



Insights into the habitat of Middle Permian pareiasaurs (Parareptilia) from preliminary isotopic analyses

AUORE CANOVILLE, DANIEL B. THOMAS AND ANUSUYA CHINSAMY

LETHAIA



Canoville, A., Thomas D.B. & Chinsamy A. 2014: Insights into the habitat of Middle Permian pareiasaurs (Parareptilia) from preliminary isotopic analyses. *Lethaia*, Vol. 47, pp. 266–274.

Pareiasaurs were an abundant group of large herbivores during Middle and Late Permian times. The habitat of pareiasaurs has proven enigmatic, and ecological interpretations from anatomical and taphonomic data have included aquatic, semi-aquatic to fully terrestrial lifestyles. Insight into the ecology of extinct taxa can also be gained from stable isotope analyses, and interpretations benefit from studies of multiple, coeval groups. Here, we report the first stable carbon and oxygen isotope analyses from the enamel, dentine and bone of pareiasaurs and contemporaneous therapsids (dinocephalians and therocephalians), in specimens recovered from the Permian *Tapinocephalus* to lower *Pristerognathus* Assemblage Zones of South Africa. Previous ecological inferences for dinocephalians (riparian to terrestrial) and therocephalians (terrestrial) are less ambiguous than reconstructions for pareiasaurs and provide an independent reference for interpreting stable isotope measurements. Oxygen isotopes of enamel carbonate were indistinguishable between pareiasaurs and therocephalians, which had higher values than dinocephalians. The data suggest that dinocephalians and pareiasaurs (megaherbivores) inhabited different ecological niches and that pareiasaurs may have shared a terrestrial habitat with therocephalians (carnivores). Our results agree with earlier suggestions of a terrestrial lifestyle among pareiasaurs and provide evidence of niche partitioning among large coeval Capitanian herbivores of South Africa. □ *Dinocephalians, Karoo Basin, palaeoecology, South Africa, therocephalians.*

Aurore Canoville [canoville.aurore08@gmail.com], and Anusuya Chinsamy [anusuya.chinsamy-turan@uct.ac.za], Department of Biological Sciences, University of Cape Town, Private Bag X3 Rhodes Gift 7707 Cape Town, South Africa; Daniel B. Thomas [thomasd@si.edu], Department of Vertebrate Zoology, National Museum of Natural History Smithsonian Institution, PO Box 37012 MRC 116 Washington, DC 20013-701, USA; manuscript received on 07/11/2012; manuscript accepted on 21/10/2013.

Pareiasaurs were a prominent tetrapod group during the Middle and Late Permian but perished during the Permo–Triassic extinction event (Lee 1997). Bereft of living descendents, the taxonomy of the Pareiasauria (Parareptilia) is uncertain, and reconstructing their life history has proven challenging (Laurin & Reisz 1995; Lee 1995; Tsuji & Müller 2009). Pareiasaurs were medium-sized to large herbivores (up to 2.5 m snout to tail; Lee 1997; Smith *et al.* 2012) and have been hypothesized as fully aquatic *Dugong*-like animals that fed on algae or plankton; a conclusion drawn from their massive bodies, stout stature and multi-cusped dentition (Case 1926; Hartmann-Weinberg 1937; Ivakhnenko 1994, 2001). Gross anatomy, bone micro-structure and taphonomic evidence have instead suggested that pareiasaurs were semi-aquatic (*Hippopotamus*-like) or riparian feeders (*Kobus*-like) that inhabited swampy environments (Boonstra 1955, 1962; Ochev 1995, 2004; Tverdokhlebov *et al.* 2005; Khlyupin 2007; Krilloff *et al.* 2008; Sumin 2009). Interestingly,

pareiasaurs have been found in flood-plain and lake deposits, in association with other aquatic or semi-aquatic vertebrates such as teleost fish and stem amphibians (Tverdokhlebov *et al.* 2005). Several specimens of *Bradysaurus* (South Africa) and *Deltavjatia* (Russia) have been discovered as complete articulated skeletons, sometimes preserved in upright positions, suggesting that those animals would have been trapped in the mud (Boonstra 1969; Ochev 1995). In contrast, studies of limb anatomy and correlations with footprint ichnofossils have suggested that pareiasaurs were fully terrestrial browsers (Lee 1994; Gubin *et al.* 2003; Benton *et al.* 2004, 2012; Valentini *et al.* 2009; Voigt *et al.* 2010).

Stable isotope analyses provide insights into the ecology of extinct taxa, and interpretations have benefited from studies of multiple, coeval groups (e.g. Clementz *et al.* 2003; Botha *et al.* 2005; Amiot *et al.* 2010). The aim of this study was to describe pareiasaur habitat from the stable isotopes of enamel carbonate with respect to coeval

synapsids. The most basal and also largest pareiasaurs such as *Bradysaurus* and *Embrithosaurus* from the *Tapinocephalus* and *Pristerognathus* Assemblage Zones (Capitanian) shared the Late Middle Permian ecosystems of the Karoo Basin of South Africa with therapsids, such as dicynodonts, dinocephalians and therocephalians (Chinsamy-Turan 2012). The dicynodonts of the Middle Permian were small fossorial herbivores, mainly represented by *Diictodon* (Smith *et al.* 2012), while the dinocephalians were large heavily built animals inhabiting mainly lowland environments (Smith *et al.* 2012) and perhaps marshy areas (Boonstra 1962). Dinocephalians were represented by the titanosaurs and tapinocephalians, which were terrestrial herbivores (Kammerer 2011; Kemp 2012; Smith *et al.* 2012), as well as by the anteosaurids, which were large terrestrial (Kammerer 2011; Kemp 2012) or riparian predators (Boonstra 1955, 1962). The contemporaneous therocephalian synapsids are exclusively interpreted as cursorial predators (Boonstra 1962; Kemp 1986). The life-history interpretations of dinocephalians and therocephalians provide a reference for the foraging ecology of pareiasaurs.

