The oldest known amniotic embryos suggest viviparity in mesosaurs

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The earliest undisputed crown-group amniotes date back to the Late Carboniferous, but the fossil record of amniotic eggs and embryos is very sparse, with the oldest described examples being from the Triassic. Here, we report exceptional, well-preserved amniotic mesosaur embryos from the Early Permian of Uruguay and Brazil. These embryos provide the earliest direct evidence of reproductive biology in Paleozoic amniotes. The absence of a recognisable eggshell and the occurrence of a partially articulated, but well-preserved embryo within an adult individual suggest that mesosaurs were viviparous or that they laid eggs in advanced stages of development. Our finds represent the only known documentation of amniotic embryos in the Paleozoic and the earliest known case of viviparity, thus extending the record of these reproductive strategies by 90 and 60 Ma, respectively.

Keywords: Mesosauridae; Early Permian; Gondwana; reproductive biology; oldest amniotic embryos; viviparity

Introduction

The origin of the amniotic egg and reproductive strategy is a key evolutionary novelty in vertebrate history (Carroll 1969), being inextricably associated with exclusively terrestrial modalities of reproduction. Nevertheless, no evidence of the amniote reproductive biology has been recorded from the Paleozoic, despite many years of intensive collecting. A partial explanation for this absence is that eggs and embryos have low fossilisation potential (Delfino and Sánchez-Villagra 2010). However, this explanation is insufficient because eggs with strongly mineralised outer membranes are common in the fossil record (Mikhailov 1991). A complementary hypothesis suggests that amniotic egg may have appeared in ancestors with extensive embryo retention that laid eggs with an unmineralised outer membrane that allowed embryo–mother interaction (Stewart 1997; Laurin and Girondot 1999). Alternatively, retention of the eggs/embryos may have facilitated the development of viviparity in early amniotes, as well as oviparity with embryo retention, which includes what was called 'ovoviviparity' in the older literature (Blackburn 1994). However, no Paleozoic fossil supports either of these hypotheses. The oldest example that directly suggests viviparity in amniotes is in the sauropterygian *Keichousaurus hui* from the Middle Triassic of China (Cheng et al. 2004). Viviparity may have

been a widespread reproductive behaviour among other sauropterygians, as suggested by isolated and clustered possible embryos of the nothosaurid *Lariosaurus* (Renesto et al. 2003). On the other hand, other examples of viviparity come from marine taxa, as documented by ichthyosaurs (Deeming et al. 1993), mosasauroids (Caldwell and Lee 2001), choristoderans (Ji et al. 2010) and plesiosaurs (O'Keefe and Chiappe 2011), and from terrestrial lizards (Wang and Evans 2011). The two latter examples are especially relevant, since they share some similarities with that we describe herein for the Early Permian mesosaurs, a clade of amniotes that has been considered as the basalmost sauropsids (Laurin and Reisz 1995) or close to parareptiles (Modesto 1999).

Here, we describe an isolated mesosaur embryo and a single embryo inside an adult mesosaur, which is presumed to be a pregnant female. We also describe several specimens that show associations between an adult and an embryo (or possibly a neonate) and suggest hypotheses that may explain this association. All these specimens belong to the aquatic Early Permian amniote *Mesosaurus tenuidens*. Our discovery fills in part a gap of 90 Ma in our knowledge of extinct amniotic reproductive strategies, from Late Carboniferous to the Middle Triassic when the earliest known viviparous reptiles are recognised in the fossil record (Bonaparte and Vince 1979; Renesto et al. 2003).

Depositional environment and preservation

The depositional conditions suggested for where the specimens were found, include waters poorly oxygenated and highly saline, to allow precipitation of gypsum crystals (Piñeiro et al. forthcoming). The mesosaur community living in that habitat was thus very peculiar, exhibiting an extremely low diversity that comprises mesosaurs, pygocephalomorph crustaceans and the trace fossil *Chondrites*. This may explain the spectacular preservation of the fossils including soft tissues, typical of Konservat-Lagestätten (Piñeiro 2006; Piñeiro et al. forthcoming).

