Dietary isotopes of Madagascar's extinct megafauna reveal Holocene browsing and grazing guilds

James P. Hansford[1,2,3,*], Samuel T. Turvey[1]

[1]*Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK*

[2]*Department of Biological Sciences, Northern Illinois University, DeKalb, Illinois 60115, USA*

[3]*School of Ocean and Earth Science, National Oceanography Centre, University of Southampton, Southampton, UK*

*Corresponding author: james.hansford@ioz.ac.uk

Abstract. Megafauna play a disproportionate role in developing and maintaining their biomes, by regulating plant dispersal, community structure and nutrient cycling. Understanding the ecological roles of extinct megafaunal communities, for example through dietary reconstruction using isotope analysis, is necessary to determine pre-human states and set evidence-based restoration goals. We use $\delta^{13}C$ and $\delta^{15}N$ isotopic analyses to reconstruct Holocene feeding guilds in Madagascar's extinct megaherbivores, which included elephant birds, hippopotami and giant tortoises that occurred across multiple habitats and elevations. We compare isotopic data from seven taxa and two elephant bird eggshell morphotypes against contemporary regional floral baselines to infer dietary subsistence strategies. Most taxa show high consumption of C_3 and/or CAM plants, providing evidence of widespread browsing ecology. However, *Aepyornis hildebrandti*, an elephant bird restricted to the central highlands region, has isotope values with much higher $\delta^{13}C$ values than other taxa. This species is interpreted as having obtained up to 48% of its diet from C_4 grasses. These findings provide new evidence for distinct browsing and grazing guilds in Madagascar's Holocene megaherbivore fauna, with implications for past regional distribution of ecosystems dominated by endemic C_4 grasses.

Keywords: Elephant bird, hippopotamus, isotope ecology, megafauna, Quaternary extinction

1. Introduction

Late Quaternary and older terrestrial ecosystems were typically dominated by megaherbivores, which shaped their environments through top-down interactions with plant communities and vegetation structure [1,2]. Megaherbivores impact diversity and structure of ecosystems by suppressing plant growth through physical disturbance and herbivory, influencing nutrient cycling within and between landscapes, and dispersing plant propagules [3,4]. However, megafauna have been disproportionately vulnerable to human-caused extinction, and many ecosystems now lack these keystone species [5]. Reconstructing the ecology of now-extinct megaherbivore guilds is essential to identify disrupted ecological processes and guide environmental management and restoration [1,6].

Late Quaternary Madagascar supported a diverse megafauna, including elephant birds, hippopotami and giant tortoises. Madagascar's megaherbivores became extinct in the late Holocene during a period of intensive anthropogenic forest clearance and conversion to open habitats around 1100-1000 BP [7-9]. Today 80% of Madagascar is covered by grassland [10,11], but the pre-disturbance distribution and extent of native grasslands remains uncertain [12,13]. Madagascar contains native grass lineages dating from the Miocene, and 40% of its grass species are unique; it contains amongst the world's highest grass diversity and endemism, with particularly diverse assemblages in the island's central highlands ecogeographic region [10,11]. However, there is limited evidence for regional existence of a late Quaternary vertebrate grazing guild, suggesting that endemic grasses may have been limited to small, low-density clearings [13-16].

Most trees, shrubs and herbs use a C_3 (Calvin) metabolic pathway for carbon fixation during photosynthesis, whereas most tropical grasses use a C_4 (Hatch-Slack) pathway [17]. Stable carbon isotope ($\delta^{13}C$) values in bones of animals that consumed these plants can indicate likely former presence of forests or grasslands, and isotope analysis is widely used for late Quaternary palaeoecological reconstruction [14]. Animals with pure C_3 diets have $\delta^{13}C$ values below -21.5‰ and pure C_4 diets above -9‰. Reported $\delta^{13}C$ values for Madagascar megaherbivores are interpreted as indicating forest environments [18-20]. However, some Madagascar megaherbivore subfossil sites are interpreted as open grassland biomes [21,22]. Using $\delta^{13}C$ values to infer open-habitat grasses as dietary resources can be confused by plants using crassulacean acid metabolism photosynthesis (CAM plants); for example, the succulent plant *Kalanchoë* exhibits flexible CAM patterns across Madagascar, with $\delta^{13}C$ values similar to C_3 plants in humid environments and to C_4 plants in dry environments [23]. The CAM-specialist extinct lemur *Hadropithecus* shows $\delta^{13}C$ values of -24.2‰ in the mesic central highlands, and -9.6 ‰ in the arid southwest [14]. However, wetland C_4 plants (rushes, sedges) also occur in Madagascar, so comparison of $\delta^{15}N$ values between co-occurring taxa can also be included in dietary assessments using $\delta^{13}C$ data; plants in arid environments have higher $\delta^{15}N$ values [24], enabling differentiation between wetland and dryland C_4 plants.

