

Why all those spines? Anachronistic defences in the Didiereoideae against now extinct lemurs

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Plants evolve physical defences, such as spines, against browsing herbivores. However, in some cases, these defences may be anachronistic because the principal consumers of protected parts of the plant are extinct. In such cases, there may be few extant species consuming heavily defended resources. Here we examine the spiny defences of Madagascar's endemic Didiereoideae, and ask whether they may be anachronistic. To accomplish this aim, we reviewed the literature to determine which species consume these plants today, and then used stable isotope biogeochemistry to determine who may have exploited Didiereoideae in the recent past. There are four major groups of browsers that are now extinct in Madagascar: giant lemurs, elephant birds (*Aepyornis* and *Mullerornis*: Aepyornithidae), pygmy hippopotamuses (*Hippopotamus*) and giant tortoises (*Aldabrachelys*: Testudinidae). Each group was evaluated for isotopic evidence of didiereoid plant consumption. Given the structure of members of this plant clade (especially *Alluaudia*), we predicted that lemurs would be their most important consumers. Three extant lemur species consume Didiereoideae. Several of the extinct lemurs, particularly *Hadropithecus stenognathus*, may have relied heavily on these spiny plants. None of the non-lemur megafaunal browsers (elephant birds, hippopotamuses and giant tortoises) were important consumers of Didiereoideae.

Motivation

Madagascar is renowned for its wealth of endemic flora and fauna. In particular, the arid south and southwest is famous for its 'spiny forests' full of spiny bushes and trees belonging to the Apocynaceae (e.g. *Pachypodium lamerei*), Euphorbiaceae (e.g. *Euphorbia stenoclada*), Fabaceae (e.g. *Acacia bellula*), Salvadoraceae (e.g. *Azima tetracantha*) and Didiereoideae,¹ an endemic subfamily of the Didiereaceae.^{2,3} Indeed, species from the latter subfamily are limited almost entirely to the Spiny Thicket and Succulent Woodland ecoregions in southern and southwestern Madagascar, which are characterised by hot temperatures and brief rainy seasons.⁴

The 12 species of the Didiereoideae belong to four genera: *Alluaudia*, *Alluaudiopsis*, *Decarya* and *Didierea*. All members of this subfamily possess sharp, thick spines along their axes which protect their leaves^{5,6}; however, none of the closely related Didiereaceae from mainland Africa (*Calyptrotheca*, *Ceraria*, *Portulacaria*) possesses spines.¹ Experimental research on plant taxa in mainland Africa has demonstrated that the spines reduce foliage loss to browsing ungulates.^{7,8} This protection suggests that the common ancestor of the Madagascan forms was subjected to intense leaf predation shortly after its arrival. Arakaki and colleagues⁹ reported a diversification estimate for Madagascan Didiereoideae of 17 million years ago (mya) based on molecular data. These data imply an earlier date for the dispersal of the basal didiereoid from continental Africa to Madagascar. According to these authors, *Alluaudia* itself began diversifying only 11 mya. Ocampo and Columbus¹⁰ support a slightly more recent radiation of Madagascan didiereoids, with the divergence of the Madagascan lineage from the closest continental African relative at around 15 mya.

Spines on these tall, emergent plants may be defences against leaf predation by climbing animals such as lemurs.⁶ Spines of most *Alluaudia* spp., for example, are found at heights above the ground (5–9 m) that were likely prohibitive for terrestrial browsers such as tortoises, hippopotamuses and elephant birds. Whereas it is conceivable that these taxa browsed juvenile forms or the lower portions of adult plants, widescale herbivory by tortoises or hippopotamuses seems unlikely. Furthermore, although the 'wiry' qualities of *Alluaudia humbertii* and *Decarya madagascariensis* may have provided some defence against elephant bird herbivory,¹¹ their spines are relatively ineffective against birds and, presumably, other animals with hard beaks that protect their mouths, such as tortoises.¹² Another reason to suspect that the spines on the Didiereoideae evolved to protect leaves against climbing animals and not against other major groups of herbivores is the timing of arrival of major herbivore groups to Madagascar. Both hippopotamuses and testudines arrived relatively recently¹³ – likely after the appearance of spines and diversification of Madagascan didiereoids. Only lemurs and elephant birds would have been present when the ancestral didiereoid arrived. If Bond and Silander¹¹ are correct in characterising elephant birds as poorly suited to exploit the leaves of the Didiereoideae, then lemurs become the most plausible contenders. Additionally, if few extant lemurs exploit these plants, then the giant extinct lemurs may be implicated.

