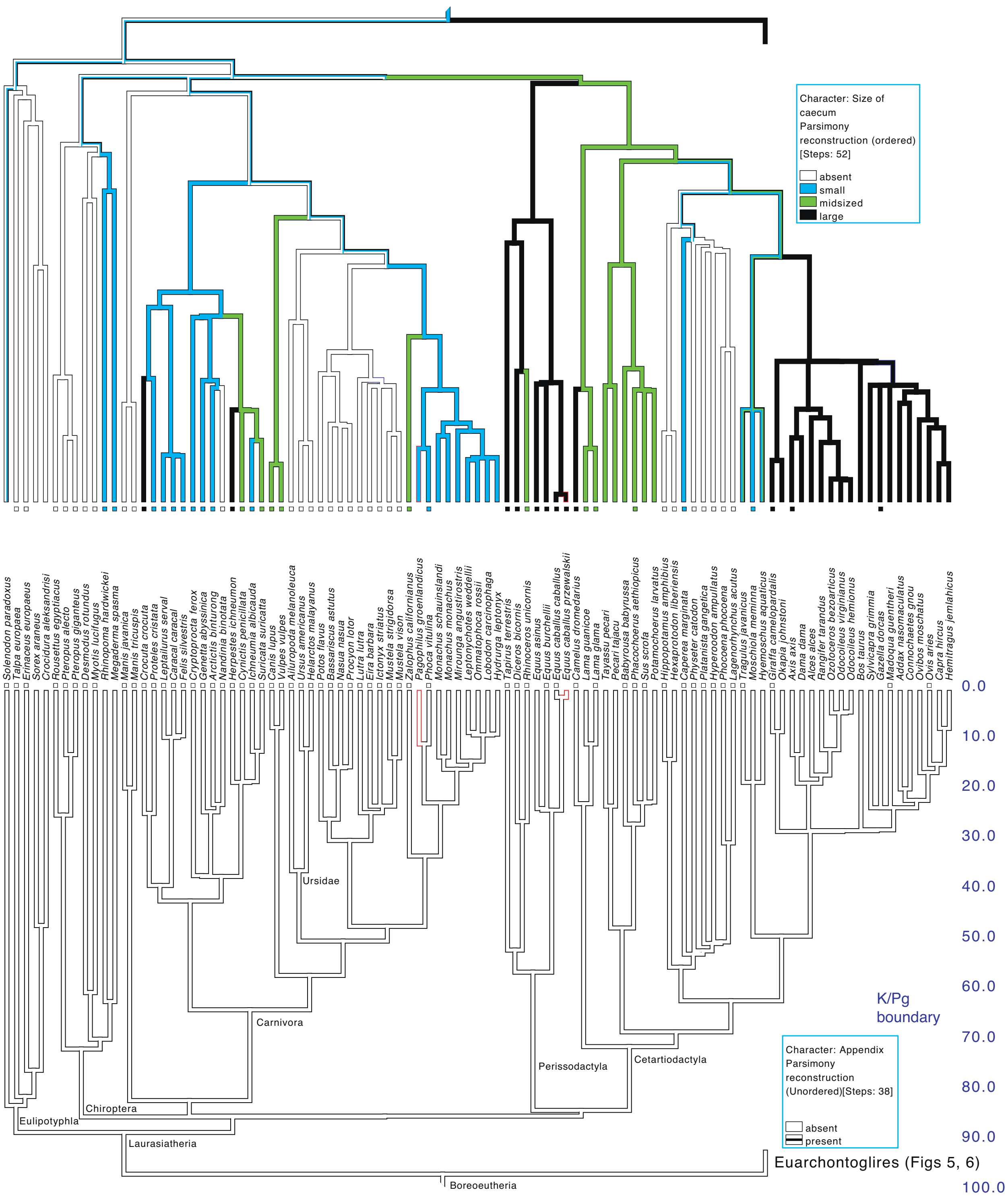

Fig. 4. Evolution of the appendix and cecum in Laurasiatheria.

Fig. 4. Évolution de l’appendice et du cæcum dans Laurasiatheria.