The mineral fractions of bone, dentine and enamel (i.e. bioapatite) contain oxygen isotopes in carbonate (CO_3^{2-}), hydroxyl (OH^-) and phosphate (PO_4^{3-}) ions. Carbonate and phosphate ions are often isolated for different analytical goals, whereas isotopes in the hydroxyl ions have received little attention (Koch 2007). Oxygen isotopes in carbonate ($\delta^{18}\text{O}_c$) are often used to describe the body water of an animal; habitat (i.e. aquatic, terrestrial), drinking water and body size can influence body water and $\delta^{18}\text{O}_c$ (Bryant & Froelich 1995; Clementz & Koch 2001). Ambient temperatures affect isotopic ratios (Longinelli & Nuti 1973), and the oxygen isotopic composition of phosphate ($\delta^{18}\text{O}_p$) has been useful for studying thermophysiology of extinct groups (e.g. Amiot *et al.* 2010). Correlations between $\delta^{18}\text{O}_p$ and $\delta^{18}\text{O}_c$ suggest that carbonate might also be informative about thermophysiology (Iacumin *et al.* 1996; Lécuyer *et al.* 2010), and recent studies have used clumped carbon and oxygen isotopes to study the body temperatures of extinct groups (e.g. Eagle *et al.* 2011). Indeed, there are a suite of biological and ecological factors that influence the oxygen isotopic compositions of bioapatite.

Here, we focus on $\delta^{18}\text{O}_c$ in bioapatite and consider habitat differences and the distribution of isotope values among Middle Permian animals. Our samples comprise four therocephalians, ten

pareiasaurs and sixteen dicynodonts, collected from locations across South Africa that spanned 3 million years of burial history. The principal caveat of our study is that we have assumed that the ecology of each group was conserved within each taxonomic order, irrespective of location or time, as well as the oxygen isotopic composition of rainwater and the palaeohydrology of the region. We were principally interested to know whether the $\delta^{18}\text{O}_c$ in pareiasaur enamel identified a semi-aquatic habitat. Similar studies have given insight into the foraging ecologies of Cenozoic mammals and Mesozoic taxa (e.g. MacLeod *et al.* 2000; Clementz *et al.* 2003, 2006, 2008; Botha *et al.* 2005; Liu *et al.* 2008; Amiot *et al.* 2010). Stable isotope studies have been reported for Middle and Late Permian dicynodonts (Thackeray *et al.* 1990; MacLeod *et al.* 2000), and the cynodonts *Diademodon* and *Cynognathus* (Botha *et al.* 2005), although similar work has not previously been reported for Middle Permian parareptiles, dinocephalians and therocephalians.

Material and methods

Specimens

Samples for stable isotope analysis were collected from 30 specimens curated at Iziko South African Museum in Cape Town, South Africa (Fig. 1; Table S1). Our sample contains the major groups of continental tetrapods from the Middle Permian of South Africa (Smith *et al.* 2012): Pareiasauria (Parareptilia, $n = 10$); Dinocephalia (Therapsida, $n = 16$); Therocephalia (Therapsida, $n = 4$). Fossils were recovered from the *Tapinocephalus* Assemblage Zone (AZ) ($n = 27$; ~263.8 Ma mid-point age), upper *Tapinocephalus* to lower *Pristerognathus* AZ ($n = 2$; ~263 Ma) and the lowermost *Pristerognathus* AZ ($n = 1$; ~261.4 Ma) (Late Middle Permian, Guadalupian–Capitanian, 265.8–260.4 Ma: from Fröbisch 2008; Smith *et al.* 2012).

Stable isotope sampling

Fossils with limited public display value and taxonomic character preservation were sampled in areas where minimal aesthetic damage would occur. Small (~20 mg) samples of enamel, dentine and bone were collected using a diamond drill bit. One canine was sampled from each therocephalian and dinocephalian and one marginal tooth for pareiasaurs. Dentine and bone samples were collected as close to enamel samples as possible.

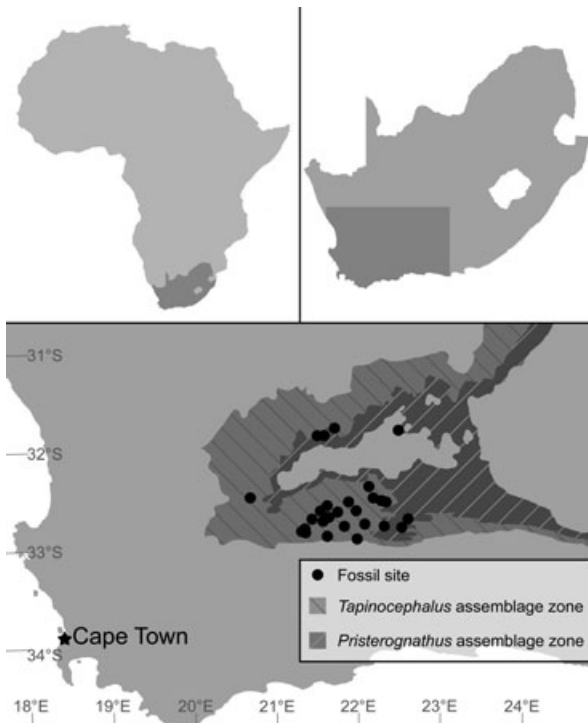


Fig. 1. Source locations for fossils analysed in the present study. Specimens had been collected from the *Tapinocephalus* ($n = 27$), upper *Tapinocephalus* to lower *Pristerognathus* ($n = 2$) and lower *Pristerognathus* Assemblage Zones ($n = 1$). The age range of the *Tapinocephalus* Assemblage Zone is ~ 266.1 Ma to ~ 261.5 Ma, and the age range of the *Pristerognathus* Assemblage Zone is ~ 261.5 Ma to ~ 259.9 Ma (age data from Fröbisch 2008; Smith *et al.* 2012).

Stable carbon ($\delta^{13}\text{C}$) and oxygen ($\delta^{18}\text{O}$) isotopes were measured using a Thermo Corporation Gas Bench II coupled to a Finnigan MAT 252 ion ratio mass spectrometer, housed in the Department of Archaeology, University of Cape Town. Approximately 10 mg of enamel, dentine or bone powder was soaked in 1 ml of $\sim 2\%$ NaOCl for one hour. Samples were subsequently rinsed in distilled water three times and left to soak overnight in 1 ml of 1 M acetic acid/calcium acetate buffer solution (Koch *et al.* 1997). Thereafter, samples were rinsed three times in distilled water and lyophilysed overnight; 2-mg aliquots were weighed for analysis by phosphoric acid reaction (CO_2 liberation occurred at 72°C using 100% H_3PO_4 ; e.g. Codron *et al.* 2008). Analysed standards included (all $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values are reported relative to PDB and SNOW, respectively), Carrara Z ($\delta^{13}\text{C} = 2.23 \pm 0.04\text{‰}$; $\delta^{18}\text{O} = 29.42 \pm 0.14\text{‰}$, $n = 25$), Cavendish marble ($\delta^{13}\text{C} = 0.34 \pm 0.07\text{‰}$; $\delta^{18}\text{O} = 21.80 \pm 0.17\text{‰}$, $n = 25$), NBS 18 ($\delta^{13}\text{C} = -5.02 \pm 0.05\text{‰}$; $\delta^{18}\text{O} = 6.93 \pm 0.16\text{‰}$, $n = 25$) and IAEA-CO-9 ($\delta^{13}\text{C} = -47.33 \pm 0.21\text{‰}$; $\delta^{18}\text{O} = 15.29 \pm 0.15\text{‰}$, $n = 11$;