Ideally, testing the hypothesis that spines served to defend the leaves of the Didiereoideae against giant lemurs requires more than compiling evidence that certain giant lemurs likely consumed these plants. We would like to know the degree to which the spines acted as a deterrent to overconsumption of small and vulnerable young leaves by giant lemurs. The latter question is challenging, at best, within the context of palaeobiology. Palaeontological evidence is often indirect, and arguments may depend on unspoken assumptions. Thus, it is important to make explicit the questions that can be addressed with the tools we have at our disposal. How, using those tools, can plant anachronisms in Madagascar be discerned?

As evolutionary biologists we can ascertain, first, whether or not the Didiereoideae are native or endemic to Madagascar (i.e. not recently introduced). Secondly, we can establish whether the presumed anachronistic spines are derived. Thirdly, we can determine whether or not the hypothesised consumers (lemurs) were present when

these features likely originated. We can probe whether spines can be understood outside the context of the proposed plant–animal interactions and we can examine palaeodistribution data to test the plausibility of the proposed interactions. Finally, we can explore whether spines serve any apparent purpose today, or were likely used in the past in a manner that no longer holds.

Our inferences in this paper are based explicitly on the combination of a literature review and stable isotope biogeochemistry that addresses these issues. We ask the following specific questions:

1. To what extent do modern lemurs feed in southern and southwestern Madagascar on C_3 , C_4 and crassulacean acid metabolism (CAM)-based plants? How much do they feed on Didiereoideae?
2. Can we distinguish Didiereoideae from other CAM plants using stable isotopes? Such discrimination is needed if we are to use stable isotope data to successfully test the hypothesis that Didiereoideae spines are anachronistic.
3. To what extent were lemurs feeding on CAM plants in the past?
4. Do stable isotopes suggest that any of the extinct non-lemur herbivores were major consumers of Didiereoideae?

Background on stable isotope biogeochemistry

Stable isotopes can be used to reconstruct the diets of living and extinct animals. The relative proportion of heavy and light isotopes (e.g. $^{13}C/^{12}C$ or $^{15}N/^{14}N$) in a substance is reported using a standardised ' δ ' notation (e.g. $\delta^{13}C$, $\delta^{15}N$). These values are measured as parts per thousand (‰) higher or lower than an international standard.

Carbon isotope ($\delta^{13}C$) values can, in some cases, be used to distinguish plants that fix carbon via C_3 photosynthesis (most trees and herbs), C_4 photosynthesis (many grasses) and CAM (stem and leaf succulents).¹⁴ Many succulents have the ability to switch between full CAM photosynthesis and C_3 photosynthesis, which can result in highly variable $\delta^{13}C$ values.¹⁴ However, in arid environments, such as those in southwestern Madagascar, carbon fixation is strongly biased towards CAM photosynthesis.¹⁵ Nitrogen isotope ($\delta^{15}N$) values in plants are affected by environmental conditions, plant physiology, nutrient availability and microbial associations.^{14,16} Nitrogen isotope values clearly distinguish plants growing in different habitats. Plants from moist, cool localities have lower $\delta^{15}N$ values than plants from dry, warm localities.^{16,17} Coastal localities can evince exceptionally high $\delta^{15}N$ values.¹⁸ Most plants obtain their nitrogen directly from soil nitrate and ammonium, and their $\delta^{15}N$ values are greater than that of air ($\sim 0\%$). Plants with symbiotic nitrogen-fixing bacteria can have $\delta^{15}N$ values close to 0‰.^{16,18} No consistent differences in $\delta^{15}N$ have been reported among the three photosynthetic groups, but CAM plants can have significantly higher $\delta^{15}N$ values than sympatric C_3 or C_4 plants.^{18,19} Differences in plant physiology or differential use of water sources may result in $\delta^{15}N$ differences between the Didiereoideae and non-spiny leaf succulents.²⁰

Isotopic patterns in plants are reflected in animal consumers with some isotopic enrichment. Carbon and nitrogen isotope values in herbivore bone collagen tend to be, respectively, ca. 5‰ and 3‰ higher than those in plants.²¹ Once we have accounted for isotopic enrichment between collagen and diet, we may be able to use carbon isotope values in consumer tissues to estimate the relative ingestion of C_3 , C_4 and CAM plants. As with plants, nitrogen isotope values in animals can be used to distinguish habitat types.^{17,22} Within a particular habitat, $\delta^{15}N$ values increase with increasing consumption of animal matter.^{21,22} However, because most of the lemur species included in our analyses are, or were, predominantly herbivorous, we do not believe our results are confounded by the effects of faunivory on nitrogen isotope values.^{23–32} The two possible exceptions are *Archaeolemur majori* (whose diet likely included some animal matter) and *Daubentonia robusta*. Extant *D. madagascariensis* consumes more animal matter than any of the other lemurs included in our study³³ and it is likely that the extinct *D. robusta*, which lived in the southwest, would have had a similar diet.³⁴ If this species consumed insects that in turn fed on CAM resources, elevated

$\delta^{15}N$ isotope values might falsely suggest CAM consumption, when in fact they really reflect trophic omnivory. We therefore omitted *D. robusta* from our analyses.

