

The sixth sense in mammalian forerunners: Variability of the parietal foramen and the evolution of the pineal eye in South African Permo-Triassic eutheriodont therapsids

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In some extant ectotherms, the third eye (or pineal eye) is a photosensitive organ located in the parietal foramen on the midline of the skull roof. The pineal eye sends information regarding exposure to sunlight to the pineal complex, a region of the brain devoted to the regulation of body temperature, reproductive synchrony, and biological rhythms. The parietal foramen is absent in mammals but present in most of the closest extinct relatives of mammals, the Therapsida. A broad ranging survey of the occurrence and size of the parietal foramen in different South African therapsid taxa demonstrates that through time the parietal foramen tends, in a convergent manner, to become smaller and is absent more frequently in euterocephalians (Akidnognathiidae, Whaitsiidae, and Baurioidea) and non-mammaliaform eucynodonts. Among the latter, the Probainognathia, the lineage leading to mammaliaforms, are the only one to achieve the complete loss of the parietal foramen. These results suggest a gradual and convergent loss of the photoreceptive function of the pineal organ and degeneration of the third eye. Given the role of the pineal organ to achieve fine-tuned thermoregulation in ectotherms (i.e., “cold-blooded” vertebrates), the gradual loss of the parietal foramen through time in the Karoo stratigraphic succession may be correlated with the transition from a mesothermic metabolism to a high metabolic rate (endothermy) in mammalian ancestry. The appearance in the eye of melanopsin-containing retinal ganglion cells replacing the photoreceptive role of the pineal eye could also have accompanied its loss.

Key words: Therapsida, pineal, parietal foramen, third eye, endothermy, Permian, Triassic, South Africa.

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Introduction

The third eye, or pineal eye, of tetrapods is a photosensitive organ that transmits variations of daylight length and brightness to the pineal gland, which in turn secretes melatonin (Stebbins and Eakin 1958; Hutchison and Kosh 1974; Quay 1979; Ralph et al. 1979). As a detector of diurnal and seasonal changes in daylight it plays an important role in regulating biological cycles related to sleep, reproduction, and body temperature (Quay 1979; Reiter 1981). The pineal eye was once a paired organ (Edinger 1955; Eakin 1973), and it is today similar to the lateral eyes in structure, ontoge-

netic development, and in the genetic pathway that controls its neurogenesis and development which strongly suggests that the pineal eye and the lateral eyes share a common genetic and embryologic basis (Quay 1979; Mano and Fukuda 2007). The evolution of the parietal foramen in extinct vertebrate species has long raised the interest of paleontologists and neurologists (Osborn 1887; Broom 1913; Eakin 1973), especially as it bears testimony to the evolution of the pineal organ, and more specifically, the pineal eye (Edinger 1955).

The pineal eye is absent in most extant vertebrates except in the lamprey, ranid frogs, the tuatara, and some lizards (Eakin 1973). However this structure was widespread in Paleozoic

and early Mesozoic taxa (e.g., stegocephales, parareptiles, and therapsids amongst others) (Edinger 1955; Quay 1979). It is well established that the parietal foramen was lost amongst derived theriodonts in the course of mammalian evolution. This absence remains a distinctive trait of mammaliaforms (Quay 1979; Rubidge and Sidor 2001), but details about this evolutionary transition are poorly known. Amongst non-mammaliaform Therapsida, it has been suggested that the parietal foramen displays great variability (Edinger 1955; Quay 1979; Roth et al. 1986; Abdala et al. 2006; Benoit et al. 2015). It can be very large, twice the size of the foramen magnum in some dinocephalians and dicynodonts, and may be absent in some dicynodonts, therocephalians, and non-mammaliaform cynodonts (Edinger 1955; Quay 1979; Roth et al. 1986; Abdala et al. 2006; Benoit et al. 2015). As such, variability in occurrence and size could have predated the complete disappearance of this foramen during premammalian evolution. As the pineal organ plays an essential role in thermoregulation and reproductive synchrony (Eakin 1973; Ralph et al. 1979; Quay 1979), one could expect that the loss of the parietal foramen, and presumably that of the pineal eye, would have had dramatic implications for the biology, physiology, and behaviour of the ancestors of mammals.

A remarkably rich fossil record of therapsids from the time expansive Karoo sedimentary succession of South Africa chronicles the emergence of the mammalian phenotype in remarkable detail (Rubidge and Sidor 2001) and provides an opportunity to document the loss of the parietal foramen/pineal eye amongst the ancestors of mammals. Our study describes the occurrence of the parietal foramen in different non-mammaliaform therapsid taxa and traces the distribution of this structure across geological periods within the phylogeny of therapsids. Special attention is drawn to non-mammaliaform Eutheriodontia (Cynodontia and Therocephalia) as they are the closest relatives of Mammaliaforms (Rubidge and Sidor 2001; Kemp 2005, 2012). This has brought new insight into the timing, causes, and biological consequences of this important morphophysiological evolutionary transition in the ancestry of mammals.

Institutional abbreviations.—AM, Albany Museum, Grahamstown, South Africa; BP, Bernard Price Evolutionary Studies Institute, Johannesburg, South Africa; RC, Rubidge Collection, Graaff Reinet, South Africa; SAM-PK, Iziko South African Museum of Natural History, Cape Town, South Africa.

Other abbreviations.—DFM, foramen magnum diameter; MD, mean width of parietal foramen; OFM, relative size of the bony orbit; RPF, relative size of the parietal foramen.

Material and methods

For this study the parietal foramen, foramen magnum and, when possible, and orbits of 663 therapsid skulls

were measured using a caliper, and the presence/absence of the parietal foramen was noted in 879 specimens from the extensive Karoo fossil collections of the Evolutionary Studies Institute (Johannesburg, South Africa), Ditsong (ex Transvaal) Museum (Pretoria, South Africa), Iziko South African Museum of Natural History (Cape Town, South Africa), Rubidge Collection (Graaff-Reinet, South Africa), Council for Geoscience (Pretoria, South Africa), Albany Museum (Grahamstown, South Africa), and National Museum (Bloemfontein, South Africa) (see SOM 1, Supplementary Online Material available at http://app.pan.pl/SOM/app61-Benoit_etal_SOM.pdf). The skulls studied represent four main lineages of non-mammaliaform therapsids (Gorgonopsia, Dicynodontia, Therocephalia, and Cynodontia). All specimens studied are from the Karoo Supergroup of South Africa, which was positioned at high latitude, 60–85° S in the late Permian; at approximately 60° S in the Early Triassic; 50–60° S in the Middle Triassic; and around 50° S in the Late Triassic (Zharkov and Chumakov 2001; Abdala and Ribeiro 2010). This is highly relevant since it has been demonstrated that in extant ectotherms, species living near the equator tend to lose their pineal eye because the lack of contrast between seasons makes the pineal eye selectively neutral (Gundy et al. 1975; Ralph 1975).

The size of the parietal foramen was measured as shown in Fig. 1. The parietal foramen is almost never perfectly round, and is often oval or slit-like. In these cases, using the average diameter (the mean of length [PL] and width [MD]), the radius, or the area of this foramen to infer the evolution of the pineal eye would be biologically meaningless (Quay 1979) because the maximum diameter of the pineal eye (if present) would have been limited by the width of the parietal foramen (Fig. 1). Accordingly we used the width of the parietal foramen (MD) as a proxy for the diameter of the pineal eye. When a specimen was too distorted, or its cranial vault was too weathered to permit a reliable measurement of the parietal foramen, only the presence or absence of the foramen was recorded. Specimens too poorly preserved or with the region of the parietal foramen crushed or eroded so that it was impossible to ascertain if the presence/absence of a visible foramen was genuine or due to the quality of preservation, such as *Kombuisia frerensis* (BP/1/430; Fröbisch et al. 2010) or *Lumkuia fuzzi* (BP/1/2669; Hopson and Kitching 2001), were excluded from the analysis.