mean \pm standard deviation). Carbon and oxygen isotope compositions were measured relative to the PDB standards. All oxygen isotope compositions were consequently converted and reported relative to SMOW according to $\delta^{18}\text{O}$ (‰SMOW) = $([\delta^{18}\text{O}$ ($\text{‰PDB})] \times 1.03092) + 30.92$ (NIST, 1992; Rohling & Cooke 1999). Student's two-tailed t-test assuming unequal variance was used for all statistical assessments of mean difference.

Diagenesis

The isotopic composition of any fossil may be a mixture of original biogenic signal and post-burial noise. Several techniques have been proposed for assessing whether a particular fossil is a high-fidelity source of biological information (e.g. Kolodny *et al.* 1996; Thomas *et al.* 2011). The method of assessing alteration that was available for this study relates carbon dioxide yield to carbonate composition (e.g. Koch *et al.* 1997). Well-preserved bioapatite in the enamel and dentine of modern mammals contains 2–5% structural carbonate (Rey *et al.* 1991; Rink & Schwarz 1995; Koch *et al.* 1997), and greater or lower percentage may be evidence for alteration. More specifically, low percentages of carbonate may indicate that a non-biogenic, secondary apatite has been incorporated into the sample. Note that the authors were unable to find carbonate percentage values for modern reptile teeth, and assume that the biogenic composition of pareiasaur teeth was around 5%. The percentage of carbonate in a bioapatite sample was interpreted from the signal amplitude (voltage) of a Faraday cup tuned to mass 44 (i.e. $^{12}\text{C}^{16}\text{O}_2$) and normalized against the amplitude of NBS-18, a calcium carbonate standard.

Results

Stable isotope measurements

The $\delta^{18}\text{O}_{\text{enamel}}$ values obtained for pareiasaurs were compared with those from dinocephalians and therocephalians. Average (\pm 95% confidence interval) $\delta^{18}\text{O}_\text{c}$ values of pareiasaurs ($14.5 \pm 1.0\text{‰}$) were significantly higher than that of dinocephalians ($11.2 \pm 0.8\text{‰}$, P -value < 0.001), but not significantly different than that of therocephalians ($15.4 \pm 0.9\text{‰}$, P -value = 0.845) (Fig. 2; Table S2). Carbon isotopes from enamel were not significantly different between pareiasaurs, dinocephalians and therocephalians, and bone and dentin samples had substantially lower carbonate percentages than enamel (Table S3).

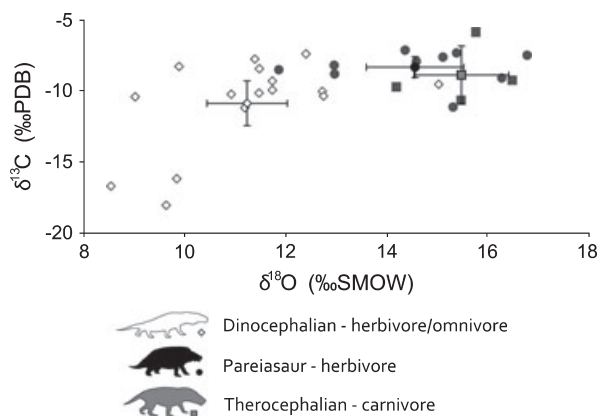


Fig. 2. Stable carbon ($\delta^{13}\text{C}$, ‰PDB) and oxygen ($\delta^{18}\text{O}$, ‰SMOW) isotopic compositions of enamel carbonate from Middle Permian tetrapods. Pareiasaurs and therocephalians have significantly higher $\delta^{18}\text{O}_{\text{enamel}}$ values compared with dinocephalians. For both open and closed symbols, measurements from individual specimens are grey-bordered, and mean \pm 95% confidence intervals are black-bordered.

Diagenesis

The normalized amplitude values (hereafter percentage carbonate values) were $5.2 \pm 0.7\%$ (mean \pm 95% confidence interval), $5.5 \pm 0.8\%$ and $5.8 \pm 3.4\%$ for the enamel of dinocephalians, pareiasaurs and therocephalians, respectively. Percentages of structural carbonate were not significantly different between dinocephalians ($n = 15$) and pareiasaurs ($n = 11$) ($P = 0.500$), and confidence intervals for both means included the expected 5% carbonate value. One therocephalian enamel sample was a substantial outlier (10.9% CO_3^{2-} : 11843, *Glanosuchus macrops*, $\delta^{18}\text{O} = 16.5\text{‰}$), and removing it from the data set lowered the mean of the remaining three samples to $4.1 \pm 0.9\%$.

A second substantial outlier was observed among the dinocephalian bone samples (11.9% CO_3^{2-} : 11591, *Struthiocephalus whaitsi*, $\delta^{18}\text{O} = -91.9\text{‰}$) and was removed from subsequent comparisons. The percentage of structural carbonate in dentine and bone was significantly lower than enamel for both dinocephalian (dentine: $3.8 \pm 0.8\%$, P -value = 0.015; bone: $3.8 \pm 0.8\%$, P -value = 0.012) and pareiasaur (dentine: $4.1 \pm 0.8\%$, P -value = 0.021; bone: $4.0 \pm 0.8\%$, P -value = 0.015) samples (Table S3).

The ca. 5% structural carbonate values for pareiasaur and dinocephalian enamel samples suggest the values have not been substantially altered. In contrast, the significantly lower values for dentine and bone suggest that some structural carbonate may have been lost from these tissues, and hence, the isotope values may not be useful for palaeoecological inferences.