Methods

To explore the extent to which modern lemurs feed on C_3 , C_4 or CAM plants, we conducted a thorough review of the literature. We examined 74 manuscripts, books and book chapters that discuss the feeding behaviour of living lemurs in southwestern Madagascar. All sources included in our survey are listed in Supplementary table 1 (see supplementary material online), and all documented observations of feeding on CAM are provided in Supplementary table 2. We used the website www.tropicos.org and Petitjean and colleagues³⁵ to identify scientific names and families for recorded food species. Succulence was assessed using species-specific isotopic or anatomical data whenever possible.^{36–41} If no data were available for particular species, we used published information at the generic or familial level.^{42,43}

To determine if the spiny Didiereoideae can be isotopically distinguished from other plants, we compared $\delta^{13}C$ and $\delta^{15}N$ values from leaves that we collected in the spiny forest at Beza Mahafaly Special Reserve (BMSR) in south-central Madagascar (taxa provided in Table 1). We included previously published isotope values for C_3 and CAM plants¹⁷ and new isotope values for C_4 plants. All plant specimens were collected between 2006 and 2009. We sampled *Alluaudia procera*, which is the only member of the Didiereoideae that occurs in abundance at the reserve. *Alluaudia* is both the most speciose and the most widespread didiereoid genus. Additionally, although carbon isotope values have been measured for a number of spiny CAM species,^{10,37,41} nitrogen isotope data have been published only for *Alluaudia procera*.¹⁷ We used an analysis of variance (ANOVA) with Tukey's tests of honestly significant differences (HSD) and Student's t-tests to test the significance of isotopic differences between C_3 , C_4 and CAM plants, and between *Alluaudia* and other CAM plants. All statistical tests were performed using JMP (version 7.0). Significance was set at $\alpha = 0.05$. We assessed the assumptions of normality and homoscedasticity of variance for all analyses. We tested for homogeneity of variances using Levene tests.

To address the extent to which lemurs and non-primate herbivores fed on CAM plants in the past, we used $\delta^{13}C$ and $\delta^{15}N$ values from bone collagen. We analysed 72 bones of extant and extinct lemurs as well as extinct giant tortoises and pygmy hippopotamuses from subfossil sites in the Spiny Thicket and Succulent Woodland ecoregions (coastal and inland). Collagen was prepared following previously published methods.⁴⁴ Samples were analysed at the Stable Isotope Laboratory at the University of California, Santa Cruz. We verified collagen preservation using collagen yield, atomic C:N ratios, and carbon and nitrogen isotope values. We added these data to our existing database of previously published isotope data.^{45–48} Raw isotope data for all individuals are presented in Supplementary table 3. Carbon isotope values for subfossil individuals were corrected to account for $\delta^{13}C$ shifts in atmospheric CO_2 following the industrial revolution (The Suess Effect).⁴⁵ Carbon isotope values for individuals younger than 150 years BP were corrected using an age-dependent correction of -0.004% per year between 1860 and 1965 AD and -0.02% per year between 1965 and 2005 (modern). All individuals older than 150 years were corrected by -1.2% . In order to avoid sampling bias, we used nonparametric Wilcoxon signed ranks tests to compare mean $\delta^{13}C$ values for subfossil extant and extinct lemur species.

We calculated mean %CAM consumption using mixing models in ISSERROR version 1.04.⁴⁹ We used mean $\delta^{13}C$ values for C_3 and CAM plants from the spiny forest at BMSR as end members, correcting for the $+5\%$ difference in $\delta^{13}C$ values between collagen and plants. We did not include C_4 plants in these models because they are relatively rare in southern Madagascar and no modern lemurs are known to consume them. If $\delta^{15}N$ values do, indeed, differentiate spiny Didiereoideae from sympatric non-spiny CAM plants, then we may be able to use mean $\delta^{15}N$ values in addition to $\delta^{13}C$ values to distinguish consumption of Didiereoideae. We corrected plant values by $+3\%$ to account for the difference in $\delta^{15}N$ values between collagen and plants.²¹ We also