Research into Madagascar's extinct vertebrate ecology has mainly focused on giant lemurs, with studies of megaherbivores hindered by poorly-resolved taxonomy [25]. However, recent taxonomic reassessments have clarified species diversity in elephant birds [26] and hippopotami [27], enabling investigation of species-specific niches and landscape ecology. Here we investigate new and published dietary isotope data for all Madagascar hippopotamus and elephant bird species and for the regionally extinct giant tortoise *Aldabrachelys* across three distinct ecogeographic zones, to determine megaherbivore dietary niches and presence of natural open grassland habitats (e.g., savannas, open wooded habitats) in Madagascar's late Quaternary ecosystems [25].

2. Material and methods

We assembled a database of 203 $\delta^{13}C$ and 118 $\delta^{15}N$ values for late Quaternary skeletal elements of all recognised Madagascar hippopotami (*Hippopotamus lemerlei*, n=15; *H. madagascariensis*, n=6), elephant birds (*Aepyornis hildebrandti*, n=8; *A. maximus*, n=3; *Mullerornis modestus*, n=9; *Vorombe titan*, n=11) and *Aldabrachelys* sp. (n=19), and for both elephant bird eggshell morphotypes ("thin

73 eggshell" representing *M. modestus*, n=9; "thick eggshell" representing *Aepyornis* or *Vorombe*, n=93;
74 [28]) (table 1)*. We include 243 published values (160 $\delta^{13}C$, 83 $\delta^{15}N$), and previously unreported data
75 for 42 specimens from accelerator mass spectrometry (AMS) dating of bone collagen [9] performed
76 at the Oxford Radiocarbon Accelerator Unit (ORAU). In total, 86 samples have associated radiocarbon
77 dates, with just one predating the Holocene (NIUTSM 01539: 14,580±460 BP). Collection localities
78 cover three Madagascan ecoregions: southern arid spiny bush (n=147, 14 localities), western
79 succulent woodland (n=25, 6 localities), and the central highlands (n=21, 4 localities) (figure 1;
80 electronic supplementary material, table S1). Ecoregions varied in megaherbivore composition;
81 although *Mullerornis* occurred in highland and lower-elevation regions, *A. hildebrandti* and *H.*
82 *madagascariensis* were largely/completely restricted to the central highlands, and most other species
83 only occurred in southern and/or western ecoregions [25,29] (table 1). *Aldabrachelys* data are only
84 available for the arid spiny bush, although specimens are also recorded from the central highlands
85 [28] (table 1)*.

86 We assessed dietary sources using mixing models in ISSOERROR v.1.04 [30] to investigate dietary niche
87 differentiation between taxa within ecoregions. We calculated proportionate consumption of C_3
88 versus CAM plants in the more arid southern and western ecoregions, and C_3 versus C_4 plants in the
89 comparatively wet central highlands, which do not support significant CAM plant biomass and contain
90 a regionally-restricted endemic C_4 plant community [10,11,14,15]. We used $\delta^{13}C$ isotope values for
91 discrimination model end-members from ref. [31]: C_3 plants, arid spiny bush and succulent woodland
92 (Beza Mahafaly), -29.4‰ (σ:2.4, n=240); central highlands (Tsinjoarivo): -28.5‰ (σ:1.8, n=49); CAM
93 plants, arid spiny bush and succulent woodland (Beza Mahafaly): -15‰ (σ:1.2, n=67). C_4 grass end-
94 member values used the global mean value of -13.1‰ [32], with substituted σ and sample size from
95 central highlands C_3 plants. $\delta^{13}C$ values used in fractionation were corrected to account for $\delta^{13}C$
96 enrichment in bone (+5‰) [33] and eggshell (+2‰) collagen [34], and by +1.22‰ to account for $\delta^{13}C$
97 shifts in atmospheric CO_2 (Suess effect; [35]).

98

99 **3. Results**

100 In arid spiny bush, elephant birds and giant tortoises show low $\delta^{13}C$ values (species means: -25.18 to -
101 28.42‰), with dietary fractionation indicating these taxa all consumed mainly C_3 plants and only
102 limited amounts of CAM plants (mean estimated proportions of CAM consumption: 0.07-0.27). *M.*
103 *modestus* (bone and thin eggshell) shows highest estimated CAM consumption (sample means: 0.21-
104 0.27). Hippopotami (*H. lemerlei* and samples unidentified to species) show higher $\delta^{13}C$ values (sample
105 means: -21.87 to -22.99‰) and correspondingly much higher estimated proportions of CAM
106 consumption, with C_3 and CAM plants both comprising about half of their diet (mean estimated
107 proportions, C_3: 0.48-0.55, CAM: 0.45-0.52) (table 1, figure 2, supplementary file S1).

