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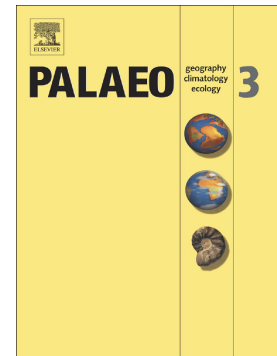
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Oxygen isotopes and ecological inferences of Permian (Guadalupian) tetrapods from the main Karoo Basin of South Africa

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Abstract

The Abrahamskraal Formation of South Africa has a diverse middle Permian (Guadalupian) fossil tetrapod fauna comprising largely basal therapsids and pareiasaurian parareptiles. Knowledge on the paleoecology of these animals is limited because inferences of many ecological traits in mid-Permian tetrapods are based on deductions from morphology. While it is widely accepted that the rhinesuchid amphibians were aquatic, the ecological lifestyle of pareiasaurs and dinocephalian therapsids remains uncertain. To investigate the ecological roles of different middle Permian tetrapod taxa we studied stable oxygen isotope compositions of phosphate from teeth and bones as a proxy for water dependence. Our results reveal similar $\delta^{18}O_p$ values for pareiasaurs, the dinocephalian *Anteosaurus,* and therocephalians, with a range of values similar to those of extant terrestrial species. The consistent $\delta^{18}O_p$ values for pareiasaurs strengthen our interpretation of a terrestrial lifestyle for these parareptiles, but the lower *Anteosaurus* $\delta^{18}O_p$ values might question the interpretation and a larger sample size may result in a more conclusive outcome. These results conclude the debate on the ecology of middle Permian pareiasaurs. A terrestrial lifestyle is applicable only to the middle Permian species and a different lifestyle is possible for pareiasaurs species that lived after the end Capitanian extinction.

1.   **Introduction**

The rich tetrapod fauna from the Abrahamskraal Formation of South Africa provides a

unique window onto high latitude fauna of Gondwana during the middle Permian

(Guadalupian). One of its major constituent taxa, dinocephalian therapsids, achieved

widespread distribution across Pangaea (Cisneros et al., 2012; Liu, 2013; Golubev, 2015);

other well-represented groups, including dicynodont therapsids, rhinesuchid temnospondyls

and pareiasaurian parareptiles, were restricted to Gondwana at this time (Golubev, 2005;

Fröbisch, 2009; Kurkin, 2011). The fauna is best known from the Capitanian-aged

Tapinocephalus Assemblage Zone from the upper Abrahamskraal Formation, which is

terminated by the extinction of 75-80% of tetrapod species, including all dinocephalians at a

global scale and all Guadalupian pareiasaur species at a local scale (Day et al., 2015).

The causes of the Capitanian mass extinction event are unclear with several

hypotheses proposed such as the Emeishan volcanism (Zhou et al., 2002; Wignall et al., 2009;

Bond and Wignall, 2014), marine anoxia (Jin et al., 1994; Hallam and Wignall, 1999; Isozaki

et al., 2008; Saitoh et al., 2013), global marine regression or even the release of methane in

the atmosphere (Krull et al., 2000; Krull and Retallack, 2000; Retallack and Jahren, 2008;

Svensen et al., 2009). Although most of these causes have been either questioned (Bond et al.,

2010; Sheldon et al., 2014) or might not have had impact on the continental fauna and flora

(Sheldon et al., 2014), with the possible exception of the volcanism in South China (Day et

al., 2015), conditions in the southwestern Karoo Basin may have been exacerbated by

regional aridification resulting from tectonic activity to the south (Rey et al., 2018). Clues to

the causes, as well as to the question of how ecosystems react to different forms of disruption,

can be found in the ecological characters of individual species and their place within food

webs. While the lifestyle and ecological niche occupation of some species can be inferred

from morphological characters (Canoville and Laurin, 2010; Canoville and Chinsamy, 2015; Jasinoski et al., 2009), this is not true for all tetrapods. Incompletely preserved skeletons lacking key parts of the anatomy, the presence of characters that are not known in extant tetrapods, or discrepancies between ecological deductions from morphology and other disciplines can all lead to uncertainty (Canoville and Chinsamy, 2017).

The pareiasaurs and dinocephalians that succumbed to the Capitanian mass extinction appear to differ in a few ecological characters: they have very disparate dentition, suggesting that the herbivores consumed different types of vegetation, and their skeletons are usually preserved under different taphonomic conditions (Boonstra, 1969), suggesting different lifestyles. The lifestyle of pareiasaurs in particular has been variously interpreted, when not inconclusive (Canoville and Chinsamy, 2017; Boitsova et al., 2019), as completely aquatic (Case, 1926; Ivakhnenko, 2001), amphibious (Boonstra, 1969; Ochev, 2004; Khlyupin, 2007; Kriloff et al., 2008), or terrestrial (Voigt et al., 2010; Benton et al., 2012; Canoville et al., 2014; Verrière et al., 2016). Similarly, the dinocephalian *Anteosaurus*, while generally accepted to have been a fully terrestrial animal (Nicolas and Rubidge, 2010; Kammerer, 2011; Kemp, 2012; Canoville et al., 2014), has also been considered to have been riparian (Boonstra, 1955, 1962), or even amphibious (Ivakhnenko, 2008).