Table 1: Plant taxa included in this study

Family	Genus	Species	N	Photosynthetic pathway	Spiny?
Apocynaceae	<i>Landolphia</i>	sp.	2	C ₃	No
Apocynaceae	<i>Pentopetia</i>	<i>androsaemifolia</i>	1	C ₃	No
Burseraceae	<i>Commiphora</i>	sp.	11	C ₃	No
Celastraceae	<i>Reissantia</i>	<i>angustipetala</i>	1	C ₃	No
Combretaceae	<i>Grewia</i>	<i>grevei</i>	5	C ₃	No
Combretaceae	<i>Terminalia</i>	spp.	9	C ₃	No
Euphorbiaceae	<i>Croton</i>	<i>geayi</i>	4	C ₃	No
Meliaceae	<i>Cedrelopsis</i>	<i>grevei</i>	5	C ₃	No
Phyllanthaceae	<i>Phyllanthus</i>	<i>decaryanus</i>	5	C ₃	No
Rhamnaceae	<i>Gouania</i>	<i>glandulosa</i>	2	C ₃	No
Salvadoraceae	<i>Salvadora</i>	<i>angustifolia</i>	3	C ₃	No
Mimosaceae	<i>Dichrostachys</i>	<i>humbertii</i>	6	C ₃	Yes
Asclepiadaceae	<i>Cynanchum</i>	<i>mahafalense</i>	1	CAM	No
Cucurbitaceae	<i>Seyrigia</i>	sp.	2	CAM	No
Cucurbitaceae	<i>Xerosicyos</i>	<i>perrieri</i>	9	CAM	No
Euphorbiaceae	<i>Euphorbia</i>	<i>tirucalli</i>	21	CAM	No
Didieraceae	<i>Alluaudia</i>	<i>procera</i>	17	CAM	Yes
Cyperaceae	<i>Cyperus</i>	sp.	1	C ₄	No
Poaceae	<i>Panicum</i>	spp.	7	C ₄	No

corrected for the small 1.6‰ difference in δ¹⁵N between coastal and inland animals (Supplementary table 4).

Results

What do modern lemurs eat in southwestern Madagascar?

Six lemur species have been observed to feed on endemic and introduced CAM plants in southern and southwestern Madagascar: *Eulemur rufifrons* (the red-fronted brown lemur), *Lemur catta* (the ring-tailed lemur), *Lepilemur leucopus* (the white-footed sportive lemur), *L. petteri* (Jean-Jaques Petter's sportive lemur), *Microcebus griseorufus* (the reddish-grey mouse lemur) and *Propithecus verreauxi* (Verreaux's sifaka) (Supplementary table 2). Historically, behavioural studies have tended to concentrate on individuals living in gallery forest habitats. However, recent research has documented significant CAM consumption by individuals living in dry or spiny forest at Berenty, Beza Mahafaly, Cap Sainte Marie and Tsimanampetsotsa.^{24,28,32,50}

Loudon et al.⁵⁰ and Gould et al.³² estimate 15% CAM consumption for *L. catta* living in spiny forest at Tsimanampetsotsa and Berenty Reserve, respectively. Consumption of CAM resources by *L. catta* at Cap Sainte Marie can be >75% during some months.²⁸ Although the vast majority of the CAM plants consumed by members of the latter population are introduced, including *Opuntia* (prickly pears), native CAM species such as *Aloe* and *Kalanchoe* can each comprise >10% of the diet of *L. catta* during some months of the year.

Only *Lemur catta*, *Lepilemur leucopus* and *Propithecus verreauxi* have been observed to consume Didiereoideae (Table 2). All but one of these published observations have involved *Alluaudia* spp. The exception was *Didierea trollii* which is consumed by *L. catta*.²⁷ Some modern lemurs have been reported to feed heavily on *Alluaudia*. For example, during certain months at Cap Sainte Marie nearly 14% of the diet of *L. catta* is *Alluaudia procera*.²⁸ *Lepilemur leucopus* was observed to rely entirely on leaves and flowers of *Alluaudia* spp. during the dry season at Berenty.^{24,25}

Table 2: Observations of lemurs feeding on Didiereoideae taxa in southwestern Madagascar