108 In succulent woodland, elephant birds (*A. maximus, V. titan*), giant tortoises and *H. lemerlei* show low
109 $\delta^{13}C$ values (species means: -26.46 to -33.52‰) and very low estimated proportions of CAM
110 consumption (0.01-0.09). In contrast, *H. madagascariensis* shows high $\delta^{13}C$ values (species mean: -
111 20.16%) and much higher mean estimated CAM consumption (0.64).

112 In the central highlands, *M. modestus* and hippopotami (*H. madagascariensis* and samples
113 unidentified to species) show high $\delta^{13}C$ values (sample means: -28.22 to -28.83‰), and are estimated
114 to have consumed almost entirely C_3 plants and minimal C_4 grasses (mean estimated proportions of
115 C_4 consumption: 0.02-0.04). Conversely, *A. hildebrandti* shows high $\delta^{13}C$ values (sample mean: -
116 21.12%) and much higher mean estimated C_4 consumption (0.48).

117 For hippopotami, *H. madagascariensis* had $\delta^{15}N$ values of 3.0-5.2‰, and *H. lemerlei* of 7.0-13.3‰. The
118 lowest hippopotamus $\delta^{15}N$ value was from Antsirabe, central highlands (1.3‰) and the highest was
119 from Beloha, arid spiny bush (13.3‰). For elephant birds, *A. hildebrandti* had $\delta^{15}N$ values of 5.0-7.8‰,

120 *A. maximus* of 11.8-15.3‰, *M. modestus* of 6.4-16.0‰, *V. titan* of 5.5-13.7‰, and "thick eggshell" of
121 8.7-17.6‰. *Aldrabrachely*s in arid spiny bush had values of 8.4-13.3‰. Across ecoregions, non-
122 overlapping δ^{15}N ranges were observed for single measurements in *A. maximus* (dry deciduous forest,
123 11.8‰; arid spiny bush, 12.7-15.3‰) and *M. modestus* (central highlands, 6.4‰; arid spiny bush, 6.5-
124 15.8‰).

125

126 4. Discussion

127 We present the first species-level dietary niche reconstruction for Madagascar's megaherbivores,
128 revealing a range of δ^{13}C and δ^{15}N values and trophic ecologies across taxa and ecoregions. Our
129 findings indicate the former existence of multiple herbivore guilds across Madagascar. These data
130 support previous identification of a widespread browsing guild, and provide the first direct evidence
131 for a megaherbivore grazing guild in Madagascar's central highlands.

132 δ^{13}C values in hippopotamus samples indicate broad trophic niches for both species, suggesting both
133 browsing and grazing behaviours. This contrasts with the mainland African hippopotamus (*H.*
134 *amphibius*), which is predominantly a terrestrial grazer [36]. Madagascar hippopotami were
135 trophically closer to Africa's extant pygmy hippopotamus (*Choeropsis liberiensis*), which is comparable
136 in size to Madagascar's extinct species, and browses on forest plants [37]. Interestingly, δ^{15}N ratios
137 indicate more aquatic feeding in *H. madagascariensis* than *H. lemerlei*. Aquatic habitats were available
138 for *H. madagascariensis* in the central highlands [25]. This result contrasts with aquatic adaptations
139 inferred from cranial morphology in *H. lemerlei* [38], but is consistent with behavioural ecology
140 (emergence onto land for feeding) in the otherwise aquatic *H. amphibius*, suggesting a similar lifestyle
141 for *H. lemerlei*. *Aldabrachelys* isotopes from arid spiny bush show a comparable δ^{13}C/δ^{15}N signal to *H.*
142 *lemerlei*, indicating a similar browsing niche in this ecoregion.

143 CAM plants comprised a substantial proportion of the diets of one or both hippopotamus species in
144 arid spiny bush and succulent woodland, but δ^{13}C values are lower in the central highlands, suggesting
145 higher reliance upon C_3 plants. CAM plants are relatively scarce in this region; however, they occur
146 across numerous biomes and elevations in Madagascar, with a range of δ^{13}C values (e.g. *Kalanchoë*, -
147 11.4 to -27.3‰) [23]. CAM plant CO_2 is fixed by the C_3 pathway in humid environments such as the
148 central highlands, producing δ^{13}C values below -22‰ [23] and thus consistent with CAM consumption
149 in this region as well. Hippopotami therefore probably consumed C_3 and CAM plants across
150 Madagascar, matching the varying δ^{13}C pattern in the CAM-specialist *Hadropithecus* across different
151 ecoregions [14].