Stable isotopic studies of hard tissues are useful to determine certain lifestyle aspects of animals. For instance, this technique can be used to reconstruct the relationship of an individual to aquatic environments. Stable oxygen isotope composition ($\delta^{18}O$) of vertebrate apatite is correlated to both body temperature and to the oxygen isotope composition of body water ($\delta^{18}O_{bw}$), which is mostly derived from the consumption of either meteoric water or plant water, or a combination of both (D'Angela and Longinelli, 1990; Kohn, 1996). Once

ingested this water undergoes fractionation, mostly caused by transcutaneous evaporation,

urine and faeces (Luz and Kolodny, 1985; Bryant and Froelich, 1995; Amiot et al., 2007).

Fractionation, which increases ^{18}O in the remaining body water, is therefore more intense for

species which do not replenish their body water by drinking often (Cerling et al., 2008) and

less intense when transcutaneous evaporation is reduced as a result of an aquatic or semi-

aquatic lifestyle (Clementz et al., 2008; Amiot et al., 2010). Consequently, for extant

mammals, species which consume large quantities of water, or spend much time in water,

have lower $\delta^{18}O_{bw}$ values than those with a strict terrestrial ecology and only consume plants

(Bocherens et al., 1996; Kohn et al., 1996; Cerling et al., 2008). For example, two studies

dedicated to the isotopic analysis of mammals from national parks in Kenya have shown that

the $\delta^{18}O$ values recorded in the apatite of extant hippopotamus are lower than those of

coexisting terrestrial mammals such as lions, elephants and even waterbuck. Depending on

the water-dependence of the terrestrial species (i.e. obligate drinkers or not), the $\delta^{18}O$ values

of the hippopotamus are about 3‰ lower than those of water-dependent animals and the

difference can be as high as 7‰ when compared with water-independent species (Bocherens

et al., 1996; Cerling et al., 2008; Fig. 1; Table 1). The $\delta^{18}O$ values of the semi-aquatic

hippopotamus, when compared with the $\delta^{18}O$ values of ingested water, fit between the

equations for terrestrial placental mammals (Amiot et al., 2004), those for crocodilians (Amiot

et al., 2007) and freshwater turtles (modified from Bocherens et al., 1996; Fig. 1). A more

complete review of $\delta^{18}O$ values for hippopotamus and co-existing vertebrates has been

compiled from extant and fossil individuals (Clementz et al., 2008) showing constant lower

values for the semi-aquatic hippopotamus. Accordingly, by comparing the apatite $\delta^{18}O$ values

of various co-existing vertebrates of contrasting lifestyles, it should be possible to interpret

the ecology of extinct taxa as has been done by Amiot et al. (2010) for spinosaurid dinosaurs.

In an earlier study Canoville et al. (2014), while undertaking research on the ecology of pareiasaurs, pointed out that most of the dinocephalians had lower oxygen isotope compositions than the rest of the tetrapod sample; however, they still interpreted dinocephalians to be fully terrestrial based on the standard deviation of their values. Rey et al. (2016) also concluded that the Guadalupian pareiasaurs were fully terrestrial, but their conclusions were only tentative as their study was undertaken on a small sample size derived from specimens curated in different museum collections in South Africa. To clarify this point, and to strengthen our comparisons, we have increased our sample size of dinocephalians, pareiasaurs and other taxa, and measured the carbon and oxygen isotope compositions of apatite carbonate as well as the phosphate oxygen isotope compositions of both bones and teeth.

2.   Material and methods

2.1.   *Sample collection*

A feature of this isotope study, apart from being the most taxonomically extensive yet undertaken in the Abrahamskraal Formation, is that the tetrapod fossils sampled are well provenanced and come from a number of different lithostratigraphic members in stratigraphic succession. This is the first time that such a stratigraphically controlled isotope study has been undertaken in the Abrahamskraal Formation, but has the disadvantage that for some of the stratigraphic members only a few specimens of particular taxa were found. Bone apatite of twenty-six tetrapod fossils were sampled, and for seventeen of them teeth were also sampled. All samples were analysed for their stable oxygen isotope composition of apatite phosphate and carbonate. The fossils were recovered from eight localities within a radius of 100 km in the Main Karoo Basin, between 21.0° and 22.1° of latitude and -32.9 and -32.2° of longitude

6

(Fig. 2; Supplementary Table 1), and are curated in the collection of the Evolutionary Studies

Institute, University of the Witwatersrand, Johannesburg. Most specimens were collected by

the authors of this study and are characterized by a secure taxonomic identification.

All sampled fossils were collected from horizons belonging to the Abrahamskraal

Formation, which is subdivided into eight members respectively named from the lowest to the

highest: Combrinkskraal, an unnamed member, Grootfontein, Leeuvlei, Koornplaats,

Swaerskraal, Moordenaars and Karelskraal members (Day et al., 2018). To compensate as

much as possible for temporal variations in isotopic values, we combined the localities from

stratigraphically equivalent horizons (Supplementary Table 1) which were divided into three

time bins. The lowest (one locality) corresponds to the upper Leeuvlei Member, the middle

bin combines four localities from the lower to lower-middle Moordenaars Member and one

between the upper Koornplaats and lower Moordenaars members, and the uppermost bin

combines two localities from the overlying Karelskraal Member.