Given the wide range of body sizes of the species sampled, for example between the gigantic *Kannemeyeria* (basal skull length 20–50 cm) and the tiny *Pachygenelus* (basal skull length 4–5 cm), we did not directly compare the MD between species. Instead, we followed Edinger (1955) and Quay (1979), and we used the ratio of the MD over the DFM (diameter of the foramen magnum, calculated as the mean of its width [FMW] and height [FMH]) for comparison between sampled species. This results in a relative size of the parietal foramen: $RPF = MD/DFM$. We preferred the DFM over direct body size estimation because it has been

demonstrated that the size of the foramen magnum is well correlated with body mass in mammals (Radinsky 1967, 1976; note that we did not use the area of the foramen magnum, but its average diameter to remain consistent with the measurement of MD; Fig. 1). Moreover, reliable estimation of body mass requires a complete skeleton which is difficult to obtain in fossil species belonging to long extinct groups (e.g., Jerison 1973; Quiroga 1984). In contrast only the braincase of a skull, even partial, is required to obtain the DFM and thus, a reasonable approximation of the body size of a given specimen (Edinger 1955; Radinsky 1967, 1976). The relative size of the bony orbit (OFM) is calculated as the quotient between the average diameter of the orbit and the DFM (Fig. 1). All data are available in the SOM 1.

The presence of a parietal foramen has long been, and is still often, used as a diagnostic character of genera and species of therapsids (e.g., Broom 1913; Camp and Welles 1956; Bonaparte 1966; Tatarinov 1968; Cox 1972; Rubidge and Sidor 2001; Kemp 2005; Abdala 2007; Fröbisch et al. 2010; Sigurdson et al. 2012; Abdala et al. 2014; Liu and Abdala 2014). Genera and/or species are sometimes partly diagnosed by the presence (e.g., *Microgomphodon oligocynus* [Abdala et al. 2014]) or absence (e.g., *Kombuisia frerensis* [Fröbisch et al. 2010]) of a parietal foramen. Hence, highlighting its variability could have important consequences for taxonomy. Moreover, lower taxonomic levels tend to minimize variability and this study was thus conducted at higher ranks. Detailed phylogenies of most therapsid groups have recently proliferated in the literature, but they are often conflicting. For example, there are discussions about the monophyly of Therocephalia (Abdala 2007 and Botha et al. 2007 against Huttenlocker 2009 and Sigurdson et al. 2012), or in the recovery of two main groups of Cynodontia, Probainognathia, and Cynognathia, against a pectined cladogram without these groups or with these groups forming a basal polytomy in eucynodonts (Hopson and Kitching 2001 and Liu and Olsen 2010 against Rowe 1993; Martinez et al. 1996; and Martinelli et al. 2005). Given these discrepancies we restricted the phylogenetic framework of this study to the most recent and best sampled (i.e., including more characters) phylogenetic hypotheses. We follow Liu and Olsen (2010) and Ruta et al. (2014) for cynodonts. Two major groups are recognized in these phylogenies: Cynognathia, including *Cynognathus* and gomphodont cynodonts (Diademodontidae, Trirachodontidae, Traversodontidae); and Probainognathia, including *Lumkuia*, Chiniquodontidae, Probainognathidae, Tritylodontidae, Tritheledontidae, and Brasilodontidae. Non-mammaliaform cynodonts basal to these clades, i.e., *Charassognathus*, *Procynosuchus*, *Cynosauros*, *Progalesaurus*, *Galesaurus*, *Thrinaxodon*, *Platycraniellus*, are here grouped into the paraphyletic basal Cynodontia (SOM 1). We used Huttenlocker (2009), and Sigurdson et al. (2012) for Therocephalia. Lycosuchidae and Scylacosauridae are here grouped together in the paraphyletic basal Therocephalia and more derived families are nested together in an unresolved polytomy inside the

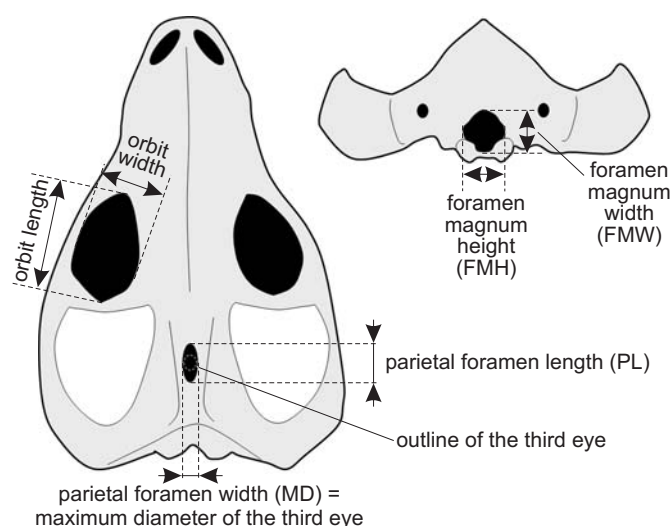


Fig. 1. Measurement protocol of foramen magnum, parietal foramen, and orbit in Therapsida.

clade Eutheriocephalia. Rubidge and Sidor (2001) are followed for the phylogenetic position of the main therapsid lineages. The taxonomic terms Therapsida, Theriodonta, Eutheriodontia, Cynodontia, Epicynodontia, Eucynodontia, and Probainognathia are not used here in their cladistic sense and thus exclude mammaliaforms unless explicitly stated. Biostratigraphy and dating follow Rubidge (2005) and Rubidge et al. (2013). Statistical tests were performed using PAST v1.01 (Hammer et al. 2001). Taxa without accurate stratigraphic records were not considered in the analysis of the evolution of the parietal foramen through time.

Results

Despite the fact that the loss of the parietal foramen is usually considered to be a synapomorphy of Probainognathia including Mammaliaformes (e.g., Rubidge and Sidor 2001; Macrini et al. 2007), the data analysed here shows that, at high taxonomic level, the presence of the parietal foramen becomes increasingly variable across therapsid phylogeny (Fig. 2). With the exception of Gorgonopsia (parietal foramen present in all 132 samples), all examined orders of therapsids comprise specimens with an absent parietal foramen (SOM 1). It is present in 97% of the Dicyodontia ($n = 440/454$), 85% of the Therocephalia ($n = 112/131$), and 80% of the Cynodontia ($n = 131/162$), whereas in Mammalia it is only pathologically present (Edinger 1933; Ferguson 2010; Labra et al. 2010).

