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Morphological evolution of the mammalian cecum and cecal appendix

Évolution morphologique de l'appendice du cæcum des mammifères

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A R T I C L E   I N F O

A B S T R A C T

The evolutionary pressures leading to the appearance of the cecal appendix, its evolutionary relationships with the cecum, and the link between these gastrointestinal characters and ecology remain controversial. We collected data on appendix presence and size, other gastrointestinal characters, ecological variables, dietary habits, and social characters hypothesized to drive appendix evolution for 533 mammalian species. Using phylogeny-informed analyses, we identified the first evidence of a positive correlation between appendix presence and cecal apex thickness, and a correlation with cecal morphology, suggesting that the appendix and cecum may be evolving as a module, the cecoappendicular complex. A correlation between appendix presence and concentration of cecal lymphoid tissue supports the hypothesis of an adaptive immune function for this complex. Other new findings include an inverse correlation between relative cecum length and habitat breadth, and positive relationships between cecum length and mean group size, and between colon length and weaning age.

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R É S U M É

Les pressions sélectives qui ont mené à l'apparition de l'appendice du cæcum, les relations entre cet appendice et le cæcum, ainsi que les liens entre ces caractères gastro-intestinaux et l'écologie demeurent discutés. Nous avons compilé des données sur la présence et la taille de l'appendice, d'autres caractères gastro-anatomiques et des variables écologiques, alimentaires et sociales pour 533 espèces de mammifères. À l'aide d'analyses prenant en compte la phylogénie, nous avons obtenu de premiers résultats en faveur d'une corrélation positive entre la présence de l'appendice et l'épaisseur de l'apex du cæcum, ainsi qu'une corrélation avec la morphologie de ce dernier, ce qui suggère que l'appendice et le cæcum évoluent comme un module, le complexe cæco-appendiculaire. Une corrélation entre la présence de l'appendice et la concentration de tissus lymphoïdes dans le cæcum corrobore l'hypothèse selon laquelle l'appendice a une fonction immunologique. D'autres résultats

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nouveaux incluent une relation inverse entre la longueur relative du cæcum et la largeur d'habitat, ainsi que des relations positives entre la longueur du cæcum et la taille moyenne du groupe, ainsi qu'entre la longueur du côlon et l'âge au sevrage.

1. Introduction

1.1. Cecal appendix

The cecal appendix is a narrow, blind extension from the terminal aspect of the cecum. We define the appendix here based strictly on morphology, as a close-ended structure projecting from the cecum that is clearly distinguished from the cecum by a distinct change in diameter (Smith et al., 2009). While there has been discussion regarding the definition of the cecal appendix (e.g., Fisher, 2000) and whether it should include considerations of lymphoid tissues and a thickened wall, these characters are also associated with the apex of the cecum in taxa that lack an appendix (e.g., Berry, 1900; Smith et al., 2009). The organ's function appears to be immunological (Berry, 1900; Bollinger et al., 2007; Gorgollon, 1978; Keith, 1912; Neiburger et al., 1976; Scott, 1980; Smith et al., 2009, 2013; Spencer et al., 1985; Zahid, 2004), involving maintenance of microbial biofilms in a location that is relatively secluded from the main flow of intestinal contents (Bollinger et al., 2007; Sanders et al., 2013). This function is postulated to be important for the recovery of the microbiome following pathogen-induced diarrheal illnesses (Im et al., 2011; Merchant et al., 2012; Sanders et al., 2013).

In contrast to the cecal appendix of humans, the cecal appendix in other species has received little attention; however, it is becoming recognized that a cecal appendix is present in a variety of non-primate mammals (e.g., Chivers and Hladik, 1980; Golley, 1960; Hume, 1999; Kotzé et al., 2010; Pereira et al., 2016; Stevens and Hume, 1995). Only recently have investigators examined the appendix across mammalian phylogeny in a comparative manner (Smith et al., 2009, 2013). As a result of this more recent work, multiple morphotypes, beyond the elongated primate "vermiform" appendix, have been described (Smith et al., 2009). These recent discoveries reveal a wide variety of appendicular diversity across a broad taxonomic range, suggesting that the cecal appendix has a complex and diverse evolutionary history. Further, an observation first made by Charles Darwin regarding the appendix (Darwin, 1871) was confirmed; increased frugivory and decreased cecal size are associated with the appearance of an appendix in hominoids (Smith et al., 2013). However, the generality of this relationship during mammalian evolution was not supported statistically by an analysis incorporating several other mammalian clades, suggesting that the appearance of an appendix concomitant with a decreasing cecum size is the exception rather than the rule in the evolution of the appendix (Smith et al., 2013).

In her study on cecal appendix presence and morphology in primates, Fisher (2000) concluded that the traditional definition of an appendix presence might be too narrow. She suggested two additional variables that may suggest an appendix-like structure is present: (1) thickening of the cecal apex wall; (2) concentration of lymphoid tissue in the cecum. Fisher suggested that these variables could be used to assess appendix presence (Fisher, 2000). However, the aforementioned study was limited taxonomically to include only primate species, and did not involve any statistical validation. Thus, to date, it has not been determined whether a consistent statistical association exists across mammals between appendix presence and both cecal apex wall thickness and lymphoid tissue concentration. Questions remain regarding whether appendix evolution has been driven by morphological evolution and anatomical traits of the cecum, or by other independent factors.

1.2. Ecological factors driving resource use and adaptation

Many studies demonstrate the impact of ecological factors, such as geographic range, group size, and habitat breadth on the anatomical and behavioral adaptations of species. Group size is typically defined as the number of conspecifics with which an animal spends the majority of its time, and there is some evidence that these individuals form a cohesive unit (e.g., Jones et al., 2009). Habitat breadth has been variably defined as the number of geographic or climatic habitats used, dietary breadth, or even abundance. In particular, geographic range size and its utilization often correlate with abundance of a species (Brown, 1984; Lawton, 1993; Pyron, 1999). Brown (1984) argued that this relationship occurs via the association between geographic range and resource abundance. Specifically, species with larger ranges typically have access to and utilize a wider variety of resources, and are consequently more likely to become widespread and abundant (Brown, 1984). In the present study, we follow Jones et al. (2009) in defining habitat breadth as the "number of habitat layers used by a species measured using any qualitative or quantitative time measure, for non-captive populations". While we recognize that there are many possible definitions for the term habitat breadth, Jones and colleagues' PanTHERIA database contains data on habitat breadth (using their definition) for close to 3000 species, so for data compilation purposes in our study, it was the logical definition to employ.

Species occupying a broad habitat tend to be prevalent and abundant (Brown, 1984; Gotelli and Graves, 1996; Pyron, 1999). They also tend to be dietarily and behaviorally versatile, able to exploit a wide variety of local resources (Brown, 1984, 1995). Species with a narrow habitat, on the other hand, tend to be habitat specialists, capable of effectively exploiting a narrow range of local resources (Brown, 1995; Pyron, 1999). These specialists are more vulnerable to environmental fluctuations or changes in resource availability (Brown, 1984). Factors influencing resource

exploitation may influence various gastrointestinal characters, and our data enable us to test this hypothesis.

By definition, species with larger groups live in closer proximity to conspecifics, consequently increasing their susceptibility to communicable diseases. Avoiding pathogens is a key ecological pressure influencing how individuals of a given species will distribute themselves in space (Brown, 1995). Therefore, it seems logical that species with larger groups may require anatomical and behavioral adaptations to living in closer proximity to one another than species with lower population density. Related variables include population group size: number of individuals that spend the majority of their time in a 24 hour cycle together; and group size (or social group size): similar to population groups size, except that there is some indication that these individuals form a social cohesive unit. If the immune functions of the appendix have played a substantial role in its evolution, we might expect it to be correlated with these characters.

Ecologists often focus on several key ecological variables when attempting to explain empirical patterns of anatomical, behavioral, and physiological diversity of species. These variables can offer valuable insights into the underlying mechanisms of ecological diversification among taxa, and help researchers explain the ecological "success" of species sharing niche space. Here, we focus on three of these variables – geographic range size, mean group size, and habitat breadth – given the influence that these factors have been found to have on socioecology and anatomical adaptation across mammals.

2. Materials and methods

2.1. Data compilation

In order to determine the morphological variation and distribution of cecal appendices in mammals, data were compiled from the literature on presence/absence, size, and morphological configuration of the cecal appendix (Tables 1 and 2; SOM 1–2). Two discrete characters represent appendix presence; the first, "observed" presence (character 2), represents scoring based on reports dealing with the species included in this study that specified whether or not the appendix is present. The second, "inferred" presence (character 3), scores more taxa (420 instead of 337) by taking into consideration statements in the literature that specify appendix distribution in larger, supraspecific taxa. As described above, we define a cecal appendix based exclusively on morphology, as a close-ended projection that is clearly differentiated from the cecum by a change in diameter.

In addition, published data were also collated on factors that have been suggested to co-vary with appendix presence, including gastrointestinal and dietary characters, as well as other ecological variables (SOM 1–2). In sum, the data included 14 discrete (Table 1, SOM 1–2) and 28 continuous characters (Table 2, SOM 1, 3). The anatomical data collected included characters of the cecum. In particular, the shape and size of the cecum, and cecal histological variables (including apex thickness and concentration of lymphoid tissue, often used in the definition

Table 1
List of discrete characters and their states. *Indicates a character from which data were derived and definition was used from Jones et al. (2009).
Tableau 1
Liste des caractères discrets et leurs états. *Indique les caractères pour lesquels les données et définitions utilisées suivent Jones et al. (2009).