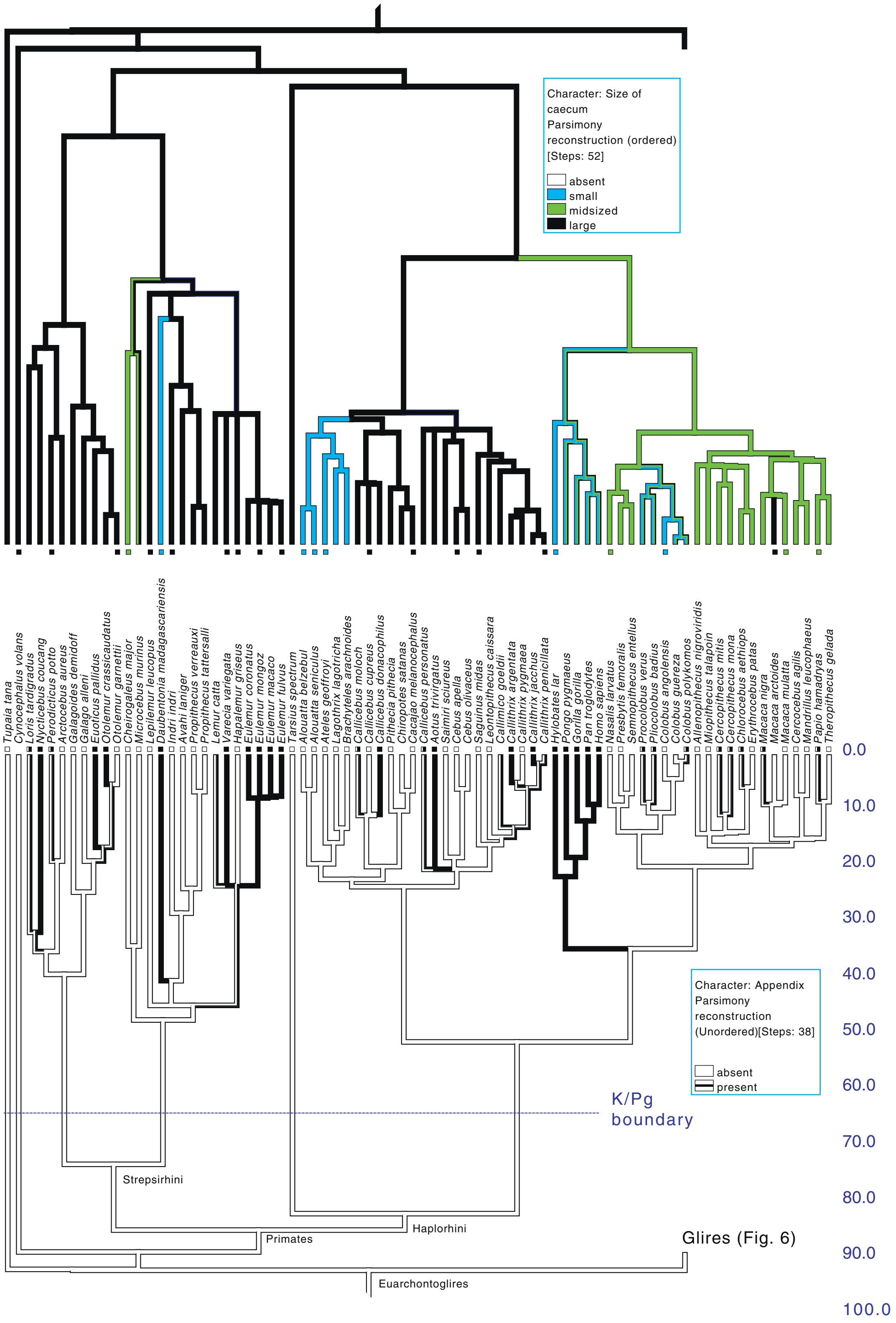

Fig. 5. Evolution of the appendix and cecum in primates and closely related taxa.

Fig. 5. Évolution de l'appendice et du cæcum chez les primates et des taxons étroitement apparentés.