Among the main lineages of eucynodonts, the Probainognathia display no parietal foramen (0%, $n = 21$). This condition is also represented in members of the group out of South Africa, such as *Brasilitherium* (Rodrigues et al. 2014), *Ecteninion* (www.Digimorph.org available from the University of Texas Digital Morphology Group), *Chiniquodon* (Kemp 2009), *Probainognathus* and *Therioherpeton* (Quiroga 1984), which do not show any trace of the parietal foramen or

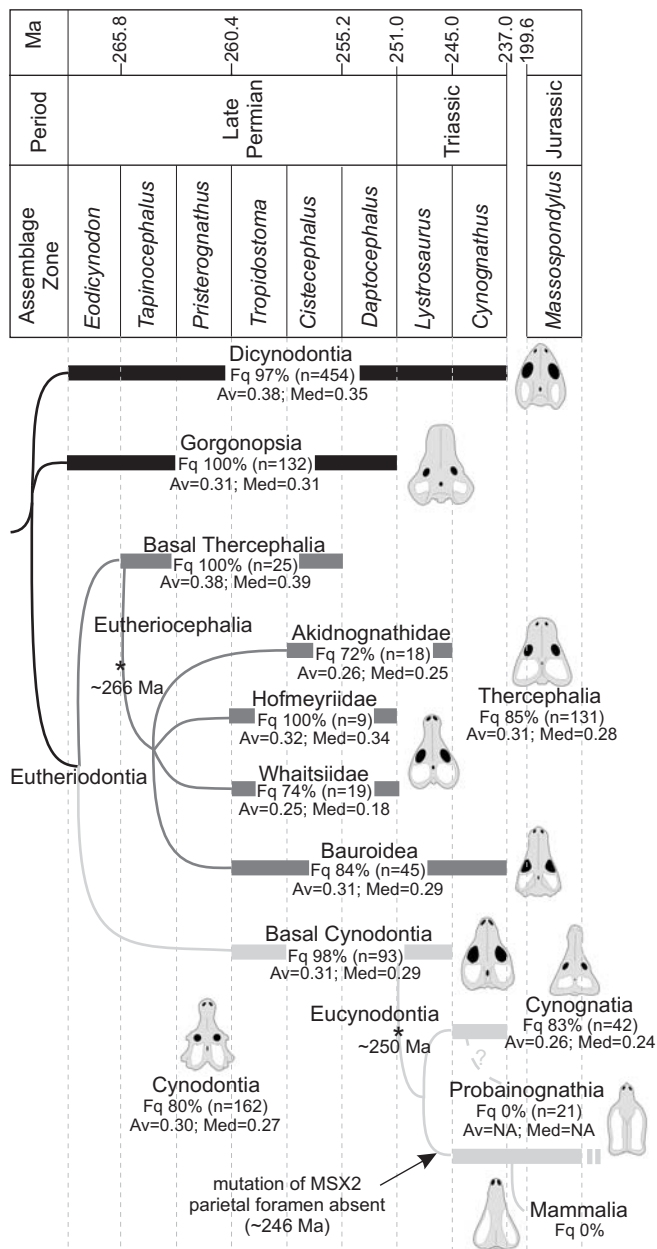


Fig. 2. Evolution of the frequency of the presence of the parietal foramen (Fq), and average (Av) and median (Med) size of the parietal foramen across the phylogeny of Therapsida. Number of specimens examined (n) is indicated for each group. Asterisks indicate the branches of the tree where a relaxation of constraints resulting from a functionless third eye is hypothesized (zone of variability). See material and methods section for the more details about the phylogenetic tree.

pineal organ on their brain-case (note that Quiroga 1984 and Kemp 2009 misinterpret a space for the cartilaginous portion of the supraoccipital bone and/or for a venous sinus, as the cast of the pineal gland; see Hopson 1979; Kielan-Jaworowska and Lancaster 2004). Amongst Cynognathia a parietal foramen is present in 83% of cases (n = 35/42). In contrast to what is observed in probainognathians, most basal cynodonts do have a parietal foramen (98% present, n = 93/95). Similarly, the parietal foramen is consistently present in the basal therocephalians Lycosuchidae and Scylacosauridae (100%,

n = 25/25) and in the Hofmeyriidae (100%, n = 9/9). This percentage decreases in the more derived Whaitsiidae (74%, n = 14/19), Baurioidea (84%, n = 38/45) and the Akidnognathidae (72%, n = 13/18).

The relative size of the RPF decreases with the decreasing frequency of the parietal foramen. In Eutheriocephalia (Hofmeyriidae, Whaitsiidae, Baurioidea, and Akidnognathidae) the average RPF is smaller than in basal Therocephalia (Wilcoxon test, $p = 0.035$), and in Eucynodontia it also decreases significantly with respect to the condition seen in basal Cynodontia (Wilcoxon test, $p = 0.0003$). This is notable in akidnognathid and whaitsiid therocephalians, and in cynognathian cynodonts, in which the average ratio falls below 0.30 (Fig. 2).

In stratigraphic succession (Fig. 3), the RPF is significantly higher in therocephalians which occurred before the beginning of the *Daptocephalus* Assemblage Zone (265.8–255.2 Ma) than later in the sequence (255.2–237.0 Ma; Viglietti et al. 2016). This means that the size of the parietal foramen in therocephalians decreases at the end of the late Permian (Wilcoxon test, $p = 0.001$). Stratigraphically upwards from the *Daptocephalus* Assemblage Zone to the extinction of therocephalians at the end of the *Cynognathus* Assemblage Zone, the average of this ratio remains below 0.30 and is accompanied by a decrease in the frequency of occurrence of the parietal foramen to around 70%. In Cynodontia the average RPF approximates 0.40 for the entire Permian, and decreases below 0.30 at the beginning of the *Lystrosaurus* Assemblage Zone (251.0 Ma). In the Early Triassic (Wilcoxon test, $p = 0.008$) it continues decreasing to 0.26 in the *Cynognathus* Assemblage Zone (~240 Ma), but this difference is statistically not significant (Wilcoxon test, $p = 0.247$). The size of the parietal foramen thus decreases significantly at the end of the Permian only in Cynodontia. In the same manner, the presence of the foramen stays above 80% for cynodonts until the *Cynognathus* Assemblage Zone (~240 Ma). It then drops to 0% in the Jurassic (199.6 Ma) (Fig. 3). The decrease in size and frequency of the parietal foramen in therocephalians and cynodonts does not occur simultaneously in the stratigraphic succession.

Based on the DFM (Table 1), dicynodont body size seems to be a little smaller in genera where the parietal foramen is absent (average DFM, 11.31; median DFM, 9.69) compared to those where it is present (average DFM, 13.02; median DFM, 10.34). In contrast, in therocephalians, when the parietal foramen is absent, the average DFM is 10.68 (median DFM, 9.95), whereas when it is present, the average DFM is 8.70 (median DFM, 7.90). However, sample size for the “absent” category is too small in both cases for statistical tests. In cynodonts, when the parietal foramen is absent, the average DFM is 7.41 (median DFM, 7.61), whereas when it is present, the average DFM is 7.28 (median DFM, 6.86). This difference is not significant (Wilcoxon test, $p = 0.068$), which means body size can be considered the same whether or not the parietal foramen is present in Cynodontia (this conclusion might be extended to Dicynodontia and Therocephalia).

