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Digital endocast of *Riograndia guaibensis* (Late Triassic, Brazil) and the evolution of the brain in non-mammalian cynodonts.

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Abstract

A digital cranial endocast of the specimen UFRGS-PV-596-T, *Riograndia guaibensis*, was obtained from μCT scan images. This is a small cynodont, closely related to mammaliaforms, from the Late Triassic of Brazil. *Riograndia* presents big olfactory bulb casts and the cerebral hemispheres region is relatively wider than in other non-mammaliaform cynodonts. Impressions of vessels were observed and a conspicuous mark on the dorsal surface was interpretated as the transverse sinus. The encephalization quotient calculated is greater than the range of the most non-mammaliaform cynodonts. The ratios between linear and area measurements of the dorsal surface suggests four evolutionary changes from a basal eucynodont morphology to mammaliaformes, evolving the increase of the relative size of the olfactory bulbs and the width of the cerebral hemispheres and cerebellum. The data supports the hypothesis of the neurological evolution of the mammlian lineage starting by a trend of the increase of the olfactory bulbs, which is associated to adaptations on the nasal cavity. This trend must be linked to the selective pressures for the small faunivorous, and probably nocturnal, animals, and represents an initial improvement of the sensory receptor system, leading to further development of the "superior" structures for sensorial processing and integration.

Keywords Mammalian brain evolution; Endocast; *Riograndia*; Cynodonts; μCT scan; Triassic

INTRODUCTION

Cranial endocasts are important tools to study the brain of some extinct taxa, and so, to make inferences about the behavior, sensorial acuity, and motor control in extinct animals. Moreover, within non-mammalian cynodonts, these paleoneurological studies can help to understand the evolutionary changes along the mammalian lineage, since living mammals are distinguished by relatively larger and more complex brains than other vertebrates (Kielan-Jaworowska et al. 2004; Kemp 2009). Although the intracranial space is occupied also for meninges and blood vessels, cranial endocasts

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show neuroanatomical external structures, especially in mammals and birds, because, contrary to small-brained non-mammalian and non-avian tetrapods, their brains largely fill the endocranial space (Jerison 1973; Starck 1979). For non-mammaliaform cynodonts, Kemp (2009) raised the possibility that their endocasts do not represent the actual shape of the brain, but in any case, some important features have been reported from some taxa as: the Early Triassic *Nythosaurus larvatus* (= *Thrinaxodon liorhinus*) and *Diademodon* (Watson 1913), the Late Triassic *Exaeretodon* (Bonaparte 1966), the Early Triassic *Trirachodon* (Hopson 1979), the Late Permian *Procynosuchus* (Kemp 1979), the Middle Triassic *Massetognathus*, *Probelesodon* (Quiroga 1979, 1980a, synonymized to *Chiniquodon*, Abdala & Giannini, 2002) and *Probainognathus* (Quiroga 1980b, c), as well as the Late Triassic *Therioherpeton* (Quiroga 1984) and *Brasilitherium* (Rodrigues et al. 2013).

Common features observed in endocasts of non-mammaliaform cynodonts

In general, the basic design of the endocast of non-mammaliaform cynodonts point to a narrow brain and commonly with tubular cerebral hemispheres, although Kemp (2009) have appointed that the region of the cerebral hemispheres could have been substantially deeper than wide, since in that region of the skull there is no bony floor for the brain cavity. The main structures usually visible in these endocasts are well-defined olfactory bulb casts and the cerebellar region, whose lateral extension is indicated by prominent paraflocculi, which fill the subarcuate fossa on the supraoccipital and periotic components of the braincase (Kemp, 2009). Moreover, in ventral view is common the identification of the hypophyseal cast, which fills the sella turcica on the basisphenoid/parasphenoid. Another structure which can be observed in non-mammalian cynodont endocasts is the vermis on the dorsal surface of the cerebellar region, indicated by Kielan-Jaworowska *et al.* (2004) for the eucynodont *Thrinaxodon*, although it is not reported in other descriptions (Watson 1913; Hopson 1979; Quiroga 1979, 1980a, b, 1984) and there is the suggestion that the dorsal depression between the parietal and interparietal bones is filled indeed by the superior sagittal sinus (Rowe et al. 1995; Kemp 2009). Also controversial is the dorsal exposure of the midbrain coliculli, reported by Quiroga (1980b, c) for *Probainognathus*, while Kielan-Jaworowska et al. (2004) warn about the possibility of marks of post-mortem changes in parts of the brain and meninges on taxa with endocasts without a clear definition of the transverse sinus marking the posterior limit of the cerebral hemispheres. Furthermore, Macrini et al.

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(2007b) claimed that the non-exposure of the midbrain should be the plesiomorphic condition for the mammalian crown-group.

Quantitative/volumetric approaches

The Encephalization Quotient (EQ) is the most widely used measure for comparison of different taxa relative to brain size, including non-mammalian cynodonts and extinct mammals (e.g. Quiroga, 1979, 1980a, b, Kielan-Jaworowska 1983, 1984; Kielan-Jaworowska and Lancaster 2004; Macrini et al. 2007b; Rowe et al. 2011; Rodrigues et al. 2013). The EQ, as developed by Jerison (1973) and discussed by Eisenberg (1981), consists of a ratio between the volume of the brain weight of an animal and the brain weight expected for an animal of the same mass, based on allometric regressions for the ratio brain weight/body weight, empirically verified for different taxa of a group defined as parameter. The use of EQs, however, has already been the target of criticism associated mainly to the significance of the relative brain size as an indicator of the capacity to integrate information (Butler and Hodos 1996). In addiction, there are methodological difficulties, specially for the study if fossils, since the body mass must be estimated (from different parameters in the fossils as skull length, postcanine teeth, lengths of long bones, mid-shaft circumferences of long bones, cross-sectional area of vertebrae, etc.), and the endocast volume (assuming that 1 cm^3 is equivalent to 1 g) cannot correspond to the actual volume occupied by the brain in the endocranial cavity (Rodrigues et al., 2013). Even so, the values of EQ former studies with non-mammalian cynodonts indicate, in general, an increased brain volume in relation to the estimated body mass in taxa successively more closely related to mammals, but it is not so clear within non-mammaliaform cynodonts: *Thrinaxodon*, 0.10 (Jerison, 1973); *Diademodon*, 0.14 (Quiroga 1980b), and 0.21 (Jerison 1973); *Massetognathus*, 0.15 and 0.22 (Quiroga 1979, 1980b); *Exaeretodon*, 0.10 and 0.15 (Quiroga 1980b), *Probelesodon*, 0.13 and 0.18 (Quiroga 1979, 1980b); *Probainognathus*, 0.12 and 0.17 (Quiroga 1980a, b); *Brasilitherium*, 0.15 and 0.22 (Rodrigues et al, 2013); *Morganucodon*, 0.32 (Rowe et al. 2011); *Hadrocodium*, 0.49 (Rowe et al. 2011); living monotremes 0.75 to 0.89 (Macrini et al. 2007a); *Triconodon*, 0.49 (Kielan-Jaworowska 1983). Regarding to the EQs of non-mammaliaform cynodonts, Rodrigues et al. (2013) suggested that the values may be higher than they should due to the equation used to estimate the body mass (in turn, then overestimated).

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The evolution of the mammalian brain among non-mammlian cynodonts

In addition to a tendency to increase in overall size, the brain evolution towards mammals presents changes in different anatomical features, whose variation can also be observed in relation to the whole endocast or the skull size. Thus, in an evolutionary sequence towards the mammalian brain, the first clear change observed from the endocasts of the non-mammaliaform cynodonts is the loss of the parietal eye (and perhaps the whole parietal-pineal complex) in most group Eucynodontia, although some eucynodonts taxa, such as *Diademodon*, *Trirachodon* and *Massetognathus*, present the primitive state (e.g., Quiroga, 1979; Hopson and Kitching 2001). Moreover, the division between the cerebral hemisphere casts by a median sulcus seems to be more defined in *Probainognathus* (Quiroga 1980a, b) than in the taxa *Thrinaxodon* (Kielan-Jaworowska et al. 2004), *Exaeretodon* (Bonaparte 1966), *Massetognathus* and *Probeleson* (*Chiniquodon*) (Quiroga 1979). Associated to this feature, the cerebral hemisphere region becomes wider, mainly near to their posterior limit in *Probainognathus* and taxa more closely related to mammals. *Brasilitherium* also presents a clear median sulcus in the cerebral hemispheres region, suggesting a phylogenetic significance, since this taxon is more nearly related to mammaliaforms than the others quoted above. Rodrigues et al. (2013), also reported for *Brasilitherium* the relative size of the olfactory bulb casts larger than in other taxa of non-mammaliaform cynodonts as well as mammaliaforms and suggesting a trend of increase of relative size olfactory bulbs among non-mammliaform cynodonts successively more related to mammals.

Among non-mammalian mammaliaforms, Luo et al. (2001) reported a progressive increase of the width of the posterior (cerebellar) region in *Sinoconodon*, *Haldanodon*, *Morganucodon*, and *Hadrocodium*, and an increase of width of the cerebral hemisphere region is also highlighted for these two last taxa in the summarization of Kielan-Jaworowska et al. (2004). These authors also indicate in the endocast of *Morganucodon* the cerebral hemisphere region relatively wider compared to non-mammaliaform cynodonts and *Sinoconodon* (in which the parietal bones are subvertically disposed, having less endocranial space in this region). Besides, they evinced the presence of marks interpreted as transversal gyres at the dorsal surface of the cerebellum cast, and, and the division of the dorsal surface in two hemispheres. Furthermore, the division of the cerebral and cerebellar cavities in *Morganucodon*, by an ossified septum, i.e., the tentorium (tentorium osseum), was suggested by Kermack et al. (1981) but this suggestion is controversial (e.g., Kielan-Jaworowska 1997).

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Kemp (2009), suggest two stages in the mammalian brain evolution, being the first represented by the relatively big size of the cerebellum in non-mammaliaforms cynodonts (and the author suggest perhaps also the midbrain structures), reporting that even a cerebellum of the modern mammal *Didelphis virginiana* fits comfortably into the hind part of the endocranial cavity of the non-mammaliamorph cynodont *Chiniquodon*. The cerebellar development could reflect the increase in the level of sophistication of the neuromuscular control of the movements associated to a more complex occlusion and the reorganization of the jaw musculature to create a bite that is simultaneously forceful and precise, as well as the locomotory system, including the more upright hindlimbs and the more mobile shoulder girdle with a wide range of possible forelimb movements. In this sense, the author mentioned the elaboration of the proprioceptive sensory input, and forward and backward feedback controls within the cerebellum (Mauk et al. 2000), suggesting new forward projections to the midbrain to be coordinated there with visual input, while the cerebral hemispheres remained primitively small, with no elaboration of cortical structures or development of a dorsal ventricular ridge. Regarding to the second stage, it is represented by the expansion of the telencephalic pallial structures to form the six-layered neocortex (isocortex) present in mammals (Kemp 2009).