1. Activity pattern*: 0 nocturnal; 1 cathemeral and/or crepuscular; 2 diurnal
2. Appendix presence (observed): 0 absent; 1 present
3. Appendix presence (inferred): 0 absent; 1 present
4. Stomach: 0 wholly glandular; 1 some squamous epithelium
5. Concentration of lymphoid tissue in cecum/appendix: 0 present; 1 absent
6. Size of colon: 0 short; 1 midsized; 2 large
7. 7a. Cecal morphology (6 states): 0 small and appendix-like; 1 spiral; 2 tapering; 3 cylindrical; 4 paired ceca/colonic appendages; 5 rounded; –absent
   7b. Cecal morphology (5 states): 0 small and appendix-like; 1 spiral; 2 tapering; 3 cylindrical; 4 paired ceca/colonic appendages or rounded; –absent
   7c. Cecal morphology (4 states): 0 small and appendix-like or spiral; 1 tapering; 2 cylindrical; 3 paired ceca/colonic appendages or rounded; –absent
   7d. Cecal morphology (4 states, alternative): 0 small appendix-like; 1 spiral; 2 tapering or cylindrical; 3 paired ceca/colonic appendages or rounded; –absent
   7e. Cecal morphology (3 states): 0 appendix-like or spiral; 1 tapering or cylindrical; 2 paired ceca/colonic appendages or rounded; –absent
8. Size of cecum: 0 absent; 1 small; 2 midsized; 3 large
9. Cecal apex thickness: 0 thin; 1 thick
10. Diet, cellulose richness, multi-state: 0 carnivory; 1 insectivory; 2 omnivory; 3 frugivory; 4 granivory; 5 gummivory; 6 folivory
11. Diet and gut adaptation, cellulose richness, binary: 0 cellulose-poor (insectivory-carnivory-omnivory-frugivory); 1 cellulose-rich (folivory-granivory-gummivory)
12. Fermentation multi-state: 0 non-ruminant foregut; 1 ruminant foregut; 2 cecal only; 3 colon (including cecal); 4 minimal
13. Fermentation binary: 0 foregut; 1 cecal or colon
14. Terrestriality*: 0 fossorial or ground-dwelling; 1 dwelling above ground
15. Habitat breadth*: 1 single layer; 2 two layers; 3 three layers; 4 four layers

1. Diagramme d'activité* : 0 nocturne ; 1 cathéméral et/ou crépusculaire ; 2 diurne
2. Présence d'appendice (observé) : 0 absent ; 1 présent
3. Présence d'appendice (déduit) : 0 absent ; 1 présent
4. Estomac : 0 entièrement glandulaire ; 1 épithélium squameux
5. Concentration de tissu lymphoïde dans le cæcum/appendice : 0 présent ; 1 absent
6. Taille de côlon : 0 étroit ; 1 de taille moyenne ; 2 grand
7. 7a Morphologie de cæcum (6 états) : 0 petit et de type appendice, 1 en spirale ; 2 fuselé ; 3 cylindrique ; 4 paire de cæcums/appendices de côlon ; – absent
   7b Morphologie de cæcum (5 états) : 0 petit ou de type appendice ; 1 en spirale ; 2 fuselé ; 3 cylindrique ; 4 paire de cæcums/appendices de côlon ou arrondi ; – absent
   7c Morphologie de cæcum (4 états) : 0 petit et de type appendice ou en spirale ; 1 fuselé ; 2 cylindrique ; 3 paire de cæcums/appendices de côlon ou arrondi ; – absent
   7d Morphologie de cæcum (4 états alternés) :0 type appendice petit ; 1 spiralé ; 2 fuselé ou cylindrique ; 3 paire de cæcums/appendices de côlon ou arrondi ; – absent
   7e Morphologie de cæcum (3 états) : 0 type appendice ou en spirale ;1 fuselé ou cylindrique ; 2 paire de cæcums/appendices de côlon ou arrondi ; – absent
8. Taille de cæcum : 0 absent ; 1 petit ; de taille moyenne ; 3 grand
9. Epaisseur de l'apex du cæcum : 0 mince ; 1 épais
10. Régime alimentaire, richesse en cellulose, état multiple : 0 carnivore ; 1 insectivore ; 2 omnivore ; 3 frugivore ; 4 granivore ; 5 gommivore ; 6 herbivore

Table 1 (*Continued*)

11. Régime alimentaire et adaptation du tube digestif, richesse en cellulose, binaire ; 0 pauvre en cellulose (insectivore-carnivore-omnivore-frugivore) ; 1 riche en cellulose (herbivore-granivore-gommivore)
12. Fermentation multi-états ; 0 partie antérieure du tube digestif non ruminant ; 1 partie antérieure du tube digestif ruminant ; 2 uniquement cæcum ; 3 côlon (incluant le cæcum) ; 4 minimum
13. Fermentation binaire : 0 partie antérieure du tube digestif ; 1 cæcum ou côlon
14. Terrestre* : 0 fossoyeur ou creusant le sol ; 1 creusant au-dessus du sol
15. Diversité de l'habitat : 1 un niveau ; 2 deux niveaux ; 3 trois niveaux ; 4 niveaux

of the appendix) were collated (Tables 1 and 2, SOM 1). Our cecal shape characters included the state "paired ceca or colonic appendages", in which we included instances of two equally-sized ceca, and instances of a second smaller "vestigial" cecum (e.g., Mitchell, 1905) and so-called "colonic appendages" (Stevens and Hume, 1995) or "distal paired caeca" (Bjornhag et al., 1994). As an outpock-eting of the intestine, it has been suggested that the cecal appendix may be affected by other gastrointestinal charac-teristics. Inclusion of numerous gastrointestinal characters in the present study, such as size of the colon and gastroin-testinal capacity, enabled us to identify possible patterns of co-variation among gastrointestinal traits (Tables 1 and 2, SOM 1).

In addition to assessing co-variation among gastroin-testinal traits, we chose to include characters that allowed us to empirically test the above-stated issues regarding: (1) a potential link between gastrointestinal characters (including appendix presence or size), and ecological variables relating to the geographic distribution and con-centration of conspecifics in space that would affect the transmission of communicable diseases, such as group size and habitat breadth; (2) the possible relationship between diet and cecal appendix presence or size, as well as other gastrointestinal characters (e.g., Darwin, 1871). We also tested a third hypothesis, that the appendix is a func-tionless evolutionary vestige, by assessing whether it has appeared significantly more times than would be pre-dicted by chance alone (based on a comparison between the number of gains and losses of the appendix). In order to test whether resource use and its corollaries, includ-ing degree of conspecific interaction, were correlated with appendix size and presence, we assessed: population den-sity, geographic range, habitat breadth (as defined by Jones et al., 2009); as the number of layers used among the following: above ground dwelling, aquatic, fossorial and ground dwelling), mean social group size, home range, group range, and activity pattern (Tables 1 and 2, SOM 1–2). The latter was tested because nocturnal species tend to be less gregarious than diurnal ones, and consequently might be expected to be exposed to lower levels of communicable pathogens, as well as differing in resource exploitation.

Dietary characters incorporated to address the dietary hypothesis for appendix evolution included: diet and gut adaptation, cellulose content of diet, type of fermenta-tion (e.g., foregut, hindgut, etc.), relative dry matter intake, particle retention time, food quality, and dietary breadth

Table 2

List of continuous characters. *Indicates a character from which data were derived and definition was used from Jones et al. (2009).

Tableau 2

Liste des caractères continus. *Indique les caractères pour lesquels les données et définitions utilisées suivent Jones et al. (2009).

Character number	Character
1	Body mass (g)
2	Mean group size*
3	Appendix length (mm)
4	Length of cecum (cm)
5	Length of colon (cm)
6	Appendix length (mm)/cubic root of body mass (g)
7	Length of cecum (cm)/cubic root of body mass (g)
8	Length of colon (cm)/cubic root of body mass (g)
9	Relative dry matter intake (rDMI in g/kg 0.75)
10	Particle mean retention time (MRT) (h)
11	Gastrointestinal (GIT) capacity (measured as total contents wet weight)
12	Food quality (g crude fibre/kg DM)
13	Adult head to body length (mm)*
14	Basal metabolic rate (BMR) (mLO$_2$/h)
15	Diet breadth*
16	Habitat breadth*
17	Home range (km^2)*
18	Population density*
19	Population group size*
20	Trophic level*
21	Weaning age*
22	Weaning body mass*
23	Precipitation mean*
24	Temperature mean (°C)*
25	Group range area (km^2)*
26	Maximum latitude*
27	Minimum latitude*
28	Mean latitude*

Numéro	Caractère
1	Masse corporelle (g)
2	Taille moyenne du groupe*
3	Longueur de l'appendice
4	Longueur du caecum (cm)
5	Longueur du côlon
6	Longueur de l'appendice (mm)/racine cubique de la masse corporelle (g)
7	Longueur du caecum (cm)/racine cubique de la masse corporelle (g)
8	Longueur du côlon (cm)/racine cubique de la masse corporelle (g)
9	Prise relative de matière sèche (rDMI ing/kg0,75)
10	Temps moyen de rétention des particules (MRT) (h)
11	Capacité gastro-intestinale (GIT), mesurée en tant que poids humide du contenu total)
12	Qualité de la nourriture (g de fibre crue/kg DM)
13	Tête par rapport à la longueur du corps chez l'adulte (mm)*
14	Taux métabolique basal (BMR) (mlO$_2$/h)
15	Souffle selon le régime alimentaire*
16	Souffle selon l'habitat*
17	Dimensions de l'habitat (km^2)*
18	Densité de population*
19	Taille du groupe de population*
20	Niveau trophique*
21	Age du sevrage*
22	Masse corporelle lors du sevrage*
23	Précipitation moyenne*
24	Température moyenne (°C)*
25	Dimensions de l'habitat du groupe (km^2)*
26	Latitude maximum*
27	Latitude minimum*
28	Latitude moyenne*

(i.e., number of dietary categories eaten by each species over a period of time) (Tables 1 and 2, SOM 1). It has recently been argued that dry matter gut contents may be a more reliable indicator of an animal's ability to process low quality food sources than is mean particle retention time [MRT] (Müller et al., 2013); however, due to the relative paucity of data on dry matter gut contents across mammals, we chose to include MRT instead. Ecological characters that could potentially affect diet and digestive strategies were also considered, including precipitation, temperature, latitude, and terrestriality (Table 2).

The correlation test that we used (see below) requires that the states be ordered according to clines. This was often straightforward, but for cecal morphology, this was less intuitive. We ordered the states along a gradient from an appendix-like, slender to a bulky cecum. Even though either end of this spectrum could have been scored "0", we selected as the state "small, appendix-like cecum" as being "0" because it is probably primitive for mammals, as shown by the fact that it is present in all sampled monotremes and some marsupials, including *Caenolestes*, which is the sister-group of the other sampled marsupials, in our reference tree (ordered parsimony unambiguously supports this interpretation). This character was scored as inapplicable for taxa lacking a discrete, recognizable cecum, both for logical reasons (the shape of an unrecognizable structure cannot be known) and because optimization of the character, with states ordered (as is most appropriate for characters with clines) or not, suggests that absence of a cecum results from a loss, and that this may occur from most states of this character. Thus, there is no logical place to insert the state "absent" into the shape morphocline. But given uncertainty about the proper ordering scheme between some states of cecal morphology, we performed a sensitivity analysis by lumping states and re-testing for correlation with the appendix; thus, the number of states was reduced from six (excluding absence, scored as inapplicable) to five, four (two alternative schemes examined), and three (Table 1).

It has been suggested that age and body size at time of weaning impacts the quantity of maternal gut bacteria that are transferred from mother to infant (Bezirtzoglou et al., 2011; De Leoz et al., 2012; Martín et al., 2003). Species that nurse their offspring for longer periods should gain a higher concentration of gut bacteria (Bezirtzoglou et al., 2011; Langer, 2003). Thus, we also tested whether weaning age and weaning body mass were correlated with appendix presence.