Sample sizes were severely limited by the availability of species from specific

geological horizons and, due to the destructive and targeted nature of sampling for stable

isotope analysis, the availability of suitable specimens with appropriate and reliable

taxonomic data. The sample collection assembled for this study included one rhinesuchid

amphibian, twelve therapsids, and thirteen pareiasaurs. The therapsid sample-set comprises

ten dinocephalians — five Titanosuchidae indet., one tapinocephalid (*Criocephalosaurus sp.*),

and four *Anteosaurus magnificus* — and two therocephalians (*Glanosuchus sp.* specimens). In

South Africa, four pareiasaur species have been recognized from the middle Permian:

Bradysaurus baini, *B. seeleyi*, *Embrithosaurus schwarzi* and *Nochelesaurus alexanderi* (Lee,

1997; Van den Brandt et al., 2019). As the pareiasaur specimens sampled are not all well

7

preserved, only six could be reliably identified with three *Bradysaurus bainii*, two *Embrithosaurus schwarzi* and one *Nochelesaurus alexanderi*; the remaining seven specimens cannot be identified to genus and are referable only to the family level (pers. com. M. Van den Brandt; see Supplementary Table 1).

Apart from the above specimens we collected, fossil data obtained from the De Bad locality were also included (Rey et al., 2016, 2017). This sample collection comprises six dinocephalians — two *Criocephalosaurus*, one addition tapinocephalid (either *Mormosaurus* or *Struthiocephalus*), one Titanosuchidae indet., two *Anteosaurus* — one therocephalian (*Glanosuchus*), and two pareiasaurs (one *Bradysaurus baini* and one unidentified).

To obviate biases resulting from environmental change over time, the stable isotope oxygen of phosphate apatite ($\delta^{18}O_p$) and the stable isotope carbon of carbonate apatite ($\delta^{13}C_c$) boxplots obtained per taxon were plotted against their respective stratigraphic members (Figs. 5 and 6).

2.2.   Analytical techniques

The phosphate radical is of interest as the molecular bond is stronger than that of carbonate and inorganic alteration at low temperature has little effect on their $\delta^{18}O_p$ values (Lécuyer et al., 1999). Comparison between the phosphate and carbonate values allows us to potentially detect isotopic compositions of diagenetic origin (Iacumin et al., 1996). In order to measure the oxygen isotope composition of the apatite phosphate radicals, we isolated these ions using acid dissolution and anion-exchange resin applying a standard protocol (Lécuyer, 2004). Once quantitatively precipitated in a thermostatic bath set at a temperature of 70 °C, the silver phosphate was filtered, washed with double deionized water, and dried at 50 °C.

Oxygen isotope compositions of the powders were measured using a high-temperature pyrolysis technique involving a VarioPYROcube[TM] elemental analyzer (EA) interfaced in continuous flow (CF) mode to an Isoprime[TM] isotopic ratio mass spectrometer (IRMS) (EA-Py-CF-IRMS technique) at the Laboratoire de Géologie de Lyon (UMR 5276, Université Claude Bernard Lyon 1). For each sample, five aliquots of 300 μg of Ag_3PO_4 were mixed with 300 μg of pure graphite powder and loaded in silver foil capsules.

Pyrolysis was performed at 1450 °C. Measurements were calibrated against the NBS120c (natural Miocene phosphorite from Florida: $\delta^{18}O$ = 21.7‰; Lécuyer et al., 1993)) and the NBS127 (barium sulfate, $BaSO_4$: $\delta^{18}O$ = 9.3‰; Hut, 1987). Silver phosphate samples precipitated from standard NBS120c were repeatedly analysed ($\delta^{18}O_p$ = 21.7‰; 1σ = 0.3; n = 12) along with the silver phosphate samples derived from fossil bioapatites to ensure that no isotopic fractionation occurred during the wet chemistry. Data are reported as $\delta^{18}O_p$ values vs. V-SMOW (in ‰ δ units).

About 10 mg of tooth or bone powder was pre-treated (Koch et al., 1997) for measuring the oxygen isotope composition of the apatite carbonate. Powders were washed with a 2% NaOCl solution to remove organic matter, then rinsed five times with double deionized water and air-dried at 40 °C for 24 hours. Potential secondary carbonate was removed by adding 0.1 M acetic acid and leaving overnight, after which the powder was again rinsed five times with double deionized water and air-dried at 40 °C overnight. The powder/solution ratio was kept constant at 0.04 $g.mL^{-1}$ for both treatments. The tubes were placed in a Thermo Finnigan Model II gas bench in a temperature controlled sampler tray set to 72°C (housed in the Stable Light Isotope Laboratory of the Archaeology Department of the University of Cape Town). For each sample, an aliquot of 2 mg of pre-treated apatite was

9

reacted with 7 drops of supersaturated orthophosphoric acid at 72 °C for at least two hours

under a He atmosphere before starting 9 measurement cycles of the isotopic composition of

the CO_2 produced with a Thermo Finnigan Delta Plus XP continuous flow isotope ratio mass

spectrometer. The measured carbon and oxygen isotopic compositions were calibrated relative

to the NBS-18, NBS-19 and an internal calcite standard 'Cavendish Marble'. Reproducibility

for the carbon and oxygen isotopic compositions of apatite carbonate is better than ±0.15‰

and ±0.20‰, respectively. The carbon and oxygen isotopic compositions are expressed as δ

values relative to V-PDB for both carbon and oxygen (in ‰ δ units). The oxygen values were

converted from V-PDB to V-SMOW following the equation from Coplen et al. (1983).