Rowe et al. (2011), in turn, indicate three "pulses" in the evolution of the mammal brain, highlighting the non-mammalian mammaliaforms *Morganucodon* and *Hadrocodium*, as examples of two first pulses of encephalization determined by a significant widening in the region of the cerebral hemispheres and cerebellum, and a third pulse associated to the origin of the mammalian crown-group, with the presence of ossified ethmoturbinals, a cribriform plate and a rigid support in the nasal cavity to the olfactory epithelium receptor (OR). The activation of OR genes, which in mammals exceed by about one order of magnitude the amount existing in the genome of most other vertebrates (Niimura, 2009), induces the growth of the olfactory epithelium and turbinals, as well as their ossification (Rowe et al., 2005). According to these authors, the elaborate visual and auditory systems of mammals evolved later than these olfactory improvements. This is corroborated by Rodrigues et al. (2013), so that a stage, related to the neurological evolution related to olfactory accuracy, could be occurred later than the first stage indicated by Kemp (2009), and prior than the three pulses suggested by Rowe et al. (2011), represented by the increasing olfactory bulbs size, associated to more complex nasal cavity, and precursor to development of "superior" brain structures (i.e.

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cerebral hemispheres) for sensorial processing and integration. Indeed, Jerison (1973) remarked that olfaction played a particularly significant role in the evolution of the mammalian brain, and associated this feature to the nocturnal habits of the small sized animals (i.e. non-mammalian mammaliamorphs).

Moreover, Kemp (2009) also highlighted the process of miniaturization in Mesozoic cynodonts and its corresponding allometric consequences, including a non-reduction of the pressure over the occlusal surface area of the dentition (since the muscle force is related to cross sectional area, not to volume), and also neurological and sensorial effects, because a relatively larger space became available for cranial expansion between the epipterygoids as the adductor musculature of the temporal region became relatively smaller. These changes coincided with the need for a relatively larger endocranial cavity. Regarding to the miniaturization, the author also suggested that the postdentary bones, reduced in mass, in addition to be free from a mechanical function as part of the lower jaw, rendered the system more sensitive to higher frequency sound.

The role of *Riograndia guaibensis* in the evolution of the mammalian brain

Riograndia is the the most abundant cynodon taxon of the upper portion of the Candelária Sequence (early Late Triassic) of the Santa Maria Supersequence (Zerfass et al. 2003). Based on its abundance, it is used as reference taxon for the *Riograndia* Assemblage Zone (Soares et al. 2011) of Norian age (FIG 1).

Moreover, *Riograndia* is an important taxon to study of the transition between the non-mammaliaform cynodonts and the mammaliforms and the evolution of aspects of the mammalian biology within non-mammalian cynodonts by its phylogenetic relationships. In the phylogenetic analysis of Liu & Olsen (2010) *Riograndia* is the sister-taxon of a clade composed by *Pachygenelus* + ((*Brasilodon* + Mammaliaformes) + Tritylodontidae)). However, according to Martinelli & Rougier (2007), in an analysis including more ichthyosaurs, *Riograndia* was positioned as a non-Tritheledontidae, but within the group Ictidosauria. In efffect, this taxon was originally described as an ictidosaur (Bonaparte et al. 2001) and it is one of the Mesozoic cynodonts that can be quoted with respect to the miniaturization and, consequently, its biological consequences. Furthermore, taking into account a sequence of the evolution of the mammalian brain observed within non-mammlian cynodonts, and the stages suggested based on the endocasts studied, as *Thrinaxodon*, *Probainoganthus*, *Brasilitherium* and different mammaliaforms, it was expected that *Riograndia* presents traits farther or

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closer to mammaliaforms, representing some intermediate stage in this evolutionary sequence.

MATHERIAL AND METHODS

The studied specimen (FIG. 3) belongs to the collections of the Laboratório de Paleontologia de Vertebrados of the Universidade Federal do Rio Grande do Sul (UFRGS) in Porto Alegre, Rio Grande do Sul state, Brazil. It was collected in the outcrop Sesmaria do Pinhal I (geographic coordinates: -29.684723°; -52.845851°; Datum WGS-84) near the town of Candelária, Rio Grande do Sul state, Brazil, and indicates the presence of the *Riograndia* Assemblage Zone (upper Candelária Sequence, Santa Maria Supersequence, Zerfass et al., 2003; Soares, et al. 2011; Horn et al. 2014). UFRGS-PV-0596-T consists of a virtually complete skull, with the right lower jaw ramus in occlusion, which is broken at the posterior half, just bellow the apex of the coronoid process, so lacking the angular process. Although the skull is well preserved, there is some parts broken and/or distorted due to post-morten deformations: (1) the zigomatic archs are missing; (2) the quadrate ramus of the pterygoid, the epipterygoid (alisphenoid) and the cultriform process of the sphenoid complex (basisphenoid + parasphenoid) are absent; (3) in ventral view, the region of the interpterigoid vacuities can not be definied, as well the otic bones (prootic and opisthotic), lateral to the basispheoid; (4) the ascendent process of alisphenoid and anterior process of prootic, can not be totally delimited on the lateral walls of the braincase, which are incomplete. Notwithstanding, these damages did not cause significant distortions in the overall endocast.

µCT scanning and digital extraction of the endocast

The images were obtained from the CT Scanner NIKON XTH225ST located at the School of Earth Sciences, Life Sciences Buildin, University of Bristol, England. From the scanning were generated 1447 slices of the skull in coronal planes, with a resolution of 1,024 x 1,024 pixels and a pixel size of pixelsize 0.0239904 x 0.0239922 mm.

The visualization of the slices, 3D rendering, measurements, segmentation and treatment of the images to separate the rocky matrix from the bony elements were performed using the softwares VGStudio Max (version 1.2.1; Volume Graphics GmbH)

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and Avizo (Standart Edition, version 7.1.0, FEI Visualization Sciences Group). The brain endocast was reconstructed using the infilling material in the cranial cavity. The slices were also manually treated in order to remove, from the segment corresponding to the endocast, external elements with similar density (gray scale) of the internal rocky matrix.

Two volumes of the endocast were calculated, one taking into account the whole cast behind the posterior limit of the nasal cavity, and other without the filling of the orbital vacuity above the pterygoids, which was digitally removed. In this way, the ventral contour of the olfactory bulb casts was setted based on this dorsal morphology, assuming that the bulbs were dorsoventrally symmetrical in lateral view. The same criterium was used by Rodrigues et al. (2013) for *Brasilitherium*, taking into account that in endocasts described in the literature the olfactory bulbs usually have a ventral contour similar to the dorsal contour in lateral view (e.g., Quiroga 1979, 1980a, b, c, 1984; Macrini et al. 2006, 2007a, b; Rowe et al. 2011), as well as the actual ventral extension of the olfactory bulbs region delimited, in non-mammaliforms, cynodonts with ossified orbitosphenoid forming a floor for this region as travesodontids (e.g. Luo 1994) and *Chiniquodon* (e.g. Kemp, 2009). In *Riograndia* UFRGS-PV-596-T, the estimated ventral extension of the olfactory bulbs region does not differ significantly from the ventral limit of the cast left by the orbital process of the frontal bones.

Quantitative analysis

To calculate the EQ for *Riograndia*, it was necessary to estimate its body mass first. Because we do not have postcranial bones associated with any of the known skulls for this taxon, the body mass was estimated from the skull length using the equation utilized by Luo et al. (2001) for the Jurassic mammaliaforms *Sinoconodon*, *Morganucodon* and *Hadrocodium* and Rodrigues et al. (2013) for *Brasilitherium*: X = 3.68Y-3.83, where X is the log10 of the body mass in grams and Y is the logarithm of the skull length in millimeters (this is a regression equation based on the scaling relationship of body mass to skull size in 64 species of living lipotyphlan insectivore mammals presented by Gingerich and Smith, 1984).

The EQ was calculated from the endocast volume (EV) and the estimated body mass (BM), which was obtained using two different equations: EQ = EV/0.12BM0.67, from Jerison (1973), and EQ = EV/0.055BM0.74, from Eisenberg (1981), as presented

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in Table 2. However, comparisons with EQs of non-mammaliamorph cynodonts are difficult, as showed by Rodrigues et al. (2013), since the endocast of *Brasilitherium* is clearly larger related to the skull, and its EQ agrees with an evolutionary sequence from non-mammaliamorph cynodonts to increasing EQs in the taxa more closely related to mammals, so that the EQs of *Diademodon* and *Massetognathus* calculed by Jerison (1973) and Quiroga (1979, 1980c), respectively, seems to be overestimated, probably due to the equation to estimate the body mass.

Thus, due to the imprecisions of the endocast volumes and, mainly, to estimate the body mass of different taxa form different equations, we try another way of comparing the relative size of the olfactory bulb cast on the brain endocasts, in order to compars the relative size of brain structures, mainly the olfactory bulbs, considering the importance of the olfactory bulbs and the increment of the olfaction sense in the transition between non-mammaliforms cynodonts and the Mammaliaformes lineage. For this purpose, given the similar endcocast general design of non-mammaliaform cynodonts, it was mesaured the length and area of the dorsal exposition of the olfactory bulbs, cerebral hemisphere and cerebellar regions, as well as of whole brain endocast in dorsal view. This measurements was realized in *Riograndia* UFRGS-PV-596-T and other non-mammlian cynodont taxa, from digitalized pictures showing the dorsal view of the endocast in other works (*Hadrocodium*, Macrini, 2006; *Brasilitherium,* Rodrigues et al. 2013; *Therioherpeton*, Quiroga, 1984; *Probainognathus*, Quiroga, 1980a, b; *Probelesodon* and *Massetognathus*, Quiroga, 1979, 1980c) - using the software Digimizer (Version 4.6.1, MedCalc Software BVBA). In order to standardize the comparison parameters, we obtained ratios of the measurements taked in relation to the skull length, as well as the measurements of the regions of the endocast between them and related to the whole endocast for each taxon. These ratios are showed in the Table 3.

DESCRIPTION AND COMPARISONS

General aspects

The endocranial cast of *Riograndia* UFRGS-PV-596T, in dorsal view (FIG. 4A), can be delimited between the foramen magnum and a groove, observed in dorsal and lateral view, which marks the anterior limit of the olfactory bulb casts. This groove results from an internal thickening of the frontal bone, which forms a ventrally directed

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ridge visible in lateral section, while their dorsal surface on the roof of the skull reamains flat, transversely aligned between the orbital processes on both sides. Thus, the nasal cavity and the brain cavity are partially separated at the roof of the skull, despite the lack of an ossified cribiform plate (which appears only in mammals; e.g. Rowe, 1988; Kielan-Jaworowska et al. 2004). However, there is not a crest in the internal surface of the skull roof which could indicate the presence of a median sulcus separating the cerebral hemispheres and the olfactory bulb casts.