152 Elephant bird δ^{13}C values from arid spiny bush and succulent woodland fall outside the range for C_4
153 consumption (open-habitat dryland grasses or wetland sedges and rushes). In arid spiny bush, δ^{13}C
154 values indicate that all elephant birds had predominantly C_3 diets, with some CAM consumption by *M.*
155 *modestus*; higher δ^{15}N values compared to sympatric hippopotami indicate that these plants were less
156 likely to be from wetlands. Differences between sympatric elephant birds may indicate further
157 species-specific dietary differences; for example, higher δ^{15}N values (e.g. in *A. maximus*) are associated
158 with frugivory or omnivory (including invertebrate or small vertebrate consumption) [31], which
159 comprise extant ratite dietary strategies [39]. Eggshell and bone values also differ in *M. modestus*,
160 possibly indicating seasonal reliance upon dietary resources during oogenesis, or that eggshell and
161 bone fractionation rates may need separate assessment.

162 Our most striking result is that δ^{13}C data for *A. hildebrandti* provide the first evidence for grazing
163 ecology in elephant birds. Although unique within Madagascar's ratites, grazing is also the primary
164 dietary strategy in greater rhea (*Rhea americana*) [40], and other large flightless birds (e.g. geese) also
165 have important regulatory effects on island grasslands [41]. Our results thus identify *A. hildebrandti*
166 as a likely top-down regulator of native grassland ecosystems in the central highlands [13,15,16]. δ^{13}C

values for this species indicate a mixed diet containing large quantities of C_4 plants (c.48%), whereas co-occurring hippopotami consumed only tiny amounts of C_4 plants (1-4%). Although *A. hildebrandti* had higher mean $\delta^{15}N$ values compared to sympatric hippopotami, this disparity is much lower than between species in other ecoregions. Indeed, lower $\delta^{13}C$ values in CAM plants within mesic conditions [23] suggest that *A. hildebrandti* might not have consumed any forest plants and was exclusively an open-habitat forager, consuming a mixture of C_4 and CAM plants. High variability in CAM plant $\delta^{13}C$ values complicates interpretation of results, but the likelihood of *A. hildebrandti* exhibiting grazing behaviour is supported by the non-matching regional $\delta^{13}C$ signature of the CAM specialist *Hadropithecus* (mean: -24.2 $\delta^{13}C$‰)[14]. This hypothesis is consistent with the small olfactory bulb in skulls assigned to *A. hildebrandti*, comparable to the neuroanatomy of extant open-habitat palaeognaths [42]. However, the taxonomic identity of these crania is uncertain; they are not associated with diagnostic postcrania or locality data, and two separate skull morphotypes have been referred to *A. hildebrandti* [43-45].

$\delta^{13}C$ data from skeletal collagen provide a comprehensive new understanding of Madagascar megaherbivore dietary ecology. Most available subfossils originate from southern Madagascar, and further research should investigate data across wider areas. For example, giant tortoises from the central highlands remain isotopically unstudied; these animals might also have been grazers, but their shell shape (associated with biomechanical advantage for grazing or browsing in extant species [46]) is poorly understood, making ecological inference difficult. However, whereas most modern-day open habitats on Madagascar are anthropogenic in origin, our results provide important evidence for former existence of native ecosystems dominated by endemic C_4 grasses. It is clear that Madagascar supported multiple megaherbivore trophic guilds with differing relationships to native vegetation, which must have played important roles in regulating diverse natural landscapes. Madagascar's ecosystems are now highly degraded, and protection and sustainable management of landscapes and ecosystem services represents a global priority for biodiversity conservation and human well-being [47,48]. Hypotheses of what constitutes a "natural" Madagascar ecosystem must therefore consider the ecologies and regulatory roles of the island's now-extinct megafauna, to support evidence-based restoration of this ecologically complex island.

References

1. Malhi Y, Doughty CE, Galetti M, Smith FA, Svenning JC, Terborgh JW. 2016 Megafauna and ecosystem function from the Pleistocene to the Anthropocene. *Proc. Natl Acad. Sci. USA* **113**, 838–846. (doi:10.1073/pnas.1502540113)

210 2.   Hopcraft JGC, Olff H, Sinclair ARE. 2010 Herbivores, resources and risks: alternating regulation
211   along primary environmental gradients in savannas. *Trends Ecol. Evol*. **25**, 119–128.
212   (doi:10.1016/j.tree.2009.08.001)

213 3.   Guimarães PR, Galetti M, Jordano P. 2008 Seed dispersal anachronisms: rethinking the fruits
214   extinct megafauna ate. *PLoS ONE* **3**, e1745. (doi:10.1371/journal.pone.0001745)