Period	Assemblage Zone	Gorgonopsia	Dicynodontia	Therocephalia	Cynodontia		
Ma	Jurassic	<i>Massospondylus</i>	NA	NA	NA		
						Fq 0% (n = 21) Av = NA; Med = NA	
199.6							
237.0	Triassic	Middle	<i>Cynognathus</i>	NA	Fq 93% (n = 15) Av = 0.58; Med = 0.56	Fq 60% (n = 5) Av = 0.28; Med = 0.19	Fq 81% (n = 34) Av = 0.26; Med = 0.24
245.0							
251.0							
255.2	Late Permian	<i>Daptocephalus</i>	Fq 100% (n = 25) Av = 0.28; Med = 0.28	Fq 96% (n = 75) Av = 0.43; Med = 0.38	Fq 70% (n = 30) Av = 0.24; Med = 0.20	Fq 90% (n = 21) Av = 0.38; Med = 0.35	
							<i>Cistecephalus</i>
260.4		<i>Tropidostoma</i>	Fq 100% (n = 6) Av = 0.32; Med = 0.32	Fq 98% (n = 46) Av = 0.33; Med = 0.33	Fq 100% (n = 6) Av = 0.40; Med = 0.39	Fq 100% (n = 2) Av = NA; Med = NA	
							<i>Priesterognathus</i>
265.8		<i>Tapinocephalus</i>	NA	Fq 100% (n = 20) Av = 0.31; Med = 0.33	Fq 100% (n = 15) Av = 0.44; Med = 0.44	NA	
							<i>Eodicynodon</i>



Fig. 3. Evolution of the frequency of the presence of the parietal foramen (Fq), and average (Av) and median (Med) size of the parietal foramen across geological times. Number of specimens examined (n) is indicated for each group. NA, no data available. Biostratigraphy (Assemblage Zones) and dates after Rubidge (2005) and Rubidge et al. (2013).

The absolute size of the parietal foramen is positively, but weakly, correlated to the DFM in Cynodontia ($R^2 = 0.421$), Therocephalia ($R^2 = 0.426$), Dicynodontia ($R^2 = 0.618$), and Gorgonopsia ($R^2 = 0.567$).

The OFM averages 2.76 (median, 2.63) in Dicynodontia, 2.73 (median, 2.63) in Gorgonopsia, 2.66 (median, 2.53) in Therocephalia, and 2.44 (median, 2.28) in Cynodontia. Amongst cynodonts, OFM is 2.17 (median, 2.27) in basal Cynodontia, 2.84 (median, 2.46) in Cynognathia, and 1.80 (median, 1.75) in Probainognathia (in this group the size of the orbit is an estimation given the lack of post-orbital bar in most species) (Table 1). When corrected for body size, there is no strong correlation between the relative width of the parietal foramen (RPF) and OFM in any lineage: dicynodonts ($R^2 = 0.25$), gorgonopsians ($R^2 = 0.08$), therocephalians ($R^2 = 0.05$), and cynodonts ($R^2 = 0.21$), or in therapsids in general ($R^2 = 0.17$).

The OFM is relatively smaller in dicynodonts that lack a parietal foramen (average OFM, 2.67; median OFM, 2.32), than in those that display a foramen (average OFM, 2.77; median OFM, 2.67). The same is true in therocephalians,

in which the average OFM equals 2.71 (median OFM, 2.60) when the parietal foramen is present, while the average OFM equals 2.14 (median OFM, 2.28) when the foramen is absent. Unfortunately, again, sample size for the “absent” category is too small in both cases for statistical tests. In cynodonts, the average OFM equals 2.49 (median OFM, 2.29) when the parietal foramen is present, while the average OFM equals 2.26 (median OFM, 2.01). Statistically, this difference is not significant (Wilcoxon test, $p = 0.215$), which suggests that the relative size of the orbit is similar in both conditions.

Discussion

Our results suggest that the loss of the parietal foramen was not a discrete event in the evolution of therapsids, but was a gradual transformation through a decrease in size and an increase in the frequency of its absence. With the noticeable exception of the Gorgonopsia, all therapsid groups exam-

ined display a certain degree of variability in the presence of the parietal foramen. In Dicynodontia, the parietal foramen is nearly always present, with a frequency averaging 97% across time. Dicynodonts display the widest range of variation in the size of the foramen (Fig. 4), from absent to slit-like, as in e.g., *Dinanomodon* (Roth et al. 1986), to twice the size of the foramen magnum in some *Lystrosaurus* specimens (SOM 1). Moreover, while in gorgonopsians the average value of the RPF equals that of the median; in dicynodonts, therocephalians, and cynodonts the median tends to be smaller than the average which implies that the parietal foramen is more often smaller than the average in these groups. This suggests that, as in therocephalians and cynodonts, the parietal foramen is more variable in size in dicynodonts than in gorgonopsians. One would expect that, given that gorgonopsians are commonly placed as the sister taxon to therocephalians and cynodonts in the clade Theriodontia (Rubidge and Sidor 2001; Kemp 2005, 2012), the gorgonopsians would share variability in both size and frequency of presence of the parietal foramen with eutheriodonts rather than the dicynodonts. However, some competing phylogenetic hypotheses (e.g., Laurin and Reisz 1990) suggest that dicynodonts may be the closest relatives of Eutheriodontia, excluding gorgonopsians from the Theriodontia.

Among the best sampled dicynodont genera ($n > 20$), variability of the presence of the parietal foramen is observed in a wide diversity of dicynodonts, in the lystrosaurid Dicynodontoidea *Lystrosaurus* (foramen absent in 4%, 2/46 specimens), in the cistecephalid Emydopidae *Cistecephalus* (foramen absent in 7%, 4/60 specimens), in the oudenodontid *Oudenodon* (foramen absent in 4%, 2/56 specimens), and the Pylaecephalid *Diictodon* (foramen absent in 2%, 1/42 specimens). In contrast, the foramen is invariably present in the eumantelliid *Pristerodon* ($n = 21$). It is noteworthy that *Cistecephalus*, a genus renowned for its morphological adaptations to fossoriality (Nasterlack et al. 2012; Laaß and Schillinger 2015), has a more frequently absent parietal foramen. Since adaptation to a subterranean habit may result in the regression of the pineal organ (Gundy and Ralph 1971), this would concur with a fossorial lifestyle, a possibility supported by the fact that *Kawingasaurus*, a highly spe-

cialized fossorial cistecephalid from Tanzania, also displays no parietal foramen (Cox 1972). However this hypothesis requires further testing because the pineal eye is noticeably retained among most extant lizards with a burrowing life-style (Gundy and Wurst 1976).

The presence of the foramen decreases to 85% of the therocephalian specimens and 80% of the cynodonts (Fig. 2). A closer view reveals that basal therocephalians (*Scylacosauridae* and *Lycosuchidae*) and basal cynodonts (i.e., *Charassognathus*, *Procynosuchus*, *Cynosaurus*, *Progalesaurus*, *Galesaurus*, *Thrinaxodon*, and *Platicraniellus*) share a high frequency of presence of the parietal foramen (100% and 98%, respectively) (Fig. 2). This implies that the last common ancestor of these two groups may have had a parietal foramen. The presence of this opening then becomes increasingly variable in Eutheriocephalia and Eucynodontia. This parallel evolution is also evidenced upwards in the stratigraphic succession (Fig. 3) and is accompanied by a significant decrease in the average size of the parietal foramen in akidnognathid and whaitsiid therocephalians, and in cynognathian cynodonts (Fig. 2).

With the occasional closure of the parietal foramen, the pineal organ would have lost its photoreceptive function and began to evolve a purely neuroendocrine role, as in extant mammals (Ekström and Meissl 2003). This would have occurred some 250 Ma in eucynodonts and around 266 Ma in eutheriocephalians (based on divergence age after Ruta et al. 2014 and Huttenlocker and Botha-Brink 2014, respectively) (Fig. 3). Parallel evolutionary trends in these two groups have been recorded for many other characters as well, the most notable being the convergent development of a secondary bony palate (e.g., Rubidge and Sidor 2001). Contrary to probainognathian cynodonts (including mammals), no therocephalian group achieves the complete loss of the parietal foramen. Among eutheriocephalians, the Hofmeyriidae is the only family in which all specimens display a parietal foramen which would support a basal position in the eutheriocephalians tree, as proposed by Abdala (2007). However, the phylogeny of Huttenlocker (2009) supports a more derived position for Hofmeyriidae, as the sister group of Whaitsiidae, which would imply more evolutionary steps.