The general shape of the dorsal surface of the endocast is anteroposteriorly elongated, being the aspect ratio between the maximum width and length approximately 0.4. The endocast is gradually wider posteriorly until the anterior limit of the cerebellar region, which presents a rounded lateral contour so that the width from this point decreases to the foramen magnum. The length of the endocranial cast, between the anterior limit of the dorsal surface of the olfactory bulbs and the foramen magnum is 16.31 mm, corresponding to 44% of the total length of the skull (35.08 mm). The maximum width is 6.88 mm, at the anterior limit of the cerebellar region, where there is a conspicuous transversal linear structure, here interpretated as a vascular cast (see the specific title, below). Behind this mark of the endocast is the cerebellar region to the foramem magnum, where is the maximum width excluding the vascular mark (6.20 mm), there are two lateral structures, corresponding to the parafloccular casts, which fill the fossa subarcuata. Contrary to *Brasilitherium* (Rodrigues, 2013), these structures are not conspicuous in *Riograndia* PV-596T.

The lateral profile of the endocranial cast of the specimen *Riograndia* UFRGS-PV-596T reveals a slightly anteroposterior convex dorsal surface at the olfactory bulbs region, so that is possible estimate their posterior limit (although the measurement of the length of the olfactory bulbs can be obtained with more precision from the 2D slices images). Behind this region, the dorsal contour becomes straight, with a slight slope in the posterior direction (5.48° relative to a hypothetical dorsal horizontal plane from the posterior limit of the olfactory bulb casts) along 6.95 mm and so the slope becomes much steeper (24.10°, from their top, and 9.12° relative to a hypothetical dorsal horizontal plane from the posterior limit of the olfactory bulb casts) for about 1.91 mm. The lowest point of this slope is interpreted as the mark of the posterior limit of the brain hemispheres region. Immediately behind that slope, there is a ridge, on a level most dorsal than the adjacent surfaces (anterior and posteriorly), on a level equivalent to

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the region of the cerebral hemispheres anterior to the slope, which corresponds to the vascular mark (discussed below). This mark in lateral view is a ridge, from which, in an antero-posterior direction, the dorsal contour of the endocast makes a slope backwards (59.24°), along 4.68 mm, to the level of the foramen magnum

The ventral limit of the anterior half of the endocast cannot be determined, due to the presence of a space not ossified between the pterygoid and the frontal (i.e. the orbital vacuity). Posteriorly to this space, in a lateral view of the skull, there is the ascendant process of the alisphenoid, and ventrally, the endocast is delimited by the basicranial bones (basisphenoid/parasphenoid, basioccipital, prootic and opisthotic). Between the orbital vacuity and the parafloculus cast, no neuroanatomical details are distinguishable in lateral view.

In ventral view, there is no relevant detail discernible on the surface, since the basicranial bones are distorted and broken. The hypophyseal fossa (filling of the sella turcica) can be observed in some 2D images (slices), and so, can be located on the 3D endocast reconstruction, but the hypophyseal cast not appears as a prominent structure as *Brasilitherium* (Rodrigues et al., 2013), but this may due to the damaged basicranium of this specimen, mainly on the basisphenoid bone. In addition, there is not a clear cast of the ventral opening of the cavum epitericum in both sides, because the quadrate rami of pterygoids and alisphenoid were broken. Thus, most of the space filling of the semilunar ganglion of the trigeminal nerve, lateral to the wall formed by an anterior extension of the prootic (i.e. the pila antotica) that delimits the intracranial space proper (Presley 1980; Maier 1987; Novacek 1993; Rougier and Wible, 2006), was not considered as compounding the volume of this endocast. The length of the ventral opening of the cavum epiptericum could be measured (= 5 mm), not on the endocast, but considering the opisthotic as the posterior limit, and the most anterior portion of the quadrate ramus of pterygoid as the anterior one. The most posterior region of the endocast, delimited by the basioccipital, has a rounded surface, with a more pronounced bulge along a central longitudinal band. This region represents the ventral surface of the hindbrain and surrounding tissues (mainly meninges), but is not possible to identify specifically the medulla oblongata and the pons.

Forebrain region

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The portions of forebrain which left visible impressions on the endocast described are the olfactory bulbs and the cerebral hemispheres. The most anterior part of the endocranial cast represent the space occupied by the olfactory bulbs and associated meninges (Macrini et al., 2007b). Despite the absence of an ossified cribriform plate in the skull, separating the nasal cavity from the braincase, the definition of the anterior limit of the olfactory bulbs could be observed in dorsal and lateral view, as mentioned above. In spite of the external surface of the skull roof, the dorsal contour of the posterior end of the nasal cavity is clearly at a level taller than the anterior limit of the olfactory bulb casts in lateral view, separated by a deep fossa in the dorsal surface of the endocast. Moreover, there is two rounded cats on the dorsal surface, corresponding to the two bulb casts, although the longitudinal groove between them is not so clear in dorsal view. Regarding this aspect, *Brasilitherium* (Rodrigues et al. 2013) presents a sulcus clearly deeper than *Riograndia*, but this taxon do not differs significantly from other non-mammaliamorph eucynodonts as *Probelesodon* and *Massetognathus*, for which Kielan-Jaworowska *et al.* (2004) indicate olfactory bulb casts more developed and better separated from each other when compared to *Thrinaxodon*.

The region of the olfactory bulbs can be delimited, both in lateral view, due to a gentle dorsal convexity in the contour, and in the dorsal view, due to a slight transversal constriction, which seems the circular fissure (sensu Loo 1930; Rowe 1996; also called transverse furrow, sensu Kielan-Jaworowska, 1986; transverse sulcus, sensu Krause and Kielan-Jaworowska 1993; or circular sulcus, sensu Luo et al. 2002). A well-defined circular fissure, although usually present in mammals (Macrini et al., 2007b), but is not described for other non-mammaliamorph cynodonts (Quiroga, 1979, 1980a, b, c), and even is ambiguous in *Brasilitherium*, in which this area has a width similar to the region immediately posterior to it (Rodrigues et al. 2013). In *Riograndia* UFRGS-PV-596-T, in turn, the width of the dorsal surface at this point is 3.85 mm, while the maximum width of the olfactory bulbs region is 4.12 mm, and the endocast becomes also wider in backward direction from this point. The olfactory bulb casts of *Riograndia* UFRGS-PV-596-T, in dorsal view, are two oval structures anteroposteriorly elongated (FIG. 4A), being the ratio of the maximum width and length equal to 0.38 and 0.34, for the bulb casts left and right, respectively. The maximum width of the two olfactory bulb casts together is 4.12 mm, measured at half the length of the bulb casts, while the width near to the anterior limit of the olfactory bulb casts is 3.64 mm.

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Due to the lack of an ossified floor in the most anterior region of the skull, there is also no impression of the olfactory tracts (Butler and Hodos, 1996), from the olfactory bulbs to the telencephalon region. In fact, the olfactory tracts do not usually leave impressions in endocasts of mammals because they are hidden by the meninges (Macrini et al., 2007b), but the posterior limit of the olfactory bulb casts immediately anterior to the cerebral hemispheres region, as viewed in *Riograndia* UFRGS-PV-596-T, allow us to infer the presence of the olfactory tracts ventral to the bulbs. With respect to this feature, we can consider as plesiomorphic within cynodonts the patter described for *Massetognathus* (Quiroga, 1979, 1980c) and *Exaeretodon* (Bonaparte, 1966), with the olfactory tracts forming a peduncle between the olfactory bulb casts and the region of the cerebral hemispheres, as occurs in many living reptiles (Hopson, 1979; Kielan-Jaworowska et al., 2004). The ventral boundary of the olfactory bulb casts can not be accurately identified due to the lack of an ossified floor for the olfactory bulbs, despite the presence of an ossified orbitosphenoid indicated by Soares et al. (2011), beacause this bone in *Riograndia*, in addition to having a more ventral position compared to other non-mmmalian cynodonts (e.g. Hopson, 1964; Sues, 1986; Kielan-Jaworowska et al., 2004; Oliveira et al., 2010), below the orbital process of the frontal , consists of a lateral lamina with no medial projection.

The region of the cerebral hemispheres in dorsal view extends 7.53 mm to the transversal vessel impression, intified as the posterior limit. The dorsal surface is flat, with a very little pronounced median sulcus (=longitudinal fissure) dividing the region into two casts of distinct hemispheres. The division in two casts becomes more evident closer to the posterior limit, at a point 6.95 mm from the limit beetween the cerebral hemispheres region and the olfactory bulb casts, from which two bulging surfaces diverge laterally, about 20° from the medial line, along 1.9 mm, leaving a very small lower central space, which forms a half-circle, whose transverse line is delimited by the elevation of the vascular cast which passes through the dorsal surface of the endocast tranversally. The point of maximum divergence is the maximum width of the cerebral hemispheres region with 5.25 mm, while the width of the endocast at the limit beetween the cerebral hemispheres region and the olfactory bulb casts is 3.9 mm. The lateral profile shows that cerebral hemispheres region is lower than the convex surface of the olfactory bulb casts and the dorsal surface is approximately straight along 5.82 mm, to a point from which an accentuated slope (24.10° from the anterior straight portion),

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corresponding to the portion of divergence of the hemisphere casts visible in dorsal view. The non-mammaform cynodonts *Thrinaxodon* (Kielan-Jaworowska et al., 2004), *Massetognathus* (Quiroga, 1979, 1980c), *Exaeretodon* (Bonaparte, 1966), and *Probelesodon* (Quiroga, 1979, 1980c) do not present a division in this region of the endocast, but the presence of two distinct hemispheres is described for *Probainognathus* (Quiroga, 1980a, b) and *Brasilitherium* (Rodrigues et al., 2013). In this last taxon the median sulcus is very pronounced (although considered not so deep as to indicate the presence of an ossified falx cerebri as different lineages of living mammals (e.g. Macrini et al., 2007b). On the other hand, the ratio between the maximum width and the length of the dorsal surface of the cerebral hemispheres region is higher in *Riograndia* than other non-mammaliaform cynodons, *Massetognathus, Probelesodon*, *Probainoganthus, Therioherpeton and Braislitherium* (see Table 3).

Since the dorsal surface of the cerebral hemispheres region is flat, there is not any mark indicating a pineal body, which is associated parietal eye and includes the portion of the epithalamus (Butler and Hodos, 1996) of the diencephalon, having thermoreceptive functions that regulate circadian rhythms and reproductive cycles (Roth et al., 1986; Butler and Hodos, 1996). In fact, the clear evidence of the pineal body in endocasts is the parietal foramen, opening to the parietal eye (Roth et al., 1986), which is absent in most eucynodonts (exceptions, *Cynognathus*, *Pascualgnathus*, and *Diademodon*, e.g., Bonaparte et al., 2005; Abdala, 2007), although a cast of a parietal tube is indicated in the parietal endocranial cast of *Massetognathus* (Quiroga, 1979, 1980c) and a pineal region, behind the median sulcus, at the most posterior region of the cerebral hemispheres, was suggested by Quiroga (1980b, c) for *Probainognathus*.