215 4.   Pires MM, Galetti M, Donatti CI, Pizo MA, Dirzo R, Guimarães PR. 2014 Reconstructing past
216   ecological networks: the reconfiguration of seed-dispersal interactions after megafaunal
217   extinction. *Oecologia* **175**, 1247–1256. (doi:10.1007/s00442-014-2971-1)

218 5.   Berti E, Svenning J-C. 2020 Megafauna extinctions have reduced biotic connectivity worldwide.
219   *Glob. Ecol. Biogeogr.* **29**, 2131–2142. (doi:10.1111/geb.13182)

220 6.   Norris K, Terry A, Hansford JP, Turvey ST. 2020 Biodiversity conservation and the earth system:
221   mind the gap. *Trends Ecol. Evol*. **35**, 919–926. (doi:10.1016/j.tree.2020.06.010)

222 7.   Burns SJ, Godfrey LR, Faina P, McGee D, Hardt B, Ranivoharimanana L, Randrianasy J. 2016
223   Rapid human-induced landscape transformation in Madagascar at the end of the first
224   millennium of the Common Era. *Quat. Sci. Rev.* **134**, 92–99.
225   (doi:10.1016/j.quascirev.2016.01.007)

226 8.   Godfrey LR, Scroxton N, Crowley BE, Burns SJ, Sutherland MR, Pérez VR, Faina P, McGee D,
227   Ranivoharimanana L. 2019 A new interpretation of Madagascar's megafaunal decline: the
228   "Subsistence Shift Hypothesis". *J. Hum. Evol.* **130**, 126–140. (doi:10.1016/j.jhevol.2019.03.002)

229 9.   Hansford JP, Lister AM, Weston EM, Turvey ST. 2021 Simultaneous extinction of Madagascar's
230   megaherbivores correlates with late Holocene human-caused landscape transformation. *Quat.*
231   *Sci. Rev.* **263**, 106996. (doi:10.1016/j.quascirev.2021.106996)

232 10. Bond WJ, Silander JA, Ranaivonasy J, Ratsirarson J. 2008 The antiquity of Madagascar's
233   grasslands and the rise of C_4 grassy biomes. *J. Biogeogr*. **35**, 1743–1758. (doi:10.1111/j.1365-
234   2699.2008.01923.x)

235 11. Vorontsova MS, Besnard G, Forest F, Malakasi P, Moat J. 2016 Madagascar's grasses and
236   grasslands: anthropogenic or natural? *Proc. Roy. Soc. B* **283**, 20152262.

237 12. Joseph GS, Seymour CL. 2020 Madagascan highlands: originally woodland and forest containing
238   endemic grasses, not grazing-adapted grassland. *Proc. Roy. Soc. B* **287**, 20201956.
239   (doi:10.1098/rspb.2020.1956)

240 13. Crowley BE, Godfrey LR, Hansford JP, Samonds KE. 2021 Seeing the forest for the trees – and
241   the grasses: revisiting the evidence for grazer-maintained grasslands in Madagascar's central
242   highlands. *Proc. Roy. Soc. B* **288**, 20201785.

243 14. Godfrey LR, Crowley BE, Muldoon KM, Kelley EA, King SJ, Best AW, Berthaume MA. 2016 What
244   did *Hadropithecus* eat, and why should paleoanthropologists care? *Am. J. Primatol.* **78**, 1098–
245   1112. (doi:10.1002/ajp.22506)

246 15. Solofondranohatra CL, Vorontsova MS, Hackel J, Besnard G, Cable S, Williams J, Jeannoda V,
247   Lehmann CER. 2018 Grass functional traits differentiate forest and savanna in the Madagascar
248   central highlands. *Front. Ecol. Evol.* **6**, 184. (doi:10.3389/fevo.2018.00184)

249 16. Godfrey LR, Crowley BE. 2016 Madagascar's ephemeral palaeo-grazer guild: who ate the ancient
250   C_4 grasses? *Proc. Roy. Soc. B* **283**, 20160360. (doi:10.1098/rspb.2016.0360)

251   17. Ehleringer JR, Monson RK. 1993 Evolutionary and ecological aspects of photosynthetic pathway
252   variation. *Annu. Rev. Ecol. Syst.* **24**, 411–439. (doi:10.1146/annurev.es.24.110193.002211)

253   18. Tovondrafale T, Razakamanana T, Hiroko K. 2014 Paleoecological analysis of elephant bird
254   (Aepyornithidae) remains from the Late Pleistocene and Holocene formations of southern
255   Madagascar. *Malagasy Nat.* **8**, 1–13.