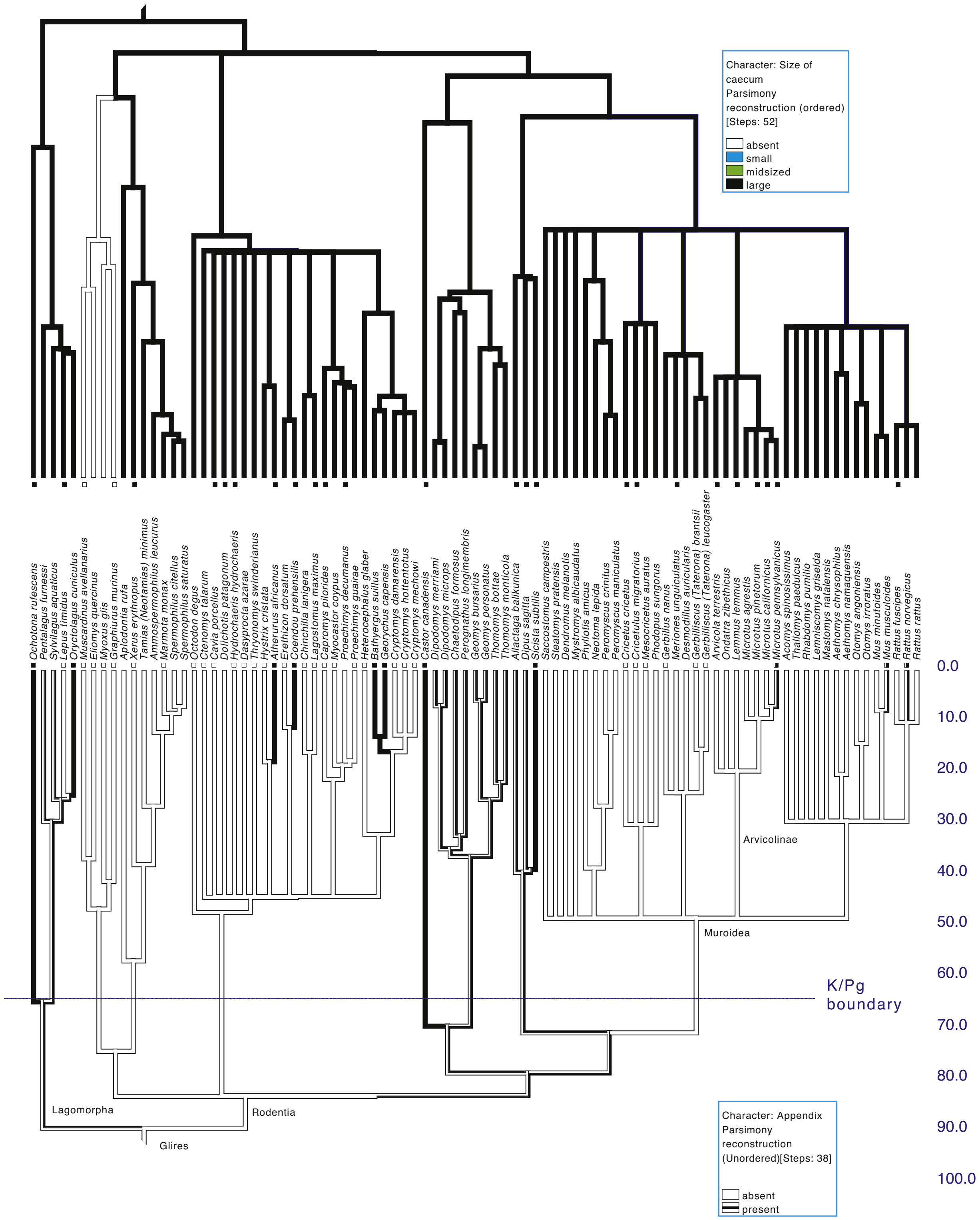

Fig. 6. Evolution of the appendix and cecum in Glires.

Fig. 6. Évolution de l'appendice et du cæcum chez les glires.

losses ($P < 0.00025$). A binomial test gives congruent results ($P < 0.0001$).

The appendix evolves at a global rate of 0.0034 transitions (gains or losses) per lineage and per million years (Ma) for mammals. However, that rate is extremely heterogeneous (Table 3), varying between 0 in Laurasiatheria (no identified transition) to 0.0071 in Euarchontoglires. Marsupials display an intermediate rate of 0.0019 transitions per lineage per Ma. These rates may be low because we have not sampled all species. Further, the rate may be underestimated since it is possible that multiple gains and losses occurred for polymorphic species, whereas we consider the transition to polymorphism as a single step. Given that polymorphism is restricted to Euarchontoglires in our matrix, the evolutionary rate in that taxon is likely more underestimated than in other taxa. The binomial test gives clear results: whether the comparison is between Euarchontoglires and Laurasiatheria, or between the former and all other placental mammals, the probability of getting such an extreme distribution if changes (per lineage and per Ma) are equally probable in all parts of the tree is less than 0.0001.