Table 1. Measurements (in mm) of the diameter of the foramen magnum and the relative size of the orbit in Therapsida. Average (Av) and median (Med) values are given for all representatives of a group (All), and for those with a parietal foramen (Present) and without (Absent). NA, no data available.

	Diameter of the foramen magnum			Relative size of the orbit		
	All	Present	Absent	All	Present	Absent
	Av / Med	Av / Med	Av / Med	Av / Med	Av / Med	Av / Med
Dicynodontia	12.97 / 10.33	13.02 / 10.34	11.31 / 9.69	2.76 / 2.63	2.77 / 2.67	2.67 / 2.32
Gorgonopsia	12.37 / 11.67	12.37 / 11.67	NA	2.73 / 2.63	2.73 / 2.63	NA
Therocephalia	9.08 / 8.16	8.7 / 7.9	10.68 / 9.95	2.66 / 2.53	2.71 / 2.6	2.14 / 2.28
Cynodontia	7.31 / 6.99	7.28 / 6.86	7.41 / 7.61	2.44 / 2.28	2.49 / 2.29	2.26 / 2.01
Basal Cynodontia	6.84 / 7.01	6.68 / 6.94	NA	2.17 / 2.27	2.15 / 2.15	NA
Cynognathia	9.16 / 7.3	9.52 / 7.22	2.76 / 7.38	2.84 / 2.46	2.85 / 2.46	2.8 / 2.4
Probainognathia	7.18 / 7.61	NA	7.18 / 7.61	1.8 / 1.75	NA	1.8 / 1.75

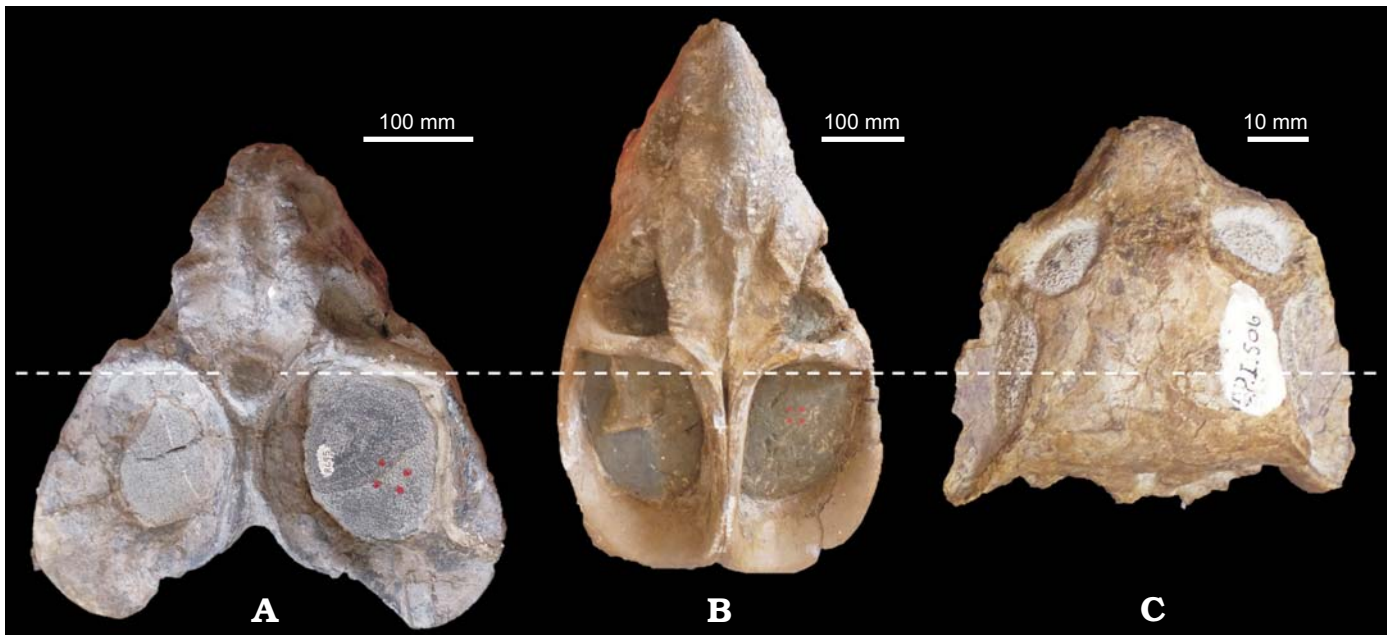


Fig. 4. Skulls of Dicyodontia illustrating the great variability of the shape and size of parietal foramen in Therapsida. **A.** Large and circular parietal foramen, with a pineal boss in *Rachiocephalus*, RC 95, Deredrif, Adendorp, South Africa, *Cistecephalus–Tropidostoma* AZ, 260–255 Ma. **B.** Slit-like parietal foramen in *Dinanomodon*, RC09, Stylkrans, South Africa, *Cistecephalus* AZ, 250–255 Ma. **C.** Absent foramen in *Cistecephalus*, BPI/1/506, Towerwater, Murraysburg, South Africa, *Cistecephalus* AZ, 250–255 Ma.

Among cynodonts, the parietal foramen is smaller and its presence is more variable in cynognathians (present in 83% of specimens) than in basal cynodonts (present in 98% of specimens) (Fig. 2). In Cynognathia, the average size of the parietal foramen is significantly smaller than in basal cynodonts. The foramen disappears completely in the branch leading to probainognathians and mammals some 245 Ma (based on the date of origin of Probainognathia after Ruta et al. 2014) (Fig. 2). Following the most recent phylogenetic hypothesis developed in the literature, which excludes traversodontid cynognathians from the ancestry of the Probainognathia (Hopson and Kitching 2001; Liu and Olsen 2010; Ruta et al. 2014), it seems that a certain degree of convergence also exists between cynognathian and probainognathian cynodonts. Published data record that late Triassic traversodontids do not have a parietal foramen (Liu and Olsen 2010; Liu and Abdala 2014) as manifested in *Exaeretodon* (Bonaparte 1966; Rodrigues et al. 2014). In *Massetognathus pascuali* the parietal foramen is present in juveniles but closes during ontogenetic development (Abdala and Giannini 2000). In the closely related species *Dadadon isaloi*, the presence of a parietal foramen has been noted in undescribed juvenile specimens while it is consistently absent in adults (Ranivoharimanana et al. 2011; Kammerer et al. 2012). Thus, in both eucynodont main lineages there is a parallel trend in the loss of the parietal foramen toward the Late Triassic. However, based on the hypothesis that cynognathians may be part of the direct ancestry of probainognathians, including mammals, the loss of the parietal foramen would constitute a synapomorphy of the clade unifying probainognathians and traversodontids (Rowe 1993; Martinez et al.

1996; Martinelli et al. 2005). In this case, the decrease in frequency of the parietal foramen in basal cynognathians would constitute an intermediate state (Fig. 2).