256   19. Parker Pearson M. 2010 *Pastoralists, warriors and colonists: the archaeology of southern
257   Madagascar*. Oxford: Archaeopress.

258   20. Clarke SJ, Miller GH, Fogel ML. Allan RC. Murray-Wallace CV. 2006 The amino acid and stable
259   isotope biogeochemistry of elephant bird (*Aepyornis*) eggshells from southern Madagascar.
260   *Quat. Sci. Rev.* **25,** 2343–2356.

261   21. Burney DA. 1999 Rates, patterns, and processes of landscape transformation and extinction in
262   Madagascar. In *Extinctions in near time: causes, contexts, and consequences* (ed. RDE
263   MacPhee), pp. 145–164. New York, NY: Kluwer Academic/Plenum.

264   22. Samonds KE, Crowley BE, Rasolofomanana TR, Andriambelomanana MC, Andrianavalona HT,
265   Ramihangihajason TN, Rakotozandry R, Nomenjanahary ZB, Irwin MT, Wells NA, Godfrey LR.
266   2019 A new late Pleistocene subfossil site (Tsaramody, Sambaina basin, central Madagascar)
267   with implications for the chronology of habitat and megafaunal community change on
268   Madagascar's Central Highlands. *J. Quat. Sci.* **34**, 379–392. (doi:10.1002/jqs.3096)

269   23. Kluge M, Brulfert J, Ravelomanana D, Lipp J, Ziegler H. 1991 Crassulacean acid metabolism in
270   *Kalanchoë* species collected in various climatic zones of Madagascar: a survey by δ^{13}C analysis.
271   *Oecologia* **88**, 407–414. (doi:10.1007/BF00317586)

272   24. Heaton THE, Vogel JC, von la Chevallerie G, Collett G. 1986 Climatic influence on the isotopic
273   composition of bone nitrogen. *Nature* **322**, 822–823. (doi:10.1038/322822a0)

274   25. Goodman SM, Jungers WL. 2014 *Extinct Madagascar*. Chicago and London: University of
275   Chicago Press.

276   26. Hansford JP, Turvey ST. 2018 Unexpected diversity within the extinct elephant birds (Aves:
277   Aepyornithidae) and a new identity for the world's largest bird. *Roy. Soc. open sci.* **5**, 181295.
278   (doi:10.1098/rsos.181295)

279   27. Weston EM, Lister AM. 2009 Insular dwarfism in hippos and a model for brain size reduction in
280   *Homo floresiensis*. *Nature* **459**, 85–88. (doi:10.1038/nature07922)

281   28. Grealy A, Phillips M, Miller G, Gilbert MTP, Rouillard JM, Lambert D, Bunce M, Haile J. 2017
282   Eggshell palaeogenomics: palaeognath evolutionary history revealed through ancient nuclear
283   and mitochondrial DNA from Madagascan elephant bird (*Aepyornis* sp.) eggshell. *Mol.
284   Phylogenet. Evol.* **109**, 151–163. (doi:10.1016/j.ympev.2017.01.005)

285   29. Agnolin FL. 2016 Unexpected diversity of ratites (Aves, Palaeognathae) in the early Cenozoic of
286   South America: palaeobiogeographical implications. *Alcheringa* **41**, 101–111.
287   (doi:10.1080/03115518.2016.1184898)

288   30. Phillips DL, Gregg JW. 2001 Uncertainty in source partitioning using stable isotopes. *Oecologia*
289   **127**, 171–179. (doi:10.1007/s004420000578)

290   31. Crowley BE, Thorén S, Rasoazanabary E, Vogel ER, Barrett MA, Zohdy S, Blanco MB, McGoogan
291   KC, Arrigo-Nelson SJ, Irwin MT, Wright PC. 2011 Explaining geographical variation in the isotope
292   composition of mouse lemurs (*Microcebus*). *J. Biogeogr.* **38**, 2106–2121. (doi:10.1111/j.1365-
293   2699.2011.02551.x)

294   32. O'Leary MH. 1988 Carbon isotopes in photosynthesis. *BioScience* **38**, 328–336.
295   (doi:10.2307/1310735)

296   33. Tykot RH. 2004 Stable isotopes and diet: you are what you eat. *Proc. Int. School Phys. "Enrico*
297   *Fermi"* **154**, 433–444. (doi:10.3254/978-1-61499-010-9-433)

298   34. Johnson BJ, Fogel ML, Miller GH. 1998 Stable isotopes in modern ostrich eggshell: a calibration
299   for paleoenvironmental applications in semi-arid regions of southern Africa. *Geochim.*
300   *Cosmochim. Acta* **62**, 2451–2461. (doi:10.1016/S0016-7037(98)00175-6)

301   35. Keeling CD. 1979 The Suess effect: ^{13}Carbon-^{14}Carbon interrelations. *Environ. Int.* **2**, 229–300.
302   (doi:10.1016/0160-4120(79)90005-9)

303   36. Cerling TE, Harris JM, Hart JA, Kaleme P, Klingel H, Leakey MG, Levin NE, Lewison RL, Passey BH.
304   2008 Stable isotope ecology of the common hippopotamus. *J. Zool.* **2**, 204–212.

