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Fossils and living taxa agree on patterns of body mass evolution: a case study with Afrotheria

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1   **Mark N. Puttick[1], Gavin H. Thomas[2]**

2   *School of Earth Sciences, Wills Memorial Building, Queen's Road, University of Bristol, Bristol, BS8*

3   *1RJ, UK*

4   *[2] Department of Animal and Plant Sciences, Alfred Denny Building, University of Sheffield, Western*

5   *Bank, Sheffield, S10 2TN, UK*

6   Author for correspondence: Mark N. Puttick

7   E-mail: mark.puttick@bristol.ac.uk

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10 **Abstract**

11 Most of life is extinct, so incorporating some fossil evidence into analyses of macroevolution
12 is typically seen as necessary to understand the diversification of life and patterns of
13 morphological evolution. Here we test the effects of inclusion of fossils in a study of the body
14 size evolution of afrotherian mammals, a clade that includes the elephants, sea cows, and
15 elephant shrews. We find that the inclusion of fossil tips has little impact on analyses of body
16 mass evolution; from a small ancestral size (~ 100 grams), there is a shift in rate and an
17 increase in mass leading to the larger-bodied Paenungulata and Tubulidentata regardless of
18 whether fossils are included or excluded from analyses. For Afrotheria, the inclusion of
19 fossils and morphological character data affects phylogenetic topology, but these differences
20 have little impact upon patterns of body mass evolution and these body mass evolutionary
21 patterns are consistent with the fossil record. The largest differences between our analyses
22 result from the evolutionary model, not the addition of fossils. For some clades, extant-only
23 analyses may be reliable to reconstruct body mass evolution, the addition of fossils and
24 careful model selection is likely to increase confidence and accuracy of reconstructed
25 macroevolutionary patterns.

28 **Introduction**

29 Body mass evolution of Mammalia has received considerable attention in the literature [1-
30 11]. Particular interest has been shown in changes in body size following the K-Pg mass
31 extinction [1], the modes of evolution [2,5], and how rates vary through geological time [3,7].
32 Many studies have approached these issues from an extant-species only perspective (e.g.
33 [3,4]), but there is an increasing awareness of the importance of including fossils in
34 macroevolutionary analyses [5,6,8-10,12].

35 Studying events in deep time using only extant taxa is problematic, as ignoring fossil data can
36 introduce biases and inaccurate reconstruction of phylogenies and macroevolutionary patterns
37 [13]. Further, when studying morphological change, the inclusion of fossils can improve
38 ancestral state estimates in deep time: models with fossil information may fit better than
39 models without [5,6,8-10] and fossil evidence can be used as prior information on ancestral
40 body mass [8]. However, there is some suggestion that studies of macroevolution may be
41 obscured by fossil evidence as it can obfuscate patterns by introducing its own biases [11].
42 One area that is particularly sensitive to the inclusion of fossils is ancestral state
43 reconstruction. Ancestral state reconstruction is generally difficult [14,15] and ignoring fossil
44 evidence can lead to over-inflated estimates of ancestral mass [6].

45 Methodological approaches, as well as the inclusion of fossils, can greatly influence
46 interpretations of macroevolution. Many methods employ a gradualistic Brownian motion
47 (BM) model to study body mass evolution [16-19] and many approaches have built on this
48 framework to study evolutionary tempo [3,20,21] and mode [17-19,22,23]. Recently,
49 parametric approaches have been employed that can model gradual evolution with sporadic
50 bursts [24,25], so these are not rooted in the gradual evolution expectation of the BM model.
51 Currently, the relative influence of model selection versus the inclusion or exclusion of
52 fossils on our understanding of evolution is unclear. Indeed, it may be that models and fossils
53 matter crucially in some circumstances, but not in others.

54 A first step to understanding the relative impacts of fossils and models on ancestral state
55 reconstruction is to reconcile extant (typically molecular) and fossil (morphological)
56 phylogenies. Recently developed methods allow for the incorporation of living and fossil data
57 in phylogenies, by enabling the concurrent analysis of molecular and morphological
58 characters [26,27]. An important step in this process is the use of fossils as tips to date
59 phylogenies [26,27] compared to traditional node dating. Total-evidence dating resolves
60 previous problems of uncertain assignment of fossils to nodes by including fossils in the
61 phylogenetic analysis [28] and it has also been suggested that molecular data improves the
62 resolution of phylogenies containing fossils [29].

63 Here we test the influence of the inclusion and exclusion of fossils on the rates and modes of
64 afrotherian body mass evolution. Using a total-evidence analysis [27], fossils were
65 incorporated from a morphological matrix [30], and evolutionary models were compared to
66 both a traditional molecular-only node-dated tree, and a total-evidence tree that had the
67 fossils removed.