4. Discussion

Character optimization of cecum size and appendix presence refutes Darwin's hypothesis for evolution of the hominoid appendix (Darwin, 1871) as an explanation for the evolution of the appendix across all mammalian clades (Figs. 2–6). Among mammals, only catarrhines, and especially hominoids, along with the manatee *Trichechus*, follow a pattern compatible with Darwin's suggestion. Darwin formulated his hypothesis regarding the evolution of the appendix from his observations in humans and other hominoids, and as such, his interpretation of the appendix as being associated with a reduction in cecal size is ultimately correct for this clade; however, this association does not necessarily reflect a causal link. Hominoids were found in the present study to possess an appendix associated with a small cecum, a pattern that is consistent with Darwin's observations. However, this pattern is atypical of other mammalian clades, indicating that Darwin's hypothesis regarding the origin of the cecal appendix in hominoids does not apply to other clades. Rather, the appendix changes isometrically with cecal and colon sizes in most clades during the course of evolution, suggesting that length of the large intestine is a more influential factor in determining the size of the appendix than any other variable evaluated here. Our results appear fairly robust because in the 10 random resolutions of the master tree, the number of positive pairs (in which both characters change in the same direction) varies from 56 to 59, whereas the number of negative pairs (in which characters vary in opposite directions, as described by Darwin) ranges from 11 to 13. It thus seems apparent that, had Darwin had access to more data regarding the occurrence of the cecal appendix in mammals, he would not have considered the evolutionary scenario for the appendix described in his seminal work. With this in mind, it seems that the time is ripe to formulate a new hypothesis about the origin of the cecal appendix.

The absence of a correlation between evolutionary changes in diet and changes in the size of the cecum or the size and presence of an appendix is perhaps at first glance surprising, and could conceivably result from lack of power in the analysis. However, a high number of taxa were scored (excluding those scored as question marks or inapplicable) for diet (337) and appendix presence (224), and the number of scored taxa for quantitative characters was relatively high (185 for the appendix, 144 for the cecum, and 106 for colon length), suggesting that this result was not due to a lack of power of the test itself. Rather, a likely explanation for this result lies in the wide breadth of our analysis, which includes species that use a wide range of digestion strategies, and lumps into a single folivorous category some species using the cecum as the sole fermentation chamber, some using primarily the colon, some with a combination of the cecum and colon, and still others utilizing the foregut. Thus, future studies which attempt to dissect the simultaneous evolution of specific dietary regimens and particular gut morphologies should probably use a finer coding of diet and separate this from digestive strategy (e.g., foregut fermentation vs. cecal fermentation). Additionally, while we sampled a wide range of species representing each of the major mammalian groups, the taxon Mammalia is very speciose, with 5676 recognized species, of which only 361 were sampled here (6.4%). Thus, lack of power may affect a few of our tests, especially those concerning the concentration of lymphoid tissue in the cecum and appendix, colonic separation mechanism, and coprophagia, which are documented in the lowest number of species in our database (25 to 78; see Table 1). The inclusion of additional species and addition of data currently missing in our database in future studies could reveal patterns that were undetected by the present analysis.

The high asymmetry between gains and losses of the appendix, with a great predominance of the gains over losses, suggests that the appendix has a biological function (that it is selectively advantageous in most taxa) or that its evolution is correlated with that of a selectively advantageous character. The present analyses provide considerable insight in that regard. First, we can say, albeit with some caveats, that the evolution of the appendix does not appear to be strictly tied to changes in diet, sociality, or as a secondary by-product of cecal reduction across all clades. Thus, we have tested for correlation with the characters that the literature suggests might be correlated with the appearance of the appendix but found no evidence for such correlations. One caveat to this is that we used group size as a proxy for sociality, although group size alone does not capture all aspects of sociality. Another caveat is that the considerations of dietary intake we utilized do not take into account potentially important aspects of digestion such as the efficiency of energy extraction from ingested food. A second conclusion, which can be stated with confidence, is that changes in the presence and relative size (adjusted for body mass) of the appendix during the course of evolution tend to correspond directly, not inversely, with changes in the relative size of the cecum and even of the colon. Thus, the size of the appendix, per se, is probably more related to factors that determine the size of the midgut and hindgut in general than to any particular

Table 3
Evolutionary rates of the appendix in several major mammalian clades.
Tableau 3
Vitesse d'évolution de l'appendice dans plusieurs clades mammaliens.