Data were collected for 533 terminal mammalian taxa comprising all speciose mammalian families (SOM 1–2). Two more taxa are included in the database, but the dog (*Canis familiaris*), and pacas (*Cuniculus*) were excluded in the first case, because the dog is a direct descendant of the wolf, which is also included in our study, and in the second case, because too few gastrointestinal and ecological data were available.

2.2. Timetree construction

The phylogeny that we used in our previous paper (Smith et al., 2013) was obtained from Bininda-Emonds et al. (2007, 2008). Here, we updated it (for topology and branch lengths), whenever we could find more recent information (which is the case for the vast majority of taxa) using Meredith et al. (2011) for all taxa included in the latter except for a few cases in which this contradicted the established consensus and other recent studies (see below). For several clades, Meredith et al. (2011) offered insufficient resolution (too few taxa were included). Thus, we also used: Phillips et al. (2009) for the divergence date between *Zaglossus* and *Tachyglossus*, Voss and Jansa (2009: fig. 35) for Didelphidae (topology only; this happened to be coherent with Bininda-Emonds et al., 2007), Meredith et al. (2008) for Macropodiformes, Meredith et al. (2009: fig. 3) for divergence times within Acrobatidae and Diprotodontia in general, Meredith et al. (2010: fig. 5) for Pseudocheiridae, Krajewski et al. (2000: fig. 4) for divergence times within *Dasyurus*, Delsuc et al. (2012) for xenarthrans, Moraes-Barros et al. (2011) for the phylogeny within *Bradypus*, Castro et al. (2013) for *Dasypus* (topology only), Poux et al. (2008) for Tenrecidae, Smit et al. (2011) for Macroscelididae, Ohdachi et al. (2006: fig. 1) for Soricidae, Esteva et al. (2010) for Sorex, Dubey et al. (2008) for Crocidura, Tougard et al. (2001) for Rhinocerotidae, Agnarsson et al. (2011) and Teeling (2009) for chiropterans, Prevosti (2010) and Agnarsson et al. (2010) for carnivoran topologies between low-ranking taxa (divergence times between these were not always available; in these cases, they were kept as close to those originally in Bininda-Emonds et al. (2007) as possible, if this was compatible with Meredith et al. (2011), and if no more detailed data were available in other studies), Slater et al. (2010) for ursid phylogeny, Patou et al. (2009) for Herpestidae, Yonezawa et al. (2007) for mustelids, Koepfli et al. (2008) for mustelids, Johnson et al. (2006) for felids, Bagatharia et al. (2013) for divergence times within *Panthera*, Hassanin et al. (2012: table 1, UNI-HARD mean column for ages) for Cetartiodactyla, Bibi (2013) for Bovidae and closely related taxa, Perelman et al. (2011) for primates, with some additions from Masters et al. (2007: fig. 4) for galagonids, and Cortés-Ortiz et al. (2003) for *Alouatta*, Honeycutt (2009) for high-level topology and divergence times among rodents, Nunome et al. (2007) for Gliridae, Mercer and Roth (2003) for Sciuridae, Vilela et al. (2009) for hystricomorphs, Hafner et al. (2007) for heteromyids, Belfiore et al. (2008) for divergence times within *Thomomys*, Jansa et al. (1999) for Nesomyidae and *Mystromys*, Lebedev et al. (2012) for topology within Dipodidae, Salazar-Bravo et al. (2013: fig. 2) for the topology of sigmodontine cricetids, with divergence times from Parada et al. (2013), Ventura et al. (2013: fig. 2) for *Chilomys instans*, Bradley et al. (2007) for *Peromyscus*, Jansa et al. (2006) and Lecompte et al. (2008) for Muridae, Galewski et al. (2006) for Arvicolinae, Neumann et al. (2006) for Cricetinae, Robovský et al. (2008: fig. 5) for *Microtus*, Steppan et al. (2004) for muroid topologies, Rowe et al. (2008) for Murinae, Steppan et al. (2005: fig. 6) and Colangelo et al. (2007) for Gerbillinae, and Verneau et al. (1998) and Robins et al. (2008) for *Rattus*. For a few taxa that were not covered in these studies, divergence times were obtained from Kumar and Hedges (2011).

For a few taxa, sources conflicted. In such cases, we opted to retain topologies that reflected the established consensus, rather than new suggestions (Fig. S1). Thus,

we placed Scandentia as sister-group of the clade that includes Cynocephalidae plus primates, as recently upheld by Lartillot and Delsuc (2012), rather than placing it as the sister-group of Glires, as recovered by Meredith et al. (2011). We deliberately did not use automated supertree construction methods, for three reasons. First, such methods are aimed at giving an automated consensus when there is significant overlap between sources and when there is no a priori reason to prefer one source tree over another, in case of conflict. This is not the case here as overlap was minimal between sources, and the few conflicts could be easily resolved by using the most recent paper, which typically had used much more data. Second, there is a problem with such methods, such as MRP (Matrix Representation Parsimony), which may yield clades that are not included in any of the source trees (Goloboff and Pol, 2002). Third, such methods typically do not incorporate branch lengths, which are critical for some of our analyses.

2.3. Detection of phylogenetic signal

We investigated the presence of a phylogenetic signal in the data to determine if phylogeny-informed tests were required (Laurin, 2004). Given that several of our characters are continuous, we used a test that uses squared-change parsimony to compare the amount of change implied by each character on the reference tree to the amount of change on a population of randomized trees. Given that squared-change parsimony is sensitive to branch lengths, the simplest way to produce such a population of random trees is to randomly reshuffle terminal taxa on the reference tree, whose topology (except for the identity of terminal taxa) and branch lengths are thus kept constant (Laurin, 2004). This procedure can also be used for discrete characters (as was done here), although other methods to produce random trees could also be used for these. These tests were performed in Mesquite (Maddison and Maddison, 2014).

2.4. Assessment of character correlation

Given that most characters display a strong phylogenetic signal (see results), we used only phylogeny-informed tests. We first checked if the continuous data could be analyzed through phylogenetic independent contrasts (PIC), the most commonly used method to analyze continuous comparative data in a phylogenetic context (Felsenstein, 1985). However, the four artifact checks implemented in Mesquite (Maddison and Maddison, 2014) indicated that most of these data departed strongly from a Brownian motion evolutionary model on the reference tree (Fig. S1). These checks consist of verifying the relationships between absolute value of standardized contrasts and expected standard deviation (based on branch lengths), between contrasts and nodal value, between contrasts and nodal height, and between nodal value and nodal height. For an ultrametric tree (i.e. with tips or terminal taxa all at the same height above the base node), none of these relationships should be statistically significant if the contrasts are adequately standardized. Most characters exhibited strong standardization artifacts (Table S1), even

after correcting for multiple tests as suggested by Canoville and Laurin (2010). To attempt to correct this problem, the data were log-transformed, but this only moderately improved standardization, with most characters still displaying very highly significant artifacts (Table S2). Therefore, PIC were not used to assess correlations between the continuous characters; instead, the non-parametric, phylogeny-informed sign test known as (phylogenetic) pairwise comparisons (Maddison, 2000; Read and Nee, 1995) was used to assess correlations between characters, continuous or discrete. This test is implemented in Mesquite (Maddison and Maddison, 2014). It consists of contrasting pairs of terminal taxa. We used mostly the pairing algorithm that contrasts taxa differing in the state of the independent character. However, the Mesquite implementation cannot handle missing data or polymorphism in the independent character, or polytomies in the tree, so the master tree was pruned to retain only terminal taxa that were scored (and non-polymorphic, and not with the "inapplicable" state). When the subtree retained a polytomy, it was resolved arbitrarily by Mesquite (without respect with character-state distribution). Contrary to our previous study (Smith et al., 2013), we did not randomly resolve polytomies ten times because our updated tree is much more resolved, with only a couple of small polytomies involving three taxa, so their impact on the analyses is negligible. In several cases, more than one pairing of terminal taxa is possible; in these cases, we averaged the probabilities of the first ten pairings found and report these values. Given the number of characters included in this study, not all possible pairs of characters were checked; instead, we focused on the possible relationships between the presence or size of the appendix and the other gastrointestinal anatomical characters, as well as between gastrointestinal anatomy and ecology.

To better assess the significance of the potential correlation between appendix presence and cecal morphology, we checked the proportion of taxa with each cecal morphology that possess an appendix. We also produced 2×2 contingency tables showing the distribution of character combinations in taxa and performed Chi^2 and Fisher's exact tests on these using Graphpad Quickcalcs (http://www.graphpad.com/quickcalcs/), to complement the pairwise comparison tests. Scatterplots are provided to support some of the results of pairwise comparisons between continuous characters (Fig. 2).

2.5. Testing for asymmetry between rates of appendix gain and loss

We compared the number of appearances and losses of the cecal appendix using parsimony optimization on the reference timetree. This test enabled us to empirically test the hypothesis that the appendix has appeared more frequently and disappeared less frequently throughout mammalian evolution than would be expected by chance alone if this character were selectively neutral. Rejection of the null hypothesis of equal rates of gains and losses would suggest that the appendix likely serves some adaptive purpose. Our results could differ substantially from those of Smith et al. (2013) because that

Table 3

Phylogenetic signal in the discrete characters, assessed using squared-change parsimony and random taxon permutations as described in Laurin (2004). The proportion of random trees that are not longer than the reference tree gives an estimate of the probability of the null hypothesis (that character states are distributed randomly with respect to the phylogeny). This was assessed using 10,000 random trees. All characters exhibit a significant phylogenetic signal (all probabilities < 0.0177 are significant after correction for multiple tests). Probabilities are rounded off to the fourth decimal.

Tableau 3

Signal phylogénétique dans les caractères discrets, établi en utilisant la parcimonie des moindres carrés et des permutations aléatoires de taxons, tel que décrit par Laurin (2004). La proportion d'arbres aléatoires qui ne sont pas plus longs que l'arbre de référence fournit une estimation de la probabilité de l'hypothèse nulle (selon laquelle les états des caractères sont répartis de manière aléatoire par rapport à la phylogénie). Ceci a été établi en utilisant 10 000 arbres aléatoires. La plupart des caractères montrent un signal phylogénétique significatif (toutes les probabilités < 0,017 sont significatives après correction pour des tests multiples). Les probabilités sont arrondies à la quatrième décimale.