Materials and methods

This study is based on several *Mesosaurus* specimens: FC-DPV (Facultad de Ciencias, Departamento de Paleonto-logía de Vertebrados) 2504 (Figures 1 and 2), an almost complete, fully articulated, very small individual (inferred total length less than 150 mm), included in a very small slab; MCN-PV (Museu de Ciências Naturais, Seção de Paleovertebrados) 2214 (Figure 3), a medium-sized, articulated adult *Mesosaurus* specimen consisting of part and counterpart of the trunk region between pectoral and pelvic girdles carrying at least one embryo in utero; and at least 26 mostly disarticulated small specimens (late embryos or hatchlings) found associated with large, partially articulated or disarticulated mesosaur skeletons (Figures 4 and 5; Tables 1 and 2). The attribution of the specimens to the species *M. tenuidens* is based on Modesto (1999, 2006, 2010) and studies performed by Piñeiro (2004, 2006) and more recently by Morosi (2011). The contributions from the last two authors suggest that most of the characters used to separate mesosaur species may be associated to ontogenetic changes and/or taphonomic artefacts and that *Mesosaurus* is the only mesosaur taxon present in Uruguay (Morosi 2011). *Brazilosaurus* may be a valid taxon if adults lack pachyostosis in the ribs, but additional studies are needed to verify these taxonomic conclusions. The biota in both formations suggests an
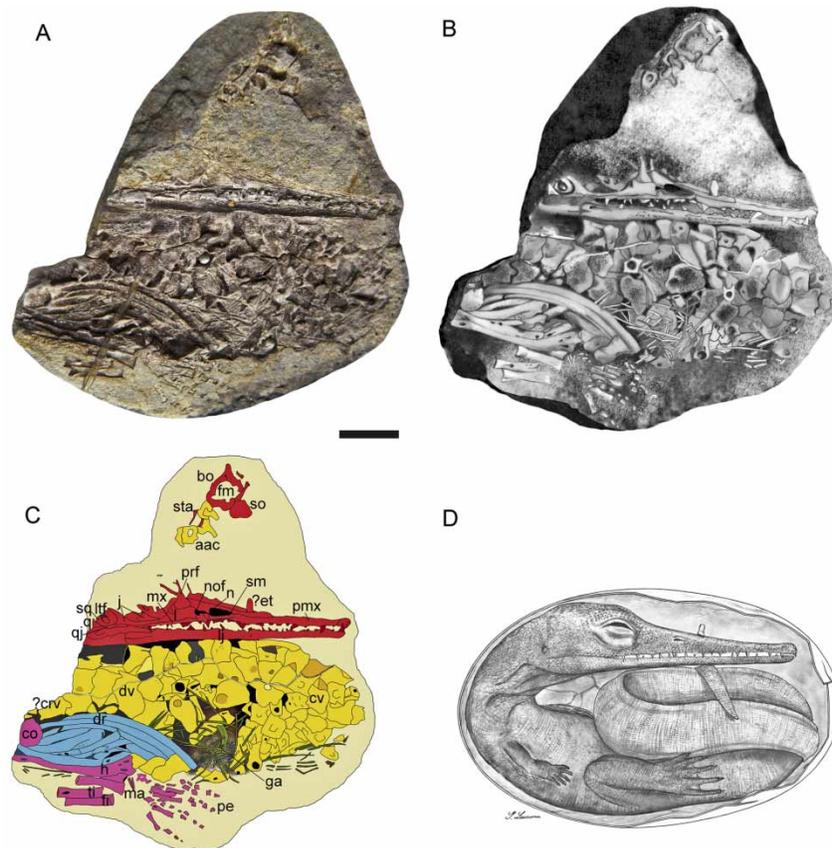

Figure 1. Mesosaur embryo from the Early Permian Mangrullo Formation of Uruguay (FC-DPV 2504): (a) picture of the specimen; (b) interpretive drawing; (c) interpretive drawing of the same specimen using colours to represent the skull (red), vertebrae (yellow), ribs (blue), elements of the appendicular skeleton (pink) and gastralia (green); (d) reconstruction. Note: Scale bar: 5 mm. aac, atlas-axis complex; bo, basioccipital; co, coracoid; ?crv, cervical vertebra; cv, caudal vertebrae; dr, dorsal ribs; dv, dorsal vertebrae; ?et, putative egg tooth; fi, fibula; fm, foramen magnum; ga, gastralia; h, humerus; j, jugal; l, lacrimal; ltf, lateral temporal fenestra; ma, manus; mx, maxilla; n, nasal; nof, nariale-obturatum foramen; pe, pes; pmx, premaxilla; q, quadrate; qj, quadratojugal; sm, septomaxilla; so, supraoccipital; sta, stapes; sq, squamosal; ti, tibia.
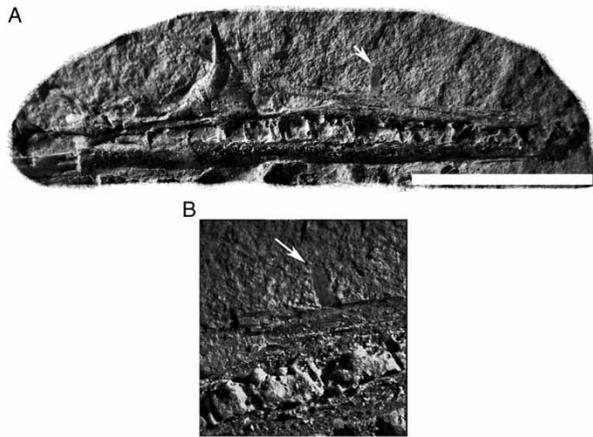
Figure 2. Possible egg tooth in FC-DPV 2504: (a) picture showing the skull and the egg tooth (white arrow); (b) close-up of (a) in the area of the egg tooth (white arrow). Note: Scale bar: 10 mm.

Early Permian, Artinskian age (278 ± 2 Ma) for these deposits, which is consistent with recently radiometric U–Pb zircon analyses (Santos et al. 2006).

Spectroscopy analyses for mineralogical composition were performed in a small fragment of the oval surface enclosing the skeleton of FC-DPV 2504, as well as for the surrounding sediment in specimens containing small mesosaurs that probably represent embryos or hatchlings. All the examined samples failed to reveal significant differences between the light oval halo and the surrounding sediment, and no crystals are visible on SEM images (data not shown). Moreover, a chi-square test and Fisher's exact test (Zar 1984) were performed to assess the statistical significance of the association between several adult (mostly large) individuals and small, disarticulated bones from very young mesosaurs (Figures 4 and 5; Table 1). We also mapped various reproductive characters on a tetrapod phylogeny (Figure 6) using a literature survey (Stewart 1997; Wilkinson and Nussbaum 1998; Laurin et al. 2000; Laurin 2005).

Results

Description of the specimens

Isolated mesosaur embryo

The exquisitely preserved isolated embryo, FC-DPV 2504 (Figure 1), consists of an almost complete, fully articulated, very small individual (inferred total length less than 150 mm). The slab on which it is preserved shows no evidence to suggest original association with an adult. Its completeness and excellent preservation, except for a natural recent fracture affecting the neck region, and some damage produced during collecting, clearly identify it as a mesosaur embryo of the species *M. tenuidens*. This identification is based upon anatomical features that diagnose this group (Figure 1), such as the relatively long snout, the retracted position of the nares and the presence of a small foramen posterior to them (the nariale obturatum foramen). The specific assignation also rests on the fact that *Mesosaurus* is the only mesosaur taxon known to be present in Uruguay (Morosi 2011), and the specimen is clearly differentiated from *Brazilosaurus* by the presence of pachyostotic ribs, which are absent in the latter.