Lemur species	Genus and species	Parts consumed	Locality	Source on feeding	Source on CAM
<i>Lemur catta</i>	<i>Alluaudia dumosa</i>	Leaves, fruit, flowers	Berenty Gallery Forest, Cap Sainte Marie	28, 52	37, 41
<i>Lemur catta</i>	<i>Alluaudia humbertii</i>	Mature leaves	Berenty Gallery Forest	27, 52	10, 37, 41
<i>Lemur catta</i>	<i>Alluaudia procera</i>	Young leaves, mature leaves, flowers	Berenty Gallery Forest and Spiny Forest; Cap Sainte Marie	27, 28, 32, 52, 53	17, 37, 41
<i>Lemur catta</i>	<i>Didierea trollii</i>	Young leaves, flowers	Berenty Gallery Forest	27, 52	10, 37, 41
<i>Lepilemur leucopus</i>	<i>Alluaudia ascendens</i>	Buds, leaves, flowers	Berenty Dry Forest	2, 24, 25	10, 37, 41
<i>Lepilemur leucopus</i>	<i>Alluaudia procera</i>	Buds, leaves, flowers	Berenty Dry Forest	2, 24, 25	17, 37, 41
<i>Propithecus verreauxi</i>	<i>Alluaudia ascendens</i>	Flowers	Hazofotsy	54	10, 37, 41
<i>Propithecus verreauxi</i>	<i>Alluaudia procera</i>	Flowers	Hazofotsy	54	17, 37, 41

On the other hand, *Lepilemur* living in the gallery forest at BMSR does not consume any Didiereoideae but relies to some degree on non-spiny *Euphorbia tirucalli*.⁵¹ These differences underscore the potential site specificity of variation in feeding observations.

Do *Alluaudia* differ isotopically from sympatric CAM plants?

Carbon isotope values differ significantly for CAM, C₃ and C₄ plants from BMSR (Figure 1; $F_{2,111}=714.9, p<0.0001$). Post-hoc HSD tests indicate that all three are distinct. Carbon isotopes cannot distinguish *Alluaudia procera* from sympatric CAM plants ($p>0.05$). However, nitrogen isotope values do clearly separate these two plant groups ($t=5.38, df=40, p<0.0001$). *Alluaudia* has distinctly elevated $\delta^{15}\text{N}$ values (Figure 1).

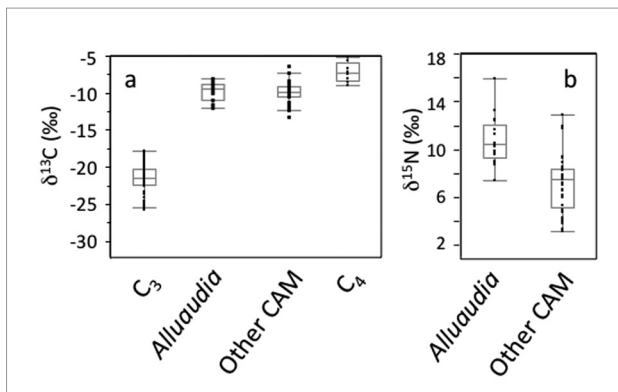


Figure 1: Box-and-whisker plots of (a) $\delta^{13}\text{C}$ values for C₃, CAM and C₄ plants and (b) $\delta^{15}\text{N}$ values for spiny Didiereoideae and non-spiny CAM from the spiny forest at Beza Mahafaly Special Reserve, Madagascar.

To what extent were lemurs feeding on CAM plants in the past?

We found no differences in mean $\delta^{13}\text{C}$ values between subfossil extant and extinct lemur species (Wilcoxon signed ranks, $S = 17, z = 0, p = 1.0$), although our subfossil sample showed greater variance. Mixing models based on $\delta^{13}\text{C}$ values suggest that subfossil individuals belonging to each of the three extant species consumed mostly C₃ resources (Table 3). CAM consumption was negligible for subfossil *Lepilemur*, but modest CAM consumption is indicated for subfossil *Lemur catta* (8.5%) and *Propithecus verreauxi* (5%). These values are slightly higher than CAM consumption estimates for *P. verreauxi* and *L. catta* living today in gallery forest,²⁷ but they are not as high as values for *L. catta* in dry forest at coastal localities in the south.^{28,32,50} Importantly, substantial CAM consumption by modern lemurs, even at coastal localities, is a seasonal phenomenon.²⁸ Because isotope values in bone collagen integrate several years of dietary input,²¹ modest %CAM estimates for subfossil individuals may reflect seasonal fluxes in CAM consumption. Among the extinct taxa living in the south and southwest, *Megaladapis edwardsi*, *M. madagascariensis*, *Pachylemur insignis* and *Palaeopropithecus ingens* show no evidence of CAM consumption (Table 3; Supplementary table 3). In contrast, our data indicate modest CAM consumption by *Archaeolemur majori* (5%) and significant CAM consumption by *Mesopropithecus globiceps* (25%) and *Hadropithecus stenognathus* (92%). In summary, while it is evident that not all southern lemurs consume CAM plants today, and it is unlikely that all consumed them in the past, some CAM consumption can be documented in a wide variety of lemur species.