2.3.   Robustness of the stable isotope record

In contrast to the structure of extant mammal teeth, tooth enamel of Permian tetrapods

is so thin that it was not possible to sample enough material suitable for isotopic analysis.

Consequently, we focused our sampling on bone and tooth dentine; however, those

bioapatites have more porous apatite than enamel, with smaller and less densely inter-grown

crystals (Mills, 1967). Thus, secondary precipitation within or on the surface of bioapatite

crystals, adsorption of ions on the surface of those crystals, or even dissolution and

recrystallization along with isotopic exchange could have altered their original isotope

composition. To differentiate apatites "isotopically-altered" by diagenesis from those partly

altered or unaltered, all samples were tested for primary preservation by comparing their

$\delta^{18}O_p$ values against their $\delta^{18}O_c$ values as recommended by Iacumin and Longinelli (1996).

In the skeletal tissue of extant mammals, the $\delta^{18}O_p$ and $\delta^{18}O_c$ are positively correlated

because apatite precipitates close to equilibrium with water for both phosphate and carbonate

components. As isotopic exchange rates between carbonate-water and phosphate-water are

highly contrasted, re-equilibration of both compounds during diagenesis is not expected to be a common process and altered enamel should show isotopic shifts from the empirical $\delta^{18}O_p$–$\delta^{18}O_c$ line (Iacumin et al., 1996). It is therefore expected that the distribution of pristine or weakly altered $\delta^{18}O_p$ and $\delta^{18}O_c$ values should display a positive regression line with a slope close to unity. At low temperature, inorganic alteration has little effect on the phosphate of the apatite, even at the scale of geological time (Tudge, 1960; Lécuyer et al., 1999), and would result in high overall carbonate content or a low discrepancies between carbonate $\delta^{18}O_c$ and phosphate $\delta^{18}O_p$ values. However, apatites can be mineralogically and isotopically altered during microbially-mediated diagenesis (Zazzo et al., 2004), which should be shown by greater $\delta^{18}O_c$–$\delta^{18}O_p$ differences. The carbonate content can be estimated from the mean magnitude of the current voltage (mV) measured during the analysis of the CO_2 by mass spectrometer (Supplementary Table 1).

3.   Results

In our dataset, most of the samples analysed by mass spectrometry yielded peaks for the mass 44 with a mean magnitude of around 1000 mV and 3000 mV, which is in accordance with the sample weights (Rey et al., 2018). However, two samples (1782-B and 7241-B) are characterized by mean magnitudes of 3900 mV and 5600 mV respectively, which are greater than the rest of the studied sample, hence suggesting an excess of carbonate in the apatite structure. Moreover, $\delta^{18}O_p$ and $\delta^{18}O_c$ values of these samples significantly deviate from the empirical regression line (Fig. 3). Therefore, we consider that those two samples lost their pristine isotopic compositions. The altered state of the sample 1782-B was also highlighted by comparing bone and tooth isotopic ratios from the same specimens. While both $\delta^{13}C_c$ and $\delta^{18}O_p$ values (Fig. 4A-C) are close to the empirical line, the $\delta^{18}O_c$ value of bone sample 1782-

11

B (Fig. 4B) is abnormally high compared to the tooth. Of the total of 43 samples, two are considered to have diagenetically altered $\delta^{18}O_c$ and $\delta^{13}C_c$ values.

Once the diagenetic and outlying values were removed from the dataset, the $\delta^{18}O_p$ and $\delta^{18}O_c$ values of the remaining weakly or non-altered samples were identified to range between 4.1‰ and 7.7‰ V-SMOW, and between 10.7‰ and 15.1‰ V-SMOW, respectively. Non-altered samples have $\delta^{13}C_c$ values that range from -13.4‰ to -6.4‰ V-PDB. These results show that the different species of pareiasaurs from neighbouring localities share similar $\delta^{18}O_p$ values. Consequently, for the purposes of this study we have not differentiated between the different pareiasaur genera.

3.1. Oxygen isotopes

For both the Moordenaars and Karelskraal Members, the $\delta^{18}O_p$ median values do not show significant differences compared to other $\delta^{18}O_p$ median values from the same horizon (Wilcoxon test p-values > 0.18; Fig. 5). This pattern most probably results from the difficulty in obtaining sufficiently large sample sizes for specimens of the same taxon from the same locality. Although no significant differences are apparent, it is noteworthy that pareiasaurs and titanosuchids have the highest mean oxygen isotope ratios for both horizons, with respectively 6.8‰ and 6.7‰ for the Karelskraal Member and 6.3‰ both for the Moordenaars Member.