Character number	Name	Number of steps of character on reference tree	Probability	Sample size (number of taxa scored)
1	Activity pattern	129	0.0000	405
2	Appendix presence (observed)	41	0.0000	337
3	Appendix presence (inferred)	41	0.0000	420
4	Stomach	13	0.0000	287
5	Concentration of lymphoid tissue in cecum/appendix	12	0.0000	115
6	Size of colon	36	0.0000	107
7	Cecal morphology	87	0.0000	309
8	Size of cecum	89	0.0000	309
9	Cecal apex thickness	7	0.0000	121
10	Diet and gut adaptation, multi-state	447	0.0000	472
11	Diet and gut adaptation, binary	86	0.0000	472
12	Fermentation, multi-state	16	0.0000	302
13	Fermentation, binary	7	0.0000	302
14	Terrestriality, binary	41	0.0000	393

previous study relied on a reference tree that had several large polytomies, which create problems with character optimization (Maddison, 1989). To deal with the ambiguity of optimization of appendix gain and loss on our tree, and considering that Smith et al. (2013) found that gains were much more numerous than losses, we used the most conservative number of gains (minimal number) and losses (maximal number) suggested by parsimony to compute the probability that both events are equally probable, which should be a conservative approach. This probability was computed using GraphPad Software (http://www.graphpad.com/quickcalcs/binomial2/). We used a two-tailed test because our initial hypothesis that gains are more probable than losses is derived from examination of the optimization, which is itself based on our data. Thus, using a one-tailed test would have introduced a bias into the study.

2.6. Testing for differences in evolutionary rates between clades

To compare evolutionary rates of the cecal appendix and habitat breadth between clades, we divided the number of transitions (appearances or losses) of the appendix by the sampled phylogenetic diversity (sum of branch lengths) of the clades (Faith, 1992), as Smith et al. (2013) did. We used a binomial test to determine the probability of the null hypothesis under which the proportion of events in each clade simply reflects its sampled phylogenetic diversity, which reflects the hypothesis that the appendix is equally likely to be gained (or lost) in all clades.

2.7. Corrections for multiple tests

Given that comparative studies often include multiple tests, corrections are required when applying the probability threshold to assess statistical significance of the results. We applied the False Discovery Rate (FDR below) procedure described by Benjamini and Hochberg (1995) because it retains more power than Bonferroni corrections, while retaining statistical validity (Curran-Everett, 2000). In this case, we applied the procedure simultaneously on all our tests, including those pertaining to the presence of a phylogenetic signal (but not the presence of statistical artifacts linked with inadequate phylogenetic contrast standardization, because most of these were strongly significant and could have biased the analysis), as well as to the correlations between characters. This allowed us to assess the significance of 170 tests (and associated probabilities) simultaneously and to eliminate about 8–9 expected false positives (170 times 0.05 probability of obtaining a significant result yields 8.5 expected spurious results; actual number of initially significant results that become nonsignificant after correction for multiple tests is reasonably close, at 5).

3. Results

3.1. Corrections for multiple tests

The false discovery rate (FDR) procedure indicates that the correct probability threshold is 0.017 (instead of the 0.05 threshold that we would have used if we had not accounted for multiple tests).

3.2. Phylogenetic signal

All discrete (Table 3) and most continuous characters (Table 4) display a strong phylogenetic signal. The only continuous character that does not display such a signal is the relative dry matter intake (9).

Table 4

Phylogenetic signal in the continuous characters, assessed using squared-change parsimony and random taxon permutations as described in Laurin (2004). Characters 29–31 were analyzed for phylogenetic signal, but not for correlations because none were expected to be found, and biological interpretation of any correlations for these would be difficult. A single character does not exhibit a significant phylogenetic signal (all probabilities < 0.017 are significant after correction for multiple tests). Probabilities are rounded off to the fourth decimal.

Tableau 4

Signal phylogénétique dans les caractères continus, établi en utilisant la parcimonie des moindres carrés et des permutations aléatoires de taxons, tel que décrit par Laurin (2004). Les caractères 29–31 ont été analysés pour le signal phylogénétique, mais non pour les corrélations, que l'on ne peut espérer trouver et dont l'interprétation biologique serait difficile. Un seul caractère ne montre pas de signal phylogénétique significatif (toutes les probabilités < 0,017 sont significatives après correction pour de tests multiples). Les probabilités sont arrondies à la quatrième décimale.

Character number	Name	Squared length on reference tree	Probability	Sample size
1	Body mass (g), ln-transformed	43.8930	0.0000	520
2	Mean group size (ln-transformed)	16.0319	0.0000	288
3	Appendix length (mm)	4.337E+03	0.0000	305
4	Length of cecum (cm)	5.010E+03	0.0000	274
5	Length of colon	5.318E+05	0.0000	190
6	Appendix length (mm)/cubic root of body mass (g)	5.411E+00	0.0000	295
7	Length of cecum (cm)/cubic root of body mass (g)	3.674E+01	0.0000	267
8	Length of colon (cm)/cubic root of body mass (g)	4.709E+02	0.0005	188
9	Relative dry matter intake (rDMI in g/kg 0.75)	1.971E+03	0.2184	90
10	Particle mean retention time (MRT) (h)	2.085E+03	0.0013	111
11	Gastrointestinal (GIT) capacity (measured as total contents wet weight), ln-transformed	22.1192	0.0000	77
12	Food quality (g crude fibre/kg DM)	8.047E+03	0.0000	94
13	Adult to head + body length (mm), ln-transformed	3.5181	0.0000	380
14	Basal metabolic rate (BMR) (mLO$_2$/h)	6.396E+08	0.0010	219
15	Diet breadth	9.732E+01	0.0001	438
16	Habitat breadth	6.981E+00	0.0000	405
17	Home range (km^2)	94.1246	0.0000	276
18	Population Density	1.071E+09	0.0000	336
19	Population group size	1.924E+09	0.0010	120
20	Trophic level	8.290E+00	0.0000	438
21	Weaning age	4.146E+05	0.0000	397
22	Weaning body mass	1.062E+10	0.0000	212
23	Precipitation mean	7.883E+04	0.0000	473
24	Temperature mean (°C)	1.909E+05	0.0000	473
25	GR area (km^2), ln-transformed	137.4395	0.0000	475
26	Maximum latitude	1.340E+04	0.0000	475
27	Minimum latitude	1.279E+04	0.0000	475
28	Mean latitude	1.069E+04	0.0000	475
29	Maximum longitude	1.435E+05	0.0000	475
30	Minimum longitude	1.174E+05	0.0000	475
31	Mean longitude	9.111E+04	0.0000	473

3.3. Character correlations

Among discrete characters, appendix presence (observed or inferred) is significantly correlated with the concentration of lymphoid tissue, cecal morphology, and with cecal apex thickness (Table 5).

The correlation with cecal morphology is strongest when the latter is coded into six to four states; it is non-significant with only three states. The relative frequency of the appendix in taxa with the various states of cecal morphology is generally congruent with our hypothesis about state orders, with a few discrepancies (Table 6). The only exceptions concern the tapering cecum, which is more frequently associated with an appendix than the spiral cecum (though we had trouble ordering these two *a priori* based on their morphology) and the rounded cecum, which is more frequently associated with the appendix than the paired ceca/colonic appendages and cylindrical ceca (though again, ordering it compared with the paired cecum/colonic appendages state was especially problematic; Fig. 1). Yet, despite these mismatches, the relationship is significant, even after accounting for corrections for

multiple tests, for more than half of the tested coding schemes, which suggests that a genuine relationship exists between cecum shape and appendix. This result is further confirmed by a classical, non-phylogenetic Chi^2 test (Table 6), whose strongly significant result ($P < 0.0001$) reflects the fact that the proportions of taxa with an appendix differ drastically between cecal morphologies.

The relationship between appendix presence and concentration of lymphoid tissue is supported by an examination of the distribution of the states, and by non-phylogenetic statistical tests (e.g., Fisher's exact test) on these (Table 7). Similarly, the relationship between appendix presence and cecal apex thickness is also supported by classical statistical tests (Table 8).

Tests between discrete (inferred appendix presence) and continuous characters yielded only one marginally significant result (with adult body length), which is no longer significant after corrections for multiple tests using FDR (Table 9).

Tests between continuous characters, namely length of appendix, cecum and colon (all divided by the cubic root of body mass to adjust for body size effects) and the other

Table 5
Correlations between discrete characters assessed using pairwise comparisons, using the pairing algorithm that contrasts taxa differing in the state of the independent character. In several cases, more than one pairing of terminal taxa is possible; in these cases, the reported probability is the average of the probabilities yielded by the first ten pairings found by Mesquite. All significant correlations (probabilities in bold type) except two remain significant after corrections for multiple tests using FDR (these are marked by an asterisk), which indicate that the appropriate threshold is 0.017. Probabilities are rounded off to the fourth decimal.

Tableau 5
Corrélations entre des caractères discrets, établies en utilisant des comparaisons de paires utilisant l'algorithme de paires qui établit un contraste entre taxons différant dans l'état de caractère indépendant. Dans certains cas, plus d'un assortiment de paires de taxons terminaux est possible ; dans ce cas, la probabilité rapportée est la moyenne des probabilités obtenues pour les dix premiers assortiments de paires trouvés par Mesquite. Toutes les corrélations significatives (probabilités en gras), à l'exception de deux, restent significatives après correction pour des tests multiples utilisant la FDR (celles marquées d'une astérisque), qui indiquent que le seuil approprié est 0,017. Les probabilités sont arrondies à la quatrième décimale.

Independent character	Dependent character	Probability	Polarity (when significant or near-significant)
C2, Appendix presence, observed	C1, Activity pattern	0.5000	
	C4, Stomach (glandular or not)	0.4750	
	C5, Concentration of lymphoid tissue	**0.0156**[*]	Positive
	C6, Colon size	0.1875	
	C7a, Cecal morphology (6 states)	**0.0112**[*]	Negative
	C7b, Cecal morphology (5 states)	**0.0112**[*]	Negative
	C7c, Cecal morphology (4 states)	**0.0112**[*]	Negative
	C7d, Cecal morphology (4 states, alternative)	0.0898	
	C7e, Cecal morphology (3 states)	0.0898	
	C8, Cecum size	0.3770	
	C9, Cecal apex thickness	**0.0156**[*]	Positive
	C10, Diet, multi-state	0.3633	
	C11, Diet, binary	0.5000	
	C12, Fermentation, multi-state	0.5000	
	C13, Fermentation, binary	0.5000	
	C14, Terrestriality	0.5000	
C3, Appendix presence, inferred	C1, Activity pattern	0.2500	
	C4, Stomach (glandular or not)	0.5000	
	C5, Concentration of lymphoid tissue	**0.0156**[*]	Positive
	C6, Colon size	0.2305	
	C7a, Cecal morphology (6 states)	**0.0065**[*]	Negative
	C7b, Cecal morphology (5 states)	**0.0065**[*]	Negative
	C7c, Cecal morphology (4 states)	**0.0065**[*]	Negative
	C7d, Cecal morphology (4 states, alternative)	0.0898	
	C7e, Cecal morphology (3 states)	0.0898	
	C8, Cecum size	0.5000	
	C9, Cecal apex thickness	**0.0156**[*]	Positive
	C10, Diet, multi-state	0.3633	
	C11, Diet, binary	0.3438	
	C12, Fermentation, multi-state	0.6500	
	C13, Fermentation, binary	0.5000	
	C14, Terrestriality	0.5469	

C: character.