Discussion

Diagenetic alteration

Interpreting $\delta^{18}\text{O}_{\text{enamel}}$ values as original, biological signals require the acceptance of certain conditions. These conditions affect how we interpret diagenetic alteration and can be summarized as: (1) all tissues preserve biogenic values; (2) bone and dentine do not preserve biogenic values; (3) enamel preserves a partial biogenic value; and (4) all three tissues preserve partial biogenic values. We suspect our data set follows scenario three or four, based on percentages of structural carbonate preserved in the samples (Table S3). We base this assessment on previous isotope studies of fossils from deep time (e.g. MacLeod *et al.* 2000; Botha *et al.* 2005), and the robustness of the $\delta^{18}\text{O}_{\text{enamel}}$ separation between pareiasaurs and dinocephalians, although we acknowledge the circularity of this approach. Hence, we will treat the observed $\delta^{18}\text{O}_{\text{enamel}}$ values as an original, biological phenomenon, under the caveat that some samples may have been completely altered, restricting interpretations to high-taxonomic-level trends.

Biological interpretation

Pareiasaur and dinocephalian $\delta^{18}\text{O}_{\text{enamel}}$ values were significantly different. We have restricted the time range of our samples to approximately 3 million years and analysed taxa from a scatter of localities. Hence, we have attempted to minimize temporal (i.e. Thackeray *et al.* 1990) and endemic biases and suggest that the observed offset is an unaltered original, biological phenomenon.

Oxygen isotopic differences between modern taxa may reflect body size (e.g. Bryant & Froelich 1995), thermophysiology (e.g. Kolodny *et al.* 1983), diet or digestive physiology (e.g. Clementz *et al.* 2009) or sources of drinking water (e.g. Amiot *et al.* 2010). *Hippopotamus* is an example of physiological fractionation regarding oxygen isotopes, as it tends to exhibit lower mean values than other megafauna in the surrounding environment (Bocherens *et al.* 1996); lower *Hippopotamus* values likely reflect high water turnover, similar to marine mammals (e.g. Hui 1981; Clementz *et al.* 2008). Isotopic compositions of enamel from extinct taxa, including the Permian tetrapods we studied, may also reflect a range of physiological or environmental factors.

Foraging niches (i.e. terrestrial, semi-aquatic, aquatic, marine) are recorded in the oxygen isotopes of taxonomically distinct animals (i.e. Cetacea and Carnivora, Clementz & Koch 2001; Anapsida and

Diapsida, Wheatley *et al.* 2012). Habitat information is provided by the mean and variance of oxygen isotope values, and variance tends to be more informative (e.g. Clementz & Koch 2001; Clementz *et al.* 2008; Wheatley *et al.* 2012). Hence, a *Hippopotamus* ecomorph for pareiasaurs would be evident as lower $\delta^{18}\text{O}_{\text{enamel}}$ values compared with dinocephalians and therocephalians. Instead, pareiasaurs have higher $\delta^{18}\text{O}_{\text{enamel}}$ values than dinocephalians. Mean $\delta^{18}\text{O}_{\text{enamel}}$ values either indicate a water source for pareiasaurs that is enriched in ^{18}O (i.e. estuarine as opposed to riverine) or a higher water turnover rate for dinocephalians (*sensu Hippopotamus*) (Fig. 2). Neither scenario is consistent with the freshwater lifestyle previously proposed for pareiasaurs. Instead, the data suggest that both dinocephalians and pareiasaurs were terrestrial and that oxygen isotopic differences reflect physiology (possibly different water turnover rates). Terrestrial lifestyles for both pareiasaurs and dinocephalians are supported by essentially similar standard deviations of enamel oxygen isotope values (1.56 and 1.63, respectively). However, we are cautious about interpreting the spread of a data set that may have been affected by alteration.

The biological and environmental factors that influence oxygen isotopic composition of carbonate in bioapatite may vary between individuals and populations, as well as between species, genera and families. Understanding the fine-scale structure in a data set of Permian isotope values would require many samples at low taxonomic levels. Instead of describing variation at low taxonomic levels, we have instead focused on determining whether there is a general trend for pareiasaurs. Specifically, we are interested in determining whether ecological factors in a semi-aquatic environment have contributed to the oxygen isotopic composition of pareiasaur enamel. The substantial enrichment in ^{18}O of pareiasaur enamel as compared to dinocephalians implies that pareiasaurs lived in terrestrial environments. The range of pareiasaur values suggests that there were differences within the terrestrial environments at low taxonomic levels. We have not had the opportunity to sample many individuals within low-level taxa, and hence our data set does not lend itself to describing fine-scale biological or ecological trends for Permian genera. Rather, we have multiple samples representing high-taxonomic levels, for which we must restrict our description of trends.

Additional considerations

Several ecological and physiological factors might contribute to the isotope signal in a biogenic tissue.

In addition to foraging ecology described above, we might also consider the effects of body size, body temperature and digestive physiology.

Body size can influence the turnover rate of water through an animal, and in turn, can influence the oxygen isotope composition of hard tissues (Bryant & Froelich 1995). We were interested in seeing whether the isotopic variation among Permian tetrapods in our data set could be explained as a function of body size. Specimens were too poorly preserved for body size estimation; instead, isotope values were regressed against previously published skull and body lengths (as available, Table S4), and length measurements were applied to congeners where data were missing (i.e. *Bradysaurus* sp. was assigned the size of *Bradysaurus seeleyi*, considering that both known species of *Bradysaurus* had a similar body size according to Lee 1997). For these regressions, we have assumed a common isotopic baseline for pareiasaurs and therapsids. Correlations between skull length and carbon and oxygen isotope value, and body length and carbon and oxygen isotope value, were all weak ($R^2 = 0.21$, P -value = 0.040; $R^2 = 0.38$, P -value = 0.004; $R^2 = 0.11$, P -value = 0.131; $R^2 = 0.23$, P -value = 0.020, respectively; Fig. S1). Hence, body size is not a strong influence on isotopic composition in our current data set, although a robust conclusion would benefit from additional sampling.

Body temperature can influence oxygen isotopic compositions of bone phosphate (Longinelli & Nuti 1973) and may affect the fractionation between body water and enamel carbonate (Lécuyer *et al.* 2010). Isotopic variation between taxa may therefore reflect differences in thermophysiology. Unfortunately, the thermophysiology of Permian tetrapods is uncertain, largely because endothermy is a feature almost completely related to soft tissue anatomy (i.e. Ruben *et al.* 2012). Fossil evidence of respiratory maxilloturbinals in the nasal cavity is the most useful feature for assessing endothermy in extinct amniotes (Clarke & Pörtner 2010; Laaß *et al.* 2011; Ruben *et al.* 2012). Maxilloturbinal ridges have been found in Upper Permian therocephalians and some cynodonts but not in pareiasaurs (Ruben *et al.* 2012); the absence of the ridges may represent a true absence or a preservation bias against the cartilaginous structure (Kemp 2006). Elevated ventilation and metabolic rates have been considered absent from Middle Permian dinocephalians or therocephalians (Ruben *et al.* 2012), although other researchers have considered these groups to have reached an incipient stage of endothermy (Kemp 2006). Furthermore, pareiasaurs were basal anapsid parareptiles (Lee 1997) and are phylogenetically bracketed by ectotherms.