Discrimination between *Mesosaurus* and *Stereosternum* based on previously suggested characters is not possible, as a recent revision (Piñeiro 2004 and
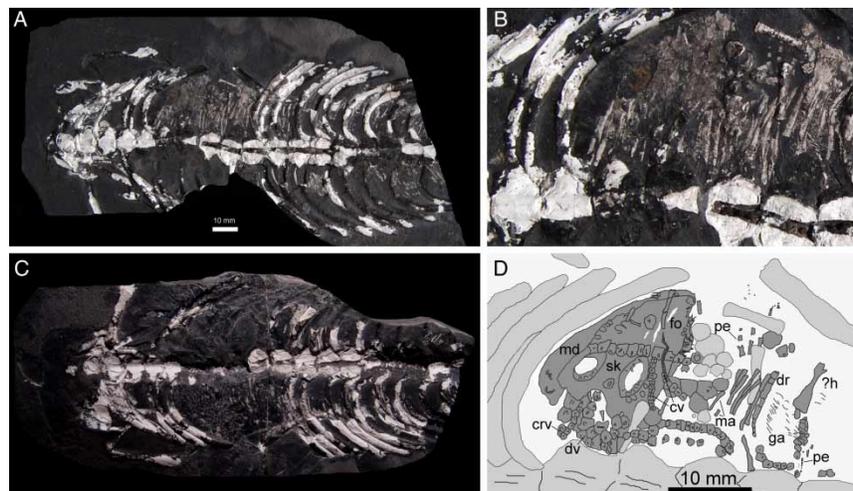

Figure 3.   Adult mesosaur specimen showing embryo in utero (MCN-PV 2214): (a) complete view of the specimen; (b) complete view of the counterpart; (c) close-up of (a) showing the embryo in utero; (d) schematic line drawing of (c), showing the partially curled arrangement of the embryo (in dark colour). The adult mesosaur (in lighter colour) was preserved in ventral view; most bones of the embryo appear to have been preserved in dorsal view. Note: Scale bar: 10 mm. crv, cervical vertebrae; cv, caudal vertebrae; dr, dorsal ribs; dv, dorsal vertebrae; fo, fontanelle openings, ga, gastralia; ma, manus elements; md, mandible; pe, pes; sk, skull; ?h, possible humerus.
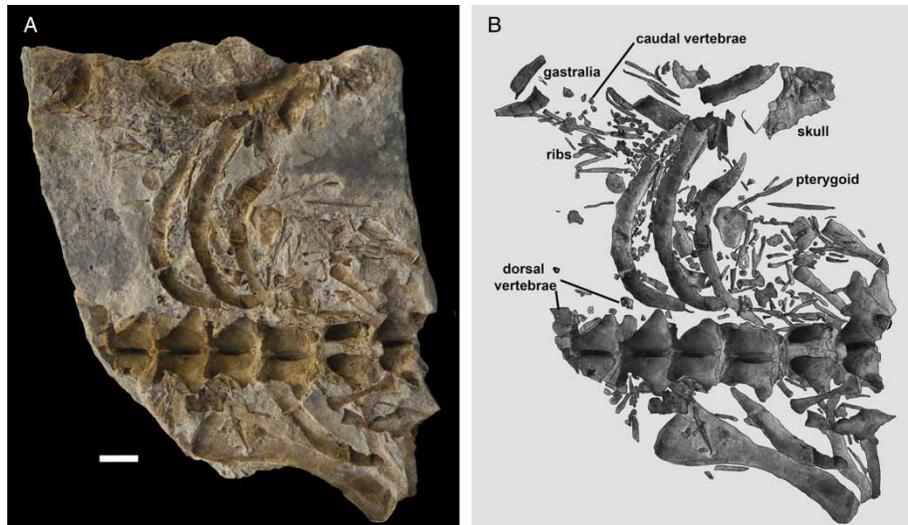
Figure 4.   Adult–juvenile association in mesosaurs: (a) FC-DPV 2545, a large, partially articulated mesosaur (probably broken by the machines that worked on the outcrop) showing small bones between the large ones; (b) edited picture of the same specimen reworked for clarity. Note: Scale bar: 10 mm. The small individual is represented by a partial, articulated skull, a disarticulated pterygoid, dorsal and caudal vertebrae, ribs, and gastralia.

unpublished data) suggests that most may reflect taphonomic artefacts, especially in specimens preserved as moulds (often part and counterpart); in other cases, the interpretation of the characters seems largely subjective. Nonetheless, the anatomical revision of these taxa suggests that while specimens assigned to *Mesosaurus* have all cervical vertebrae of equivalent length, specimens identified as *Stereosternum* have some of the anterior cervical vertebrae longer than the posterior ones, a feature also present in specimens assigned to *Brazilosaurus* (see Shikama and Ozaki 1966; Sedor and Ferigolo 2001).

The small size of FC-DPV 2504 (the specimen is about 10% the average size of an adult *Mesosaurus*) and its coiled disposition are reminiscent of an embryo in an egg (Figures 1 and 7). The original shape and ornament of the bones are perfectly discernible in this specimen, which allows distinguishing of skeletal features commonly encountered in embryos and immature specimens of various amniotes. All bones show a porous surface, as is commonly seen in osteologically immature skeletons (Johnson 1977); this presumably reflects very active growth (de Margerie et al. 2004). Most skull elements are ossified, but they are not sutured to each other. The vertebral centra have open, very large notochordal canals, and the neural arches seem to lack dorsal spines (at best, they are poorly developed). Of the pectoral and pelvic girdles, only a probable coracoid can be observed, near the left broken edge of the slab (Figure 1(b), (c)) It is unclear whether the remaining girdle elements were not preserved (possibly because they were not ossified), whether they are hidden underneath the curled vertebral column or whether they were preserved in the missing counterpart of the specimen. Although pachyostotic ribs, observed in

the specimen, occur rarely in amniote embryos, this condition is commonly present in very young *Mesosaurus* individuals, thus suggesting that this feature was already an adaptive advantage at very early stages of development.