Table 6
Relative and absolute frequency of the appendix in taxa with the various states (of character 7a, with 6 states) of cecal morphology. Taxa for which appendix presence had not been scored have been ignored in the count. To assess the proportion of taxa with a cecal appendix, taxa in which the appendix is uniformly present were scored as "1", whereas polymorphic taxa were scored as "0.5". A Chi^2 test on these data, excluding polymorphic taxa, shows that this pattern is highly significant (Chi^2 of 59.941, 1 df, $P < 0.0001$).

Tableau 6
Fréquence relative et absolue de l'appendice dans les taxons d'états variés (du caractère 7a à 6 états) de la morphologie du caecum. Les taxons pour lesquels la présence n'a pas été notée ont été ignorés dans le comptage. Pour établir la proportion des taxons à appendice cécal, les taxons dans lesquels l'appendice est uniformément présent ont été notés « 1 », tandis que les taxons polymorphiques ont été notés « 0,5 ». Un test Chi^2 sur ces données, excluant les taxons polymorphiques, montre que cette configuration est hautement significative (Chi^2 de 59, 941, 1 df, $p < 0,0001$).

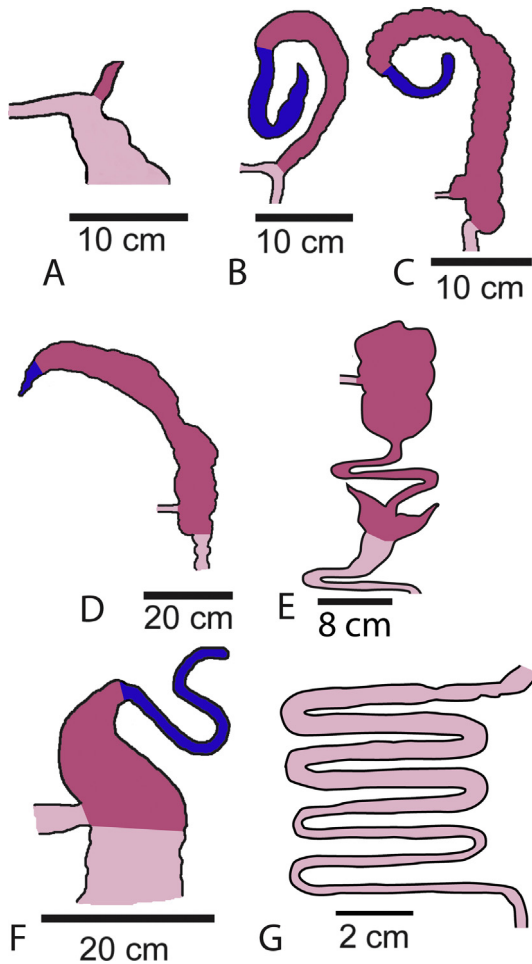| State number	Cecal morphology	Number of taxa for which appendix presence is scored	Number of taxa with appendix uniformly present (inferred)	Number of taxa with appendix uniformly absent (inferred)	Number of taxa polymorphic for appendix	Proportion with appendix
0	Small and appendix-like	6	6	0	0	1.00
1	Spiral	14	5	9	0	0.36
2	Tapering	42	19	23	1	0.46
3	Cylindrical	100	6	94	4	0.08
4	Paired ceca/colonic appendages	14	1	13	0	0.07
5	Rounded	41	7	34	4	0.22
	Total	217	44	173	9	0.22

Fig. 1. Illustration of the seven cecal character states included in this study (see also Table 1, character 8): (A) appendix-like cecum of a common wombat (*Vombatus ursinus*); (B) spiral-shaped cecum of a common brushtail possum (*Trichosurus vulpecula*); (C) elongated, tapering cecum of a rabbit (*Oryctolagus cuniculus*); (D) cylindrical cecum of a North American beaver (*Castor canadensis*); (E) paired ceca (or colonic appendages) in a rock hyrax (*Procavia habessinica*); (F) rounded cecum of an orangutan (*Pongo pygmaeus*); (G) absent cecum in a bush-tailed phascogale (*Phascogale tapoatafa*). The cecum (dark pink or dark grey) and appendix (dark blue or darkest grey) are oriented toward the top of each drawing, the distal end of the small intestine toward the left, and the proximal end of the large intestine toward the bottom (both light pink or light grey). A cecal appendix is most frequently found in association with spiral (B) and tapering (C) cecal shapes. Images redrawn from Stevens and Hume (1995) and Hume (1999). Figure by Brent Adrian.

Fig. 1. Illustration de sept états de caractères inclus dans cette étude (voir aussi le Tableau 1, caractère 8) : (A) caecum ressemblant à un appendice chez le wombat commun (*Vombatus ursinus*) ; (B) caecum en spirale du phalanger-renard (*Trichosurus vulpecula*) ; (C) long caecum fuselé du lapin (*Oryctolagus cuniculus*) ; (D) caecum cylindrique du castor nord-américain (*Castor canadensis*) ; (E) paire de caecums (ou appendices du côlon) pair du daman du Cap (*Procavia habessinica*) ; (F) caecum sphérique de l'orangoutan de Bornéo (*Pongo pygmaeus*) ; (G) absence de caecum du phascogale à queue en brosse (*Phascogale tapoatafa*). Le caecum (rose foncé ou gris foncé) et l'appendice (bleu foncé ou le gris le plus foncé) sont placés vers le haut de chaque dessin, la partie distale du petit intestin est vers la gauche, et la partie proximale du gros intestin est situé vers le bas (les deux rose ou gris pâle). Un appendice du caecum est le plus fréquemment associé à un caecum de forme en spirale (B) ou fuselé (C). Redessiné par Brent Adrian de Stevens et Hume (1995) et Hume (1999).

gastrointestinal and ecological characters, yielded several significant correlations, four of which remain significant after corrections through FDR (Table 10). Of the ecological variables of particular interest to the research questions, there is a positive correlation between cecum length and group mean size ($P = 0.0013$; Fig. 2), a negative relationship between cecum length and habitat breadth ($P = 0.0073$), and a significant inverse correlation between colon length and weaning age ($P = 0.0101$; Table 10). There are no significant correlations between appendix length and home range, dietary breadth, or any of the other dietary or ecological variables (Table 10). Among the gastrointestinal variables, unsurprisingly, we found a positive relationship between cecum and colon length ($P = 0.0003$).

3.4. Asymmetry between rates of appendix gain and loss

Parsimony suggests a minimum of 29 gains and a maximum of 12 losses of the cecal appendix. Note that not a single loss event is unambiguously supported by our tree, so that there might actually be 41 gains and 0 losses. However, even the most conservative estimate of 29 gains and 12 losses, if we hypothesize that both events are equally probable, the test yields a probability of 0.0115 (two-tailed test) of finding such asymmetrical results. This leads us to reject the null hypothesis, and we conclude that gains in the appendix are more probable than losses.

3.5. Differences in evolutionary rates between clades

These data support a great heterogeneity in evolutionary rates of the cecal appendix between clades (Table 11). The probability that the actual evolutionary rates are equal in Laurasiatheria (which show no transitions) and Euarchontoglires (which show 36 transitions) is inferior to 0.00001. Even the less spectacular difference in evolutionary rates between Euarchontoglires and Metatheria is statistically very highly significant ($P = 0.00094$).

4. Discussion

4.1. Character correlations

4.1.1. Cecal characters and the appendix

We identified evidence of a correlation between the cecal appendix and various characters relating to cecal morphology, including cecal shape, apex thickness, and lymphoid tissue concentration. While these links between the appendix and cecal factors have been suggested before, this is the first time they have been statistically validated. The association between appendix presence and lymphoid tissue provides support for the immune hypothesis of appendix evolution. The present study also documents correlations between the cecal appendix and other gastrointestinal characters. Among these correlations are those between the appendix on one hand, and cecal morphology and cecal apex thickness on the other hand (Table 5). The fact that lymphoid tissue is associated with the appendix in humans has been previously documented (Berry, 1900; Bollinger et al., 2007), but we have now confirmed that this relationship also exists in other species,

Table 7
Number of taxa with an appendix (character 2, appendix observed; state 0, absence; state 1, presence) and with lymphoid tissue (character 5; state 0, absence; state 1, presence). Statistical tests performed only on cells representing known, non-polymorphic scores (bold type) indicate that this pattern is highly significant (Fisher's exact test: $P < 0.0001$; Chi^2 with Yates correction: 69.27, DF = 1, $P < 0.0001$). Chi^2 without Yates correction: 73.82, DF = 1, $P < 0.0001$).

Tableau 7
Nombre de taxons avec appendice (caractère 2, appendice observé ; état 0, absence ; état 1, présence) et tissu lymphoïde (caractère 5 ; état 0, absence ; état 1, présence). Les tests statistiques réalisés seulement sur les cellules représentant des scores connus non polymorphiques (en gras) indiquent que cette configuration est hautement significative (test exact de Fisher : $p < 0,0001$; Chi^2 avec correction Yates : 69,27, DF = 1, $p < 0,0001$). Chi^2 sans correction Yates : 73,82, DF = 1, $p < 0,0001$.

Number of taxa	Ch 5, state 0	Ch 5, state 1	Ch 5,?	Total
Ch. 2, state 0	**75**	**5**	202	282
Ch 2, state 1	**1**	**22**	21	44
Ch 2,?	0	4	191	195
Ch2, 0&1	2	6	7	15
Total	78	37	421	536

DF, degrees of freedom; ?, score unknown.

and more importantly, that both characters are correlated in mammalian evolution, thus providing additional support to the idea that the appendix serves an immunological function in mammals that have one. Additionally, Fisher argued that cecal apex thickness and concentration of lymphoid tissue, both of which were found to be significantly associated with an appendix here, represent alternate characters that should be incorporated into the definition of whether a species possesses an appendix (Fisher, 2000). Our finding that these variables are indeed statistically correlated supports the suggestion that these traits are associated and should be considered in conjunction with appendicular presence/absence.