Furthermore, as would be expected, there is no mark of the rhinal fissure, which corresponds to the ventral boundary of the isocortex (neocortex), nor markings indicating the presence of cerebral gyri and sulci, the convolutions of the cortex and the grooves between the gyri, respectively (Butler and Hodos, 1996). Despite the possibility of endocasts do not reflect the convolutions of the real brains (e.g., *Tursiops truncates*, Colbert et al., 2005), it is expected that non-mammalian cynodonts have had lissencephalic surfaces (smooth cortical brain surfaces) because this condition is considered plesiomorphic for the mammalian crown-group (Kielan-Jaworowska et al., 2004). Regarding to the rhinal fissure, the presence of a neocortex (isocortex) in a non-mammaliaform cynodont was suggested only for *Probainognathus* by Quiroga (1980b),

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based on the presence of a groove which would mark the posterior limit of that region, but it has been contested and the groove has been considered as a mark of a blood vessel in the external surface of the brain (Kielan-Jaworowska, 1986). A rhinal fissure can also be present in brains without appearing in the respective endocasts (Jerison, 1991), but is also expected that it is present only among taxa of the mammalian crown-group (Macrini et al., 2007b). Morevore, in ventral view, no feature of the forebrain can be observed.

Midbrain Region

The portion of the midbrain exposed on the dorsal surface of the endocasts corresponds mainly to the anterior and posterior colliculi. Although these structures may be exposed on the dorsal surface of the brain but does not appear in endocasts, even in living taxa (e.g. *Didelphis virginiana*, Dom et al., 1970; *Monodelphis domestica*, Macrini et al., 2007a; *Tenrec ecaudatus*, Bauchot and Stephan, 1967), the presence of casts of these structures is described for several extinct mammals e.g., *Labidolemur* (Silcox et al., 2011), *Kennalestes* (Kielan-Jaworowska, 1984, 1986), *Asioryctes* (Kielan-Jaworowska, 1984), and *Zalambdalestes* (Kielan-Jaworowska, 1984, 1986). However, the midbrain may not be visible in endocasts beacause it is covered by meninges, blood sinuses or due to the presence of a telencephalon posteriorly expanded or an anteriorly expanded cerebellum (Edinger, 1964).

Regarding to non-mammalian cynodonts, the only suggestion of midbrain exposition in a non-mammalian cynodont is for *Probainognathus* by (Quiroga 1980b, c), but according to Macrini et al. (2007b), the plesiomorphic condition for the mammalian crown-group should be the no exposure of the midbrain. For *Brasilitherium*, Rodrigues et al. (2013) found no evidence of exposure of the midbrain, considering it more likely to be covered by telencephalon, since the casts of the cerebral hemispheres appear to extend to the cerebellar region in dorsal view. Moreover, the median sulcus appears continuous along the the cerebral hemispheres region of *Braislitherium*, without a central wider depression, which is observed in *Probainognathus* (Quiroga 1980b), between the hemispheres casts at their most posterior portion.

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In *Riograndia* UFRGS-PV-596-T, there is no direct evidence of midbrain exposition, as well as a pineal region as suggested by Quiroga (1980b, c) for *Probainognathus.* The small lower space amid the lateral divergence between the hemispheres casts at their posterior end could have a little extension posteriorly, perhaps becoming wider and representing the midbrain dorsal exposition, however, according to our interpretation, the transversal vascular cast (tranversal ridge on the endocast) covers the surface between the posterior boundary of the cerebral hemispheres and the most anterior dorsal surface of the cerebellar region.

Hindbrain Region

The hindbrain region appears, in dorsal view, posteriorly to the slope of the cerebral hemipheres casts, just behind the transverse ridge (vascular cast, see below). The parafloccular cast, which fills the fossa subarcuata at the ascendant process of prootic, is posterior to the lateral continuity of the ridge, as structures that detach (0.72 mm at left side, and 0.74 mm at right side) from the lateral surface of this region of the endocast, constituting its widest point. From the parafloccular cast to the foramen magnum lateral contour is rounded. The dorsal surface is convex laterally, with no detail conspicuous (as could be, for example, a division of cerebellar hemispheres, which was indicated in *Probainognathus* (Quiroga, 1980a, b), although it is not clear in others non-mammaliaform cynodonts).

In any case, the dorsal surface has a slight bulging in the more central portion (approximately 1/3 of the endocast width on this region), which could correspond to the vermis of the cerebellum, as suggested for *Brasilitherium* (Rodrigues et al, 2013). The vermis, as well as the lateral cerebellar hemispheres are not characterized as specialized structures, functionally distinct from the rest of the cerebellum (Butler and Hodos, 1996). However, they are structures that can be marked in endocasts, and their presence within non-mamamlian cynodonts can be associated with the degree of development of the cerebellum in different taxa along the evolution of the mammalian lineage.Thus, although a vermis has not been identified in many descriptions of endocasts of non-mammalian cynodonts (Watson 1913; Hopson 1979, Quiroga 1979, 1980a, b, c, 1984), Kielan-Jaworowska et al. (2004) indicate its presence in the endocast of *Thrinaxodon*, represented by a protuberance in the posterior part of the dorsal surface, which fills a non-ossified area covered by the parietal and partly by the interparietal. However, Rowe

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et al. (1995) suggested that the dorsal space between the parietal and interparietal bones marks the position of a superior sagittal sinus. As metioned below, we interpretated the filling of this unossied zone as a vascular cast, so that the vermis could be dorsally exposed behind this mark, represented by the central bulging, probably partially covered by the blood vessels.

Regarding to the parafloccular casts, they are not so conspicuos as in *Brasilitherium* (Rodrigues et al. 2013). Although the degree of filling of the fossa subarcuata can be variable in different mammals (e.g. Sanchez-Villagra 2002), this feature, in endocasts, is associated with the degree of development of the paraflocculi lobes (Kielan-Jaworowska et al. 2004), which is associated with coordination, balance and vestibular sensory acquisition (Butler and Hodos, 1996). The parafloccular casts are absent in the endocasts of some non-therapsid cynodonts, often due to the poor ossification of this region of the skull (Kielan-Jaworowska et al., 2004), but the presence of prominent parafloculi is a plesiomorphic condition for eucinodonts, as is described for *Thrinaxodon* (Rowe, 1996) and *Nythosaurus* (Hopson 1979), and all other non-eucinodont mamaliaformes with endocasts described (e.g., Quiroga, 1979, 1980a, b, c, - indentified as "flocculi" in these works). Although less prominent than *Brasilitherium*, the lateral projection of the endocast of *Riograndia* UFRGS-PV-596-T seems to be similar to the parafloccular casts of *Probelesodon* and *Probainognathus*, but Quiroga (1980a, b) reconstitute this strucures as a more elongated projection, directed more posteriorly, as visible in the endocast itself of *Massetognathus* (Quiroga, 1979, 1980c), while in *Riograndia* they appear as a lateral prominence. In *Brasilitherium*, the parafloccular casts are perfectly defined and not so posteriorly directed (posterolaterally oriented away from the longitudinal axis of the endocranial cast by approximately 40°-60°, Rodrigues et al, 2013)

Vascular marks on the endocast

As already mentioned, the endocast of *Riograndia* presents a ridge, which passes through the endocast transversely, in dorsal view, just behind the cerebral hemipheres casts, and, in lateral view, is on a level most dorsal than the adjacent surfaces, i.e. the slope (24.10°) of the posterior end of the cerebral hemisphere casts, anteriorly, and the slope of the dorsal surface of the cerebellar region (59.24°) to the foramen magnum. The 2D slices of this portion of the skull in lateral and coronal planes shows (FIG. 5)

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that is the region of the unossified zone, delimited anteriorly and dorsally by the parietal, and posteriorly by the parietal and supraoccipital, , having, most of the space, a position more dorsal than the last. This space is a common feature of the non-mammaliaform cynodonts, a dorsal region of the supraoccipital roofed by the parietal and perhaps partly the interparietal. (e.g. Kemp, 2009) and, as mentioned above, it can left a mark on the endocast of non-mammlian cynodonts interpretated as a cerebellar vermis cast (e.g. Kielan-Jaworowska et al., 2004) or the sagittal sinus (Rowe et al. 1995).

In *Riograndia* UFRGS-PV-596-T, a transversal ridge is visible in dorsal view, whose posterior slope extends by 1.66 mm, but only in the medial portion, corresponding to about 1/3 of the width of the endocast in this region. and covering part of the posterior adjacent dorsal surface of the endocast, which forms a slope of 4.68 mm to the foramen magnum. In addition, this central structure has lateral continuity, 1/3 of the ridge each side in dorsal view, as a narrower transversal ridge, which extends to the lateral walls of the endocast. These transversal continuities of the ridge are slightly curved lateroposteriorly and extend ventrally approaching the parafloccular casts.

We suggest that the transversal ridge as a vascular cast, corresponding to the transverse sinus. In addiction, taking into account this transversal vascular cast, the most central portion, which extends backwards, and fills the unossified zone, it is interpretated here as the transverse sinus, which is a dorsal continuation of the prootic sinus (e.g. Wible & Hopson 1995), but there is no mark of the cast of this vessel on the lateral wall of the endocast of *Riograndia* UFRGS-PV-596-T. The prootic sinus cast was identified in the endocast of *Brasilitherium* (Rodrigues et al. 2013).

Relative size of the brain cast and their structures

The volume of the whole endocast is 445.45 mm³ and the volume of the endocast with the filling of the orbital vacuity above the pterygoids digitally removed is 404.20 mm³ (FIG 4D). Thus, from a body mass estimated in 71.15 g, we obtained four values of EQs, two using the equation of Jerison (1973), 0.22 and 0.20, and two through the equation of Eisenberg (1981), 0.30 and 0.33 (Table 2).

Comparatively, the EQs calculated for *Riograndia* are higher (0.20 without the filling of the orbital vacuity, and 0.22, endocast complete) than the EQ calculated

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through the same equation for the non-mammaliamorph cynodonts for *Thrinaxodon* (0.10; Jerison, 1973), *Diademodon* (0.14, Quiroga, 1980b), *Massetognathus* (0.15; Quiroga, 1979, 1980c); *Exaeretodon* (0.10 and 0.15; Quiroga, 1980b), *Probelesodon* (0.13 and 0.18; Quiroga, 1979, 1980c), *Probainognathus* (0.12 and 0.17; Quiroga 1980a, b). However, the values of *Diademodon* (0.21) calculated by Jerison (1973), and one of the values, based on another body mass estimate, calculated by Quiroga (1979, 1980c) for *Massetognathus* (0.22), are higher than the EQs of *Riograndia*. Among mammaliamorphs, the EQs of *Riograndia* are higher than *Brasilitherium* (0.15 and 0.22, for the equations of Jerison, 1973, and Eisenberg, 1981, respectively) and the value of the endocast complete (0.33) exceeds *Morganucodon* (0.32, Rowe et al. 2011), using the same equation to calculate the EQ. Furthermore, *Riograndia* presents a lower EQ compared to other mammaliaforms (Table 2).