68   Afrotheria, which includes elephants, hyraxes, and tenrecs, consists of around 77 extant
69   species [31-33]. The general consensus on their relationships is that Afrotheria comprises two
70   clades: Afroinsectiphilia, including Tubulidentata (aardvark), Afrosoricida (Chrysochloridae
71   plus Tenrecidae) and Macroscelidea (elephant shrews), and the generally larger-bodied
72   Paenungulata, including elephants and hyraxes [30,33]. Fossil afrotheres are known
73   throughout the Cenozoic [34], and living forms are known to have a wide variation of body
74   size that spans six orders of magnitude.

75   Surprisingly, we find the inclusion or exclusion of fossil tips has little impact on analyses of
76   body mass macroevolution: with all phylogenies there is a relatively small ancestral body size
77   for Afrotheria, and a branch-based shift in rate leading to Paenungulata and Tubulidentata.
78   No datasets support Brownian motion models of evolution, and parametric rate-variable
79   approaches indicate a smaller ancestral mass compared to Brownian motion estimates. The
80   addition of fossil tips on the phylogeny here has little impact on evolutionary rate analyses,
81   but there are differences attributable to model selection. Whilst inclusion of morphological
82   characters and fossil species alters phylogenetic topology, these differences result in
83   negligible differences in patterns of body mass evolution or ancestral body mass estimation.
84   In some cases of macroevolutionary analyses, as here, it may be possible to reconstruct
85   evolutionary history whilst using extant species only, although the addition of fossils will
86   increase confidence of reconstructed patterns.

87   **Methods**

88   Taxa

89   We recognise a total of 77 extant afrotherian species (see electronic supplementary material,
90   S1) [31], and we used a morphological matrix of fossil and extant afrotheres [30, 35]. The
91   matrix contains a sample of fossil taxa across Afrotheria, and these fossils are generally
92   early-diverging members of crown clades, so it is likely that they give good estimates of
93   ancestral morphology and timing of diversification [27, 35]. We sample a total of 39
94   afrotherian fossils based on morphological data only and a further seven taxa for which
95   molecular data is available (see below). For Afrotheria, the morphological data samples all
96   extant orders, as well as fossil members of extant orders. Within Afrotheria, these fossil taxa
97   are believed to be stem or crown members of extant familes, with the possible exception of
98   *Chambius kasserinensis* and *Herodotius pattersoni* [35]. Extant outgroup taxa were selected
99   from Xenathra (3 species), Boreoeutheria (13 species), and marsupials (3 species).
100   Additionally, we sampled two fossil crown placentals (*Montanalestes keeblerorum* and
101   *Prokennalestes trofimovi*) (see electronic supplementary material, S1).

102   Genetic Data

103   Genetic data were taken for six nuclear and four mitochondrial loci from Genbank [33, 36].
104   Genetic data were aligned using ClustalW [37], with protein-coding genes aligned by codons
105   and non-protein genes by nucleotide. Unalignable regions were removed from non-coding
106   sequences using GBlocks (version 0.91b) [38].

107   The following genes were used in the analyses: Growth Hormone Receptor (GHR), Alpha-2B
108   Adrenergic Receptor (ADRA2B), Androgen Receptor (AR), von Willebrand Factor (vWF),
109   Interphotoreceptor Retinoid-Binding Protein (IRBP), and Brain-Derived Neurotrophic Factor
110   (BDNF) were the nuclear protein-coding genes, and Cytochrome b (cyt*b*) and Nicotinamide
111   Adenine Dinucleotide (NADH2) were the two mitochondrial protein-coding genes.
112   Additionally, sequence data from the mitochondrial 12s and 16s genes were collected. The
113   dataset differs from Kuntner *et al.* [33] by the addition to Brain-Derived Neurotrophic Factor
114   and some additional data for some species (see Supplementary Material S1). Of the 77 extant
115   species recognised we have genetic data for 60 (approximately 78% of the total). When
116   extinct species that have genetic information are included, coverage for Afrotheria species
117   ranges from 67% for GHR to 25% for AR.

118   Data were also collected for extinct species in the analysis. As with Kuntner *et al.* [33], we
119   gathered information on the proboscideans *Elephas antiquus falconeri*, *Elephas cypriotes*,
120   *Elephas maximus asurus*, and *Elephas* sp., and an undetermined species from Tilos island
121   [32]. We also included the mastodon *Mammut americanum,* and the mammoths *Mammuthus*
122   *primigenius* and *Mammuthus columbii* and Steller's sea cow (*Hydrodamalis gigas*).