The skull is very large relative to the postcranium and extends over the entire length of the coiled body with the snout directed caudally. A sharp, tooth-like structure situated at mid-length of the dorsal side of the snout may represent an egg tooth (Figures 1 and 2). However, this interpretation is tentative, as we acknowledge that the position of the possible egg tooth was not mechanically optimal to break the outer egg membrane. Most marginal teeth are short, intercalated with larger ones, already displaying the growth pattern that later in ontogeny, allowed for regular tooth replacement. The size of the skull represents more than 90% of the length of the trunk, a ratio which is consistent with data provided by Renesto et al. (2003) for embryos of the aquatic, middle- to large-sized nothosaurid *Lariosaurus*, a Triassic sauropterygian. However, the snout-vent length of FC-DPV 2504 represents barely 10% of the average for adult mesosaurs (Figure 7), which is smaller than in the diapsid taxa surveyed by Renesto et al. (2003: Table 2). The forelimbs are folded either under the thoracic region or against the sides of the body, judging from the position of the manus. The latter is disarticulated, but appears to have been very small relative to the pes (Figure 1). Hind limbs are partially visible, with their large feet folded under the body and overlapping each other.

All these features are consistent with the interpretation of this specimen as an embryo, probably in a terminal stage of development. Other evidence includes the relatively short snout (around half of the skull length) and the large size of the orbits (Figure 1).
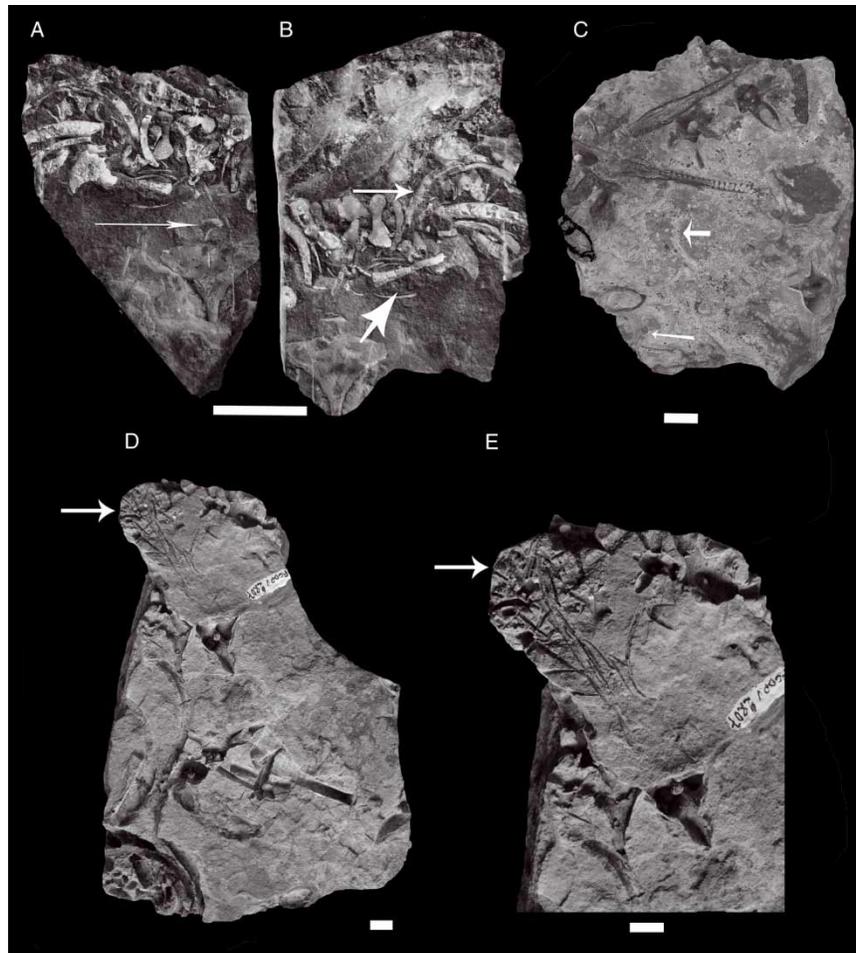
Figure 5.   Additional evidence for adult–juvenile association in mesosaurs: (a, b) FC-DPV 2494, part and counterpart of a large, partially preserved articulated mesosaur associated to both a set of totally disarticulated smaller bones, possibly from a younger individual (horizontal arrow), and to tiny bones of a very young, possible hatchling individual (large, oblique arrow); (c) FC-DPV 2611, distorted skull and mandible of a large mesosaur associated with bones of a possible hatchling specimen (horizontal thick arrow); the oblique thin arrow indicates the presence of a large pygocephalomorph in association with both mesosaurs; (d) FC-DPV 2207, a partially articulated large mesosaur skeleton in association with a small specimen (white arrow); (e) enlarged view of the hatchling mesosaur in FC-DPV 2207. Note: Scale bar: 10 mm.

Table 1.   Number of isolated and associated mesosaur specimens, sorted into ontogenetic stages.