Dinocephalians and therocephalians were synapsids (Sidor 2001), for which the crown taxa (mammals) are endothermic. Extending the thermophysiological state of crown taxa approximately 260 million years into deep time, and drawing on considerations about maxilloturbinal ridges, we might consider pareiasaurs and dinocephalians to have had different body temperatures, which may explain the variation observed in the carbonate isotope data set. We can compare isotopic offsets between modern endotherms and ectotherms to gain some insight into the effect of body temperature on oxygen isotopes in Middle Permian tetrapod enamel. Ideally, we would compare enamel isotopes from herbivorous, terrestrial anapsids (i.e. tortoises) and herbivorous, terrestrial synapsids (i.e. ungulate mammals). Tortoise isotope data are not available. We therefore refer to oxygen isotopic compositions of carbonate component of bone from marine anapsids (i.e. turtles) and synapsids (i.e. dugongs). We have found a single report for the oxygen isotopic composition of carbonate in turtle bone (Wheatley *et al.* 2012): $1.3 \pm 0.6\text{‰}$ PDB (i.e. 32.26‰ SMOW), loggerhead turtle (*Caretta caretta*). The mean isotopic composition from loggerhead turtle bone is significantly higher than for dugongs, manatees (Clementz & Sewell 2011) and other marine mammals (Roe *et al.* 1998; Clementz & Koch 2001). However, ectothermic diapsids (e.g. alligators, crocodiles, iguanas) exhibit substantial variation in their oxygen isotopic compositions, and bones from marine iguanas (*Amblyrhynchus cristatus*) and American alligators (*Alligator mississippiensis*) have values that are substantially different than loggerhead turtle bone, but not significantly different than many marine mammals (Roe *et al.* 1998; Clementz & Koch 2001; Clementz & Sewell 2011; Wheatley *et al.* 2012). We therefore conclude that thermophysiology is not easily classified with stable isotopes of carbonate. Furthermore, the $\delta^{18}\text{O}$ of pareiasaur enamel is higher than the composition of dinocephalian enamel and not significantly different than the composition of therocephalian enamel. Therocephalia is a clade within Synapsida, and hence, the ancestral state inference of endothermy for dinocephalians would also apply to therocephalians. A thermophysiology effect that does not separate pareiasaur and therocephalian values is unlikely to provide the dominant explanation for a carbonate isotopic offset between pareiasaurs and dinocephalians.

An offset in $\delta^{13}\text{C}$ has been observed between herbivores and carnivores in modern and Cenozoic communities (e.g. Clementz *et al.* 2009). We did not recover a $\delta^{13}\text{C}$ difference between herbivores and carnivores in our Palaeozoic samples, although we

did not have the opportunity to study many well-defined carnivores (four therocephalians and possibly two dinocephalians). The enamel carbonate of carnivores and herbivores may exhibit oxygen isotopic offsets (after Kohn 1996), owing to different water sources within a shared environment. For example, many small herbivores may heavily rely on plants as a source of water, whereas carnivores might drink more surface waters (Kohn 1996). The enamel of therocephalian synapsids (carnivores) had higher $\delta^{18}\text{O}$ values compared with dinocephalian synapsids (herbivores/omnivorous), which might be attributed to dietary differences. The isotope values of herbivorous pareiasaurs and carnivorous therocephalians were not significantly different, however. Diet may explain variation within sample groups (i.e. the range of dinocephalian values), but the oxygen isotopic segregation of herbivorous pareiasaurs and herbivorous/omnivorous dinocephalians is unlikely to reflect diet.

Water conservation is another physiological factor that may influence fractionation of oxygen isotope values in enamel. Modern animals that conserve water tend to live in terrestrial, water-limited environments and have more enriched oxygen isotope values in bioapatite (e.g. kangaroo rats; Smith *et al.* 2002). The relative high $\delta^{18}\text{O}$ values of pareiasaur compared with dinocephalians may be interpreted as water conservation, which would also contradict the semi-aquatic lifestyle proposed for pareiasaurs.

Permian Karoo ecosystem

The stable isotopes of modern taxa provide insight into both foraging environment and trophic position, and we sought similar information from Middle Permian megaherbivores and carnivores. We observed significant $\delta^{18}\text{O}_{\text{enamel}}$ differences between pareiasaurs ($n = 10$) and dinocephalians ($n = 16$, P -value < 0.001), but not between herbivorous pareiasaurs ($n = 10$) and carnivorous therocephalians ($n = 4$, P -value = 0.214). Although these data are limited, they suggest that pareiasaurs may have shared their habitat with therocephalians. Mean $\delta^{13}\text{C}_{\text{enamel}}$ values were only significantly different between dinocephalians ($n = 16$) and pareiasaurs ($n = 10$, P -value = 0.009). Unfortunately, the variation within each small group limits meaningful conclusions.

The isotope values we recovered are consistent with previous measurements of Permian animals. Thackeray *et al.* (1990) measured $\delta^{13}\text{C}_{\text{dentine}}$ from *Diictodon* sp. (Synapsida; Dicynodontia) from the Middle and Late Permian. The five herbivorous