Table 3: Descriptive statistics including number of specimens analysed, and mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values $\pm 1\sigma$ for each species

Genus and species	Carbon			Nitrogen	
	N	Mean $\delta^{13}\text{C} \pm 1\sigma^a$	%CAM ^b	N	Mean $\delta^{15}\text{N} \pm 1\sigma^d$
<i>Lemur catta</i>	25	-20.5 \pm 1.4	8.5	25	10.6 \pm 2.0
<i>Lepilemur leucopus</i>	8	-21.9 \pm 1.7	0	8	9.5 \pm 0.7
<i>Propithecus verreauxi</i>	29	-20.9 \pm 1.0	5	29	9.6 \pm 2.1
<i>Archaeolemur majori</i>	23	-20.9 \pm 1.4	5	19	11.3 \pm 1.9
<i>Hadropithecus stenognathus</i>	9	-10.8 \pm 1.5	92	7	13.8 \pm 3.2
<i>Megaladapis edwardsi</i>	8	-22.0 \pm 0.5	0	5	11.5 \pm 2.6
<i>Megaladapis madagascariensis</i>	14	-21.7 \pm 1.2	0	12	11.3 \pm 1.4
<i>Mesopropithecus globiceps</i>	4	-18.6 \pm 2.9	25	3	13.1 \pm 1.8
<i>Pachylemur insignis</i>	18	-22.2 \pm 1.4	0	16	11.4 \pm 1.9
<i>Palaeopropithecus ingens</i>	30	-21.7 \pm 0.7	0	27	13.5 \pm 2.0
<i>Aepyornis</i> spp.	60	-24.4 \pm 0.9 ^c	11 ^c		
<i>Aldabrachelys</i> spp.	19	-20.6 \pm 3.3	8	16	10.3 \pm 1.4
<i>Hippopotamus lemerlei</i>	14	-21.1 \pm 2.0	3	14	9.0 \pm 1.8

^a%CAM values were calculated using ISSOERROR version 1.04.⁴⁹

^bCollagen $\delta^{13}\text{C}$ values have been corrected to account for $\delta^{13}\text{C}$ shifts in atmospheric CO₂ following the industrial revolution.

^cMean %CAM consumption was estimated using $\delta^{13}\text{C}$ values from C₃ and CAM plants from the spiny forest at Beza Mahafaly Special Reserve (-21.5‰ and -9.9‰, respectively). Plant $\delta^{13}\text{C}$ values were corrected by +5‰ to account for the isotopic difference between collagen and plants.²¹

^d $\delta^{13}\text{C}$ values and %CAM estimates are from the organic portion of eggshell.⁵⁴ Carbon isotope values were corrected for the isotopic difference between eggshell and plants.⁵⁷ We corrected carbon isotope data for eggshells to account for atmospheric changes in $\delta^{13}\text{C}$ (-1.2‰).

^ePlant $\delta^{15}\text{N}$ values were corrected by +3‰ to account for the isotopic difference between collagen and plants.²¹

Because isotope values in plants are reflected in their animal consumers, we may be able to use differences in $\delta^{15}\text{N}$ between *Alluaudia* and sympatric CAM plants to identify lemurs that consumed Didiereoideae in the past. Among those species identified as CAM consumers by their $\delta^{13}\text{C}$ values, differing $\delta^{15}\text{N}$ values suggest varying degrees of *Alluaudia* consumption (Figure 2). Nitrogen isotope values indicate that Didiereoideae were not a dominant element of *L. catta* or *P. verreauxi* diets. Among the extinct taxa, *A. majori* may have consumed small amounts of Didiereoideae. However, *M. globiceps* may have consumed substantial amounts of Didiereoideae, and *H. stenognathus*, which is characterised by exceptionally high $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, may have relied heavily on Didiereoideae (Figure 2).