For the Upper Leeuvlei Member, only one specimen of each species was available for sampling, thus precluding any comparison between taxon median oxygen isotope values. The rhinesuchid amphibian has the lowest $\delta^{18}O$ value and the therocephalian *Glanosuchus* has the highest, while the dinocephalian *Anteosaurus* lies in between.

12

3.2. Carbon isotopes

Similar to the oxygen values, the $\delta^{13}C_c$ median values are not significantly different from each other (Wilcoxon test p-values > 0.06). This outcome could result from the small sample size, but we note that the highest value documented in the Karelskraal Member corresponds to a titanosuchid, whilst the lowest one corresponds to a specimen of the therocephalian *Glanosuchus*; the opposite pattern was found for the lower-middle Moordenaars Member (Fig. 6). Pareiasaurs and dinocephalians have a consistent relationship throughout all horizons, with the pareiasaurs having higher median values than all dinocephalians. The Leeuvlei Member is characterized by almost identical values for the rhinesuchid amphibian and *Glanosuchus* while *Anteosaurus* is represented by a $\delta^{18}O$ value 1‰ higher.

4. Discussion

Among all the sampled taxa, only one rhinesuchid temnospondyl individual represents the aquatic ecology. Indeed, the hypotheses for rhinesuchids being aquatic predators (Schoch and Milner, 2000) has been strongly supported by bone microstructure analysis (McHugh, 2014). Accordingly, a lower stable isotope oxygen value for the rhinesuchid relative to those for all the rest of the terrestrial taxa is expected from this individual (Fig. 5). The value difference between the rhinesuchid and *Anteosaurus* (0.79±0.68‰), or even *Glanosuchus* (1.46±0.92‰) (Fig. 5; Supplementary Table 1), is lower than those observed between semi-aquatic and terrestrial extant mammal species (around 3‰ to 7‰; Cerling et al., 2008), but within the range observed for extinct reptiles. Indeed, a study on the aquatic ecology of spinosaurids (Amiot et al., 2010) showed differences of less than 2‰ between terrestrial theropods and aquatic crocodilians and turtles.

13

4.1. *Was* Anteosaurus *a fully terrestrial therapsid?*

The arguments given by Ivakhnenko (2008, p. 969) in favour of an amphibious lifestyle for *Anteosaurus* was that it possessed a "high skull with considerably elevated nares and orbits, and thin, gracile skeleton with wide manus and poorly ossified epiphysial regions of tubular bones […] and the postcranial skeleton corresponds to the aquatic mode of life". The arguments in favour of a terrestrial lifestyle has also made reference to morphology (Kammerer, 2011, pp. 295-296): "Aquatic and semiaquatic piscivores generally have a relatively small cranium (excluding the jaws), as a heavy skull would reduce acceleration during pursuit of evasive prey (Taylor, 1992) […], the non-jaw region of the skull is massive, extremely so in the giant taxa *Titanophoneus* and *Anteosaurus*. The postcranial anatomy of anteosaurids is also not suggestive of amphibious habits. […] anteosaurids have relatively elongate limbs for dinocephalians (Boonstra, 1955)."

Considerations of taphonomy have tended towards the same conclusion "anteosaur fossils are usually found in deltaic facies (Olson, 1962; Rubidge, 1995), taphonomic evidence suggests that, at least for the South African taxa, these remains were washed in from upland habitats (Boonstra, 1969; Kitching, 1977)." (Kammerer, 2011, p. 296).

In the Karelskraal Member, *Anteosaurus* has the lowest $\delta^{18}O_p$ value (Fig. 5), which could indicate that this therapsid had a closer relationship to water than did the other sampled therapsids (Cerling et al., 2008; Amiot et al., 2010). This observation is consistent with the data from the upper Leeuvlei Member, where the $\delta^{18}O_p$ value obtained for *Anteosaurus* is intermediate between that of the therapsid *Glanosuchus* and the rhinesuchid temnospondyl. However, this pattern may be the result of a small sample size because $\delta^{18}O_p$ values for

Anteosaurus sampled from the lower-middle Moordenaars Member have a similar range to those measured in other sampled tetrapod taxa from this stratigraphic interval for which larger sample sizes are available (n≥4), i.e. pareiasaurs and titanosuchids (Fig. 5). The range of $\delta^{18}O_p$ values for *Anteosaurus* also overlaps values obtained for the tapinocephalid *Criocephalosaurus* (n=2) and *Glanosuchus* (n=1) and even includes the highest recorded value for $\delta^{18}O$ (7.5‰). Therefore, while the value from the Leeuvlei Member belongs to the same range of values from the Moordenaars Member, the very low value from the Karelskraal Member might need more careful consideration as it may not be reflective of the lifestyle of the species.

Distinguishing between a riparian or other terrestrial lifestyles for dinocephalians cannot be tested with stable oxygen isotopes. Extant riparian mammal species, such as the waterbuck *Kobus ellipsiprymnus* (Pacini and Harper, 2008), show values similar to those of other terrestrial species (Cerling et al., 2008). The "stable isotope tracer" is sensitive to water dependency and not to the habitat of the species. Therefore, based on the few sampled *Anteosaurus* from the Karoo Basin, our isotopic results tend to favour the terrestrial hypotheses, but cannot determine if the species was riparian or an in-land dweller.