Moreover, regarding to the relative size of the olfactory bulbs, Macrini et al. (2007b) used the ratio, in relation to the total volume of the endocranial cast, as a character in their phylogenetic analysis, defining the states discreetly, with reference to an average of 6%, and the primitive state corresponding to a size greater than or equal to 6%. However, the authors state that the plesiomorphic condition corresponds to the primitive mammalian morphology, and the derivate state were acquired convergently in those lineages and are presumably related to the reduced sense of smell in aquatic mammals (Negus, 1958; Pirlot and Nelson, 1978; Meisami and Bhatnagar, 1998; Macrini et al. 2007b).On the other hand, as highlighted by Rodrigues et al. (2013), among non-mammaliaform cynodonts there is a great variation, with the volume of the olfactory bulb casts corresponding to 5% of the endocranial cast in *Massetognathus*, 19.7% in *Exaeretodon*, 7.8% in *Probelesodon*, 6.4% in *Probainognathus* (Quiroga, 1980b), and 35.8% in *Brasilitherium* (Rodrigues et al., 2013), while the percent of endocast composed by olfactory bulb casts is 14.22 in the non-mammalian mammaliaform *Hadrocodium* (Macrini 2006). In *Riograndia*, the volume of the olfactory bulbs region (with the ventral contour digitally delimited) is 79.49 mm^3, corresponding to 19.67% of the volume of the endocast without the the rocky filling of the orbital vacuity.

Besides, the area of the dorsal exposure of the olfactory bulbs region is 19.4% of the dorsal surface of the whole endocast, being the regions of the cerebral hemispheres and cerebellum, 66.7% and 13.9% of the dorsal surface of the endocast, respectively.

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The comparison with other cynodont taxa, regarding to the area of dorsal exposure of the endocast and their regions related to the skull length is showed in the Table 3.

The results of the approach proposed here (evolving three non-prozostodontia cynodonts, three prozostodontian non-mammaliaforms, one basal mammaliform, and one crown mammalian) are summarized on the Table 3, are remarked separately, for the endocasts and the different regions of the brain identified in dorsal view.

The whole endocast

Related to the skull length, the endocast length is, in general, relatively larger the smaller the skull, corresponding to a larger nasal cavitity (related to the legth of the endocranial space) the larger the size of the skull. This ratio is better adjusted in a linear regression with the exception of *Therioherpeton*, which has the relatively longer endocast among the taxa sampled, as well as *Riograndia* and *Brasilitherium*, in which the relatively more elongated nasal cavity. Moreover, regarding to the aspect ratio taking the maximum width and length, the relationship is also proportional to the size of the skull, so that smaller skulls have relatively wider endocranial space. However, in this case, a phylogenetic influence may be suggested, since a regression line with a high coefficient of determination (R^2 = 0.98) can be obtained for non-mammaliaform cynodonts, while *Hadrocodium* (the smallest sample skull) and *Vincelestes* (with a skull of intermediate size between the specimens compared, similar to *Probainoganthus*) present relatively wider endocasts (see graphic in the Appendix 1).

The olfactory bulbs region

The length of the olfactory bulbs region related to the skull length is also relatively larger the smaller the skull (R^2=0.93) Thus, the bulbs are relatively longer in relation to the skull the longer the relative length of the endocranial space, but, regarding to this relantionship, *Brasilitherium* stands out above the regression line, which can corroborate the suggestion of Rodrigues *et al*. (2013) that this taxon presents the largest relative size of olfactory bulbs within cinodonts, including mamaliaformes, with endocasts described.

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In addiction, *Brasilitherium* presents the highest value for the ratio of the area of the olfactory bulbs region on dorsal view and the area of the dorsal surface of the whole endocast (0.35). In effect, this ratio appear to be, in general, higher for non-mammaliaform cynodonts successively more related to mammals, although the value of *Probelesodon* (0.29) is higher than *Probainognathus* (0.22) and of *Therioherpeton* (0.34) is higher than *Riograndia* (0.30). Among mamaliaformes, this same ratio becomes much lower in *Hadrocodium* and even lower in *Vincelestes*.

The cerebral hemispheres region

The ratio of the area of the cerebral hemispheres region on dorsal view and the area of the dorsal surface of the whole endocast, seem to present a clear change among mammaliaformes, in which the relative area of the cerebral hemisphere region in dorsal view is larger, being *Vincelestes* higher than *Hadrocodium*. On the other hand, *Riograndia* present the lowest value of this ratio. Anyway, the non-mammlialiaform cynodonts taxa in general presents similar values fot this ratio, without a significant correlation, neither with the phylogenetic proximity to the mammals nor with the length of the skull.

However, it is interesting to note that among non-mammalian cynodonts there seems to be a trend of increase of the width of the cerebral hemispheres region according as the taxa are more closely related to mammals, indicated by the values of the aspect ratio (maxium width/length): *Massethognathus*, 0.35; *Probelesodon*, 0.32; *Probainognathus*, 0.57; *Therioherpeton*, 0.80; *Riograndia*, 0.84; *Brasilitherium*, 0.63; and *Hadrocodium*, 1.4 (the only one wider than long), while this ratio for the Theria *Vincelestes* is 0.59. Regarding to the non-mammliaforms cynondont, it is worth noting the aspect ratio of the cerebral hemipheres region of *Brasilitherium*, lower than *Therioherpeton* and *Riograndia*, as well as *Probelesodon*, which present the lowest value among all taxa, although close to the value of *Massetognahtus*, do not fit in a phylogenetic sequence among mammaliamorphs more closely related to mammals. There is also a relantionship between the aspect ratio of the cerebral hemipheres region and the skull length, so that the width is larger the smaller the skull, with the sole excpetion of *Riograndia*, in which the cerebral hemisphere region is relatively wider than *Therioherpeton,* but the specimen of the last taxa is smaller.

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Moreover, a variation in the morphology of the cerebral hemispheres region can be represented by the ratio between the width measured near the posterior limit of the region and the width taken at half its length: *Massethognathus*, 1.24; *Probelesodon*, 1.22; *Probainognathus*, 1.29; *Therioherpeton*, 1.59; *Riograndia*, 1.27; *Brasilitherium*, 1.59; and *Hadrocodium*, 1.00; and *Vincelestes*, 0.72. Following a phylogenetic sequence, these ratios are similar among non-mammaliamprph cynodonts and *Riograndia*, becomes higher in *Therioherpeton* and *Brasilitherium*, and then decays in *Hadrocodium* and – still more - in *Vincelestes*.

The cerebellar region

The cerebellar region does not present a correlation with skull length or phylogenetic position regarding to the aspect ratio and related to the area of the endocast. Even so, a variation that may have some phylogenetic significance is observed with respect to the cerebellar width comparativelly to the width of the cerebral hemispheres at the half of their length: *Massetognathus*, 1.56; *Probelesodon*, 1.43; *Probainognathus*, 1.16; *Therioherpeton*, 1.68; *Riograndia*, 1.45; *Brasilitherium*, 1.73; and *Hadrocodium*, 0.96; and *Vincelestes*, 0.71. Following a phylogenetic sequence, the ratios decay among non-mammaliamprph cynodonts, then increase in *Therioherpeton*, *Riograndia* (the lowest value within non-mammaliaform mammaliamorphs) and *Brasilitherium*, and decays again in *Hadrocodium* and - more - in *Vincelestes*.

DISCUSSION

The study of the endocast of *Riograndia* reveals some important features. Within the characters commonly inditified in the endocasts of the non-mammlian cynodonts, it is interting the not so much conspicuous paraflocculars casts. The visision between cerebral hemispheres is also lesse visible than in *Braslitherium* but the aspect ratio of thos region clearly indicate that it is not narrow as other non-mammliamorph cynodonts, so that the not deep median sulcus must be due to a single concavity in the internal surface of the frontal and parietal bones without a bony median ridge, but should not represents a primitive condition (the actual brain must have been divided in two cerebral hemispheres in this region).

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Furthermore, It should be noted the marks suggested as vessel casts, since there is not mention of the transverse sinus for endocasts of non-mammliaform cynodonts in previous works (Jerison 1973; Bonaparte, 1966; Quiroga, 1979, 1980a, b, c, 1984; Rodrigues et al. 2013), although it is the continuation of the prootic sinus, whose cast was identified in the endocast of *Brasilitherium* (Rodrigues et al., 2013). In addiction, the ridge indetified as the transverse sinus presents a clear continuity with the filling of the unossified zone (dorsal region of the supraoccipital roofed by the parietal and perhaps partly the interparietal) at the more central portion of the dorsal surface of the endocast. This mark in endocasts of non-mammlian cynodonts is interpretated as a cerebellar vermis cast (e.g. Kielan-Jaworowska et al., 2004). However, due to the continuity with a vascular mark, we interepretate the filling of the unossified zone (dorsal region of the supraoccipital roofed by the parietal and perhaps partly the interparietal) as the sagittal sinus, following (Rowe et al. 1995), although the vermis could have been present in *Riograndia*, covered by the vessel and extending posteriorly as central bulging visible on the dorsal surface of the cerebellar region, similar to the morphology described for the endocast of *Brasilitherium* (Rodrigues et al., 2013).

Regarding to the quantititive approches, the EQs calculated can fit on a evolutionary sequence as it would be exepected for *Riograndia* due to its phylogenetic position related to other taxa with EQs reported, taking into account the data of mammaliaform taxa, although the values are higher than the EQ calculated for *Brailitherium* by Rodrigues et al. (2013). This sequence can be corroborated by the most data of the non-mammaliamorph cyndonts, but the reported values for *Diademodon* reported by Jerison (1973) and, among the taxa studied by Quiroga (1979, 1980a, b, c, 1984), at least the EQ of *Massetognathus*, disregarding all EQs calculated from one of the equations that the last author used to estimate body mass. These EQs appears overestimated, not only by the phylogenetic relationship of the taxa, but by the morphology observed, as noted by Rodrigues et al. (2013, see FIG. 10). We can artibute these overstimated EQs due to inaccuracy in endocast volume measurement (considering that the works used natural endocasts, extracted form the skull, although posterior studies can use CT scan), as well as the body mass estimates from The mass estimates from little or a single factor (usually skull length), for specimens of different size (and hence, body proportions). The equation that we used to estimate the body mass

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of *Riograndia* seems to be more realistc, since the equation is used for mammaliaforms and based on insectivores, being all taxa of small size and similar body design.