The correlation between appendix presence and cecal morphology (Table 5) is intriguing and suggests that the shape of the cecum may play a role in whether it has an associated appendix (Fig. 2). Specifically, a cecal appendix is most commonly found in association with tapering and spiral cecal shapes (Fig. 2), in decreasing relative frequency, and is least commonly associated with paired ceca/colonic appendages or rounded ceca. This is supported by examination of the data. Thus, all six species with an appendix-like cecum have an appendix, whereas out of the 37 species with a rounded cecum that are also scored for appendix, only six have an appendix, and four more are polymorphic for appendix presence. The difference in results between the two coding schemes with four states suggests that an important difference in appendix frequency occurs between taxa with a tapering and cylindrical cecum. In lagomorphs and several rodent taxa, the cecal appendix is found almost exclusively in association with a long tapering cecum. However, the relationship between cecal shape and appendix incidence is more variable among primates, in which cecal appendices are variably but inconsistently found in conjunction with rounded, cylindrical, tapering, and spiral-shaped ceca. It should be noted that there exists within the literature a discrepancy regarding whether the "paired ceca" present in, for example, the rock hyrax (*Procavia habessinica*) represent two separate ceca (Rübsamen et al., 1982; Stevens and Hume, 1995), or two colonic appendages projecting off a large colonic sac (Björnhag et al., 1994). Given this disagreement, we have treated these structures as paired ceca or colonic appendages in the present study. However, if future histological evidence suggests that they are true cecal appendices, our data would need to be reinterpreted in that light.

As we reported previously, primates are the most variable large mammalian clade with regard to appendicular and cecal morphology (Smith et al., 2009). There is extensive variation in appendix presence such that some primate species are variable, with some individuals of the species possessing an appendix while others do not. It is unclear whether this variation partially reflects the fact that primate anatomy tends to be relatively well studied and extensively documented compared to many other mammalian taxa, or whether primates are indeed the most polymorphic taxon in this regard. Either way, the variation that has been documented in cecal and appendicular

Table 8
Number of taxa with an appendix (character 2, appendix observed: state 0, absence; state 1, presence) and with cecal apex thickness (character 9: state 0, thin; state 1, thick). Statistical tests performed only on cells representing known, non-polymorphic scores (bold type) indicate that this pattern is highly significant (Fisher's exact test: $P < 0.0001$; Chi^2 with Yates correction: 91.24, DF = 1, $P < 0.0001$; Chi^2 without Yates correction: 97.752, DF = 1, $P < 0.0001$).

Tableau 8
Nombre de taxons avec appendice (caractère 2, appendice observé ; état 0, absence ; état 1, présence) et tissu lymphoïde (caractère 5 ; état 0, mince ; état 1, épais). Les tests statistiques réalisés seulement sur les cellules représentant des scores connus non polymorphiques (en gras) indiquent que cette configuration est hautement significative (test exact de Fisher : $p < 0,0001$; Chi^2 avec correction Yates : 91,24, DF = 1, $p < 0,0001$). Chi^2 sans correction Yates : 97,752, DF = 1, $p < 0,0001$.

Number of taxa	Ch 15, state 0	Ch 15, state 1	Ch 15,?	Total
Ch. 2, state 0	**91**	**2**	189	282
Ch 2, state 1	**0**	**18**	26	44
Ch 2,?	0	4	191	195
Ch 2, 0&1	0	6	9	15
Total	91	30	415	536

DF: degrees of freedom; ?: score unknown.

Table 9

Correlations between inferred appendix presence (a discrete character) and continuous characters, assessed using pairwise comparisons, using the pairing algorithm that contrasts taxa differing in the state of the independent character (appendix presence). In several cases, more than one pairing of terminal taxa is possible; in these cases, the reported probability is the average of the probabilities yielded by the first ten pairings found by Mesquite. A single correlation is significant (probability in bold type), but it does not remain significant after corrections for multiple tests, which indicate that the appropriate threshold is 0.017. Probabilities are rounded off to the fourth decimal.

Tableau 9

Corrélations entre présence d'appendice (caractère discret) et caractères continus, établies en utilisant des comparaisons de paires utilisant l'algorithme de paires qui établit un contraste entre taxons différant dans l'état de caractère indépendant. Dans certains cas, plus d'un assortiment de paires des taxons terminaux est possible ; dans ces cas, la probabilité rapportée est la moyenne des probabilités obtenues pour les dix premiers assortiments de paires trouvés par Mesquite. Une corrélation significative est figurée en gras, mais elle ne reste pas significative après correction pour des tests multiples, qui indiquent que le seuil approprié est 0,017. Les probabilités sont arrondies à la quatrième décimale.

Dependent character	Probability	Polarity (when significant or nearly so)
C1, Body mass	0.0539	
C2, Mean group size	0.4018	
C4, Cecum length	0.1814	
C7, Length of cecum (cm)/cubic root of body mass (g)	0.2120	
C8, Length of colon (cm)/cubic root of body mass (g)	0.3115	
C9, Relative dry matter intake (rDMI in g/kg 0.75)	0.5000	
C10, Particle mean retention time (MRT) (h)	0.5000	
C11, Gastrointestinal (GIT) capacity (measured as total contents wet weight)	0.5000	
C12, Food quality (g crude fibre/kg DM)	0.3500	
C13, Adult to head + body length (mm)	**0.0318**	Positive
C14, Basal metabolic rate (BMR) (mLO$_2$/h)	0.0569	
C15, Diet breadth	0.3201	
C16, Habitat breadth	0.5338	
C17, Home range (km^2)	0.1554	
C18, Population density	0.5000	
C19, Population group size	0.3125	
C20, Trophic level	0.3770	
C21, Weaning age	0.1436	
C22, Weaning body mass	0.6047	
C23, Precipitation mean	0.1538	
C24, Temperature mean (°C)	0.3605	
C25, GR area (km^2)	0.2122	
C26, Maximum latitude	0.2706	
C27, Minimum latitude	0.4845	
C28, Mean latitude	0.3362	

C: character.

morphology and the statistically high frequency of appendices in this clade is intriguing and suggests that the appendix is of particular adaptive significance in primates.

The significant correlation between the appendix and cecal morphological traits also suggests that the cecum and appendix may be co-evolving. Since the appendix develops as an outpocketing of the cecum, it is reasonable to hypothesize that these anatomical structures form an evolutionary module. Modularity has been extensively documented in mammals, although in other organs, such as the skull (e.g., Koyabu et al., 2014). Thus, we propose the term "cecoappendicular complex", to refer to these anatomical structures as a functional, developmental, and evolutionary module.

4.1.2. Appendix morphology and ecological variables

A central question in this study is whether ecological factors, especially those that could affect the incidence of gastrointestinal infection, might influence the evolution of the appendix. In particular, we wanted to assess whether geographic range, group size, or habitat breadth were associated with cecal appendix size or presence. Given that the vermiform appendix is apparently a safe house for bacteria (Bollinger et al., 2007; Sanders et al., 2013), it could be hypothesized that factors affecting maintenance of the microbiome drive the evolution of the appendix. In humans, the single most influential factor adversely

affecting the microbiome in pre-industrial societies is the nature of the water supply and the need for water. Indeed, diarrhoeal illness due to water contamination is a leading cause of childhood death in developing countries. Although our study cannot document a strictly equivalent character in other mammals, the fact that we failed to find a link between appendix presence (or size) and the dietary, ecological, and social characters is puzzling.

4.1.3. Cecal morphology and ecological variables

This study also reveals a multifaceted relationship between ecology and cecal morphology that has not been previously recognized. Relative cecal length was found to correlate with mean group size (Fig. 2). Given the apparent function of the lymphoid tissue within the cecum and appendix in the preservation of the microbiome, these new findings support the view that preservation of the microbiome may be more challenging in the face of larger groups. We also revealed an inverse relationship between cecal length and habitat breadth, which may relate to the phenomenon described above.

Species with larger groups necessarily spend more time in the presence of other individuals, which may in turn cause a greater exposure to communicable diseases and pathogens. Similarly, species with narrow habitat usage may also be forced into closer proximity with conspecifics, than more dispersed generalist species that can spread out

Table 10

Correlations between continuous characters assessed using pairwise comparisons, using the pairing algorithm that finds the greatest number of pairs. In several cases, more than one pairing of terminal taxa is possible; in these cases, the reported probability is the average of the probabilities yielded by the first ten pairings found by Mesquite. The polarity of all significant correlations (before corrections for multiple tests) is indicated. Of the initially significant correlations (probabilities in bold type), only those inferior to 0.017 remain significant after corrections for multiple tests using FDR (marked by an asterisk). Probabilities are rounded off to the fourth decimal.

Tableau 10

Corrélations entre caractères continus, établies en utilisant des comparaisons de paires, avec l'algorithme qui trouve le plus grand nombre de paires. Dans certains cas, plus d'un assortiment de paires de taxons terminaux est possible ; dans ces cas, la probabilité rapportée est la moyenne des probabilités obtenues pour les dix premiers assortiments de paires trouvés par Mesquite. La polarité de toutes les corrélations significatives (avant correction pour de multiples tests) est indiquée. Parmi les corrélations initialement significatives (probabilités en gras), seules celles inférieures à 0,017 restent significatives après correction pour tests multiples utilisant FDR (marquées par une astérisque). Les probabilités sont arrondies à la quatrième décimale.