305   37. Robinson PT, Flacke GL, Hentschel KM. 2017 *The pygmy hippo story: West Africa's enigma of the*
306   *rainforest*. New York: Oxford University Press.

307   38. Stuenes S. 1989 Taxonomy, habits, and relationships of the subfossil Madagascan hippopotami
308   *Hippopotamus lemerlei* and *H. madagascariensis*. *J. Vertebr. Paleontol.* **9**, 241–268.
309   (doi:10.1080/02724634.1989.10011761)

310   39. Davies SJJF. 2002 *Ratites and tinamous*. Oxford: Oxford University Press.

311   40. Pereira JA, Quintana RD, Monge S. 2003 Diets of plains vizcacha, greater rhea and cattle in
312   Argentina. *J. Range Manage*. **56**, 13–20.

313   41. Summers RW, McAdam JH. 1993 *The upland goose: a study of the interaction between geese,*
314   *sheep and man in the Falkland Islands*. Huntingdon, UK: Bluntisham.

315   42. Torres CR, Clarke JA. 2018 Nocturnal giants: evolution of the sensory ecology in elephant birds
316   and other palaeognaths inferred from digital brain reconstructions. *Proc. Roy. Soc. B* **285**,
317   20181540. (doi:10.1098/rspb.2018.1540)

318   43. Wiman C, Edinger T. 1941 Sur les cranes et les encephales d'*Aepyornis* et de *Mullerornis*. *Bull.*
319   *Acad. Malgache* **42**, 1–50.

320   44. Monnier L. 1913 Les Aepyornis. *Paleontologie de Madagascar* **8**, 125–172.

321   45. Lamberton C. 1934 Ratites subfossiles de Madagascar, Les Mullerornithidae. *Mem. Acad.*
322   *Malgache* **17**, 125–168.

323   46. Gerlach J. 1999 Feeding behavior and the saddleback shell of *Dipsochelys arnoldi*. *Chelonian*
324   *Conserv. Biol.* **3**, 496–500.

325   47. Gillespie TW, Lipkin B, Sullivan L, Benowitz DR, Pau S, Keppel G. 2012 The rarest and least
326   protected areas in biodiversity hotspots. *Biodivers. Conserv*. **21**, 3597–3611.
327   (doi:10.1007/s10531-012-0384-1)

328   48. Robuchon M, Pavoine S, Véron S, Delli G, Faith DP, Mandrici A, Pellens R, Dubois G, Leroy B.
329   2021 Revisiting species and areas of interest for conserving global mammalian phylogenetic
330   diversity. *Nature Commun*. **12**, 3694. (doi:10.1038/s41467-021-23861-y)

331   49. Yoder AD, Campbell CR, Blanco MB, Dos Reis M, Ganzhorn JU, Goodman SM, Hunnicutt KE,
332   Larsen PA, Kappeler PM, Rasoloarison RM, Ralison JM. 2016 Geogenetic patterns in mouse
333   lemurs (genus *Microcebus*) reveal the ghosts of Madagascar's forests past. *Proc. Natl. Acad. Sci.*
334   *USA* **19**, 8049-8056.

336 **Figure 1.** Madagascar ecoregions, showing localities for specimens in this study. Adapted from ref.
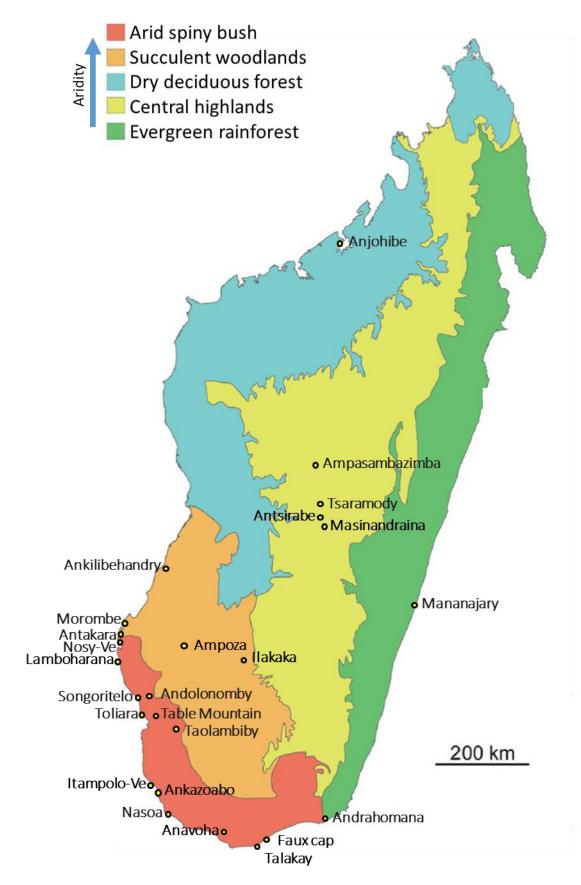337 [49].