Anyway, in addition to the finding of relative increase in overall brain size, we consider it important to seek to identify which structures are responsible for volumetric growth in general and how these variables can be related. In this sense, in spite of the limitations of sampling, as well as possible inaccuracies of measurements from photographs of other works and the restriction itself because only the surface dorsally exposed in 2D is being evaluated, at least the three main regions (i.e. olfactory bulbs, cerebral hemispheres and cerebellum) dorsally visible could be studied comparatively with respect to the relative size. In fact, the methodological limitations do not seem to us greater than the inaccuracies of volumetric studies with extinct taxa and mass estimates.Due to the covariation of these strucutures within the taxa, several different ratios were calculated in order to isolate variables, and the results obtained must indicate some neurological adaptations along the evolution of the lineage of the cynodonts, as pointed out below.

Thus, we can suggest a sequence of changes in cynodonts successively more closely related to mammals, from the primitive pattern represented by *Massetognathus* and *Probelesodon*. The changes suggested evolve basically increasing of regions of the endocast in relation to both the whole endocast and the skull:

1) The increase in the relative size of the olfactory bulbs among non-mammalian cynodonts - a continuous increase, but more pronunced within Prozostodontia;

2) The increase in the relative width of the cerebral hermispheres at the posterior portion – a continuous increase from *Probainoganthus* to mammaliaformes;

3) The increase of the relative width of the whole cerebral hemispheres region - mainly from Prozostodontia;

4) The increase of the width of the cerebellar region related to the skull (not necessarly relative to the whole endocast because of the growth of the regions mentioned above) - from Prozostodontia.

Thus, taking into account the different ratios showed in the Table 3, we can suggest the trend of increase if the olfactory bulbs, being the appex represented by

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Brasiliterium. The value for the relative area of the dorsal surface of this region, higher in *Probelesodon* than *Probainognathus*, could be explained by the fact that the width of the cerebral hemispheres region is increased from *Probainognathus* to other taxa more clessely related to mammaliaforms, as indicated by the aspect ratio of the cerebral hemispheres region, changing from 0.35 in *Massethognathus* and 0.33 *Probelesodon* to 0.57 in *Probainognathus,* and so 0.80, in *Therioherpeton* and 0.84 in *Riograndia Brasilitherium*, 0.63. Indeed, it is clear the increase of the width of the cerebral hemispheres region, from a narrow and apparently tubular morpholgy, without division in two hermispheres on the endocast, the condition represented by *Massethognathus* and *Probelesodon* (although, in the last taxon, already appears the increase in the size of the bulbs) to a morphology with clear divided cerebral hemipheres, and increasing the width as the taxa are closer to mammals.

Still regarding to the aspect ratio of the cerebral hemispheres, it is intersting the value of *Brasilitherium*, the lowest among non-mammliaform mammaliamorphs, indicating elongated cerebral hemispheres. However, this taxon, in addition to having higher values than the non-mammliamorph cynodonts, presents the lowest values related to the dorsal exposition of the cerebellar region, which can suggest a posterior elongation covering the cerebellum and not a condition near to the narrow brains of the non-mammalimorphs cynodonts. Moreover, *Brasilitherium* presents a conspicuous division between the cerebral hemispheres (see Rodrigues et al., 2013) and the clear increase of the width of the posterior portion of this region. In this regard, the ratio between the width of the cerebral hemispheres region near to the posterior limit, and the width of the region at the half of the length, indicate values about 1.2 for *Massetognathus* and *Probelesodon*, increasing to near of 1.3 in *Probainognathus* and *Riograndia*, and reaching about 1.6 in *Therioherpeton* and *Brasilitherium*. Within the mammaliaforms, this ratio is 1.0 in *Hadrocodium*, evidencing a relative growth in width at the median portion of the cerebral hemispheres, until it exceeds the width of the posterior limit in *Vincelestes* (0.7).

In view of the foregoing, we can suggest an evolutionary sequence from the narrow brains the non-mammliamorphs cynodonts with more basal position related to mammals, starting by a gradual increase of the olfactory bulbs, wich must be associated to the changes in the nasal cavity evolving the closure and posterior extension of the ossified secondary palate, as well as the complex of the nasal turbinates (e.g. Hillenius,

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1994; Kielan-Jaworowska et al. 2004; Ruf et al 2014). These changes, in turn, are associated to the breathing with an endothermic metabolism (e.g. Hillenius, 1992, 1994), at the same time that they enable an improvement in olfaction sense. Thus, the selective pressure to improve the olfaction seems to be an important factor in the beginning of the evolution of the mammalian brain, being this sense, as mentioned by Jerison (1973), a very significant feature to the nocturnal habits of the small sized animals (i.e. non-mammalian mammaliamorphs).

This study of *Riograndia*, as well as the taxa using for comparison, corroborate this trend, being the maximum development of the olfactory bulbs related to the endocast represented by *Brasilitherium*, just the taxon that appears as the sister-group of mammaliaforms in phylogentic analysis (e.g. Bonaparte et al 2003; Martinelli & Rougier 2007; Abdala 2007; Liu & Olsen, 2010.).Among mammliaforms, the relative size of olfactory bulbs seems to decay, which must be associated to the enlargement of the cerebral hemispheres, which corresponds to the first and second of the three "pulses" indicated for Rowe et al. (2011) in the evolution of the mammal brain, represented by *Morganucodon* and *Hadrocodium*, respectively.

As already hypothesized by Rodrigues et al (2013), it seems to have been a neurological evolution of the mammlian lineage starting by the improvement of the sensory receptor system (adaptations on the nasal cavity) and increase of the primary processing strucures (i.e. olfactory bulbs), leading to further development of the "superior" structures of the neurological system, for sensorial processing and integration in the brain (i.e. cerebral hemispheres). After, following the sequence indicated by Rowe et al. (2011), the third pulse is represented in the origin mammalian crown-group, associated to other adaptations of olfactory reception: the ossified ethmoturbinals, a cribriform plate and a rigid support in the nasal cavity to the olfactory epithelium receptor (whose genes exceed by about one order of magnitude the amount existing in the genome of most other vertebrates; Niimura, 2009).

Regarding the evolution of the cerebral hemispheres, we can suggest an initial increase in width at the posterior portion, possibly associated to the increease in width of the cerebellum. This trend can be not the same indicated by Kemp (2009), regarding to the size of the cerebellum, which is represented already by cynodonts of basal position related mammalimorphs. This initial cerebellar development mentioned by Kemp (2009) can be reflect the improvement of the neuromuscular control associated to

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a complex occluding posterior teeth, jaw musculature to a bite forceful and precise, more upright hindlimbs and mobility of the shoulder girdle. In turn, the increase of the cerebellar width among mammaliamorphs, mainly in non-mammlian mammaliforms, can be associated to the adaptations of the auditory system, reflected by the development of the cochlea and the petrosal promontorium, at least from *Brasilitherium* (Rodrigues et al. 2013).

Indeed, the reduced number of specimens compared does not allow an accurate distinction between phylogenetic influence and some allometric relation with skull size, which would require studies involving ontogenetic series. It would be also interesting because there is some correlation between the closer phylogenetic relationship with mammals, and the small body size, since the neurological and sensorial adaptations of the mammalian lineage among non-mamlian cynodonts are also concomitant with the evolutionary process of the miniaturization in Mesozoic cynodonts.

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Length of the endocast, measured from the anterior limit of the dorsal surface of the olfactory bulb to the foramen magnum	17.67 mm
Maximum width	6.88 mm
Maximum height, measured from the ventral limit of the hypophyseal cast to the dorsal surface above it	8.71 mm
Maximum height, corresponding to the distance between the ventral limit of the hypophyseal cast and the most dorsal point of the olfactory bulb casts)	10.08 mm
Volume of the whole endocast	445.44 mm^3
Volume of the endocast with the rocky filling of the orbital vacuity digitally removed	404.20 mm^3
Lenght of the dorsal surface of the two olfactory bulb casts	7.10 mm
Maximum width of the olfactory bulb casts together	4.12 mm
Estimated volume for the region of the olfactory bulb region *	79.49 mm^3
Width of the endocast at the limit between the olfactory bulb casts and cerebral hemispheres region	3.90 mm
Lenght of the dorsal surface of the region of the cerebral hemisphere casts	7.53 mm
Maximum width of the dorsal surface at the region of the cerebral hemisphere casts	5.26 mm
Anteroposterior lenght of the dorsal surface (slope) of the cerebellar region	4.68 mm
Anteroposterior lenght of the cerebellar region taking a horizontal line	1.90 mm
Width of the cerebellar region (between the paraflocular casts)	6.20 mm
Distance between the apex of the left parafloccular cast and the adjacent lateral surface	0.72 mm
Distance between the apex of the right parafloccular cast and the adjacent lateral surface	0.74 mm
Lenght of the ventral opening of the cavum epiptericum (left side)	5 mm

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*Values based on the ventral contour and anterior limit estimated for the olfactory bulbs by removing the infilling of the orbital vacuity in order to make the ventral contour becomes similar to the dorsal contour.

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Taxon	EQ[1]	EQ[2]	Source
Thrinaxodon,	0.1		Jerison (1973)
Diademodon	0.21		
	0.14		Quiroga (1980b)
Massetognathus	0.15; 0.22		Quiroga (1979, 1980b)
Exaeretodon	0.10; 0.15		Quiroga (1980b)
Probelesodon	0.13; 0.18		Quiroga (1979, 1980b)
Probainognathus	0.12; 0.17		Quiroga (1980a, b)
Riograndia	0.22	0.33	This study
	0.20	0.30	
Brasilitherium	0.15	0.22	Rodrigues et al. (2013XX)
Morganucodon		0.32	Rowe et al. (2011)
Hadrocodium		0.49	
Obdurodon		1.00	Macrini et al. (2007a)
living monotremes		0.75-0.89	
Triconodon		0.49	Kielan-Jaworowska (1983)
Chulsanbaatar		0.55	
Kryptobaatar		0.71	Kielan-Jaworowska and Lancaster (2004)
Ptilodus		0.49	Kielan-Jaworowska (1983)
Vincelestes		0.37	Macrini et al. (2007b)
Kennalestes		0.36	Kielan-Jaworowska (1984)
Asioryctes		0.56	
Zambdalestes		0.7	
didelphids		0.5-1.09	Eisenberg and Wilson (1981)