Independent character	Dependent character	Probability	Polarity (when significant or near-significant)
C6, Appendix length/cubic root of body mass	C1, Body mass	0.5982	
	C2, Mean group size	0.1094	
	C7, Length of cecum (cm)/cubic root of body mass (g)	0.3036	
	C8, Length of colon (cm)/cubic root of body mass (g)	0.2905	
	C9, Relative dry matter intake (rDMI in g/kg 0.75)	0.4063	
	C10, Particle mean retention time (MRT) (h)	0.5188	
	C11, Gastrointestinal (GIT) capacity (measured as total contents wet weight)	0.4375	
	C12, Food quality (g crude fibre/kg DM)	0.2500	
	C13, Adult to head + body length (mm)	0.5226	
	C14, Basal metabolic rate (BMR) (mLO$_2$/h)	0.6875	
	C15, Diet breadth	0.5000	
	C16, Habitat breadth	0.3438	
	C17, Home range (km^2)	0.1719	
	C18, Population density	0.0898	
	C19, Population group size	0.0625	
	C20, Trophic level	0.2539	
	C21, Weaning age	0.1334	
	C22, Weaning body mass	0.6230	
	C23, Precipitation mean	**0.0287**	Positive
	C24, Temperature mean (°C)	0.5628	
	C25, GR area (km^2)	0.0898	
	C26, Maximum latitude	0.2120	
	C27, Minimum latitude	0.1938	
	C28, Mean latitude	0.3953	
C7, Length of cecum (cm)/cubic root of body mass (g)	C1, Body mass	0.5000	
	C2, Mean group size	**0.0013***	Positive
	C8, Length of colon (cm)/cubic root of body size (g)	**0.0003***	Positive
	C9, Relative dry matter intake (rDMI in g/kg 0.75)	0.5000	
	C10, Particle mean retention time (MRT) (h)	0.1537	
	C11, Gastrointestinal (GIT) capacity (measured as total contents wet weight)	0.2517	
	C12, Food quality (g crude fibre/kg DM)	0.2266	
	C13, Adult to head + body length (mm)	0.5482	
	C14, Basal metabolic rate (BMR) (mLO^2/h)	0.1744	
	C15, Diet breadth	0.2051	
	C16, Habitat breadth	**0.0073***	Negative
	C17, Home range (km^2)	0.0672	
	C18, Population density	0.1945	
	C19, Population group size	0.5000	
	C20, Trophic level	0.1537	
	C21, Weaning age	**0.0284**	Positive
	C22, Weaning body mass	0.5598	
	C23, Precipitation mean	**0.0392**	Negative
	C24, Temperature mean (°C)	0.0937	
	C25, GR area (km^2)	0.2592	
	C26, Maximum latitude	0.5044	
	C27, Minimum latitude	0.3319	
	C28, Mean latitude	0.3686	
C8, Length of colon (cm)/cubic root of body size (g)	C1, Body mass	0.5000	
	C2, Group mean size	0.2706	
	C9, Relative dry matter intake (rDMI in g/kg 0.75)	0.5881	
	C10, Particle mean retention time (MRT) (h)	0.3103	
	C11, Gastrointestinal (GIT) capacity (measured as total contents wet weight)	0.2517	
	C12, Food quality (g crude fibre/kg DM)	0.2539	

Table 10 (*Continued*)

Independent character	Dependent character	Probability	Polarity (when significant or near-significant)
	C13, Adult to head + body length (mm)	0.1208	
	C14, Basal metabolic rate (BMR) (mLO$_2$/h)	0.0609	
	C15, Diet breadth	0.3444	
	C16, Habitat breadth	0.1537	
	C17, Home range (km^2)	**0.0197**	**Positive**
	C18, Population density	0.4900	
	C19, Population group size	0.0835	
	C20, Trophic level	0.3388	
	C21, Weaning age	**0.0101**[*]	**Positive**
	C22, Weaning body mass	0.4427	
	C23, Precipitation mean	0.2025	
	C24, Temperature mean (°C)	0.3978	
	C25, GR area (km^2)	0.2199	
	C26, Maximum latitude	0.0753	
	C27, Minimum latitude	0.5000	
	C28, Mean latitude	0.0753	

C: character.

across a greater proportion of the environment. Since the avoidance of pathogens is a key ecological pressure affecting species distribution and ecospace occupation (Brown, 1984), the cecoappendicular complex may have evolved as an adaptive immune response to pathogen avoidance in species with large group sizes and/or narrow habitat breadth. It is also possible that animals living in larger groups may need to be more dietarily flexible (which a large cecum would facilitate) in order to minimize competition for resources with conspecifics.

4.1.4. Dietary factors

The relative length of the cecum and colon are highly significantly correlated with each other (P = 0.00154), which presumably reflects their common digestive function (Table 10). However, we did not reveal any other significant dietary associations. Appendix presence, size, and cecal morphology were not found to correlate with any of our dietary variables. Thus, this study did not provide any evidence in support of the dietary hypothesis of cecal appendix evolution. This suggests that the correlation observed between cecum length and habitat breadth is not an indirect result from a correlation between group size and diet.

4.1.5. Phylogenetic vs. non-phylogenetic correlations

The relationship between cecum length and mean group size constitutes a very interesting pattern in that it is not apparent on a simple bivariate plot (Fig. 2A),

but it becomes apparent when phylogeny-informed comparisons are made (Fig. 2B). This pattern appears to be fairly rare; it is the first time that we encounter it in our careers. The reverse pattern, in which a relationship appears to be significant on a bivariate plot but is no longer significant when phylogeny is taken into consideration, appears to be far more common, judging from the literature, and from our own experience (e.g., Canoville and Laurin, 2010), but we encountered this in nearly all our studies involving character correlation), and it is in fact the case for which phylogenetic comparative methods were developed (e.g., Felsenstein, 1985: fig. 7). A greater prevalence of cases in which relationships can be detected through non-phylogenetic methods than by phylogenetic methods is also suggested by simulations, which showed that simple linear regressions had more power, in a large variety of cases, than phylogenetic independent contrasts (Laurin, 2010: fig. 5), and that non-phylogenetic regressions also recovered a far greater number of false correlations (Laurin, 2010: table 2). This was shown to be true for the three tested evolutionary models (the most commonly used ones), namely Brownian evolution (Brown, 1828), Ornstein-Uhlenbeck (Uhlenbeck and Ornstein, 1930), and speciational (Rohlf et al., 1990) evolution (for a review of these evolutionary models, see Garland et al., 1993). Our data show that at least occasionally, phylogenetic comparative methods can detect relationships that would remain unnoticed using other statistical methods.

Table 11
Evolutionary rate of the appendix presence in various mammalian clades. The events can be gains or losses; of these, the number of gains implied by parsimony is comprised between 29 and 41 (including both extremes of that range).
Tableau 11
Taux évolutif de présence de l'appendice dans divers clades de mammifères. Les évènements peuvent être des gains ou des pertes et, parmi ceux-ci, le nombre de gains établi par parcimonie est compris entre 29 et 41 (incluant les deux extrêmes de cette fourchette).

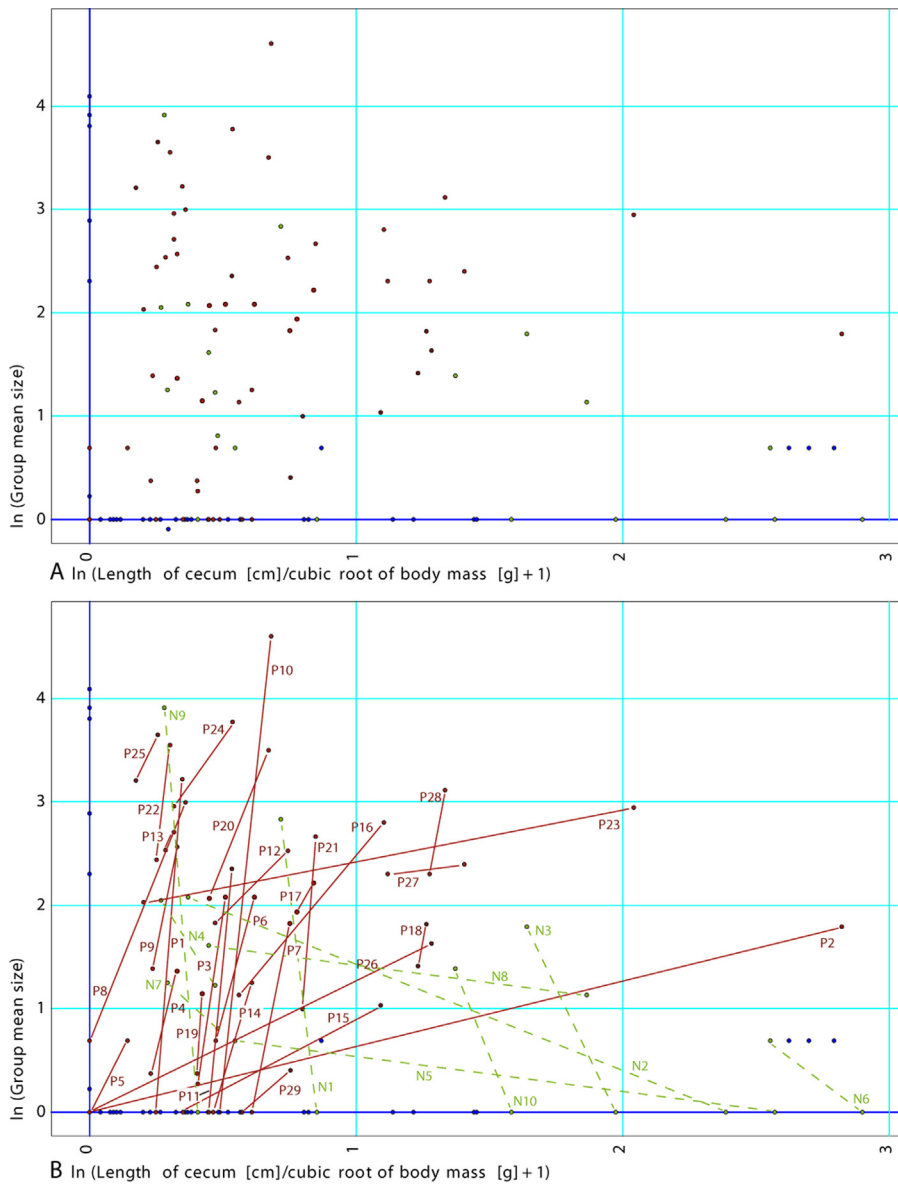| Character	Taxon	Number of events	PDI (My)	Evolutionary rate (events/My)
Appendix presence (observed)	Mammalia	41	11,244	0.003646
	Metatheria	3	1726	0.001737
	Laurasiatheria	0	3816	0.000000
	Euarchontoglires	36	4104	0.008771

My: million years.

Fig. 2. Relationship between body size-corrected cecum length (*x*-axis) and mean group size (*y*-axis). A. Simple scattergram, in which no relationship is obvious. B. Same, with pairs of taxa compared by the algorithm drawing the highest number of pairs in Mesquite. Pairs that have a positive slope (solid red lines, each of which is identified by "P" followed by a number) are far more numerous (29) than pairs that have a negative slope (10; green dashed lines, each of which is identified by an "N" followed by a number). Uninformative pairs (in which a single character changes, or in which one character has missing data) are not shown. Taxa (represented by circles) are color-coded after the polarity (positive or negative) of their pair. This pattern is highly significant ($P = 0.0013$, for the average of examined pairing schemes). This illustrates the need for a phylogeny-informed test of comparative data. Note that there are several ways in which pairs can be selected, but all of them yield significant results, with probabilities ranging from $5.8 \cdot 10^{-5}$ to 0.01185 (this range in probabilities reflects the fact that the exact number of positive and negative pairs varies a bit). This particular pairing scheme corresponds to a probability of intermediate significance ($P = 0.0017$).