Amongst the examined cynodont genera, the absence of the parietal foramen is randomly distributed between specimens of various sizes (see *Cynosaurus*, *Cynognathus*, and *Diademodon* in SOM 1), weakening the hypothesis of an ontogenetic influence on the variability of the parietal foramen in the taxa we sampled. In *Cynosaurus*, the parietal foramen is absent in the largest specimen examined (SOM 1), but a larger specimen is known (SAM-PK-4333) which displays traces of a parietal tube (Benoit et al. 2015). In addition MD does not correlate well with DFM in the best sampled cynodonts some of which do not have a foramen, i.e., *Diademodon* ($R^2 = 0.006$, the foramen is absent in six specimens of various sizes) and *Cynognathus* ($R^2 = 0.114$, the foramen is absent in the smallest specimen sampled), which suggests that the size of the parietal foramen does not decrease with growing body size. Since most of the specimens without a foramen are large adults, a pattern of ontogenetic closure of the parietal foramen similar to that in *Massetognathus pascuali* has been hypothesized for the therocephalian *Theriognathus microps* (Huttenlocker and Abdala 2015), but we document the absence of a parietal foramen in both large and small specimens of *Theriognathus* (SOM 1). Although three of the six specimens which lack a parietal foramen are among the largest skulls sampled, there appears to be no correlation between the MD and DFM in this genus ($R^2 = 0.229$). As a consequence, there does not appear to be a correlation between the absence or the size of a parietal foramen and body size in the taxa examined here.

This contrasts with observations in *Massetognathus* and, perhaps, *Dadadon*.

Interspecific variability of the parietal foramen in therapsids has been emphasized in the past (Edinger 1955; Hopson 1979; Roth and Roth 1980; Roth et al. 1986) and recent reports of variation in the size and shape or the presence or absence of a parietal foramen within species has shown that a large amount of variability exists among therapsids. In cynodont lineages that usually have a parietal foramen, the abnormal absence of this feature has been reported in the holotype of *Trirachodon berryi* AM461 (Abdala et al. 2006; but note that CT-scanning revealed that a conspicuous parietal foramen is indeed present on this specimen; Fig. 5), one specimen of an adult *Cynosaurus suppostus* (Benoit et al. 2015; Fig. 5), and in the largest specimens of *Massetognathus pascuali* (Abdala and Giannini 2000). In contrast, Ranivoharimanana et al. (2011) mentioned the presence of a parietal foramen in some undescribed juveniles of *Dadadon isaloi*, whereas this species reportedly does not have one (Kammerer et al. 2012). Amongst Therocephalia, the absence of a parietal foramen has been reported in some specimens of *Theriognathus microps* (Huttenlocker and Abdala 2015) (Fig. 5). In contrast, the presence of a parietal opening identified as a “fontanelle” has been reported in some specimens of *Tetracynodon darti*, a species which reportedly does not have a parietal opening (Sigurdson et al. 2012: 1118; Fig. 5).

Here, for the first time, we provide insight into both the intra- and interspecific variability of the parietal foramen in non-mammaliaform therapsids. Even species considered to have a parietal foramen, such as *Diademodon tetragonus*, displays no foramen in some specimens (Fig. 5), whereas species without a parietal foramen, such as *Bauria cynops*, do have a small one in some specimens (Fig. 5; SOM 1). Our analysis shows that these variations are not randomly distributed, but they are structured through time and phylogeny (Figs. 2, 3). In particular our data shows a gradual and convergent trend toward a less frequent presence of the parietal foramen and the size reduction of this opening in eucynodonts and eutheriocephalians. In addition, many representatives of these groups display a very small (SOM 1), often slit-like foramen (e.g., *Bauria*; Fig. 5) which implies that if a pineal eye was present, it might have not been fully functional. Such a condition might be considered closely related to the persistence of a small fronto-parietal fontanelle in the adult, since ossification of the parietal foramen is part of the ossification of the fronto-parietal fontanelle in extant reptiles (Quay 1979; Roth et al. 1986). Such variability of presence and size of the foramen suggests that the role originally played by the pineal eye in eucynodonts and eutheriocephalians became dispensable and consequently this organ began to evolve under weak selective pressure. A similar situation is encountered in extant lizards, in which 40% of genera include species that do not have a pineal eye (Gundy et al. 1975; Ralph 1975). Amongst the Iguanidae, Agamidae, Scincidae, and Lacertidae, 83–98% of the genera lack a pa-

rietal foramen (Quay 1979). Those genera which lack a parietal foramen live in more equatorial environments where there is less contrast in seasonal temperature, rendering the pineal eye functionless (Gundy et al. 1975; Ralph 1975).

The multiple evolutionary losses of the pineal eye in lizards mirrors that in eutheriocephalia and eucynodontia, and suggests that the pineal eye was already vestigial in some of these therapsid lineages as the parietal foramen began evolving under relaxed selective pressures (Camp and Welles 1956; Benoit et al. 2015). Geological and palaeogeographical evidence suggests marked temperature and seasonal fluctuations for the Karoo in the late Permian (Rubidge 1995; Smith 1995). This would have put strong selective pressure against the loss of the parietal foramen on South African therapsids if their third eye was functional. Thus the trend toward the loss of the parietal foramen in eutheriodonts during the Permian and Early Triassic seems counterintuitive.

Since it has been shown that extant ectotherms that live in equatorial environments tend to display a pineal opening less frequently, it could be hypothesized that the convergent diminishing frequency and reduction in size of the parietal foramen in Therocephalia and Cynodontia was the consequence of climatic or environmental changes, for example, driven by the northward drift of Africa during the Permo-Triassic (Zharkov and Chumakov 2001; Abdala and Ribeiro 2010). The influence of environment on the distribution of the parietal foramen in eutheriodonts is evidenced by the more frequent absence of a parietal opening in species that lived in lower latitudes.

In *Dvinia*, a basal cynodont from the late Permian of Russia, *Sinognathus*, a cynognathian from the Middle Triassic of China, and in all Middle–Late Triassic Argentinian gomphodonts, a parietal opening is absent while it is present in most of their South African relatives (Tatarinov 1968; Liu and Olsen 2010; Ivakhnenko 2013). Additionally, amongst the eleven Russian therocephalian species for which enough cranial material is documented, only five of them have a parietal foramen (Ivakhnenko 2011). This is consistent with the lower latitude (close to 30° N) of the Russian deposits during the late Permian and Early Triassic (Zharkov and Chumakov 2001). However, this relaxation of selective pressures in the evolution of the parietal foramen in therapsids does not seem to reflect environmental change: firstly, because the trend toward the loss of the parietal foramen was not contemporaneous in Cynodontia and Therocephalia (Fig. 3); secondly, because this trend is confined to Cynodontia and Therocephalia as the frequency of presence of the parietal foramen in Dicyodontia and Gorgonopsia remains relatively constant (Fig. 3); and finally, because South Africa was located close to the polar region throughout the Permian and Triassic, and seasonality remained pronounced during this extended period (Rubidge 1995; Smith 1995). The trend toward loss of the parietal foramen was thus probably not driven by climate or environmental changes. If the pineal eye began to evolve under weak selective pressure in cynodonts

and therocephalians, the cause could have been intrinsic modifications in the physiology of these species.