123   All alignments were checked by eye. PartitionFinder (version 1.1.1) [39] was used to select
124   the partitions of genes and models of evolution for the genetic data. For most genes the best-
125   fitting substitution model was the General Time-Reversible (GTR) model with gamma
126   distributed rate variation between sites and a proportion of invariant sites. Exceptions to this
127   model were the GTR with gamma distributed rate variation and no invariant sites (cytB), the
128   Kimura 82 model (GHR), and the Kimura 82 model with a proportion of invariant sites
129   (BDNF).

130   Phylogenies

131   Phylogenies were constructed and dated in MrBayes 3.2.5 [40]. All phylogenetic analyses
132   were run for twenty million generations sampling every 1000 generations, with four chains
133   and four independent runs for each analysis. The heating parameter was set to 0.05 for
134   analyses that included fossils and 0.1 for analyses that did not include fossils. Priors were set
135   using established protocols [27] (see electronic supplementary material, S1), and convergence
136   was judged using in-built diagnostics of MrBayes and Tracer [41].

137   An initial non-clock analysis was run on the entire dataset of fossils and extant species, with
138   no calibration on ages (see Supplementary Materials S5, and Supplementary Fig. S2).

139   Time-calibrated analyses

140   We conducted three sets of dating analyses (i) node and tip dating using both morphological
141   and molecular data (total-evidence analysis), (ii) node only dating using molecular data only
142   (node-dating analyses), and (iii) node only dating using both morphological and molecular
143   data. For both the total-evidence and node-dating analyses, the following nodes were
144   calibrated at Theria (root), Marsupialia, Placentalia (crown), Boreoeutheria, Atlantogenata,
145   Xenarthra, Afrotheria, Paenungulata, and Macroscelidea. Node dates were set as offset-

146 exponential distributions with dates primarily taken from a published source [42]. For the
147 total-evidence analysis, tip dates came from 41 unconstrained species believed to be
148 Afrotheria and from two stem placentals. Tip dates for fossils were set as uniform
149 distributions, with dates taken from the FossilWorks [43] portal which accesses data in the
150 Paleobiology Database [44] (see electronic supplementary material, S6). However these data
151 were further checked using the primary literature (see Supplementary Table S2). For the
152 total-evidence analyses there were 50 dating points on the phylogeny (41 tips dates and nine
153 node dates). In MrBayes, we set the fossilised birth-death model [45] as tree prior. The
154 fossilised birth-death model relaxes the assumption of a uniform prior between the timing of
155 nodes and incorporates estimates of speciation, extinction, and fossil sampling rates into the
156 tree prior. In this model we assumed that fossil tips are sampled as branching lineages
157 ('Samplestrat=fossiltip') but not as direct ancestors sitting on branches as is used in some
158 models (i.e., not in the implementation in [46]). Priors for the speciation, extinction, and
159 sampling rates were set at their defaults as according to MrBayes 3.2.5: the speciation rate
160 prior ('SpeciationPr') was set to an exponential distribution with rate 1, and the relative
161 extinction rate prior ('Extinctionpr') and the relative fossilisation rate ('FossilizationPr') were
162 both set to a Beta distribution (mean = 1, shape = 1) which gives a uniform prior between 0-
163 1.

164 For the total evidence analysis the following topological constraints were applied:
165 Marsupalia, Boreoeutheria, Atlantogenata, Xenarthra, crown Placentalia, Afrotheria,
166 Paenungulata, Proboscidea, Sirenia, Hyracoidea, Macroscelidea, crown Macroscelidea, and
167 Chrysochchloridae. These clade memberships were based upon an initial unconstrained non-
168 clock phylogenetic analysis.

169 Body Mass Data

170 Measurements of body mass were obtained for extant and extinct species in the phylogeny.
171 Body mass data for extant species were predominantly taken from published estimates (see
172 electronic supplementary material, S12). For the extinct species, the preferred data sources
173 were from previously published mass estimates; when published data were not available,
174 body masses were mainly estimated from regression equations on molar area [47] (see
175 electronic supplementary material, S12).

176 Models of Body Mass Evolution

177 Models of body mass evolution were tested on a selection of trees to assess the impact of
178 fossils. For a direct comparison of the effects of fossils, body mass evolution was tested on
179 the total-evidence phylogeny (i), and on the total-evidence phylogeny with fossils removed
180 (ii). Furthermore, models were tested on the molecular-only node-dated phylogeny (iii), as
181 this reflects the classic approach to construct time-calibrated phylogenies for comparative
182 analyses. Additionally, models were tested on the node-dated phylogeny constructed using
183 molecular and morphological data (iv).