4.2.   Were middle Permian South African pareiasaurs aquatic or terrestrial?

Previous published suggestions for a semi-aquatic lifestyle in pareiasaurs are even more subjective. "The mode of life of pareiasaurs was similar to that of the hippopotamus: they spent most of the time in water, occasionally coming out" (Khlyupin, 2007, p. 3) or "Ivakhnenko's (2001) believes that details of the skeleton structure of *Scutosaurus* point to its aquatic mode of life and its poor terrestrial locomotion" (Gubin et al., 2003, p. 522). Kriloff et al. (2008) interpreted *Pareiasaurus* as an aquatic animal, based on their models from bone

microanatomy, but changed to semi-aquatic on the basis of morphological characters such as "its morphology (i.e. its well-ossified skeleton, and possibly graviportal adaptations) suggests adequate terrestrial locomotor abilities, and this taxon will be considered as amphibious (rather than aquatic) for the optimizations" (Kriloff et al., 2008, p. 817). Their argument continues further with "The bones of the pareiasaur *Bunostegos* have been described as spongy and pachyostotic (Sidor et al., 2003, p. 46), which is also compatible with an amphibious or aquatic habitat" (Kriloff et al., 2008, p. 817).

The terrestrial hypothesis is supported by ichnofossils ascribed to pareiasaurs (Gubin et al., 2003; Valentini et al., 2009; Voigt et al., 2010). Other publications support the purported terrestrial lifestyle of pareiasaurs based on limb morphology such as "the numerous postcranial autapomorphies of *Bunostegos* suggest a suite of derived features related to a relatively parasagittal (i.e., nonsprawling) forelimb posture" (Turner et al., 2015, p. 12). Earlier carbonate stable oxygen isotope analysis also suggests that "pareiasaurs were terrestrial browsers and are inconsistent with the earlier semiaquatic ecomorph hypothesis proposed" (Canoville et al., 2014, p. 7).

Teeth and bones of middle Permian pareiasaurs, which were sampled from both the Moordenaars and the Karelskraal Members of the Abrahamskraal Formation, have similar $\delta^{18}O_p$ values. The range of these values, approximately 2‰, is similar to that of extant mammal species ranging from 2‰ (lion, waterbuck) to 5‰ (rhino, buffalo) (Cerling et al., 2008), a bracket which includes all values obtained for the taxa in our sample. The absence of a significant difference between the median values of the different groups (Wilcoxon test p-values > 0.18; Fig. 5) indicate that dinocephalians, therocephalians, and the pareiasaurs could have shared a similar terrestrial lifestyle.

This study of the lifestyle of pareiasaurs through a geochemical proxy, the $\delta^{18}O_p$ values from the phosphate group of the apatite, is in alignment with previously published studies that interpreted pareiasaurs as a fully terrestrial taxon. It is important to stress that this study focused only on middle Permian pareiasaurs from the Karoo Basin of South Africa, which are the oldest pareiasaurs known (Van den Brandt et al., 2019), and that different results might be obtained from analyses of later species.

5.   Conclusions

Stable oxygen and carbon isotope compositions of phosphate apatite of teeth and bones were analyzed to investigate the debated ecological lifestyle of middle Permian pareiasaurs and the dinocephalian *Anteosaurus*. Our results demonstrate that the aquatic ecology of the rhinesuchid specimen is strongly supported by its low $\delta^{18}O_p$ value, the lowest of the horizon. For *Anteosaurus*, our results favour the terrestrial hypotheses, but a single low $\delta^{18}O_p$ value questions the robustness of this interpretation. A future isotope study focusing on the ecology of *Anteosaurus* with an increased sample size may result in a more conclusive outcome. The middle Permian pareiasaurs, on the other hand, are represented by values all indicating a terrestrial way of life, which support the outcomes of previous taphonomic and geochemical studies.

Acknowledgments

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Figure captions

Figure 1: Mean oxygen isotope compositions of apatites versus drinking waters of mammals

from two Kenyan parks (Bocherens et al., 1996; Cerling et al., 2008) showing difference in

$\delta^{18}O_p$ values between semiaquatic hippopotamuses and terrestrial herbivorous mammals

(error bars are ±1σ). V-SMOW—Vienna standard mean ocean water. Phosphate-water

fractionation lines of terrestrial placental mammals, freshwater turtles, and crocodilians are

displayed for comparison. Equations from Coplen et al., (1983) and Lécuyer et al., (2010)

were used to respectively convert $\delta^{18}O_c$ (V-PDB) values from Cerling et al. (2008) into $\delta^{18}O_c$

(V-SMOW) values and $\delta^{18}O_c$ (V-SMOW) values from Bocherens et al. (1996) and Cerling et

al. (2008) to $\delta^{18}O_p$ (V-SMOW) values. Updated from Amiot et al., (2010).

Figure 2: Studied localities along with the main lithostratigraphic units of the western part of

the Main Karoo Basin bordering the Cape Fold Belt. Farm name abbreviations are B:

Buffelsvlei; DB: De Bad; DD: Die Drift; R: Rietfontein; SMK: Schoppel Maay Kraal; Sp: Spinnekopkraal; St: Stinkfontein; W: Wolwehoek.