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Ratios	length/skull length				area/ endocast area			maximum width / length				width/CH mid width		
Taxa (skull lenght)	**E**	**OB**	**CH**	**CR**	**OB**	**CH**	**CR**	**E**	**OB**	**CH**	**CR**	**OB**	**CH post.**	**CR**
Vincelestes (63 mm)	0,545	0,169	0,322	0,061	0,194	0,667	0,139	0,474	0,836	0,589	3,034	0,546	0,727	0,716
Hadrocodium (12 mm)	0,627	0,219	0,322	0,096	0,230	0,603	0,167	0,768	1,094	1,488	4,504	0,492	1,001	0,958
Brasilitherium (38 mm)	0,450	0,182	0,265	0,038	0,352	0,563	0,085	0,402	0,625	0,626	4,738	1,048	1,585	1,730
Riograndia (35 mm)	0,443	0,169	0,188	0,082	0,300	0,443	0,256	0,405	0,668	0,839	2,188	0,912	1,274	1,448
Therioherpeton (31 mm)	0,680	0,283	0,346	0,059	0,336	0,538	0,126	0,429	0,807	0,797	4,927	1,317	1,593	1,682
Probainognathus (65 mm)	0,583	0,158	0,323	0,131	0,222	0,556	0,223	0,316	0,793	0,570	1,267	0,899	1,285	1,161
Probelesodon (120 mm)	0,406	0,127	0,267	0,034	0,287	0,576	0,137	0,223	0,575	0,325	3,166	1,073	1,221	1,430
Massetognathus (95 mm)	0,517	0,125	0,303	0,109	0,217	0,570	0,213	0,260	0,724	0,353	1,235	1,048	1,237	1,558

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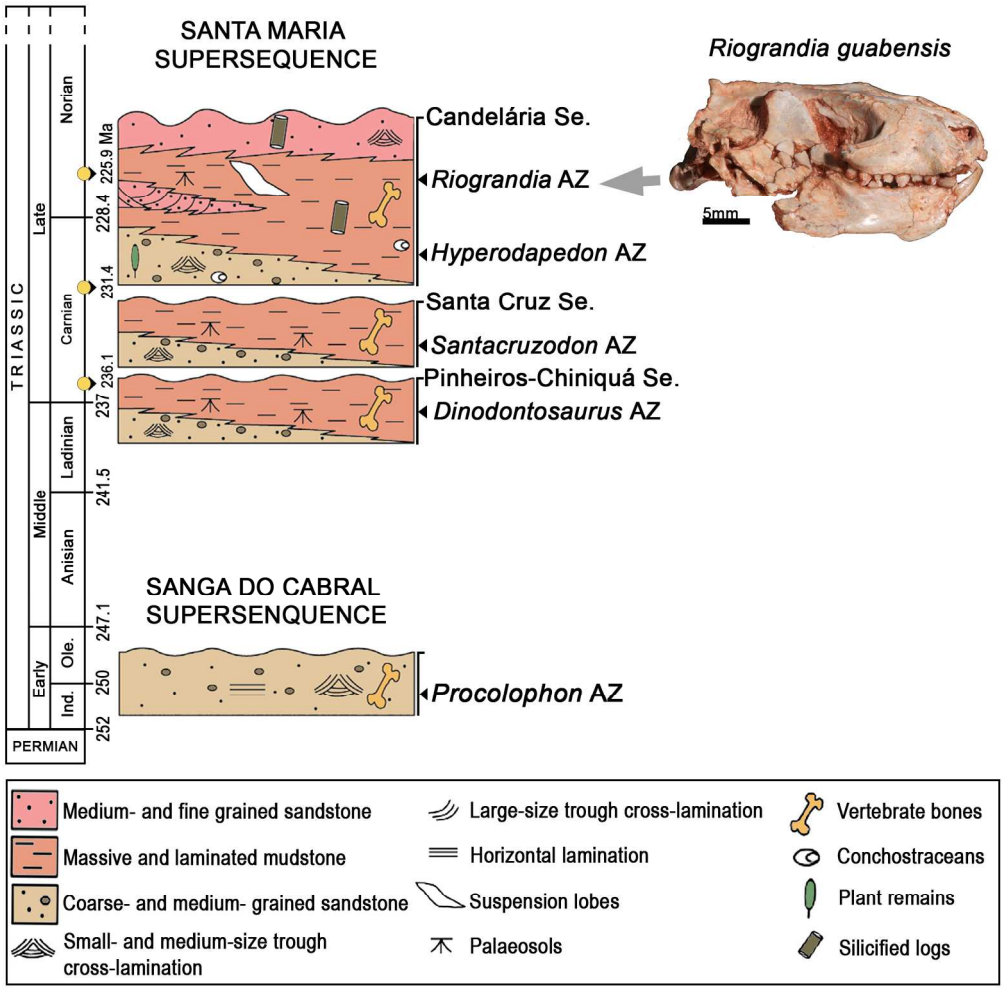

Figure 1 - Chronoestratigraphy of the Triassic of Southern Brazil with vertebrate biozones (modified from Horn et al. 2014).

179x178mm (300 x 300 DPI)

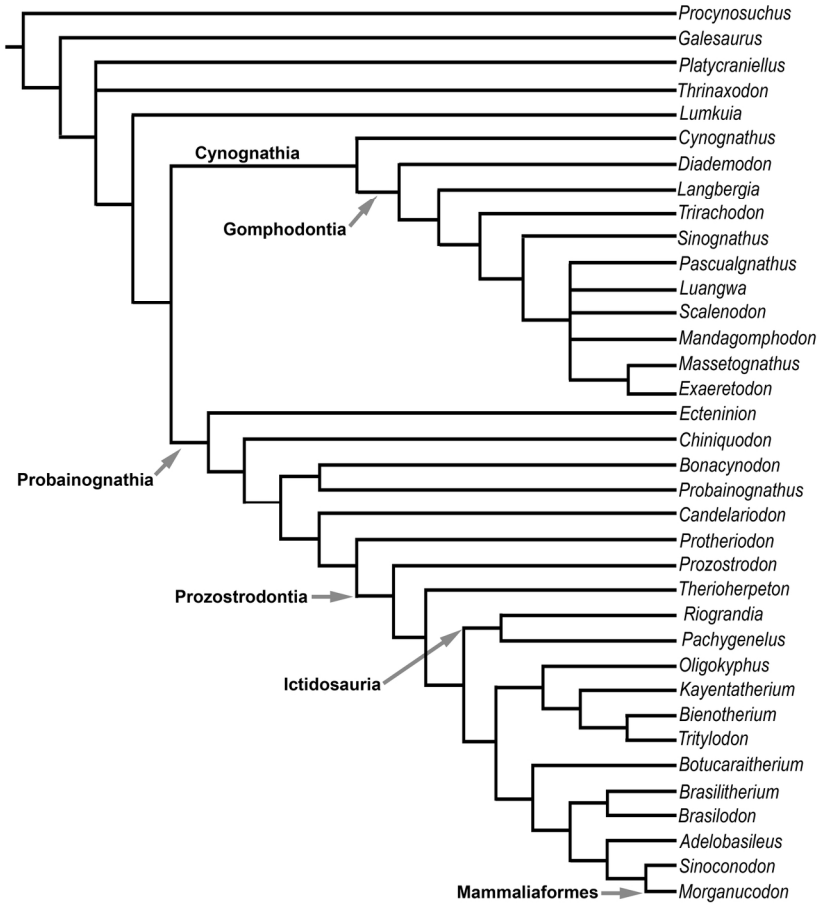

Figure 2 - Cladogram showing phyogenetic relationship among cynodont taxa, inluding Mammalia, according to the analysis of Martinelli et al. (in press).

166x152mm (300 x 300 DPI)

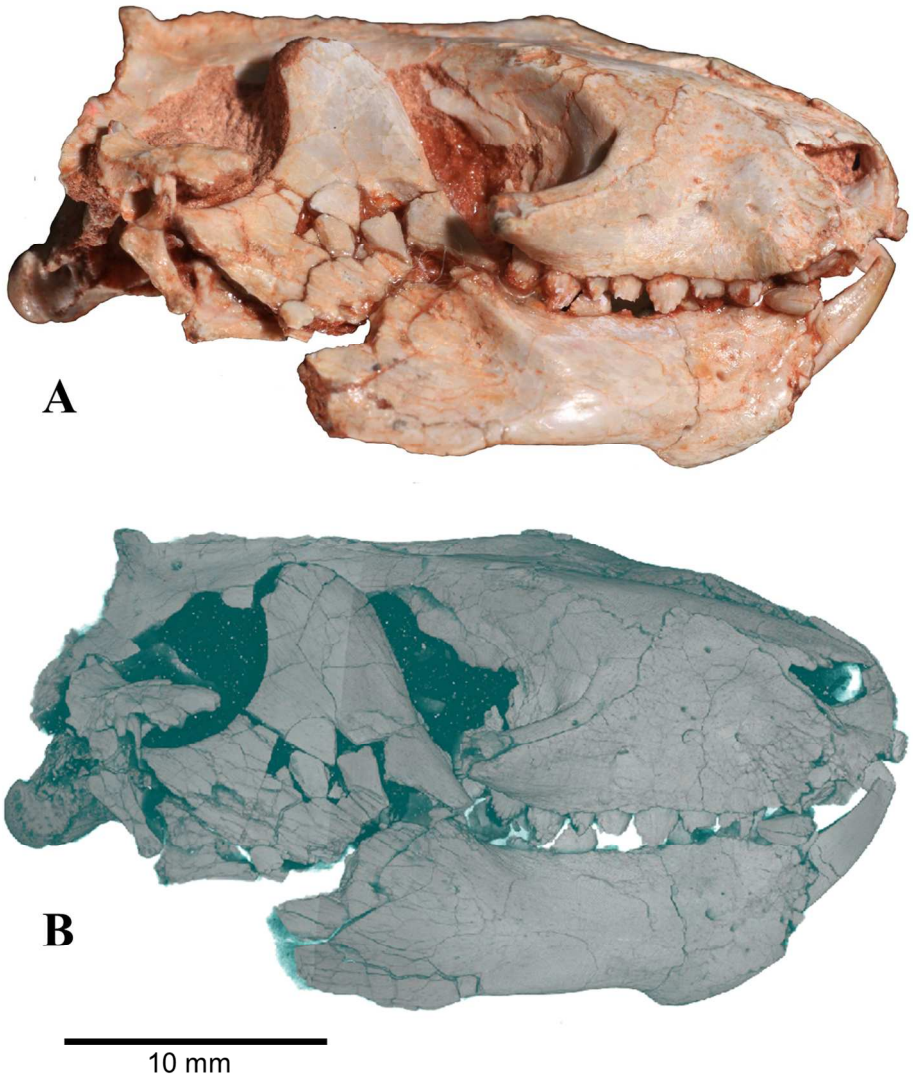

Figure 3 – The specimen *Riograndia guaibensis* UFRGS-PV-596-T in lateral view. Photograph (A) and the 3D image from CT scan (B).