184 The BM model is commonly used either to model trait evolution on phylogenies directly or
185 as a basis for more complex models. The BM model assumes, on a phylogeny with branch

186 lengths scaled to time, that variation in trait data accumulates proportionally through time,
187 with a mean expectation of zero change in the value of the trait per unit time. However, the
188 model makes assumptions that may be unrealistic [24,25]. The nature of the model means
189 that variance, and therefore rates, are finite and do not change in the phylogeny [25].
190 Therefore, to incorporate any rate variation the model must be extended with extra
191 parameters to model changes in rate [3,20,21,48]. If this is performed over the entire
192 phylogeny with each branch permitted to take a unique rate [48], the result is that the model
193 has too many parameters for justifiable inference – a new rate on every branch in a fully
194 bifurcating phylogeny results in nearly as twice as many parameters (2n-2 where n is tips) as
195 data points (values at the tips). An alternative to modelling specific changes in rates is to use
196 parametric models that do not assume constant rates, by sampling rates from a heavy-tailed,
197 rather than normal, distribution [24, 25]. This achieves two objectives: these models do not
198 require a homogeneous gradual model of evolution, and they allow for an ancestral trait
199 reconstruction with a model of rate evolution that is not over-parameterised.

200 We use the software StableTraits to parametrically model gradual evolution with intermittent
201 bursts and to reconstruct ancestral size estimates and model rates through time [25].
202 StableTraits samples from a symmetrical, mean zero distribution which is defined by its
203 index of stability (α): for BM $\alpha=2$, which results in a normal distribution, but when $\alpha<2$ this
204 results in a shallower distribution with heavy tails which allows for a more unpredictable
205 evolutionary trajectory. For all trees, results from a heavy tailed distribution in which the α is
206 allowed to vary from BM were compared to a BM model in terms of the rates through time,
207 ancestral size estimation, and the model fit [25]. The MCMC chain was run for 2000000
208 iterations with four runs, until the Potential Scale Reduction Factor went below 1.01. The
209 burn-in was set to 10%, with the output containing the calculated rates, ancestral states, and
210 maximum posterior probability. The model was tested against a model fixed to BM by re-
211 running the analyses with $\alpha = 2$, and then comparing the Bayesian Predictive Information
212 Criterion (BPIC) [25]. Subsequent data processing and plotting were carried out in R [49].

213 Prior information on ancestral mass

214 To introduce further information for the ancestral mass estimation for Afrotheria, an arbitrary
215 outgroup tip was added and set a given mass to represent knowledge from the fossil record or
216 ancestral estimates from previous studies; this outgroup was separated from Afrotheria by
217 either 5 Myr (the edge leading to the tip of the outgroup was 0.01 Myr). 5 Myr was the
218 original length separating the Afrotheria from the Xenarthra and would allow prior
219 information to influence the root, but the mass value could change over the length. In
220 different analyses the outgroup was given a mass of 0.1, 0.5, 1, 5, 10, and 20 kg respectively.
221 The values incorporate estimates for late Cretaceous mammals from the fossil record, ~80g
222 [1], as well as larger estimates for ancestral Afrotheria from genomic studies, ~0.5–30 kg
223 [e.g, 4].

224 **Results**

225 Topology and divergence times

226 The total-evidence phylogeny (figure 1) and non-clock phylogeny (see electronic
227 supplementary material, figure S2) are very similar, and the composition of all the major
228 clades is identical.

229 Larger differences are seen when morphological data is included compared to molecular-only
230 topologies: in all analyses with the morphological cladistic matrix Afroinsectiphilia is not
231 monophyletic as Macroscelidea is closer to Paenungulata. The composition of crown families
232 is consistent, but the position of fossil taxa do vary between analyses. For example, the fossils
233 *Chambius* and *Herodotius* move from sister of Paenungulata plus Tubulidentata in the non-
234 clock topology to being in a basal polytomy with Macroscelidea in the total-evidence
235 analysis.

236 Ages from the total-evidence analysis that includes fossils (figure 1) are older than the ages
237 from node-dating analysis (Table 1).

238 Ancestral States

239 For all analyses, neither rates nor ancestral body size reconstructions are strongly influenced
240 by the inclusion of in-group fossils. Additionally, for all analyses the StableTraits model
241 provided a better fit for the data than BM.

242   In the total-evidence based approach with no-outgroups and rate heterogeneous
243 (StableTraits) model the ancestral size at the origin of the Afrotheria is estimated to be 0.10
244 kg (95% CIs 0.02-0.95 kg). In contrast, the BM estimate is an order of magnitude larger 1.45
245 kg (95% CIs, 0.31-6.82 kg); however, the broad confidence intervals overlap with those of
246 the rate heterogeneous model (table 2; figure 2). The fit of the heavy tailed rate
247 heterogeneous model ($\alpha = 1.77$, 1.47-1.94) was superior to the BM model ($\alpha = 2$) (ΔBPIC =
248 21.8).