Figure 3: Phosphate $\delta^{18}O_p$ values plotted against corresponding carbonate $\delta^{18}O_c$ values, together with the empirical isotopic equilibrium line (Iacumin et al., 1996) with a slope close to unity (bold line). The altered samples are within the circle. The reduced major axis regression lines are represented for all samples (dash line) except unaltered ones (full line).

Figure 4: Bone values ($_b$) are plotted against tooth values ($_t$) of the same individual and compared to a line with a slope of 1, for their $\delta^{18}O_p$ values (A), $\delta^{18}O_c$ values (B) and $\delta^{13}C_c$ values (C). V-PDB: Vienna PeeDee Belemnite; V-SMOW: Vienna standard mean ocean water. The circled $\delta^{18}O_c$ value corresponds to the sample 1782-B considered as altered (see text).

Figure 5: Box plot of apatite $\delta^{18}O_p$ values of each taxon plotted within their respective stratigraphic Member. V-SMOW: Vienna standard mean ocean water. A: *Anteosaurus*; C: *Criocephalosaurus*; G: *Glanosuchus*; P: Pareiasauridae; R: Rhinesuchidae; Ta: Tapinocephalidae; Ti: Titanosuchidae.

Figure 6: Box plot of apatite $\delta^{13}C_c$ values of each taxon plotted within their respective stratigraphic Member. V-PDB: Vienna PeeDee Belemnite. A: *Anteosaurus*; C: *Criocephalosaurus*; G: *Glanosuchus*; P: Pareiasauridae; R: Rhinesuchidae; Ti: Titanosuchidae.

Table 1: Mean oxygen isotope compositions of apatites of mammals from two Kenyan parks (Bocherens et al., 1996; Cerling et al., 2008). V-PDB: Vienna PeeDee Belemnite; V-SMOW: Vienna standard mean ocean water. Equations from Coplen et al., (1983) and Lécuyer et al., (2010) were used to respectively convert $\delta^{18}O_c$ (V-PDB) values into $\delta^{18}O_c$ (V-SMOW) values and $\delta^{18}O_c$ (V-SMOW) values into $\delta^{18}O_p$ (V-SMOW) values. SD correspond to $\pm 1\sigma$.

Declaration of interests

32

☒ The authors declare that they have no known competing financial interests or personal

relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be
considered as potential competing interests:

Table 1.

Mean oxygen isotope compositions of apatites of mammals from two Kenyan parks								
Common name	Species name	Tsavo National Park			Ambolesi Park			
		$\delta^{18}O_c$	$\delta^{18}O_p$	SD	$\delta^{18}O_c$	$\delta^{18}O_c$	$\delta^{18}O_p$	SD
		(‰, V-SMOW)			(‰, V-PDB)	(‰, V-SMOW)		
Terrestrial species								
Buffalo	*Syncerus caffer*	30.9	21.8	1.4	1.7	32.7	23.5	0.9
Elephant	*Loxodonta africana*	29.8	20.7	0.9	0.5	31.4	22.3	0.6
Giraffe	*Giraffa camelopardalis*				4.3	35.3	26.1	0.8
Lion	*Panthera leo*				0.7	31.6	22.5	0.5
Oryx	*Oryx beisa*				2.4	33.4	24.2	1.7
Rhinoceros	*Diceros bicornis*	29.5	20.4	1.3	0.7	31.6	22.5	1.4
Warthog	*Phacochoerus aethiopicus*	31.0	21.9	-				
Waterbuck	*Kobus ellipsiprymnus*				2.4	33.4	24.2	0.9
Wildebeest	*Connochaetes taurinus*	33.4	24.2	0.9				
Zebra	*Equus burchelli*	31.4	22.3	1.5	3.2	34.2	25.0	1.6
Semi-aquatic species								
Hippopotamus	*Hippopotamus amphibius*	26.9	17.9	1.6	-2.7	28.1	19.1	0.8

V-PDB: Vienna PeeDee Belemnite.

V-SMOW: Vienna standard mean ocean water.

Equations from Coplen et al., (1983) was used to convert δ18Oc (V-PDB) values into δ18Oc (V-SMOW) values.

Equations from Lécuyer et al., (2010) was used to convert δ18Oc (V-SMOW) values into δ18Op (V-SMOW) values.

SD correspond to ±1σ.

33

34

Highlights:

- First application of $\delta^{18}O_p$ of apatite to South African tetrapods for paleoecology.

- The lifestyle of middle Permian pareiasaurs is strongly shown to be terrestrial.