Taxon	Number of transitions	PDI	Transition rate
Mammalia	38	11280.6219	0.0034
Metatheria	3	1543	0.0019
Laurasiatheria	0	3220.1219	0[a]
Euarchontoglires	33	4672.9	0.0071[a]

PDI: Phylogenetic Diversity Index; Transition rate: events/lineage per Ma.

[a] Transition rates of the Euarchontoglires and Laurasiatheria are significantly different ($P < 0.00001$).

factor dealing with immune function. A strong asymmetry between gains and losses is akin to an evolutionary trend, although trends are usually studied in continuous rather than in discrete characters (Laurin, 2010a; McNamara, 1980; McShea, 2000). Nevertheless, we interpret the apparent trend towards appearance of the appendix as lending support to the hypothesis that the appendix is selectively advantageous in most mammalian taxa because if it were selectively neutral, losses should be about as common as gains.

The apparent appendix found in absence of a cecum in monotremes and some marsupials is an intriguing detail. Metatheria and Prototheria represent the sister-group of eutherian mammals and of Therians, respectively; therefore, the evidence suggests that a small appendix appeared early in some mammals (in monotremes, probably in the Mesozoic). As we noted previously (Smith et al., 2009), in these taxa, this small narrow structure is unlikely to serve any digestive function, and as such, it is difficult to posit any evolutionary scenario beyond its function as an immune structure.

The rapid evolutionary rate of the appendix in Euarchontoglires, and the great heterogeneity of this rate in various mammalian clades lend support to the previous suggestion that the appendix is a "recurrent character" (Laurin et al., 2011), at least in Euarchontoglires, and potentially in marsupials. If this is correct, Euarchontoglires in particular are likely to have been more prone to evolve an appendix than species in other clades, even given similar selection pressures. Thus, the presence or absence of an appendix may have more to do with intrinsic propensity to evolve an appendix than any other factor, including diet or social status. Further, since cecal appendix morphotypes vary dramatically among taxa (Smith et al., 2009), it is likely that different clades have evolved an appendix as a response to different adaptive pressures, or that a wide range of morphologies can perform similar functions. The idea that the apex of the cecum in mammals without an appendix is homologous to the appendix (Fisher, 2000; Palva and Palva, 1966) strongly supports the latter view.

5. Conclusions

Substantial evidence supports the view that the cecal appendix is an immune structure primarily functioning as a safe-house for beneficial bacteria, and comes from a range of disciplines, including medicine, epidemiology, immunology, and microbiology (Laurin et al., 2011). Corroborating this view that the appendix has an adaptive function is the finding in this study that the appendix has evolved a minimum of 32 times in mammals. The fact that the appendix is unknown in many other taxa may reflect an evolutionary constraint. For instance, if the appendix is a recurrent phenotype as recently argued (Laurin et al., 2011), some peculiarity of mammals may have facilitated appendix appearance. Alternatively, it is possible that the appendix occurs in other taxa but has not yet been described or recognized. At the same time, the results lend additional support for the previous assertion (Laurin et al., 2011) that Euarchontoglires in particular and perhaps some marsupials have a propensity to evolve an appendix more so than other mammalian clades because our results are based on a much more extensive taxonomic sampling and slightly more sophisticated analytical methods (incorporating branch lengths into the calculations, for instance) than previous studies. The results tend to refute the idea that individual factors such as social behavior or diet strongly influence the evolution of the appendix, although the possibility that some combination of these and perhaps other factors are important has not been ruled out. Finally, this study demonstrates that the size of the cecal appendix tends to evolve isometrically with the size of the rest of the large intestine, indicating that an evolutionary transition from a large cecum without an appendix to a smaller cecum with an appendix, as found in hominoids by Darwin, is an exception rather than the rule. Given all of the information available, a new working hypothesis might be developed in which the appendix has evolved as a microbial safe-house under selection pressure from gastrointestinal pathogens potentially transmitted via a range of mechanisms rather than via a single mechanism dominated by a particular dietary or social factor.