249 Removal of fossils caused little difference in the ancestral size estimation of Afrotheria
250 (0.13kg) but had a marked effect on the confidence intervals, which became much wider
251 (0.02-12.48 kg). For the molecular-only node dating analysis, the ancestral size for estimate
252 Afrotheria was 0.11 kg (95% CIs, 0.02-761.4 kg). Similar results were found for the
253 combined morphological-molecular node-dating analysis (Table 2).

254 Evolutionary Rates from StableTraits

255 In all StableTraits analyses there is an increase in the rate of body mass evolution leading to
256 the Tubulidentata plus Paenungulata (figure 2). For the total-evidence analysis, the increase
257 leading to Tubulidentata plus Paenungulata is 137.7 times the original branch length (length
258 of the identical branch on the time-scaled input phylogeny) (figure 2), compared to an
259 increase of 117.0 times the original length when fossils are removed from the phylogeny. The
260 rate increases are less dramatic for the molecular-only node-dated phylogeny (35.2 times the
261 original rate) and the morphology and molecular node-dated phylogeny (19.9 times the
262 original rate). On the morphology and molecular node-dated tree with only extant taxa there
263 is also a further increase (37.3 times the original rate) leading to the Proboscidea plus Sirenia.

264   Impact of prior information

265   The addition of outgroups of variable mass (0.1 to 20 kg) had little impact on estimates of
266   ancestral mass for Afrotheria (Supplementary Table S2 and S3) or rates through time
267   (Supplementary Figure S7). Even when the outgroup represents a body mass that is much
268   larger than those known from the fossil record (e.g., 20 kg), the mass estimates from ancestral
269   Afrotheria are relatively small (~ 2 kg), indicating the stability of the reconstructed patterns
270   in this study.

271   **Discussion**

272   Congruent patterns of body mass evolution are produced when fossil tips are included or
273   excluded. The addition of fossil tips to analyses has little effect on the analyses of ancestral
274   mass estimation and rates of body mass evolution through time. A number of studies have
275   argued that fossils are vital to understand patterns of body mass evolution [6,8-10], but results
276   from analyses in Afrotheria are consistent if fossil tips are included or excluded from
277   phylogenies. The minor impact of fossil tips on macroevolutionary interpretations in this case
278   may be expected: the afrotherian fossil record is biased towards Paenungulata [34], and none
279   of the fossils in the clades is larger or smaller than extant members of those clades.
280   Furthermore, there is generally a bias in the fossil record of the two groups: with the
281   exception of Macroscelidea, the fossil record of Afroinsectiphilia is not as comprehensive as
282   the record of Paenungulata [34], but there is fossil representation of all the major clades
283   included in our analyses. There is no evidence to suggest that earlier afroinsectiphilians
284   (excluding tubulidentates) were much larger than today's species, whereas some extinct
285   hyraxes were indeed much larger than their extant relatives. Fossils, or at least morphological
286   character data, do have large impacts on the topology of Afrotherian phylogeny. However,
287   these differences in topology do not have a large impact on analyses of body mass evolution
288   in this study, but instead show how different data types and fossil inclusion can change our
289   interpretations of evolution. More evident than the inclusion or exclusion of fossils is the
290   impacts of model selection.

291   Despite the minor impact of fossils in estimating ancestral body size in the Afrotheria, we do
292   not suggest that these results should be taken as grounds to ignore fossil data. Previous
293   studies have demonstrated the need for phylogenetically informed sampling for ancestral
294   state reconstruction [50]. Recent studies have suggested the results here – that fossils have
295   little impact upon reconstructions of morphological evolution - may not be applicable to other
296   clades, such as birds [51], or even all mammals [6,8-10]. As noted above, the distribution of
297   fossil tips and sizes may explain their minor impact in this specific case. The omission or
298   misplacement of taxa, whether fossil or extant, can effect estimates of evolutionary rates and
299   ancestral states. Moreover, our results suggest that inclusion of fossil data may increase
300   confidence in ancestral state estimates. Fossils may still be very important in studies of body
301   mass evolution, but exploration of alternative evolutionary models can also be important. A
302   recent study has shown that careful model selection can elucidate body mass evolution
303   patterns from extant data that have previously only been shown in fossils [52]; here we
304   support that the evolutionary model can have a large impact on our interpretations of

305   evolution. It will often be difficult to judge *a priori* whether fossils or the evolutionary

306   model will matter more and as such both should be assessed wherever possible.