Diictodon samples analysed from the *Tapinocephalus* and *Pristerognathus* Assemblage Zones had a mean $\delta^{13}\text{C}_{\text{dentine}}$ value of around -11‰ ; the mean $\delta^{13}\text{C}_{\text{dentine}}$ value from all 21 specimens between the *Tapinocephalus* and *Dicynodon* Assemblage Zones was around -12‰ , and the mean $\delta^{18}\text{O}_{\text{dentine}}$ value across all Assemblage Zones was around 14‰ . The values of Thackeray *et al.* (1990) are essentially identical to the dentine values we observed in three Permian tetrapod groups, but were less consistent with our enamel values. A subsequent study of Late Permian dicynodonts (Synapsida: Dicynodontia), including *Diictodon* sp., *Dicynodon* sp. and *Lystrosaurus* sp., found $\delta^{13}\text{C}_{\text{dentine}}$ values of around -12‰ , -14‰ and -13‰ , respectively, and $\delta^{18}\text{O}_{\text{dentine}}$ values of around 12‰ for each genus (MacLeod *et al.* 2000). Early Triassic *Cynognathus* sp. and *Diademodon* sp. (Synapsida: Cynodontia) had $\delta^{13}\text{C}_{\text{enamel}}$ values around -11‰ and -13‰ , and $\delta^{18}\text{O}_{\text{enamel}}$ values around 18‰ and 16‰ , respectively (Botha *et al.* 2005). Botha *et al.* (2005) attributed the lower $\delta^{13}\text{C}_{\text{enamel}}$ values of Early Triassic cynodonts to a trophic system based upon C_3 plants; an explanation which may apply to $\delta^{13}\text{C}_{\text{dentine}}$ and $\delta^{13}\text{C}_{\text{enamel}}$ values from Middle and Late Permian tetrapods (i.e. this study; Thackeray *et al.* 1990; MacLeod *et al.* 2000). $\delta^{18}\text{O}$ values from enamel and dentine are also substantially lower (e.g. this study, Thackeray *et al.* 1990; MacLeod *et al.* 2000; Botha *et al.* 2005) than values from modern tetrapods (e.g. Clementz & Koch 2001; Codron *et al.* 2008; Wheatley *et al.* 2012). The lower oxygen isotope values may reflect isotopic fractionation at high palaeolatitudes (latitude information from Scotese 2002).

Conclusions

We have sampled individuals from a wide range of genera within three high-taxonomic-level groups; our data set provides a coarse perspective on Middle Permian palaeoecology, particularly, large-scale isotopic differences between pareiasaurs and dinocephalians. Pareiasaurs had previously been hypothesized to be aquatic, semi-aquatic or fully terrestrial on the basis of anatomical and taphonomic data. Stable oxygen isotopes from pareiasaur enamel were found to be as or more enriched in ^{18}O when compared with isotopes from coeval therapsid enamel. This pattern suggests that pareiasaurs were terrestrial browsers and are inconsistent with the earlier semi-aquatic ecomorph hypothesis proposed. Isotopic differences between herbivorous dinocephalian

therapsids and pareiasaurs may also reflect higher water turnover rates in the former group (by analogy to modern mammals), which informs about niche partitioning in Capitanian megaherbivores.

Acknowledgements. – We thank Roger Smith and Sheena Kaal (Iziko South African Museum, Cape Town) for access to specimens, and Ian Newton (Department of Archaeology, University of Cape Town) for processing isotope samples. We received helpful comments from two anonymous referees and from Romain Amiot and Juan Carlos Cisneros. The Claude Leon Foundation, the URC and the Research and Innovation Department of the University of Cape Town as well as, the National Research Foundation of South Africa are acknowledged for research support for A. Canoville, D. Thomas and A. Chinsamy-Turan, respectively. Last but not least, we thank *Lethaia* editorial staff for its efficient handling of this manuscript.

References

- Amiot, R., Buffetaut, E., Lécuyer, C., Wang, X., Boudad, L., Ding, Z., Fourel, F., Hutt, S., Martineau, F., Medeiros, M.A., Mo, J., Simon, L., Suteethorn, V., Sweetman, S., Tong, H., Zhang, F. & Zhou, Z. 2010: Oxygen isotope evidence for semi-aquatic habits among spinosaurid theropods. *Geology* 38, 139–142.
- Benton, M.J., Tverdokhlebov, V.P. & Surkov, M.V. 2004: Ecosystem remodelling among vertebrates at the Permian–Triassic boundary in Russia. *Nature* 432, 97–100.
- Benton, M.J., Newell, A.J., Khlyupin, A., Shumov, I.S., Price, G.D. & Kurkin, A.A. 2012: Preservation of exceptional vertebrate assemblages in Middle Permian fluviolacustrine mudstones of Kotel'nich, Russia: stratigraphy, sedimentology, and taphonomy. *Palaeogeography, Palaeoclimatology, Palaeoecology* 319–320, 58–83.
- Bocherens, H., Koch, P.L., Mariotti, A., Geraads, D. & Jaeger, J.-J. 1996: Isotopic biogeochemistry (^{13}C , ^{18}O) of mammalian enamel from African Pleistocene hominid sites. *Palaios* 11, 306–318.
- Boonstra, L.D. 1955: The girdles and limbs of the South African Dinocephalia. *Annals of the South African Museum* 42, 185–327.
- Boonstra, L.D. 1962: The dentition of the titanosuchian dinocephalians. *Annals of the South African Museum* 46, 57–112.
- Boonstra, L.D. 1969: The fauna of the *Tapinocephalus* Zone (Beaufort beds of the Karoo). *Annals of the South African Museum* 56, 1–73.
- Botha, J., Lee-Thorp, J. & Chinsamy, A. 2005: The palaeoecology of non-mammalian cynodonts *Diademodon* and *Cynognathus* from the Karoo Basin of South Africa, using stable light isotope analysis. *Palaeogeography, Palaeoclimatology, Palaeoecology* 223, 303–316.
- Bryant, J.D. & Froelich, P.N. 1995: A model of oxygen isotope fractionation in body water of large mammals. *Geochimica et Cosmochimica Acta* 59, 4523–4537.
- Case, E.C. 1926: Environment of tetrapod life in the late Paleozoic of regions other than North America. *Carnegie Institution of Washington Publication* 375, 1–211.
- Chinsamy-Turan, A. 2012: *The Forerunners of Mammals: Radiation, Histology, Biology*, 330 pp. Indiana University Press, Bloomington & Indianapolis.
- Clarke, A. & Pörtner, H.O. 2010: Temperature, metabolic power and the evolution of endothermy. *Biological Reviews* 85, 703–727.
- Clementz, M.T. & Koch, P.L. 2001: Differentiating aquatic mammal habitat and foraging ecology with stable isotopes in tooth enamel. *Oecologia* 129, 461–472.
- Clementz, M.T. & Sewell, J.O. 2011: Latitudinal gradients in greenhouse seawater $\delta^{18}\text{O}$: evidence from Eocene sirenian tooth enamel. *Science* 332, 455–458.