- The carnivorous dinocephalian *Anteosaurus* was probably living a terrestrial ecology.
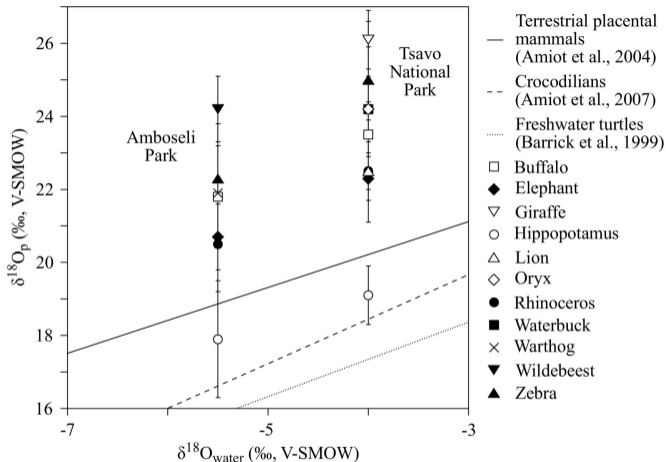
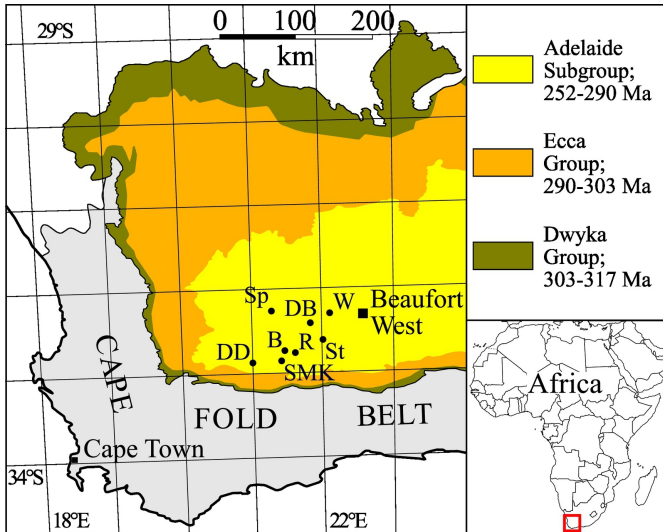- Knowing the ecology of major species helps understand the nature of the extinction.
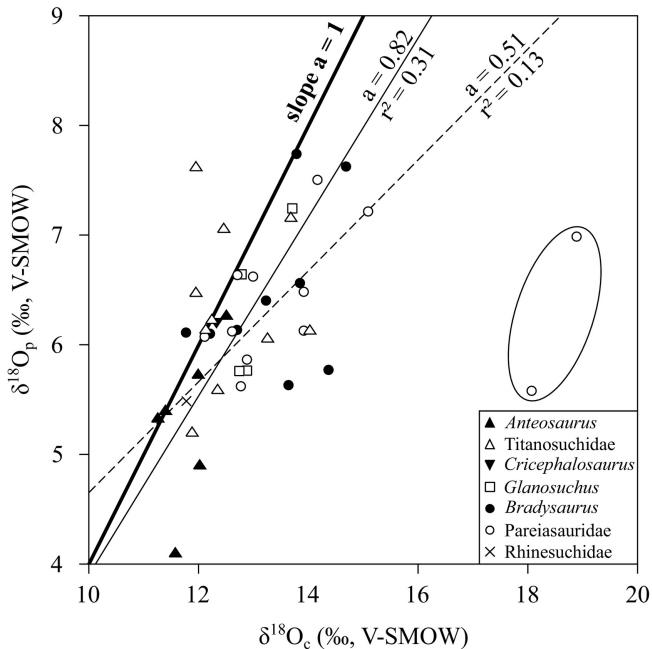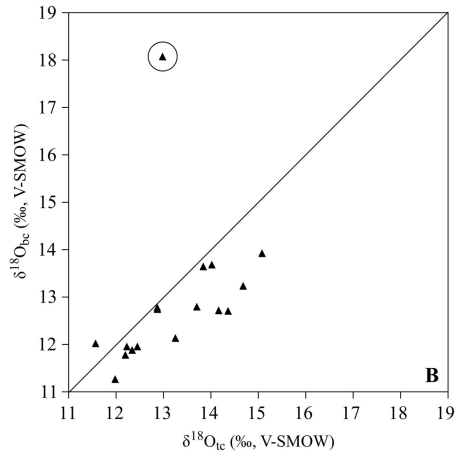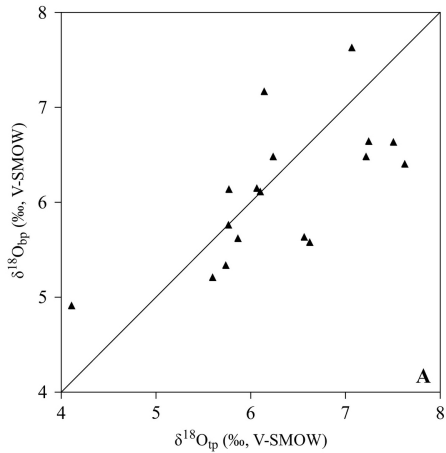
Figure 1

Figure 2

Figure 3
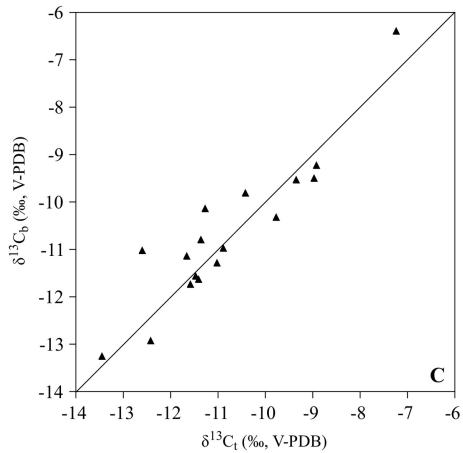
Figure 4

Figure 5

Figure 6