	Isolated	Associated	Total
Adult	211 (195.46)	24 (43.76)	234
Juvenile	2 (22.76)	26 (5.24)	28
Total	213	49	262

Note: Specimens are considered associated if their bones are intermingled, if the skeletons were located adjacent to each other (i.e. the individuals would have been in physical contact when they died) or if the juvenile is located in the adult specimen. The tested null hypothesis is that specimens are randomly distributed into isolated and associated categories; it implies that associated specimens occur in the same proportion in juveniles and adults. It is rejected by a chi-square test based on the contingency table ($\chi^2 = 110.76$, df $= 1$, $p = 0.0000$) and by Fisher's exact test ($p = 0.0000$), both done in Statistica 7. Our own calculations of the chi-square test in Excel spreadsheets using critical values reported in a standard statistical textbook [20] also confirm that the probability is very low ($p \ll 0.001$). In parentheses, the expected frequency in each cell under the null hypothesis is indicated.

SEM analyses failed to reveal significant differences with the surrounding sediment, and no crystals are visible on SEM images. However, this may be because FC-DPV 2504 is an external mould and none of the original hard parts have been preserved.

The in utero specimen

The specimen MCN-PV 2214 from the Brazilian Iratí Formation (Figure 3) consists of part and counterpart of the trunk region in ventral view. The skeleton, preserved between pectoral and pelvic girdles, represents a medium-sized, articulated *Mesosaurus* specimen associated with the skeleton of a very small mesosaur. It is here interpreted as a gravid mesosaur female carrying a single embryo in utero. The very small specimen was, without doubt, intimately associated with the large one, as it occupies a

Table 2. Size of juvenile specimens associated with adults.

Specimen	Bone and measurement	Dimension (mm)	Relative size, compared with 2504	Average relative size of specimen
FCDPV 2504	Rib distal (maximal) diameter	0.919	1.000	
FCDPV 2504	Vertebral centrum height	1.287	1.000	
FCDPV 2207	Rib distal (maximal) diameter	0.326	0.355	0.403
FCDPV 2207	Vertebral centrum height	0.580	0.451	
FCDPV 2614	Rib distal (maximal) diameter	0.463	0.504	
FCDPV 2614	Vertebral centrum height	0.849	0.660	0.582
FCDPV 2084	Vertebral centrum height	0.804	0.624	0.624
FCDPV 2037	Rib distal (maximal) diameter	0.878	0.955	0.742
FCDPV 2037	Vertebral centrum height	0.680	0.528	
FCDPV 2597	Rib distal (maximal) diameter	0.673	0.733	0.766
FCDPV 2597	Vertebral centrum height	1.030	0.800	
FCDPV 2608	Rib distal (maximal) diameter	0.980	1.067	0.856
FCDPV 2608	Vertebral centrum height	0.830	0.645	
FCDPV 2607	Rib distal (maximal) diameter	0.984	1.071	0.887
FCDPV 2607	Vertebral centrum height	0.906	0.704	
FCDPV 2522	Rib distal (maximal) diameter	1.086	1.182	0.908
FCDPV 2522	Vertebral centrum height	0.815	0.633	
FCDPV 2545	Rib distal (maximal) diameter	0.916	0.997	
FCDPV 2545	Vertebral centrum height	1.158	0.900	0.948
FCDPV 2613	Rib distal (maximal) diameter	0.927	1.008	
FCDPV 2613	Vertebral centrum height	1.158	0.900	0.954
FCDPV 2285	Rib distal (maximal) diameter	1.308	1.424	1.002
FCDPV 2285	Vertebral centrum height	0.748	0.581	
FCDPV 2611	Rib distal (maximal) diameter	1.224	1.332	1.047
FCDPV 2611	Vertebral centrum height	0.980	0.761	
FCDPV 2612	Rib distal (maximal) diameter	1.036	1.127	1.081
FCDPV 2612	Vertebral centrum height	1.332	1.035	
FCDPV 2606	Rib distal (maximal) diameter	1.167	1.269	
FCDPV 2606	Vertebral centrum height	1.904	1.479	1.176
FCDPV 2606	Vertebral centrum height	1.167	0.907	
FCDPV 2606	Vertebral centrum height	1.351	1.050	
FCDPV 2536	Rib distal (maximal) diameter	1.180	1.284	1.245
FCDPV 2536	Vertebral centrum height	1.553	1.207	
FCDPV 2609	Rib distal (maximal) diameter	1.417	1.542	1.278
FCDPV 2609	Vertebral centrum height	1.304	1.013	
FCDPV 2605	Rib distal (maximal) diameter	1.373	1.493	1.335
FCDPV 2605	Vertebral centrum height	1.513	1.176	
FCDPV 2494	Rib distal (maximal) diameter	1.965	2.138	1.351
FCDPV 2494	Vertebral centrum height	1.834	1.425	
FCDPV 2494	Rib distal (maximal) diameter	1.913	2.081	
FCDPV 2494	Rib distal (maximal) diameter	0.522	0.568	
FCDPV 2494	Vertebral centrum height	0.696	0.541	
FCDPV 2496	Rib distal (maximal) diameter	1.297	1.411	1.411
FCDPV 2205	Rib distal (maximal) diameter	1.679	1.827	1.448
FCDPV 2205	Vertebral centrum height	1.374	1.068	
FCDPV 2049	Rib distal (maximal) diameter	1.823	1.983	1.558
FCDPV 2049	Vertebral centrum height	1.458	1.133	
FCDPV 2413	Rib distal (maximal) diameter	1.775	1.931	1.598
FCDPV 2413	Vertebral centrum height	1.627	1.265	
FCDPV 2202	Rib distal (maximal) diameter	2.820	3.068	2.301
FCDPV 2202	Vertebral centrum height	1.974	1.534	