- Clementz, M.T., Hoppe, K.A. & Koch, P.L. 2003: A paleoecological paradox: the habitat and dietary preferences of the extinct tethythere *Desmostylus*, inferred from stable isotope analysis. *Paleobiology* 29, 506–519.
- Clementz, M.T., Goswami, A., Gingerich, P.D. & Koch, P.L. 2006: Isotopic records from early whales and sea cows: contrasting patterns of ecological transition. *Journal of Vertebrate Paleontology* 26, 355–370.
- Clementz, M.T., Holroyd, P.A. & Koch, P.L. 2008: Identifying aquatic habits of herbivorous mammals through stable isotope analysis. *Palaïos* 23, 574–585.
- Clementz, M.T., Fox-Dobbs, K., Wheatley, P.V., Koch, P.L. & Doak, D.F. 2009: Revisiting old bones: coupled carbon isotope analysis of bioapatite and collagen as an ecological and palaeoecological tool. *Geological Journal* 44, 605–620.
- Codron, D., Brink, J.S., Roussouw, L. & Clauss, M. 2008: The evolution of ecological specialization in southern African ungulates: competition- or physical environmental turnover? *Oikos* 117, 344–353.
- Eagle, R.A., Tütken, T., Martin, T.S., Tripathi, A.K., Fricke, H.C., Connely, M., Cifelli, R.L. & Eiler, J.M. 2011: Dinosaur body temperatures determined from isotopic (^{13}C – ^{18}O) ordering in fossil biominerals. *Science* 333, 443–445.
- Fröbisch, J. 2008: Global taxonomic diversity of anomodonts (Tetrapoda, Therapsida) and the terrestrial rock record across the Permian-Triassic boundary. *PLoS ONE* 3, 1–14.
- Gubin, Y.M., Golubev, V.K., Bulanov, V.V. & Petuchov, S.V. 2003: Pareiasaurian tracks from the Upper Permian of Eastern Europe. *Paleontological Journal* 37, 514–523.
- Hartmann-Weinberg, A. 1937: Pareiasauriden als Leitfossilien. *Problemy Paleontologii* 2, 649–712.
- Hui, C.A. 1981: Seawater consumption and water flux in the common dolphin *Delphinus delphis*. *Physiological Zoology* 54, 430–440.
- Iacumin, P., Bocherens, H., Mariotti, A. & Longinelli, A. 1996: Oxygen isotope analysis of co-existing carbonate and phosphate in biogenic apatite: a way to monitor diagenetic alteration of bone phosphate. *Earth and Planetary Science Letters* 142, 1–6.
- Ivakhnenko, M.F. 1994: A new Late Permian dromasaur (Anomodontia) from Eastern Europe. *Paleontologicheskii Zhurnal* 1, 77–84.
- Ivakhnenko, M.F. 2001: Tetrapods from the East European Placket, a Late Paleozoic natural territorial complex. *Trudy Paleontologicheskogo Instituta, Rossiiskaya Akademiya Nauk* 283, 1–200.
- Kammerer, C.F. 2011: Systematics of the Anteosauria (Therapsida: Dinocephalia). *Journal of Systematic Palaeontology* 9, 261–304.
- Kemp, T.S. 1986: The skeleton of a baurioid therocephalian therapsid from the Lower Triassic (*Lystrosaurus* Zone) of South Africa. *Journal of Vertebrate Paleontology* 6, 215–232.
- Kemp, T.S. 2006: The origin of mammalian endothermy: a paradigm for the evolution of a complex biological structure. *Zoological Journal of the Linnean Society* 147, 473–488.
- Kemp, T.S. 2012. The origin and radiation of therapsids. In Chinsamy-Turan, A. (ed.): *Forerunners of Mammals: Radiation, Histology Biology*, 3–28 pp. Indiana University Press, Bloomington.
- Khlyupin, A.Y. 2007: Cemetery of the Permian reptiles. *Paleomir* 1, 50–57.
- Koch, P. 2007: Isotopic study of the biology of modern and fossil vertebrates. In Michener, R. & Lajtha, K. (eds): *Stable Isotopes in Ecology and Environmental Science*, 2nd Edition, 63–92 pp. Wiley-Blackwell, Oxford.
- Koch, P.L., Tuross, N. & Fogel, M.L. 1997: The effects of sample treatment and diagenesis on the isotopic integrity of carbonate in biogenic hydroxylapatite. *Journal of Archaeological Science* 24, 417–429.
- Kohn, M.J. 1996: Predicting animal delta ^{18}O : Accounting for diet and physiological adaptation. *Geochimica et Cosmochimica Acta* 60, 4811–4829.
- Kolodny, Y., Luz, B. & Navon, O. 1983: Oxygen isotope variations in phosphate of biogenic apatites, I. Fish bone apatite – rechecking the rules of the game. *Earth and Planetary Science Letters* 64, 398–404.
- Kolodny, Y., Luz, B., Sander, M. & Clemens, W.A. 1996: Dinosaur bones: fossils or pseudomorphs? The pitfalls of physiology reconstruction from apatitic fossils. *Palaeogeography, Palaeoclimatology, Palaeoecology* 126, 161–171.
- Krilloff, A., Germain, D., Canoville, A., Vincent, P., Sache, M. & Laurin, M. 2008: Evolution of bone microanatomy of the tetrapod tibia and its use in paleobiological inference. *Journal of Evolutionary Biology* 21, 807–826.
- Laaß, M., Hampe, O., Schudack, M., Hoff, C., Kardjilov, N. & Hilger, A. 2011: New insights into the respiration and metabolic physiology of *Lystrosaurus*. *Acta Zoologica* 92, 363–371.
- Laurin, M. & Reisz, R.R. 1995: A reevaluation of early amniote phylogeny. *Zoological Journal of the Linnean Society* 113, 165–223.
- Lécuyer, C., Balter, V., Martineau, F., Fourel, F., Bernard, A., Amiot, R., Gardien, V., Otero, O., Legendre, S., Panczer, G., Simon, L. & Martini, R. 2010: Oxygen isotope fractionation between apatite-bound carbonate and water determined from controlled experiments with synthetic apatites precipitated at 10–37°C. *Geochimica et Cosmochimica Acta* 74, 2072–2081.
- Lee, M.S.Y. 1994: *Evolutionary Morphology of Pareiasaurs*. Unpublished Ph.D. thesis, University of Cambridge, Cambridge.
- Lee, M.S.Y. 1995: Historical burden in systematics and the interrelationships of parareptiles. *Biological Reviews* 70, 459–547.
- Lee, M.S.Y. 1997: A taxonomic revision of pareiasaurian reptiles: implications for Permian terrestrial palaeoecology. *Modern Geology* 21, 231–298.
- Liu, A.G.S.C., Seiffert, E.R. & Simons, E.L. 2008: Stable isotope evidence for an amphibious phase in early proboscidean evolution. *PNAS* 105, 5786–5791.
- Longinelli, A. & Nuti, S. 1973: Revised phosphate-water isotopic temperature scale. *Earth and Planetary Science Letters* 19, 373–376.
- MacLeod, K.G., Smith, R.M.H., Koch, P.L. & Ward, P.D. 2000: Timing of mammal-like reptile extinctions across the Permian-Triassic boundary in South Africa. *Geology* 28, 227–230.
- National Institute of Standards and Technology 1992: Report of investigation. *Reference materials*, 8543–8546 pp.
- Ochev, V.G. 1995: Mysterious Kotel'nich. *Priroda*, 53–59.
- Ochev, V.G. 2004. Materials to the tetrapod history at the Paleozoic-Mesozoic boundary. In Sun, A. & Wang, Y. (eds): *Sixth Symposium on Mesozoic Terrestrial Ecosystems and Biota, Short Papers*, 43–46 pp. China Ocean Press, Beijing.
- Rey, C., Renugopalakrishnan, V., Shimizu, M., Collins, B. & Glimcher, M.J. 1991: A resolution enhanced Fourier transform infrared spectroscopic study of the environment of the CO_3 ion in the mineral phase of enamel during its formation and maturation. *Calcified Tissue International* 49, 259–268.
- Rink, W.J. & Schwarz, H.P. 1995: Tests for diagenesis in tooth enamel: ESR dating signals and carbonate contents. *Journal of Archaeological Science* 22, 251–255.
- Roe, L.J., Thewissen, J.G.M., Quade, J., O'Neil, J., Bajpai, S., Sahni, A. & Hussain, S.T. 1998. Isotopic approaches to understanding the terrestrial-to-marine transition of the earliest cetaceans. In Thewissen, J.G.M. (ed.): *The Emergence of Whales*, 399–422 pp. Plenum Press, New York.
- Rohling, E.J. & Cooke, S. 1999: Stable oxygen and carbon isotopes in foraminiferal carbonate shells. In Sen Gupta B.K. (ed.): *Modern Foraminifera*, 239–258 pp. Kluwer Academic, Dordrecht.
- Ruben, J.A., Hillenius, W.J., Kemp, T.S. & Quick, D.E. 2012: The evolution of mammalian endothermy. In Chinsamy-Turan A. (ed.): *Forerunners of Mammals: Radiation, Histology, Biology*, 273–286 pp. Indiana University Press, Bloomington.
- Scotese, C.R. 2002: <http://www.scotese.com>, (PALEOMAP web-site).
- Sidor, C. 2001: Simplification as a trend in synapsid cranial evolution. *Evolution* 55, 1419–1442.