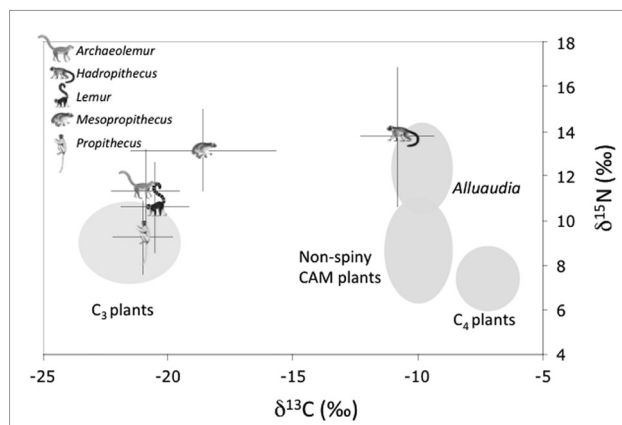
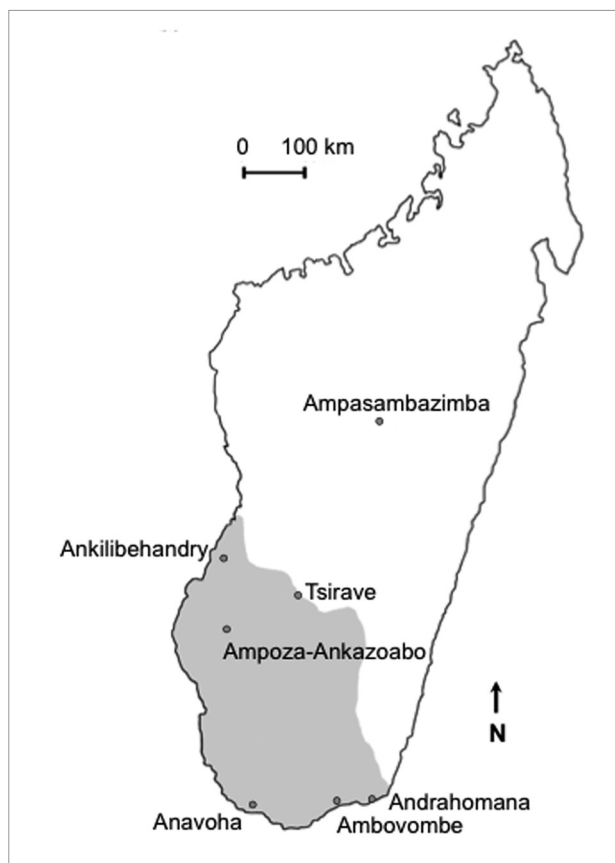


Figure 2: Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values $\pm 1\sigma$ for lemur species that likely consumed varying degrees of non-spiny and spiny CAM plants. Bubbles represent mean carbon and nitrogen isotope values $\pm 1\sigma$ for C_3 , C_4 , non-spiny CAM plants and *Alluaudia procera*. Subfossil carbon isotope values for subfossils were corrected for the post-industrial shift in atmospheric $\delta^{13}\text{C}$ values. Carbon and nitrogen isotope values in plants were corrected by $+3\text{‰}$ and $+5\text{‰}$ to account for the isotopic difference between collagen and plants. Finally, nitrogen isotope values in plants were shifted $+1.6\text{‰}$ to account for the mean isotopic difference between inland Beza Mahafaly Special Reserve and coastal localities (Supplementary table 3) in Madagascar.

The living lemur most reliant on Didiereoideae is likely *Lemur catta*. This species may have consumed more Didiereoideae in the past than it currently does in moist gallery forests.^{23,45} Goodman and colleagues⁵⁵ noted the distributional overlap of *L. catta* and the Didiereoideae. They suggested that this lemur species may have evolved in dry forests and subsequently moved into moister riparian forest, where didiereoid taxa do not exist. In fact, even today, in some arid habitats where *L. catta* still thrives and CAM resources abound (e.g. Tsimanampetsotsa, Cap Sainte Marie), these lemurs consume substantial amounts of Didiereoideae and other CAM plants.^{28,50} More research is needed to document the degree to which *L. catta* exploits Didiereoideae as opposed to other CAM plants.

The fact that both *Alluaudia* and *Hadropithecus* have extreme $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values is striking. The geographic overlap of the Didiereoideae and *Hadropithecus stenognathus* is also remarkable (Figure 3). With the exception of Ampasambazimba in Central Madagascar, all subfossil localities yielding *Hadropithecus* fall within the modern distributional range of the Didiereoideae. Compellingly, $\delta^{13}\text{C}$ values for the two *H. stenognathus* individuals sampled from Ampasambazimba suggest a pure C_3 -based, rather than a CAM-based, diet ($\delta^{13}\text{C} < -22\text{‰}$). This geographic overlap combined with the match for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between *Alluaudia* and *Hadropithecus*, strongly suggests that Didiereoideae was a staple in the diet of *Hadropithecus* in the Spiny Thicket and Succulent Woodland ecoregions of Madagascar.