Note: Both the absolute linear dimensions and relative size compared with the potentially isolated embryo (FC-DPV 2504) are given because the latter probably represents a late pre-hatchling phase. Associated specimens are sorted by increasing size. Specimens smaller than FC-DPV 2504 are considered as unhatched embryos. Larger specimens may be juveniles that were still being cared by the parents when they died, or they may simply be larger embryos that were still in utero. Given the depositional environment, it is unlikely that they represent laid eggs because amniotic eggs must be laid on land. The measurements are not very precise because these small, fragile, poorly mineralised specimens are subject to significant distortion and flattening, but there is no doubt that the smallest is much smaller than FCDPV 2504, whereas the largest one is much larger. Thus, these specimens constitute a partial embryonic (and potentially early juvenile) growth series.
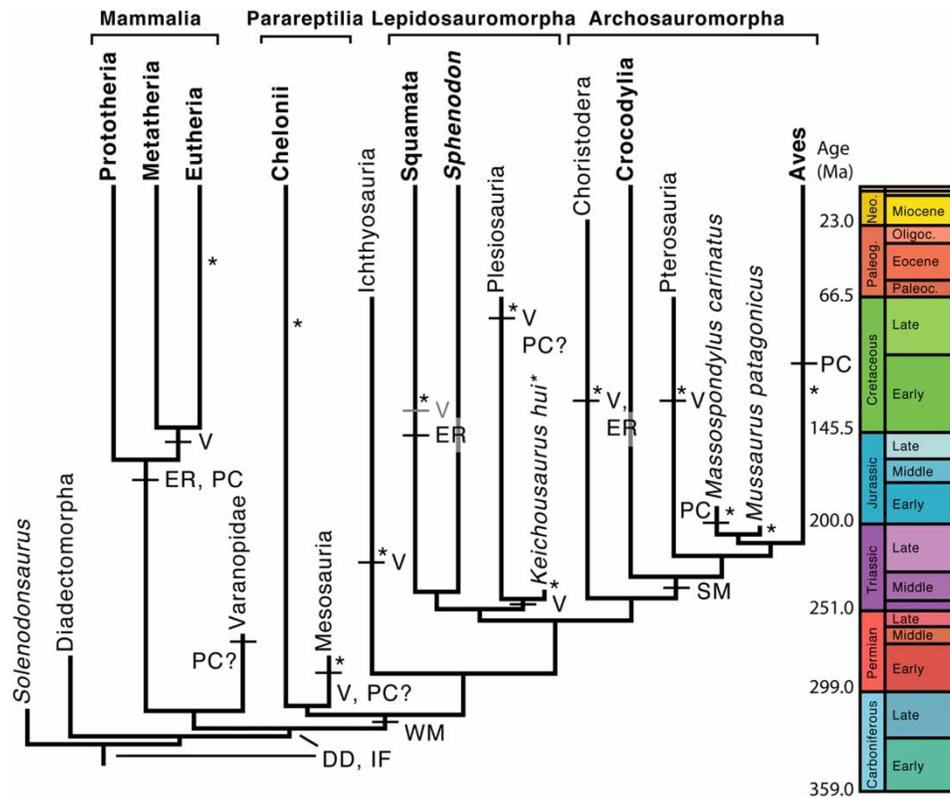
Figure 6.   Reptiliomorph phylogeny showing the evolution of relevant developmental characters. Data on reproductive characters and on the topology are taken from papers cited above and other sources (Bonaparte and Vince 1979; Laurin and Reisz 1995; Lin and Rieppel 1998; Cheng et al. 2004; Maxwell and Caldwell 2003; Wang and Zhou 2004; Zhou and Zhang 2004; Botha-Brink and Modesto 2007; Reisz et al. 2005; Jackson and Schmitt 2008; Ji et al. 2010; Sánchez-Villagra 2010; O'Keefe and Chiappe 2011; Wang and Evans 2011). Note: Characters that occur only in some taxa are in grey. DD, direct development; ER, embryo retention; IF, internal fertilisation; PC, parental care; SM, strongly mineralised eggshell; V, viviparity; WM, weakly mineralised eggshell; ∗earliest fossilised embryos along each branch of the tree.

short area inside the rib cage of the latter, at the level of the left forelimb, whose different bones can be seen intercalated. Although apparently slightly disrupted, it looks like a very small mesosaur, consisting in a very large skull and several small vertebrae, along with part of the hind limb, including an almost complete foot. Other taxonomic interpretation for the small specimen can be ruled out, as no other tetrapods co-occur with mesosaurs in either the Mangrullo or Iratí shales, or the specimen is much too small to have been preyed by other mesosaur. Besides, no bone damage is visible, but gastric acid would quickly leave traces and should be visible if the specimen had been feed upon. MCN-PV 2214 is about half the size of the isolated Uruguayan embryo, which probably represents a very late pre-hatchling stage. This may explain why MCN-PV 2214 is not completely coiled and thus resembles, in this respect, embryos of viviparous Mesozoic marine reptiles (Cheng et al. 2004). The skull is proportionally very large (probably more than 100% of the trunk length, although this is uncertain because the trunk is incompletely preserved). The left mandible, although poorly preserved, is articulated and in anatomical

orientation. Teeth appear to have been incipiently developed, and most of them are very fragile structures. Some bones of the hind limb, including the diminutive foot, as well as part of the axial skeleton, are articulated and easily recognisable, whereas other elements have been displaced, or were probably not yet ossified. The small size of the limb bones, the loose attachment of the skull elements and the occurrence of a central fontanelle (Figure 3) clearly demonstrate its early embryonic stage.