Fig. 2. Relations entre longueur du caecum relative à la taille corporelle (abscisse) et taille moyenne du groupe (ordonnée). A. Diagramme de dispersion simple, dans lequel aucune relation n'est évidente. B. Le même, avec les paires de taxons comparées par l'algorithme établissant le plus grand nombre de paires dans Mesquite. Les paires qui montrent une relation positive (lignes rouges continues identifiées par un « P » suivi d'un nombre) sont bien plus nombreuses (29) que les paires qui montrent une relation négative (10 ; lignes vertes pointillées identifiées par un « N » suivi d'un nombre). Les paires non informatives (dans lesquelles un seul des deux caractères change) ne sont pas montrées. Les taxons (représentés par des cercles) sont colorés selon la polarité (positive ou négative) de leur paire. Ce patron est fortement significatif ($p = 0,0013$, pour la moyenne des sélections de paires examinées). Ceci illustre le besoin de tests prenant en compte la phylogénie dans les études comparatives. À noter qu'il existe plusieurs façons de sélectionner les paires, mais toutes donnent des résultats significatifs, avec des probabilités allant de $5,8 \cdot 10^{-5}$ à 0,01185 (cet intervalle reflète le fait que le nombre de paires positives et négatives varie un peu). Le patron illustré ici correspond à une probabilité de valeur intermédiaire ($p = 0,0017$).
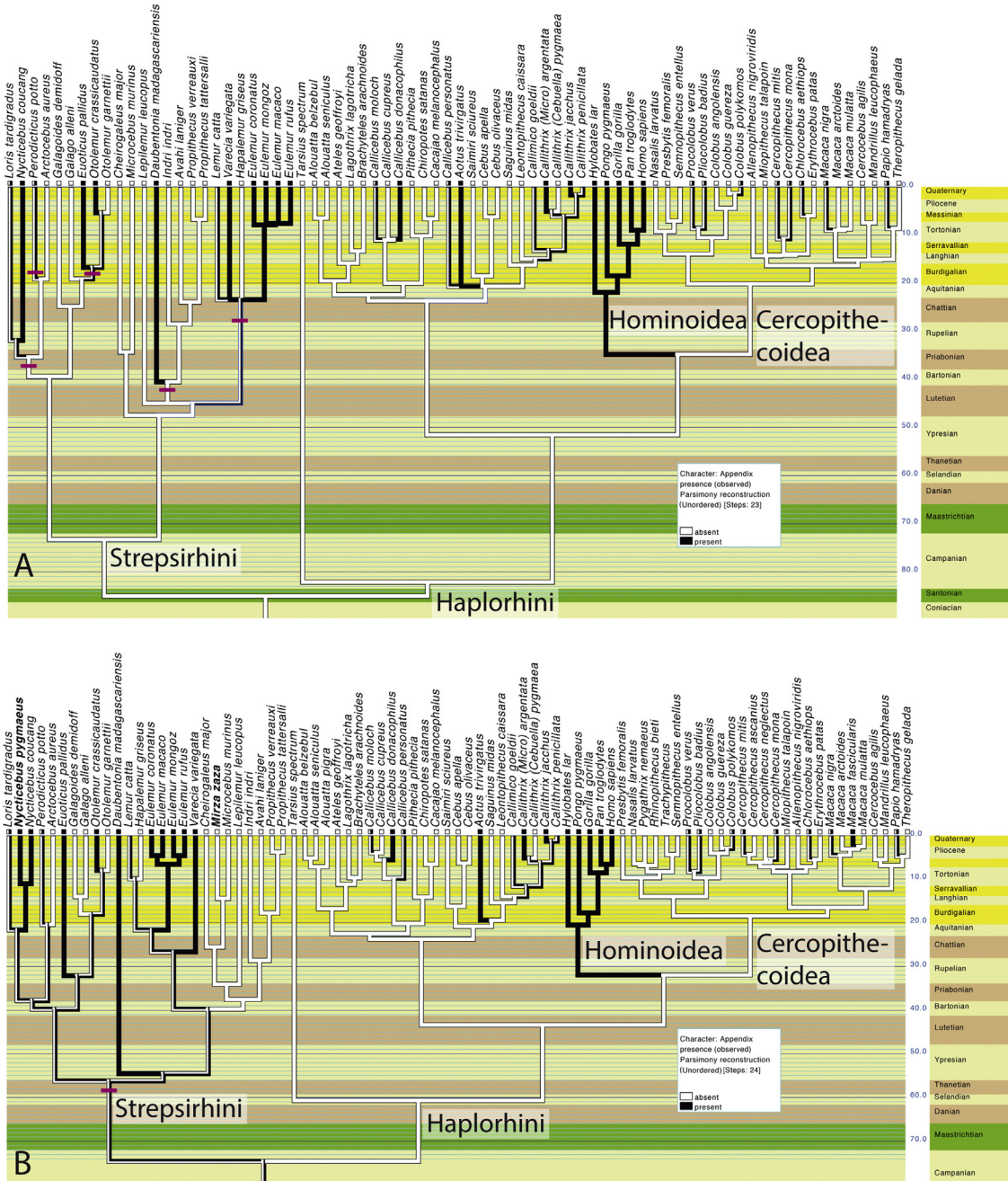
Fig. 3. Evolution of the cecal appendix in primates, according to two reference trees. A. Reference tree of 74 primate species used by Smith et al. (2013). B. Reference tree of 83 primate species used in this study. Note that the tree above (A) implies at least five appearances (pink horizontal bar) of the appendix in strepsirhines, at least under some resolutions of the polytomies. The updated tree (below, part B) implies at least one appearance of the appendix in the same clade. In part B, we added, among strepsirhines, *Nycticebus pygmaeus* and *Mirza zaza* (set in bold type). Note that, in both cases, the actual number of appearances may be greater (up to eight in both cases). Appendix distribution in haplorhines is shown to better document the polarity of the character; gains of the cecal appendix are not marked by a horizontal line in this clade, to avoid excessive detail. This figure is a slightly edited output from Mesquite (Maddison and Maddison, 2014) with the Stratigraphic Tools (Josse et al., 2006). The geological timescale has been added in Mesquite (Maddison and Maddison, 2014) with the Stratigraphic Tools (Josse et al., 2006).

Fig. 3. Évolution de l'appendice du caecum chez les primates, selon deux arbres de référence. A. Arbre de référence de 74 espèces de primates utilisé par Smith et al. (2013). B. Arbre de référence de 83 espèces de primates utilisé dans cette étude. Notez que l'arbre du haut (A) implique au moins cinq apparitions (barres roses horizontales) de l'appendice chez les strepsirhiniens, au moins selon certaines résolutions des polytomies. L'arbre mis à jour (en dessous, partie B) implique au moins une apparition de l'appendice dans ce clade. Dans la partie B, nous avons ajouté, parmi les strepsirhiniens, *Nycticebus pygmaeus* et *Mirza zaza* (en caractères gras). Notez que, dans les deux cas, le nombre d'apparitions réel peut être supérieur (jusqu'à huit, dans les deux cas). La distribution de l'appendice chez les haplorhiniens est montrée pour mieux documenter la polarité de ce caractère ; les apparitions de l'appendice ne sont pas indiquées dans ce clade pour éviter un encombrement excessif. Cette illustration est une version légèrement adaptée d'un arbre exporté de Mesquite (Maddison et Maddison, 2014) avec les *Stratigraphic Tools* (Josse et al., 2006).

4.2. Asymmetry between rates of appendix gain and loss

4.2.1. Asymmetry across the entire mammalian phylogeny

This expanded dataset reveals that the cecal appendix has evolved a minimum of 29 times, possibly as many as 41 times, throughout mammalian evolution, while it has only been lost a maximum of 12 times. This statistically strong evidence that the appearance of the appendix is significantly more probable than its loss suggests a selective value for this structure. Thus, we can confidently reject the hypothesis that the appendix is a vestigial structure with little adaptive value or function among mammals.

The minimal number of gains (29) is lower than in our previous study (32, in Smith et al., 2013), despite the addition of several taxa. This results mostly from changes in topology, which, in some cases, yields ambiguous optimization where, in our previous study, several appearances of the appendix had been hypothesized. The strepsirhines illustrate this. The reference tree of our previous study implied five gains of the appendix in strepsirhines (Fig. 3A), at least under some resolutions compatible with the polytomy. The updated tree, with two additional strepsirhine species (26 instead of 24; we added *Nycticebus pygmaeus* and *Mirza zaza*) implies at least one gain only, because the optimization at the root of Strepsirhini is now ambiguous (Fig. 3B). Note that in both cases, as many as eight gains are compatible with one of the most parsimonious optimizations. However, note that as expected, the increased amount of data and greater resolution of our tree result in a greater number of evolutionary events. Smith et al. (2013) had identified 38 events (including 32 to 38 gains), whereas we have now identified 41 events (including 29 to 41 gains).

4.2.2. Differences in evolutionary rates between clades

The great differences in evolutionary rates, and especially the high evolutionary rate of the cecal appendix in Euarchontoglires, support our earlier suggestion (Laurin et al., 2011; Smith et al., 2013) that this character is a recurrent phenotype, *sensu* West-Eberhard (2003). It is particularly recurrent within Euarchontoglires, with numerous transitions in rodents and primates, and it is essentially ubiquitous in lagomorphs. Interestingly, the heterogeneous pattern of this recurrence appears to be explained better by the phylogeny than by the ecological factors tested. This is shown by the fact that Laurasiatheria, which does not display a single appendix appearance, displays only a little less habitat breadth than Euarchontoglires (rodents, which are euarchontoglires, appear to have one of the highest mean habitat breadth among major mammalian taxa). Some laurasiatherian species surveyed here (*Solenodon paradoxus, Condylura cristata, Neomys fodiens*, among Eulipotyphla and *Cryptoprocta ferox* among Feliformia), have great habitat breadth, but nevertheless lack an appendix. The fact that the evolutionary rates in habitat breadth evolution do not differ significantly between Euarchontoglires and Laurasiatheria, along with the lack of a shift in inferred ancestral habitat breadth near the divergence between both clades, also lends support to the hypothesis that the lack of an appendix in the latter clade is not attributable solely to habitat breadth differences, or differences in the other ecological factors that we analyzed. Whether this represents a purely phylogenetic effect, or in part a correlate of an ecological factor that we have not considered remains to be determined.

The finding that there have been three separate evolutionary gains of the appendix within the Metatheria (marsupials) and probably another in monotremes suggests that the appendix has a deep evolutionary history in mammals. Interestingly, all marsupials and monotremes with an appendix possessed the morphotypes of a long tapering cecum or a small appendix/appendix-like cecum, while monotremes with a rounded or cylindrical cecum were not found to have an appendix. Additionally, several possessed a possible appendix in the absence of any apparent cecum. This raises the question of whether the cecum evolved as an expansion of the appendix, or vice versa, and provides additional support for considering the two structures together, as the cecoappendicular complex.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.crpv.2016.06.001.

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