307   The largest difference in reconstructions of body mass evolution in Afrotheria is not when

308   fossils are included or excluded, but when comparing alternative evolutionary models.

309   Mesozoic mammals, including early Placentalia, have been shown to be generally small (~80

310   g) [1] and high morphological rates of change are found early in the evolution of clades (Raia

311   et al. 2013). In contrast, genomic studies have indicated a larger ancestral mass for Afrotheria

312   [4,53]. Our results are congruent with the fossil record, whether fossils or included or

313   excluded (figure 2). Furthermore, other studies have found similarly small ancestral sizes for

314   the Afrotheria (0.36 kg) using the same method (StableTraits) but different data [25]. There is

315   a ~10 fold difference in estimates from StableTraits and BM (Table 2); this suggests that

316   model selection, rather than inclusion of fossils has a greater impact in reconstructed

317   ancestral body mass. However, it should be noted that in all cases the confidence intervals for

318   StableTraits and BM ancestral size estimates overlap (Table 2). Whilst there are general

319   difficulties in reconstructing ancestral mass [14-15], fossil tips do not necessarily impact on

320   either the best fitting evolutionary model or the ancestral state estimates. Our results appear

321   to be robust to the possibility of undiscovered afrotherian species with extreme body sizes as

322   demonstrated by the very minor effect of manipulating a proxy prior on the root. The main

323   effect of an informed prior, such as previous estimates (e.g., [4]), is to tighten the confidence

324   intervals for ancestral state estimates.

325   Previously total-evidence data have been shown to produce both younger and older ages than

326   node dating [27, 54], but other studies (e.g. [55]) are congruent with the results here in that

327   the majority of node ages are older in the total-evidence analyses (see electronic

328   supplementary material, figure S5). Here the evidence strongly suggests that fossils are

329   pushing median dates back in time; a similar result has been found generally for all mammals

330   [55]. While these ages are larger than large-scale molecular estimates [56], they are not

331   implausible [55] and there is still an overlap in the posterior distributions of ages on the major

332   nodes and root; thus there is no significant effect from the morphological matrix on

333   divergence time estimation. Additional studies that have employed the fossilised birth-death

334   model [46] have found that employing a method that allows for sampling fossils as direct

335   ancestors generally results in age estimates that are more congruent with the fossil record [46,

336   57-59]. However, many of these studies (e.g. [57]) find that traditional node-constraints can

337   result in ages that are congruent with the fossil record, which appears to be the case here.

338   **Conclusions**

339   Fossils have a vital role to play in the understanding of macroevolution. However, it is

340   important to note that the addition of fossils will not always produce results that contradict

341   analyses based on extant taxa. Data from fossils, in some cases, will agree with data from

342   living species, so other factors, such as the choice of evolutionary model, are likely to be also

343   important when elucidating patterns of evolution. Therefore, it may be possible to trust

344   analyses based on extant taxa only, but incorporating fossil information and careful model

345   selection can increase confidence in our interpretations.

352   **Data accessibility:** input files for phylogenetic analysis, and the body mass data are available
353   in the electronic supplementary materials.

Authors' contributions

357   MNP and GHT devised the project, MNP carried out the analyses, MNP wrote the first draft
358   and MNP and GHT revised and edited subsequent drafts of the manuscript.

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	Total-evidence	Node-Dating (molecular and morphological data)	Node Dating (molecular only)
Afrotheria	**106.3** (91.3, 123.9)	**96.7** (78.6, 116.9)	**92.9** (74.3, 114.5)
Paenungulata	**99.3** (85.3, 115.4)	**61.5** (55, 74.8)	**61.8** (55, 76.2)
Afroinsectiphilia	NA	NA	**90.2** (71.6, 110.7)
Proboscidea	**29.5** (18.9, 41.1)	**23.9** (14.6, 33.8)	**24.5** (15.3, 34.5)
Sirenia	**29.6** (17.6, 43.9)	**26.9** (15.9, 39.7)	**27.3** (15.5, 39.4)
Hyracoidea	**21.9** (12.1, 33.3)	**18.5** (8.4, 29.1)	**18.7** (8.4, 28.8)
Afrosoricida	**91.6** (77.1, 109.0)	**89.5** (71.5, 109.1)	**85.1** (67.3, 106.1)
Tenrecidae	**77.5** (62.2, 92.2)	**78.8** (62.0, 97.8)	**76.7** (59.5, 96.3)
Chrysochloridae	**34.3** (23.4, 46.7)	**39.6** (27.6, 53.3)	**40.4** (27.8, 56.0)
Macroscelidea	**57.8** (45.0, 71.9)	**75.2** (58.0, 95.7)	**77.5** (58.6, 98.4)

517

518   **Table 1** Dates from the total-evidence analyses are older than the node-dating analysis but
519   the 95% posterior density shows overlap for crown Afrotheria.