Adult–juvenile association

Contrary to the record of a pregnant female in the Iratí Formation, no undisputable record suggesting viviparity has been found in the Mangrullo Formation, despite the several hundreds of individuals already collected, including some exquisitely preserved specimens, such as FC-DPV 2504. However, at least 26 mostly disarticulated small specimens (late embryos or neonates) were found associated with large, partially articulated or mostly disarticulated mesosaur skeletons (Table 2; Figures 4 and 5). Despite the variable degree of disarticulation of the
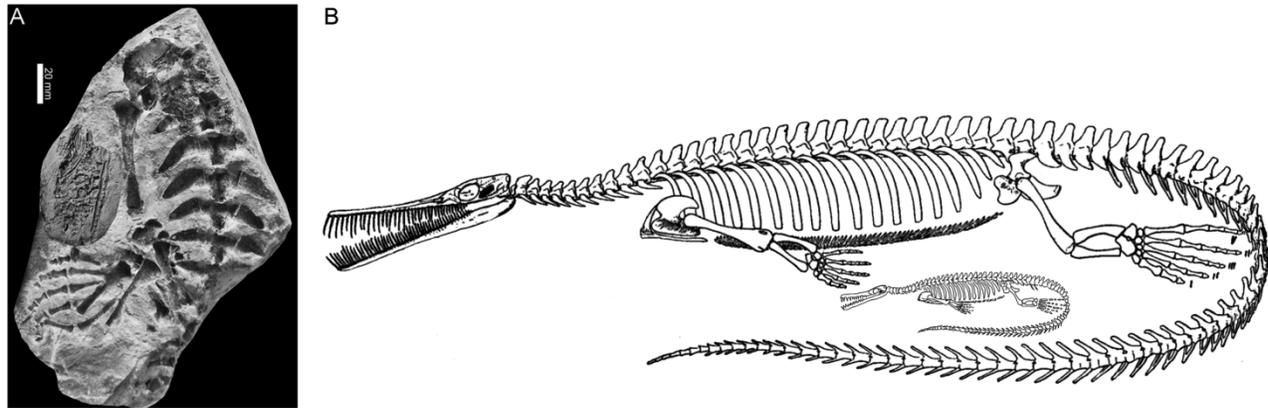
Figure 7. Mesosaur embryo size: (a) composite figure to show the small size of the isolated embryo (FC-DPV 2504) in relation to FC-DPV 2041, a large mesosaur from the same levels; (b) graphic reconstruction of an adult and a posthatched *Mesosaurus tenuidens* (modified from McGregor 1908) to show the putative average-sized adult/neonate relation. Rate was based on the inferred measurement of FC-DPV 2504, an embryo in advanced stage of development.

skeletons, the associations seem to include an adult with a single small individual, as evidenced by preservation of paired postcranial bones and skull fragments. Among the 26 specimens detailed in Table 2, 10 appear to be smaller than FC-DPV 2504 (the smallest is less than half its size, in linear dimensions) and undoubtedly represent embryos. Two other specimens are about the same size as FC-DVP 2504, whereas the remaining 13 are larger (up to twice as large, in linear dimensions). The latter may also represent late embryos, considering there is a large variation in size among reptile embryos, where the largest neonate could be twice the size of the smallest within a species (Deeming et al. 1993). Another possibility is that they are hatchlings that remained with their parents and died simultaneously. In any case, the performed chi-square test and Fisher's exact test (Table 1) both reject the hypothesis that this association is random ($p \ll 0.001$).

Three hypotheses could explain this association. First, high mortality rates were widespread among pregnant mesosaur females, and as a consequence, there were high mortality rates in mesosaur embryos and their delicate skeletons underwent post-mortem disruption. Second, mesosaurs may have displayed oviparous egg retention, as in some extant squamates, in which eggs with a poorly mineralised or unmineralised membrane are laid shortly before hatching (Smith and Shine 1997; Andrews and Mathies 2000, p. 230). Third, hatchling or juvenile mesosaurs remained along with their parents some time after hatching. The last two hypotheses are compatible with parental care in mesosaurs, to the form of egg care and care for the hatchlings. Cannibalism is a putative fourth hypothesis, as this behaviour was proposed to have existed in mesosaurs (Raimundo-Silva and Ferigolo 2000; Piñeiro et al. forthcoming). However, we reject that hypothesis because most of the juvenile specimens are too well preserved to represent gastric content. If the association

resulted from cannibalism, we should expect the bones to show mechanical breakage resulting from the passage through the narrow esophagus, and surface damage produced by contact with gastric acid. Additionally, it is known that gastric contents usually include more than one type of dietary elements (Maxwell and Caldwell 2003), a condition which is actually observed in some mesosaur specimens that preserved gut content consisting in minute indeterminable particles associated with recognisable crustacean remains and disarticulated eroded small mesosaur bones (Piñeiro et al. forthcoming). Thus, although we do not rule out that some associations result from cannibalism, it can explain only the most poorly preserved and fragmentary materials.

Evolution of outer egg membranes

According to the parsimony optimisation of outer egg membrane, gnathostomes, actinopterygians, actinistians, dipnoans, lissamphibians and monotremes have unmineralised outer egg membranes, and this must have been the condition in at least some of the earliest sauropsids (Figure 6). A weakly mineralised outer egg membrane is a synapomorphy of reptiles, but such membranes do not usually fossilise (Laurin et al. 2000). Indeed, this may explain the absence of amniotic eggs in the abundant Paleozoic fossil record of amniotes. The fossil embryo from Uruguay lends additional support for this hypothesis because no shell has been preserved, although it is uncertain whether it reflects a lack of outer egg membrane mineralisation, or whether the shell was dissolved.

Discussion

The interpretation of these fossils is not without difficulties. On the one hand, we have strong evidence

for extensive embryo retention, which is most commonly expressed as viviparity in mesosaurs, as indicated by the adult specimen carrying a well-developed, partially ossified embryo; on the other hand, we have an isolated embryo (FC-DPV 2504) and several specimens that show adult–embryo/hatchling association. The isolated embryo could be interpreted either as an aborted egg or as a laid 'egg' that may have required only a short period of incubation before hatching. The last hypotheses are supported by the apparent lack of a shell, although the specimen is preserved as a mould of the original skeleton, and in the place where the shell should have been, a light halo of unknown significance encloses the embryo.