Authors' contributions

HFS helped design the study, conducted the data collection, and drafted much of the manuscript. WP conceived the study, helped interpret the results, and wrote sections of the manuscript. SK contributed insight into mammalian gastrointestinal anatomy and appropriate methods, helped interpret the results, and edited the manuscript. ML conceived the tests of the study, compiled the reference phylogeny, analyzed the data, and helped draft the paper. All authors read and approved the final version of the manuscript.

Acknowledgements

This work was supported in part by start-up funds from Midwestern University (HFS) and the CNRS and the French Ministry of Research to the UMR 7207 (ML). Figure 1 was created by Brent Adrian. The authors would like to thank Brent Adrian, Verne Simons, and Priyanka Purnanada for assistance with reference management and literature searches, and Nathan L. Sanders for proofreading of the manuscript.

Appendix A. Supplementary data

Supplementary data (Supplementary online material 1. Data matrix used in the present study in Mesquite NEXUS format, Supplementary online material 2. Data matrix used in the present study in Microsoft Excel format, Supplementary online material 3. Source of the data on the gastrointestinal tract of the mammalian species included in the present study (Stevens and Hume, 1995), Supplementary online material 4. Phylogenetic time-calibrated tree showing character optimization for appendix presence, cecum presence, and size. This file should be viewed on-screen under high magnification, because of the high number of taxa) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.crpv.2012.12.001.

References

Arvy, L., 1972. L'appendice vermiforme ou caecal. Mammalia S1, 1–68.

Barboza, P.S., Hume, I.D., 1992. Digestive tract morphology and digestion in the wombats (Marsupialia: Vombatidae). J. Comp. Physiol. B 162, 552–560.

Barker, D.J.P., Osmond, C., Golding, A., Wadsworth, M.E.J., 1988. Acute appendicitis and bathrooms in three samples of British children. Br. Med. J. 296, 956–958.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. Roy. Stat. Soc. B Met. 57, 289–300.

Berry, R.J.A., 1900. The true caecal apex, or the vermiform appendix: its minute and comparative anatomy. J. Anat. Physiol. 35, 83.

Bininda-Emonds, O.R.P., Cardillo, M., Jones, K.E., MacPhee, R.D.E., Beck, R.M.D., Greyner, R., Price, S.A., Vos, R.A., Gittleman, J.L., Purvis, A., 2007. The delayed rise of present-day mammals. Nature 446, 507–512.

Bininda-Emonds, O.R.P., Cardillo, M., Jones, K.E., MacPhee, R.D.E., Beck, R.M.D., Grenyer, R., Price, S.A., Vos, R.A., Gittleman, J.L., Purvis, A., 2008. The delayed rise of present-day mammals. Nature 456, 274 (corrigendum).

Bockman, D.E., 1983. Functional histology of the appendix. Archiv. Histol. Japonicum 46, 271–292.

Bollinger, R.B., Barbas, A.S., Bush, E.L., Lin, S.S., Parker, W., 2007. Biofilms in the large bowel suggest an apparent function of the human vermiform appendix. J. Theor. Biol. 249, 826–831.

Boroda, C., 1961. The incidence of acute appendicitis in African patients. East Afr. Med. J. 38, 83.

Bremner, C.G., 1964. Ano-rectal disease in the South African Bantu I. Bowel habit and physiology. South Afr. J. Surg. 2, 119–122.

Bürgi, O., 1905. Blinddarm und Wurmfortsatz bei den Wirbeltieren. Schweiz. Arch. Tierheilkd. 47, 173–194.

Burkitt, D.P., 1969. Related disease-related cause? Lancet ii, 1229–1231.

Burkitt, D.P., 1971. The aetiology of appendicitis. Br. J. Surg. 58, 695–699.

Cave, A.J.E., 1936. Appendix vermiformis duplex. J. Anat. 70, 283–292.