338

Figure 2. Observed isotope ‰ for Madagascar's megaherbivores: A, $\delta^{13}C$ values. B, $\delta^{13}C$ and $\delta^{15}N$ values. Key: green star, *Aepyornis hildebrandti*; pink triangle, *A. maximus*; orange square, *Mullerornis modestus* (bone); blue triangle: *Vorombe titan*; open square: thin eggshell (*M. modestus*); open triangle: thick eggshell (*Aepyornis* or *Vorombe*); blue diamond: *Hippopotamus lemerlei*; purple cross: *H. madagascariensis*; open diamond: *Hippopotamus* sp.; filled circle: *Aldabrachelys* sp.
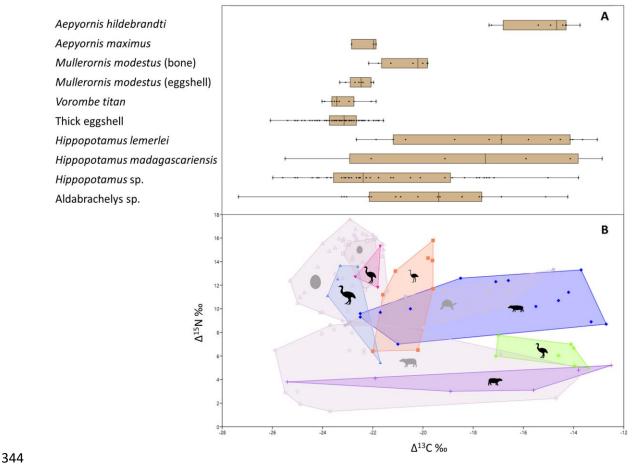
Table 1. Mean isotope values and dietary proportion estimates for Madagascar's megaherbivores, inclusive of bone/eggshell correction and Suess correction. Asterisk indicates SD not available and arid spiny bush value used.

1. Arid spiny bush

Taxon	No. of specimens	δ¹³C (mean)	SD	Est. diet proportion (mean) C₃ plants	Est. diet proportion (mean) CAM plants	SE
Aepyornis maximus	2	-28.42	0.71	0.93	0.07	0.04
Mullerornis modestus (bone)	7	-26.44	0.81	0.79	0.21	0.02
Mullerornis modestus (eggshell)	9	-25.58	0.48	0.73	0.27	0.01
Thick eggshell	93	-26.35	0.92	0.79	0.21	0.01
Hippopotamus lemerlei	10	-21.87	2.49	0.48	0.52	0.06
Hippopotamus sp.	18	-22.99	2.33	0.55	0.45	0.04
Aldabrachelys sp.	18	-25.18	2.75	0.7=1	0.29	0.05

2. Succulent woodland

Taxon	No. of specimens	δ¹³C (mean)	SD	Est. diet proportion (mean) C₃ plants	Est. diet proportion (mean) CAM plants	SE
Aepyornis maximus	1	-28.02	0.71*	0.9	0.1	0.05
Vorombe titan	11	-29.26	0.72	0.99	0.01	0.02
Hippopotamus lemerlei	4	-28.13	0.73	0.91	0.09	0.03
Hippopotamus madagascariensis	3	-20.17	1.56	0.36	0.64	0.06
Hippopotamus sp.	15	-26.07	3.71	0.77	0.23	0.07
Aldabrachelys sp.	1	-33.52	2.75*	1	0	0.19

3. Central highlands

Taxon	No. of specimens	δ¹³C (mean)	SD	Est. diet proportion (mean) C₃ plants	Est. diet proportion (mean) C₄ plants	SE
Aepyornis hildebrandti	8	-21.12	1.42	0.52	0.48	0.03
Mullerornis modestus (bone)	1	-28.22	0.81*	0.98	0.02	0.06
Hippopotamus madagascariensis	3	-28.27	3.25	0.99	0.01	0.12
Hippopotamus sp.	13	-27.83	4.29	0.96	0.04	0.08