This fossil preservation parallels what has been observed in the aquatic choristoderan *Hyphalosaurus* from the Early Cretaceous Yixian Formation of China (Gao et al. 2000). This taxon contains two species, *Hyphalosaurus lingyuanensis* and *Hyphalosaurus baitai-gouensis*, that only differ in the number of cervical vertebrae (19 and 24–26, respectively; Ji et al. 2004; Hou et al. 2010), a condition also suggested as diagnostic for the recognition of the mesosaur monotypic genera (Araújo 1976; Modesto 1996). As for *M. tenuidens* among mesosaurs, associations of adult and juvenile/embryo individuals are known only for *H. baitaigouensis* among hyphalosaurids. These associations in the latter include pregnant females carrying embryos, adults associated with eggs containing embryos, as well as isolated embryos, some even preserved at the moment of hatching (Ji et al. 2004, 2006, 2010; Hou et al. 2010). Even though these fossils suggest the apparent representation of several reproductive strategies, viviparity has been suggested as the more plausible reproductive behaviour for *H. bataigouensis* (Ji et al. 2010). Such a conclusion was based on the record of a gravid specimen carrying 18 terminal stage embryos, and the lack of a shell in the 11 eggs associated to the holotypic specimen (Ji et al. 2010). However, the recent report of two isolated eggs with flexible, poorly mineralised eggshell containing embryos and hatched neonates of *H. bataigouensis* (Hou et al. 2010) indicates a different (oviparous) strategy for the same species. As in mesosaurs, the eggs and the embryos of *H. bataigouensis* are very small compared to the size of the adult average. Large adults are not common in the Yixian Formation, compared to the high preservation of juvenile skeletons, which thus led the authors to suggest low survivorship rates (presumably of juveniles) for *H. bataigouensis*, as occurs in most extant semi-aquatic reptiles (Hou et al. 2010).

High hatchling mortality in mesosaurs, within an environment highly favourable for fossil preservation, would explain the very frequent adult/embryo (or hatchling) association found in the Mangrullo Formation. If at least some *Mesosaurus* populations were oviparous with extensive embryo retention, they would presumably have laid eggs on land, most likely in coastal, moist areas, and may have buried the eggs to avoid desiccation. These inferences are drawn because amniotic eggs are almost universally laid on land, because the lack of mineralised eggshell implies a fairly high water conductance of the membrane (Hou et al. 2010), and because most soil at moderate depth includes enough interstitial water to prevent eggs from dehydrating (Skulan 2000). Contrary to the condition in fully aquatic viviparous reptiles (Cheng et al. 2004), the anatomical structure of the mesosaur pelvic region, including the fusion of the ribs to the two sacral vertebrae, as well as an articulation (although weak) between these ribs and the iliac blade, suggests that mesosaurs had terrestrial locomotory capabilities required to deposit eggs on land, plausibly close to water. Thus, mesosaurs may be the oldest known amniotes that displayed extended embryo retention, either as viviparity or as oviparity with extended embryo retention, two reproductive strategies that are compatible with the available materials and which may vary between mesosaurid populations or taxa. This would not be so surprising, as shown by extant squamates, in which reproductive strategies are especially labile (Blackburn 1995; Chapple 2003) and in which a few species have both viviparous and oviparous populations (Smith and Shine 1997).

The evidence presented above suggests that gravid females typically carried only one, and more rarely two embryos. This normally occurs in taxa that display K-type reproductive strategies (as in mammals, birds and some rare squamates), where parental care is a common behaviour. K-selected reproduction is often associated with a large size of neonates (O'Keefe and Chiappe 2011), although marsupials and monotremes provide counter-examples (Manger et al. 1998; Smith 2001). According to the inferred size of FC-DPV 2504, here interpreted as a late embryo, hatchling mesosaurs may have been fairly small, about 10% of adult body length. Despite this small size, comparisons with other amniotes such as marsupials and monotremes raise the possibility that parental care of the offspring or of the eggs until hatching occurred in mesosaurs. On the other hand, there are few examples of co-occurrence of viviparity and parental care reported for extant and fossil reptiles. These examples are linked to complex social, stable aggregations of closely related individuals (e.g. 'family' groups), including males, females and young individuals that remain in contact in response to co-habitation in available favourable settings and refugia. Such social aggregations were extensively studied and better known in scincid lizards of the genus *Egernia* (Chapple 2003), and as the aggregation is constrained by habitat availability and ecological benefits, there could be social and solitary species in a single lineage. Within the social species, parental care could be a consequence of living in group (Chapple 2003). Other

potential examples are related to a K-selected reproductive strategy producing reduced litter size and relatively large progeny, as suggested by a recently described marine plesiosaur specimen that shows association between an adult and a single, large fetus (O'Keefe and Chiappe 2011).

The origin and evolution of parental care and viviparity (Figure 4) are poorly understood aspects of the early amniote reproductive biology because they leave virtually no trace in the fossil record. The oldest known case of parental care is represented by Middle Permian varanopid synapsids (Botha-Brink and Modesto 2007), about 20 Ma after the mesosaur record. While adult–embryo (or hatchling) associations are commonly found in the Mangrullo Formation, there is only one known instance of this association (MCN-PV 2214; pregnant female) in the Iratí Formation, where, instead, clustered or isolated very young individuals have been found (Schultz, personal communication, 2010). This may provide support for viviparity, but not for parental care in Brazilian mesosaurs. In Uruguay, *Mesosaurus* populations may have developed either viviparity or oviparity with extensive egg retention, and perhaps, some kind of parental care may have existed, maybe controlled by ecological constraints that favoured social aggregations as has been postulated to occur in some extant squamates (Chapple 2003).

The fossils described herein represent the geologically oldest record of extensive embryo retention in amniotes, as well as equivocal evidence of parental care. Mesosaur reproductive biology is now the most thoroughly documented among Paleozoic amniotes.

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