Cho, I., Blaser, M.J., 2012. The human microbiome: at the interface of health and disease. Nat. Rev. Gen. 13, 260–270.

Clark, W.E.L.G., 1971. The antecendents of man. An introduction to the evolution of primates. Quadrangle Books, Chicago.

Cubo, J., Azagra, D., Casinos, A., Castanet, J., 2002. Heterochronic detection through a function for the ontogenetic variation of bone shape. J. Theor. Biol. 215, 57–66.

Curran-Everett, D., 2000. Multiple comparisons: philosophies and illustrations. Am. J. Physiol.: Regul. Integr. Comp. Physiol. 297, 1–8.

Darwin, C., 1871. The descent of man and selection in relation to sex. John Murray, London.

Eggeling, H.V., 1920. Inwieweit ist der Wurmfortsatz am menschlichen Blinddarm ein rudimentäres Gebilde? Anat. Anz. 53, 401–428.

Everett, M.L., Palestrant, D., Miller, S.E., Bollinger, R.B., Parker, W., 2004. Immune exclusion and immune inclusion: a new model of host-bacterial interactions in the gut. Clin. Appl. Immunol. Rev. 5, 321–332.

Faith, D.P., 1992. Conservation evaluation and phylogenetic diversity. Biol. Conserv. 61, 1–10.

Felsenstein, J., 1985. Phylogenies and the comparative method. Am. Nat. 125, 1–15.

Fisher, R.E., 2000. The primate appendix: a reassessment. Anat. Rec. 261, 228–236.

Gelfand, M., 1956. The Sick African. Stewart, Cape Town.

Gluckmann, F., 1939. Sur l'évolution et la signification de l'appendice des Primates, 209, 1008–1010.

Gluckmann, F., 1947a. Aperçu synthétique de l'évolution de l'appendice caecal. Évolution de l'appendice et phénomène d'adaptation. C. R. Seances Acad. Sci. (Paris) 224, 1181–1183.

Gluckmann, F., 1947b. L'appendice vrai et le faux appendice du cæcum. Définition de l'appendice caecal. C. R. Seances Acad. Sci. (Paris) 224, 156–158.

Groves, C.P., 1986. Systematics of the great apes. Comparative primate biology. Alan R. Liss, Inc., New York, pp. 187–218.

Guerrant, R.L., Hughes, J.M., Lima, N.L., Crane, J., 1990. Diarrhea in developed and developing countries: magnitude, special settings, and etiologies. Rev. Infect. Dis. 12, S41–S50.

Hill, W.C.O., 1972. Evolutionary biology of primates. Academic Press, London.

Im, G.Y., Modayil, R.J., Lin, C.T., Geier, S.J., Katz, D.S., Feuerman, M., Grendell, J.H., 2011. The appendix may protect against *Clostridium difficile* recurrence. Clin. Gastroenterol. Hepatol., In Press.

Jacobshagen, E., 1922. Zur Morphologie des menschlichen Blinddarms. Anat. Anz. 56, 97–113.

Janssens, P.G., de Muynck, A., 1966. Appendicular pathology in the African Negro. Trop. Geogr. Med. 18, 81–96.

Jones, F.W., 1929. Man's place among the mammals. Edward Arnold & Co, London.

Jones, K.E., Bielby, J., Cardillo, M., Fritz, S.A., O'Dell, J., Orme, C.D.L., Safi, K., Sechrest, W., Boakes, E.H., Carbone, C., et al., 2009. PanTHERIA: a species-level database of life history, ecology, and geography of extant and recently extinct mammals. Ecology 90, 2648.

Josse, S., Moreau, T., Laurin, M., 2006. Stratigraphic tools for Mesquite. http://mesquiteproject.org/packages/stratigraphicTools/

Keith, A., 1912. The functional nature of the caecum and appendix. Br. Med. J. 2, 1599–1602.

Kostanecki, K., 1913. Zur vergleichenden Morphologie des Blinddarmes unter Berücksichtigung seines Verhältnisses zum Bauchfell. I Teil. Anatomische Hefte. I. Abteilung 48, 309–386.

Kostanecki, K., 1926. Le caecum des Vertébrés (y compris l'Appendice vermiculaire). Morphologie et signification fonctionelle. Bull. Int. Acad. Polonaise Sci. Lett., 1–295, Cracovie, Suppl.