- Smith, K.F., Sharp, Z.D. & Brown, J.H. 2002: Isotopic composition of carbon and oxygen in desert fauna: investigations into the effects of diet, physiology, and seasonality. *Journal of Arid Environments* 52, 419–430.
- Smith, R., Rubidge, B. & van der Walt, M. 2012: Therapsid biodiversity patterns and paleoenvironments of the Karoo Basin, South Africa. In Chinsamy-Turan, A. (ed.): *Forerunners of Mammals: Radiation, Histology, Biology*, 31–62 pp. Indiana University Press, Bloomington.
- Sumin, D.L. 2009: Hibernation as a factor responsible for preservation of the Pareiasauria in the Kotel'nich locality. In Shishkin M.A., Tverdokhlebov V.P. (eds): *Researches on paleontology and biostratigraphy of ancient continental deposits (Memories of Professor Ochev, V.G.)*, 173–176 pp. ONauchnaya Kniga Publishers, Saratov.
- Thackeray, J.F., Van der Merwe, N.J., Lee-Thorp, J.A., Sillen, A., Lanham, J.L., Smith, R., Keyser, A. & Monteiro, P.M.S. 1990: Changes in carbon isotope ratios in the late Permian recorded in therapsid tooth apatite. *Nature* 347, 751–753.
- Thomas, D.B., McGoverin, C.M., Fordyce, R.E., Frew, R.D. & Gordon, K.C. 2011: Raman spectroscopy of fossil bioapatite — a proxy for diagenetic alteration of the oxygen isotope composition. *Palaeogeography, Palaeoclimatology, Palaeoecology* 310, 62–70.
- Tsuji, L.A. & Müller, J. 2009: Assembling the history of the Parareptilia: phylogeny, diversification, and a new definition of the clade. *Fossil Record* 12, 71–81.
- Tverdokhlebov, V.P., Tverdokhlebova, G.I., Minikh, A.V., Surkov, M.V. & Benton, M.J. 2005: Upper Permian vertebrates and their sedimentological context in the South Urals, Russia. *Earth-Science Reviews* 69, 27–77.
- Valentini, M., Nicosia, U. & Conti, M.A. 2009: A re-evaluation of *Pachypes*, a pareiasaurian track from the Late Permian. *Neues Jahrbuch für Geologie und Paläontologie, Abhandlungen* 251, 71–94.
- Voigt, S., Hminna, A., Saber, H., Schneider, J.W. & Klein, H. 2010: Tetrapod footprints from the uppermost level of the Permian Ikakern Formation (Argana Basin, Western High Atlas, Morocco). *Journal of African Earth Sciences* 57, 470–478.
- Wheatley, P.V., Peckham, H., Newsome, S.D. & Koch, P.L. 2012: Estimating marine resource use by the American crocodile *Crocodylus acutus* in southern Florida, USA. *Marine Ecology Progress Series* 447, 211–229.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Collection, curatorial and taxonomic details of study specimens. All the specimens come from the Iziko South African Museum in Cape Town, South Africa.

Table S2. Stable carbon ($\delta^{13}\text{C}_\text{c}$; ‰PDB) and oxygen ($\delta^{18}\text{O}_\text{c}$; ‰SMOW) isotopic compositions measured from fossils. Specimen identifications correspond with taxon and location data in Table S1. Isotopic differences (Δ) between tissues are presented as absolute values.

Table S3. Percentage carbonate calculated for each specimen. Specimen identifications correspond with taxon and location data in Table S1.

Table S4. Body size parameters.

Figure S1. Correlations between isotope values and body size parameters. Sources of body size data given in Table S4.