One possible way to explain the disappearance of the parietal foramen in therapsids would be that the gradual evolution of photosensitive melanopsin-containing retinal ganglion cells in the lateral eyes and the accompanying retinohypothalamic tract (Berson et al. 2002) began to replace and compensate for the role of the pineal eye amongst eucynodonts and eutheriocephalians. The spectral sensitivity of melanopsin overlaps that of cone type pigments, suggesting that the mammalian melanopsin-containing retinal ganglion cells evolved in correlation with the loss of colour vision in early Mammaliaforms during the “nocturnal bottleneck”, as they adapted to increasingly nocturnal ecological niches during the Late Triassic (Davies et al. 2010; Gerkema et al. 2013). Gerkema et al. (2013) consider that the pineal complex might have played a significant role in the evolution of nocturnality because the evolution of mammals was accompanied by the loss of certain proteins involved in circadian rhythms. Therefore, the melanopsin-containing retinal ganglion cells of extant mammals might have originated after the divergence of cynodonts and therocephalians, but both might have convergently adapted to nocturnality. Angielczyk and Schmitz (2014) suggested that some synapsids, particularly some pelycosaur and birmosuchians, may have adapted to a nocturnal niche well before the alleged “nocturnal bottleneck” event. Their taxon sampling does not completely overlap with ours, therefore the two datasets are difficult to compare. Nevertheless their dataset indicates that therocephalians and cynodonts were often nocturnal or semi-diurnal whereas dicynodonts and gorgonopsians were diurnal (Angielczyk and Schmitz 2014). This difference in lifestyle could account for our results on the distribution of the parietal foramen: groups displaying a variable occurrence of the parietal foramen would be those adapted to a nocturnal lifestyle.

Under this assumption, following the principle of proper mass (Jerison 1973), it could be expected that the loss of nervous tissue and fibres which once innervated the pineal eye (the pineal eye itself, the paraphysis and the pineal nerve) would be compensated for by a gain of sensitive tissues and nervous fibres in the lateral eyes (i.e., the retinal ganglion cells). This could result in an increase of the size of the orbit in forms where the parietal foramen is reduced or absent; however, analysis of our dataset (Table 1) demonstrates that the size of the orbit varies independently of the parietal foramen, and that the orbit is not larger in those therapsids which lack a parietal foramen. Based on this line of evidence, the hypothesis of functional compensation seems unlikely (but it should be stressed that orbit size is not the best proxy to infer eyeball size; Angielczyk and Schmitz 2014). Additionally, the nocturnal hypothesis would not explain why taxa such as *Dimetrodon*, *Cynosaurus*, or *Ictidosuchoides* were likely scotopic according to Angielczyk and Schmitz (2014) while they invariably display a parietal foramen. In fact, what these authors mainly show is that synapsids displayed

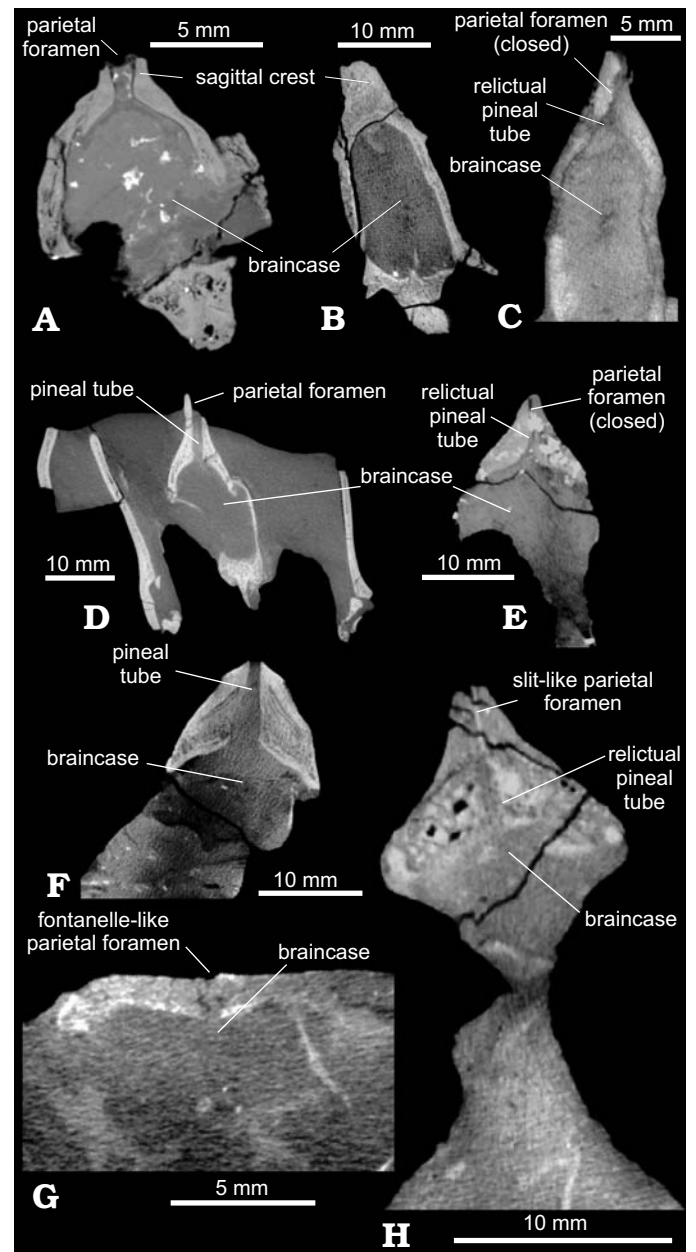


Fig. 5. CT-sections through the sagittal crest in Cynodontia (A–D) and Therocephalia (E–H) illustrating the variability of the parietal foramina. **A.** *Cynosaurus suppostus* Schmidt, 1927, BPI/1/1563 (voxel size: 0.0291 mm); Ringsfontein, Murraysburg, South Africa, *Daptocephalus* AZ, 255–251 Ma. **B.** *Cynosaurus suppostus* Schmidt, 1927, BPI/1/3926 (voxel size: 0.0708 mm); Tweefontein, Nieu Bethesda, South Africa, *Daptocephalus* AZ, 255–251 Ma. **C.** *Diademodon tetragonus* Seeley, 1895, BPI/1/3776a (voxel size: 0.0801 mm); Cragievar, Burgersdorp, South Africa, *Cynognathus* AZ, 245–237 Ma. **D.** *Trirachodon berryi* Seeley, 1895, AM461 (voxel size: 0.0668 mm); Burgersdorp, South Africa, *Cynognathus* AZ, 245–237 Ma. **E.** *Theriognathus microps* Owen, 1876. BPI/1/512 (voxel size: 0.0801 mm); Suurplaas, Graaf-Reinet, South Africa, *Daptocephalus* AZ, 255–251 Ma. **F.** BPI/1/100 (voxel size: 0.0756 mm); Vlakteplaas, Graaf-Reinet, South Africa, *Daptocephalus* AZ, 255–251 Ma. **G.** *Tetracynodon darti* Sigogneau 1963, NMQR3756 (voxel size: 0.0445 mm); Fairydale, South Africa, *Lystrosaurus* AZ, 251–245 Ma. **H.** *Bauria cynops* Broom 1909, BPI/1/3770 (voxel size: 0.0728 mm); Cragievar, Burgersdorp, South Africa, *Cynognathus* AZ, 245–237 Ma.

signs of adaptation to nocturnality well before the process of disappearance of the parietal foramen and the forthcoming “nocturnal bottleneck”. Conversely, the fact that the platypus still has the SWS2 opsin pigment (while it is lost in therian mammals) suggests that the last common ancestor of extant mammals still had a trichromatic vision and was thus diurnal (Davies et al. 2007). This implies that nocturnality occurred far more recently in the phylogeny of mammals. Finally, the study of extant reptiles reveals that the absence of a parietal foramen is not more frequent in nocturnal species (Gundy et al. 1975; Quay 1979). Thus, although the hypothesis of a convergent adaptation to nocturnality sounds likely, it is not supported by current knowledge.