Laurin, M., 2004. The evolution of body size. Cope's rule and the origin of amniotes. Syst. Biol. 53, 594–622.

Laurin, M., 2010a. Assessment of the relative merits of a few methods to detect evolutionary trends. Syst. Biol. 59, 689–704.

Laurin, M., 2010b. The subjective nature of Linnaean categories and its impact in evolutionary biology and biodiversity studies. Contrib. Zool. 79, 131–146.

Laurin, M., Everett, M.L., Parker, W., 2011. The cecal appendix: one more immune component with a function disturbed by post-industrial culture. Anat. Rec. 294, 567–579.

MacFayden, B.V., Mercer, D.W., Potts, J.R., 2000. Small intestine and appendix. In: Lawrence, P.R., Bell, R.M., Dayton, M.T. (Eds.), Essentials of general surgery. Williams & Wilkins, Philadelphia: Lippincott, pp. 241–264.

Maddison, W.P., 1991. Squared-change parsimony reconstructions of ancestral states for continuous-valued characters on a phylogenetic tree. Syst. Biol. 40, 304–314.

Maddison, W.P., 2000. Testing character correlation using pairwise comparisons on a phylogeny. J. Theor. Biol. 202, 195–204.

Maddison, W.P., Maddison, D.R., 2011. Mesquite: a modular system for evolutionary analysis. Version 2.75. http://mesquiteproject.org

McNamara, K.J., 1980. Evolutionary trends and their functional significance in chasmopine trilobites. Lethaia 13, 61–78.

McShea, D.W., 2000. Trends, tools and terminology. Paleobiology 26, 330–333.

Midford, P., Garland, T.J., Maddison, W.P., 2008. PDAP Package for Mesquite. Version 1.12. http://mesquiteproject.org/pdap_mesquite/index.html

Muthmann, E., 1913. Beiträge zur vergleichenden Anatomie des Blinddarmes und der Lymphoiden Organe des Darmkanals bei Säugetieren und Vögeln. Anatomische Hefte 144, 67– 113.

Napier, J.R., Napier, P.H., 1985. The natural history of the primates. MIT Press, Cambridge.

Pagel, M., 1994. Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. Proc. R. Soc. Lond. B Bio. 255, 37–45.

Palva, T., Palva, A., 1966. Size of the human mastoid air cell system. Acta Otolaryngol. 62, 237–251.

Read, A.F., Nee, S., 1995. Inference from binary comparative data. J. Theor. Biol. 173, 99–108.

Scott, G.B.D., 1980. The primate caecum and appendix vermiformis: a comparative study. J. Anat. 131, 549–563.

Smith, H.F., Fisher, R.E., Everett, M.L., Thomas, A.D., Bollinger, R.B., Parker, W., 2009. Comparative anatomy and phylogenetic distribution of the mammalian cecal appendix. J. Evol. Biol. 22, 1984–1999.

Snipes, R.L., 1984. Anatomy of the cecum of the West Indian manatee (Mammalia Sirenia). Zoomorphology 104, 67–78.

Sonnenburg, J.L., Angenent, L.T., Gordon, J.I., 2004. Getting a grip on things: how do communities of bacterial symbionts become established in our intestine? Nat. Immunol. 5, 569–573.

Stevens, C.E., Hume, I.D., 1995. Comparative Physiology of the Vertebrate Digestive System, 2nd ed. Cambridge University Press, New York.

Swofford, D.L., Maddison, W.P., 1987. Reconstructing ancestral character states under Wagner Parsimony. Math. Biosci. 87, 199–229.

Thomas, A.D., Parker, W., 2010. Cultivation of epithelial-associated microbiota by the immune system. Future Microbiol. 5, 1483–1492.

Trowell, H.C., 1960. Non-Infective Disease in Africa. Edward Arnold, London.

Walker, A.R.P., Walker, B.F., Richardson, B.D., Woolford, A., 1973. Appendicitis, fibre intake and bowel behaviour in ethnic groups in South Africa. Postgrad. Med. J. 49, 243–249.

Wilson, D.E., Reeder, D.M., 2005. Mammal Species of the World. A Taxonomic and Geographic Reference. Johns Hopkins University Press, 2142.