255x310mm (150 x 150 DPI)

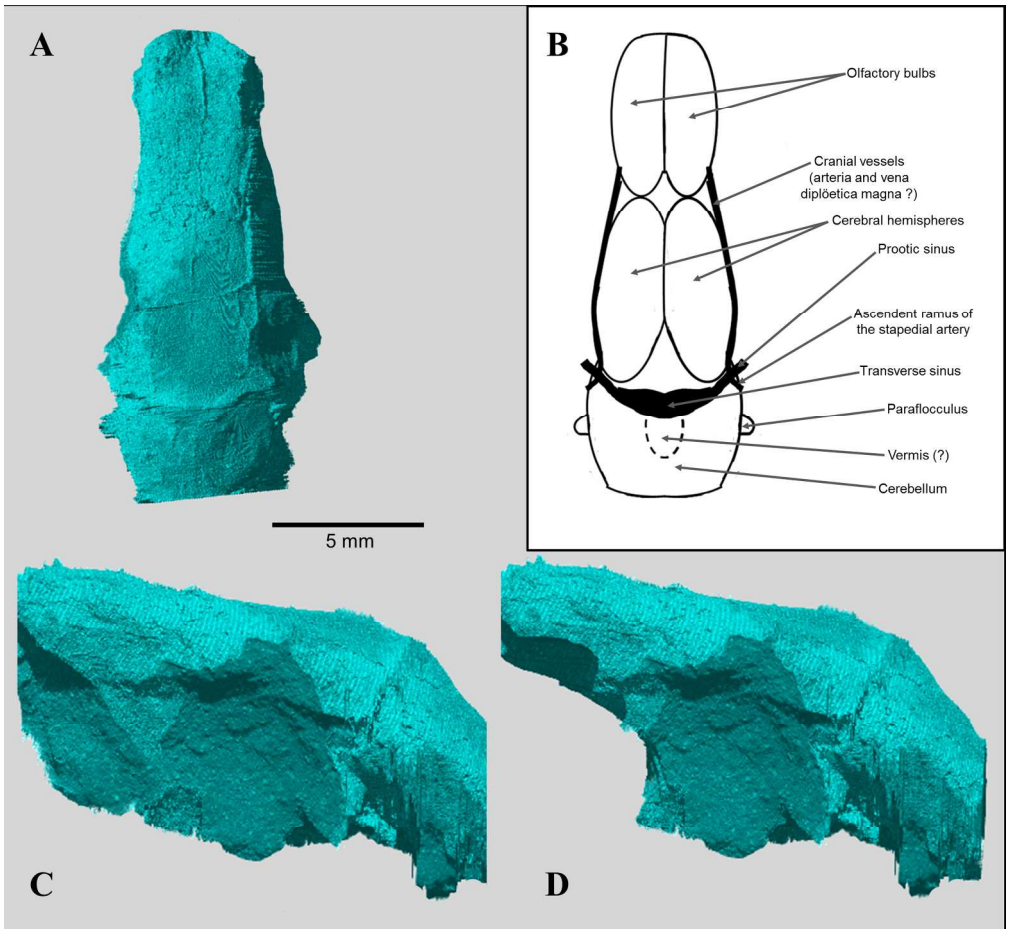

Figure 4 – Digital enndocast of *Riograndia* UFRGS-PV-596-T in dorsal (A) and lateral view (C and D) and a schematic reconstruction of the dorsal view in B. with The filling of the orbital vacuity digitally removed in D.

369x340mm (150 x 150 DPI)

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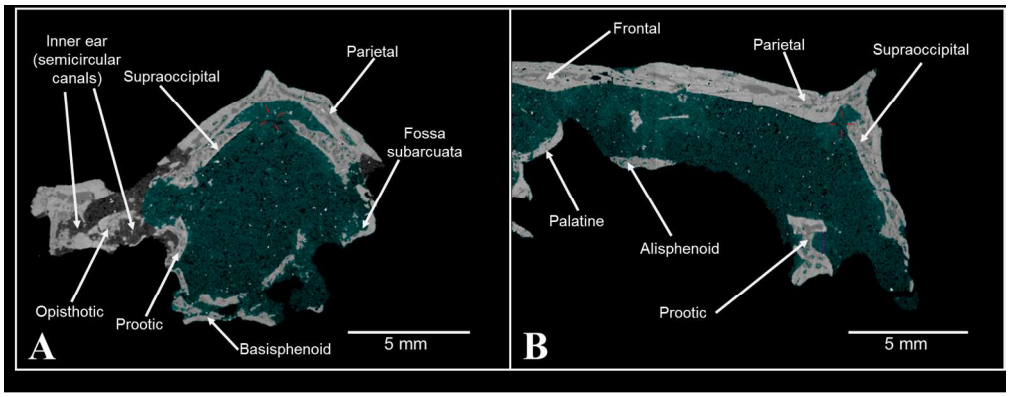

Figure 5 – Slices in coronal (A) and sagittal planes showing the unossified zone (the red mark corresponds to the same point in the two planes).

323x125mm (150 x 150 DPI)

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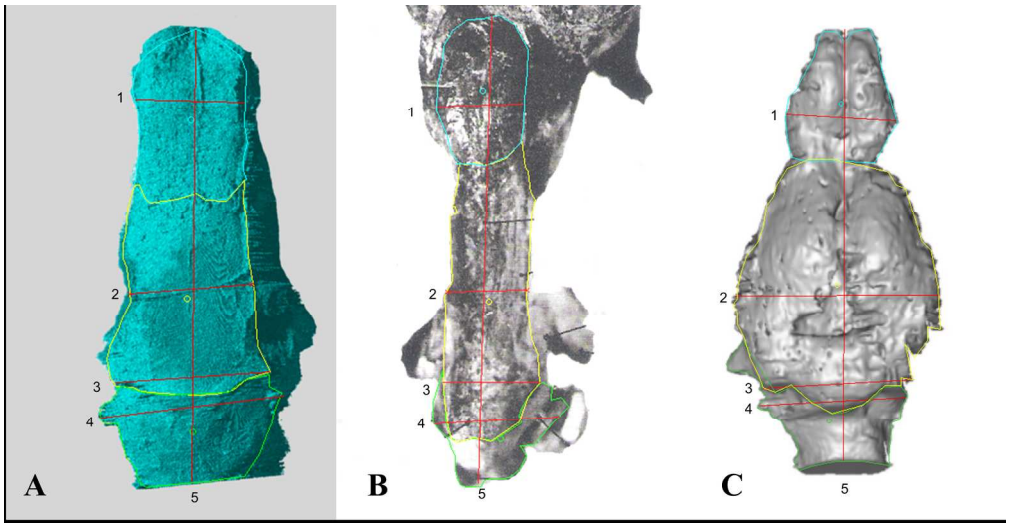

Figure 6 – Examples of the measurements taked on the dorsal surface of the endocast of *Riograndia* (A) and other taxa from images of other works, *Probelesoson* (modified from Quiroga, 1979; B) and *Vincelestes* (modified from Macrini, 2006; C): areas of the regions of olfactory bulbs region (line blue), cerebral hemispheres (line yellow), and cerebellum (line green); maximum width of the olfactory bulbs region (1), width of the cerebral hemispheres region at the half of the length (2), width of the cerebral hemispheres region near to the posterior limit (3), width of the cerebellar region (4), and the endocast length (5).

397x205mm (150 x 150 DPI)

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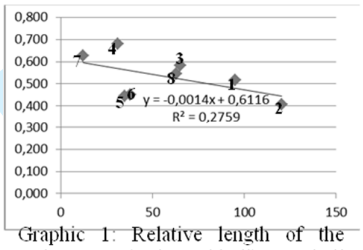
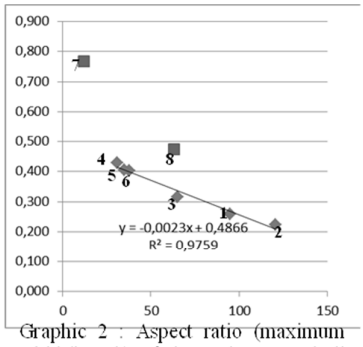
Appendix 1

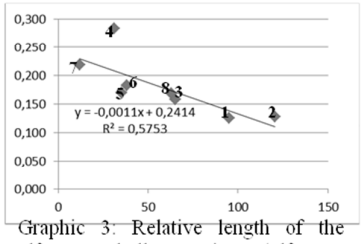
Graphics showing relationships between the ratios of t he measurements of the endocast and skull for different cynodont taxa (1, *Massetoganthus*; 2, *Probelesodon*; 3, *Probainognathus*; 4, *Therioherpeton*; 5, *Riograndia*; 6, *Brasilitherium*; 7, *Hadrocodium*; 8, *Vincelestes*).



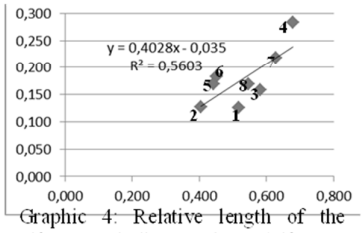
Graphic 1: Relative length of the endocast (endocast/skull) x skull length.



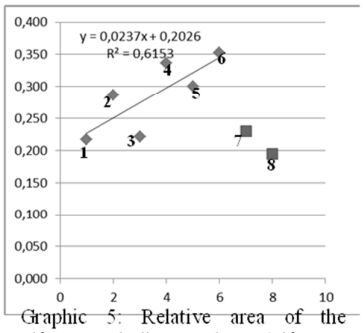
Graphic 2: Aspect ratio (maximum width/length) of the endocast x skull length (7 and 8 excluding from the trendline).



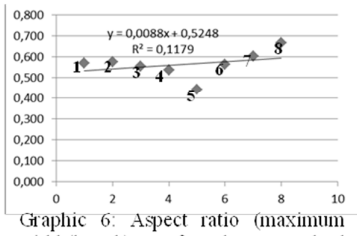
Graphic 3: Relative length of the olfactory bulbs region (olfactory bulbs/endocast) x skull length.



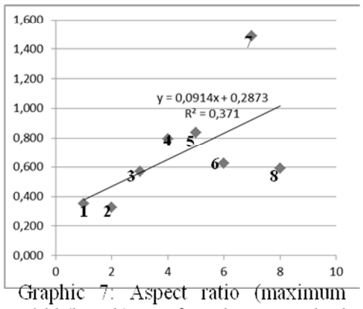
Graphic 4: Relative length of the olfactory bulbs region (olfactory bulbs/endocast) x relative length of the endocranial space (endocast/skull).



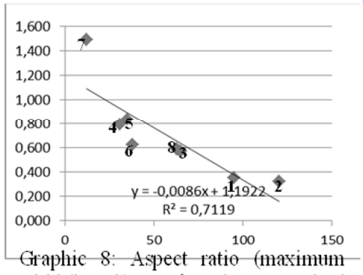
Graphic 5: Relative area of the olfactory bulbs region (olfactory bulbs/endocast) x phylogenetic proximity to mammals (7 and 8 excluding from the trendline).



Graphic 6: Aspect ratio (maximum width/length) of the cerebral hemispheres region x phylogenetic proximity to mammals.



Graphic 7: Aspect ratio (maximum width/length) of the cerebral hemispheres region x phylogenetic proximity to mammals



Graphic 8: Aspect ratio (maximum width/length) of the cerebral hemispheres region x skull length

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