Finally a large pineal eye in ectotherms is believed to permit faster and more efficient adaptation to diurnal and seasonal temperature variations (Edinger 1955; Gundy et al. 1975; Ralph 1975; Ralph et al. 1979). Lizards have the largest pineal eye and pineal complex of all extant ectothermic vertebrates, and they are the most active and fine-tuned thermoregulators. This role in fine-tuned thermoregulation is played by the pineal eye as demonstrated by surveys showing that blinding the pineal eye of extant lizards increases exposure to sunlight and that removing the pineal eye affects their thermoregulatory capacity (Hutchison and Kosh 1974; Eakin 1973; Ralph et al. 1979; Reiter 1981). Given that South African therapsids lived in seasonally contrasting environments (Rubidge 1995; Smith 1995), it is fair to assume that the acquisition of a condition close to endothermy would have stabilized body temperature and resulted in making the role of the pineal eye in behavioural thermoregulation far less crucial (Quay 1979; Roth et al. 1986). Accordingly, since the therapsid lineage lead to the origin of mammals, they may have evolved better thermoregulation capacities and consequently it would be surprising if the loss of the parietal eye was completely independent from the evolution of endothermy in therapsids (Roth and Roth 1980; Roth et al. 1986; Benoit et al. 2015).

Studies from several independent fields of research such as bone histology, presence of nasal turbinates, predator/prey ratios, and paleoenvironmental analysis (reviewed in Bennett and Ruben 1986; Hillenius and Ruben 2014) suggest that most therapsids were not endotherms but were spread on a continuum between ectothermy and endothermy, a condition that is called mesothermy (Roth et al. 1986). All these studies suggest the same big picture of evolution of endothermy in mammalian ancestry: from full ectothermy in “pelycosaurs” to various degrees of the intermediate mesothermic condition in non-mammaliaform therapsids, toward full endothermy in some non-mammaliaform cynodonts and mammals (Roth et al. 1986; Ruf et al. 2014; Botha-Brink et al. 2012; Hillenius and Ruben 2014). The evolution of the parietal foramen, with its gradual increase in variability both in size and in frequency in cynodonts and therocephalians, would thus reflect the same gradual transition toward a condition approaching mammalian endothermy. This “zone of variability” (Bever et al. 2011) of the parietal foramen in the

phylogeny of therapsids (Fig. 2) testifies to the loss of function of the pineal eye because of the acquisition of better thermoregulatory capacities and stable body temperature, a critical step in the evolution of mammalian endothermy.

Against this hypothesis, one could claim that most ectothermic reptiles (crocodilians, chelonians, and snakes) do not have a parietal eye (Bennett and Ruben 1986). In these taxa, as in mammals, the pineal gland still exists (except in crocodiles) and is involved in the regulation of natural cycles (e.g., reproduction cycle) and body temperature. However, it should be noted that under certain conditions some representatives of these ectotherms, such as incubating pythons and leatherback turtles, are capable of non-behavioural increase of body temperature which, coupled with the fact that there is good evidence that crocodiles were formerly endothermic (Seymour et al. 2004) and that it has recently been proved that the Tegu lizard (*Salvator merianae*) is capable of non-behavioural thermoregulation during the reproduction season (Tattersall et al. 2016), suggest that the evolution of ectothermy in extant Sauropsida was much more complex than the simplistic model of an inherited plesiomorphic condition. In addition, despite the fact that they no longer have a parietal foramen, most sauropsids (including birds) retain photoreceptive pinealocytes in their pineal organ (Eakin 1973; Ralph 1975; Ralph et al. 1979; Ekström and Meissl 2003).

Accordingly we hypothesise that one of the possible sources of the relaxation of natural selection on the parietal foramen and the corresponding pineal eye was linked to the acquisition of better control of body temperature in Eucynodontia. In this group, the pineal eye would have become less dominant functionally and would have begun to regress when the body began to generate sufficient heat to stabilize body temperature. This is supported by the fact that the mutation of *Msx2*, the gene responsible for the ossification of the fronto-parietal fontanelle in mice (and by extension, for the closure of the parietal foramen in therapsids), is also involved in the maintenance of hair, a major contributor to body insulation in mammals (Garcia-Miñaur et al. 2003; Satokata et al. 2000; Ferguson 2010).

This hypothesis is also supported by numerous long bone paleohistological studies which show less vascularized cortical bone with well-marked growth marks in basal therapsids and the basal cynodont *Procynosuchus*, thus indicating seasonally dependent and slow growth (Botha and Chinsamy 2000, 2004, 2005; Ray et al. 2004; Botha-Brink et al. 2012). In contrast the absence of growth rings and high vascular density in more derived epicynodonts indicates that they experienced sustained growth and were not particularly susceptible to environmental fluctuations (Botha and Chinsamy 2000, 2004, 2005; Botha-Brink et al. 2012). Sustained growth rates and the absence of growth marks are not direct evidence for endothermy since they can be the consequence of hibernation, even in endotherms (Bennett and Ruben 1986). However in highly seasonal climatic conditions such as those of the Permian and Triassic of South Africa, the absence of these features strongly sug-

gests that only vertebrates with high metabolic rates could have achieved continuous growth (Botha and Chinsamy 2000, 2004, 2005). In addition, in Probainognathia (*Trucidocynodon*, *Chiniquodon*, *Tritylodon*) the bone histology is virtually identical to that of the basal-most mammaliaform *Morganucodon* (Botha-Brink et al. 2012).

Amongst theriocephalians, the increase in the frequency of the absence of the parietal foramen in derived forms could also be the result of independent development of their own kind of endothermy since histological data shows that highly vascularized cortical bone with fewer growth marks evolved in Triassic species of Baurioidea and Akidnognathidae (Huttenlocker and Botha-Brink 2014). However, the overlap between the evolution of the parietal foramen and paleohistology is not perfectly matched since some dicynodonts and, for example, the basal epicynodont *Thrinaxodon*, always have a parietal foramen (SOM 1) and also display a significant amount of fibro-lamellar tissue in their bones (Chinsamy and Rubidge 1993; Ray et al. 2004; Botha-Brink et al. 2012). These discrepancies emphasize, (i) that bone paleohistology, which documents growth rates and various other life history traits, is only indirectly and thus imperfectly correlated to the evolution of metabolic rate (Bennett and Ruben 1986); and (ii) that there is a multiplicity of roles played by the pineal eye in the physiology and behaviour of ectothermic animals (as diverse as orientation, Foà et al. 2009; or aggressive behaviour, Phillips and Howes 1987) and that its evolution might have been driven by multiple factors (Quay 1979).

Conclusion

Our study suggests that most therapsids required an efficient pineal organ to receive and transmit information on daylight and seasonal variations. Because of increasing variability in the size and shape of the parietal opening in eucynodonts and eutheriocephalians and its disappearance in probainognathians in the upper horizons of the Karoo stratigraphic succession, we hypothesise that a gradual and convergent relaxation of evolutionary constraints on the pineal eye in both theriocephalians and cynodonts took place around 266 and 245 Ma respectively, maybe because of the loss of the photoreceptive function of the pineal organ. The evolution of the typical mammalian melanopsin-containing retinal ganglion cells or that of a high metabolic rate could equally explain this increase of variability. Parallel evolution of characters is not uncommon in these groups and the acquisition of a similar metabolism would partially account for these convergent evolutionary trends. For example, the closure of the secondary palate isolates the nasal cavity and enables turbinates to more efficiently warm the air (Hillenius and Ruben 2014). A convergent evolution to nocturnality cannot be ruled out and both hypotheses (endothermy and nocturnality) certainly are not mutually exclusive since they are related to functions monitored by the pineal organ.

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