Our subfossil isotope data do not support the notion that *Lepilemur* consumed large quantities of *Alluaudia* in the past. This finding might be considered curious, because *Lepilemur* is the only living lemur that has been reported to consume large quantities of *Alluaudia* today. The diet of *Lepilemur* has been studied in detail only at two localities in southern and southwestern Madagascar: the spiny forest at Berenty Private Reserve where *Alluaudia* exists, and the gallery forest at BMSR, where didiereoid taxa do not exist. *Alluaudia* spp. may comprise close to 100% of this species' diet at Berenty Private Reserve at least during the dry season.^{2,24,25} Yet $\delta^{13}\text{C}$ values for *Lepilemur* from multiple subfossil sites in the southwest indicate negligible CAM consumption in the past (Table 3; Supplementary table 3).



Source: adapted from www.tropicos.org.

Figure 3: Map of Madagascar including localities where *Hadropithecus stenognathus* remains have been found. The distribution of the Didiereoideae is shaded in grey.

Because our subfossil *Lepilemur* specimens come from several, geographically widespread, localities (two inland, one coastal), it is unlikely that this result reflects sampling bias. Instead it would appear that modern individuals might have recently shifted their diet at Berenty to include a resource that was inconsistently exploited (if at all) in the past. Recent transitions in diet or habitat may be widespread among modern lemurs living in southwestern Madagascar.⁴⁵ Isotope values for subfossil *Lepilemur* do not differ significantly from those for extinct *Archaeolemur majori*, *Megaladapis edwardsi*, *M. madagascariensis*, *Palaeopropithecus ingens* or *Pachylemur insignis*. Four of these (all except *Archaeolemur*) have a 0% CAM signal, as does subfossil *Lepilemur*. Of these four, the two *Megaladapis* species have dental topography much like *Lepilemur*,²³ as well as relatively small infraorbital foramina²⁹ and dental microwear^{30,56} that suggest dominant foliage consumption.

Did now extinct non-lemur herbivores consume Didiereoideae?

Estimated CAM consumption for extinct hippopotamuses, tortoises and elephant birds is minor compared to that for *Mesopropithecus* and *Hadropithecus*. Mixing models suggest that, on average, *Hippopotamus* spp. and the giant tortoise *Aldabrachelys* spp. consumed only 3% and 8% CAM, respectively (Table 3). Clarke et al.⁵⁷ used $\delta^{13}\text{C}$ values in *Aepyornis* eggshells to estimate that elephant birds consumed ca. 11% CAM. Nitrogen isotope values are similar in subfossil *Hippopotamus*, *Aldabrachelys*, *Lemur catta* and *Propithecus verreauxi* (Table 3; Supplementary table 3), indicating nominal consumption of Didiereoideae for these species. Nitrogen isotope values do not exist for *Aepyornis*. However, their $\delta^{13}\text{C}$ values suggest that elephant birds did not exploit large amounts of Didiereoideae.

Conclusions

Stable isotope data do not support significant CAM consumption by non-climbing extinct herbivores such as elephant birds, giant tortoises or pygmy hippopotamuses, but they do support significant CAM consumption in several extinct lemur lineages. It seems likely that spines evolved in the ancestral didiereoid as a defence against lemur folivory. At the very least, as didiereoids diversified to include relatively large spiny trees in southern and western Madagascar, they must have been exploited by climbing herbivores of some kind. Because so many herbivores in the south and southwest have become extinct, one might hypothesise that the spines on these plants are today anachronistic. The unusual isotopic signal of these plants allows us to test the plausibility of this hypothesis, and to offer new insights into likely past consumers. Our data support the conclusions that the herbivores exploiting the leaves of *Alluaudia* were largely climbing lemurs, and that the loss of giant climbing lemurs has rendered the spines of didiereoid plants, such as *Alluaudia*, increasingly anachronistic. With the exceptions of *Lepilemur* and *Lemur catta* in some locations, lemur species today consume little CAM. However, carbon isotope values indicate that both extant and now-extinct lemurs may have consumed more CAM plants in the past, including didiereoid taxa such as *Alluaudia*. In particular, *Lemur catta*, *Mesopropithecus globiceps*, and especially *Hadropithecus stenognathus*, may have relied heavily on Didiereoideae in the recent past. If indeed the dominant consumers of *Alluaudia* leaves are now extinct, these plants may no longer require formidable defence.

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Authors' contributions

Both authors participated in the planning and design of the research. L.R.G. conceived of the project and B.E.C. conducted analyses. Both authors wrote the manuscript.

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