Phylogeny	StableTraits	Brownian motion	Best-fitting model	ΔBPIC
Total Evidence	**0.10** (0.02, 0.95)	**1.45** (0.31, 6.82)	StableTraits	**21.76725**
Extant Only	**0.13** (0.02, 12.48)	**1.59** (0.28, 8.94)	StableTraits	**53.5555**
Node Dating (molecular only)	**0.11** (0.02, 761.4)	**0.77** (0.14-3.99)	StableTraits	**57.42925**
Node Dating (molecular and morphological data)	**0.09** (0.02, 0.62)	**0.53** (0.10, 2.75)	StableTraits	**42.499**

520

521   **Table 2.** Reconstruction of ancestral body size using StableTraits indicate the minimal
522   impact of fossil tips on root mass estimates.

523

524

525   **Figure 1.** The dated total-evidence phylogeny of the Afrotheria indicates a late Cretaceous
526   origin for Afrotheria. Tubulidentata and Macroscelidea form successive outgroups to the
527   Paenungulata (Sirenia, Proboscidea, Hyracoidea), and so the Afroinsectiphilia
528   (Macroscelidea, Afrosoricida) is non-monophyletic. All major clades are highlighted:
529   Proboscidea (purple), Sirenia (brown), Hyracoidea (navy), Tubulidentata (red),
530   Macroscelidea (yellow), Chrysochloridae (blue), and Tenrecidae (green). Animal images
531   public domain, except for the following from Wikipedia, and covered by Creative Commons
532   licenses that are attributed to the following authors: Elephant (Ikiwaner), Hyrax (D. Gordon
533   E. Robertson), Aardvark (Masur), Elephant shrew (Joey Makalintal), Golden mole (Hohum),
534   and Tenrec (Wilfried Berns).

535

536   **Figure 2.** The effects of model selection are more evident than the inclusion of fossils. If
537   fossils are included or excluded, there is a large increase in the morphological rate of
538   evolution leading to the Paenungulata plus Tubulidentata (red branch) (a,b). The
539   reconstructed body size is comparable between the total evidence and neontological studies
540   that use the StableTraits models (c,d), and BM model (e,f).

Calcochloris leucorhina
Chrysochloris stuhlmanni
Cryptochloris wintoni
Chrysochloris asiatica
Eremitalpa granti
Calcochloris obtusirostris
Chrysospalax villosus
Chrysospalax trevelyani
Chlorotalpa sclateri
Chlorotalpa duthieae
Carpitalpa arendsi
Neamblysomus julianae
Neamblysomus gunningi
Amblysomus septentrionalis
Amblysomus robustus
Amblysomus marleyi
Amblysomus hottentotus
Amblysomus corriae
Potamogale velox
Micropotamogale lamottei
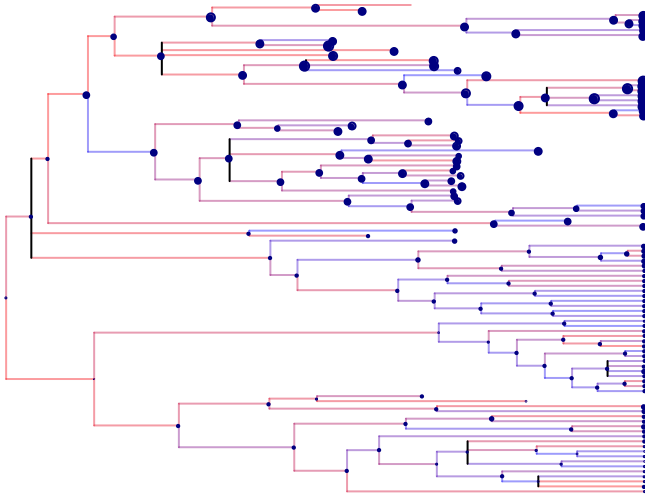*Protenrec tricuspis*
Widanelfarasia bowni
Dilambdogale gheerbranti
Tenrec ecaudatus
Hemicentetes semispinosus
Setifer setosus
Echinops telfairi
Geogale aurita
Oryzorictes hova
Microgale brevicaudata
Microgale taiva
Microgale parvula
Limnogale mergulus
Microgale talazaci
Microgale jobihely
Microgale cowani
Microgale pusilla
Microgale principula
Microgale longicaudata
Herodotius pattersoni
Chambius kasserinensis
Metoldobotes sp nov
Elephantulus fuscipes
Elephantulus revoili
Elephantulus fuscus
Rhynchocyon petersi
Rhynchocyon chrysopygus
Rhynchocyon udzungwensis
Rhynchocyon cirnei
Macroscelides proboscideus
Petrodromus tetradactylus
Elephantulus rozeti
Elephantulus myurus
Elephantulus edwardii
Elephantulus rupestris
Elephantulus intufi
Elephantulus rufescens
Elephantulus brachyrhynchus
Myorycteropus africanus
Orycteropus afer
Protosiren smithae
Prorastomus sirenoides
Hydrodamalis gigas
Dugong dugon
Trichechus senegalensis
Trichechus manatus
Trichechus inunguis
Moeritherium spp
Phosphatherium escuilliei
Daouitherium rebouli
Numidotherium koholense
Barytherium spp
Arcanotherium savagei
Omanitherium dhofarensis
Phiomia spp
Mammut americanum
Elephas maximus asurus
Elephas cypriotes
Elephas sp.
Elephas antiquus falconeri
Elephas maximus
Mammuthus primigenius
Mammuthus columbi
Loxodonta cyclotis
Loxodonta africana
Dimaitherium patnaiki
Microhyrax lavocati
Seggeurius amourensis
Thyrohyrax pygmaeus
Thyrohyrax meyeri
Thyrohyrax domorictus
Dendrohyrax dorsalis
Procavia capensis
Heterohyrax brucei
Saghatherium bowni
Selenohyrax chatrathi
Saghatherium antiquum
Afrohyrax championi
Antilohyrax pectidens
Titanohyrax angustidens
Titanohyrax sp nov
Titanohyrax andrewsi
Thyrohyrax litholagus
Megalohyrax sp nov
Megalohyrax eocaenus
Bunohyrax fajumensis
Pachyhyrax crassidentatus
Bunohyrax major
Geniohyus mirus
Geniohyus diphycus

Chrysochloridae
Tenrecidae
Macroscelidea
Tubulidentata
Sirenia
Proboscidea
Hyracoidea

Afrosoricida
Paenungulata

106 90 70 50 30 10
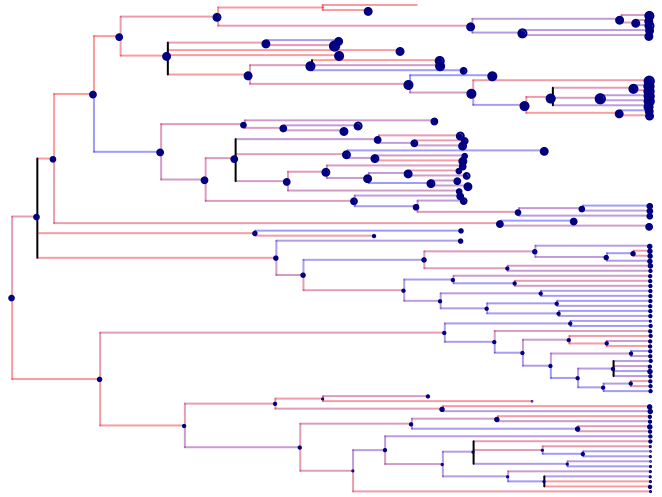
Ma

(a) Total Evidence − stableTraits Rates

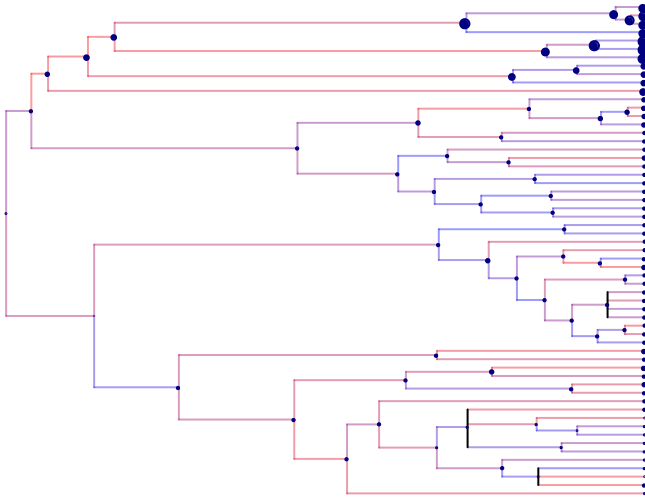*(b)* Extant only − stableTraits Rates

(c) Total evidence ancestral states − stableTraits

(d) Total evidence ancestral states − bm

(e) Extant species ancestral states − stableTraits

(f) Extant species ancestral states − bm