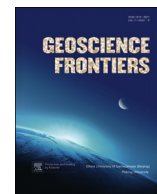




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Focus paper

The first pterosaur 3-D egg: Implications for *Pterodaustro guinazui* nesting strategies, an Albian filter feeder pterosaur from central Argentina



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ABSTRACT

The increasing number of fossil pterosaur eggs sheds light on nesting environments and breeding behaviors of these extinct flying reptiles. Here we report the first partial three-dimensional egg of the pterosaur, *Pterodaustro guinazui*, from central Argentina. The specimen was discovered from the same Albian deposits as the exceptional *P. guinazui* embryo described in 2004. Microscopic characterizations indicate a pristine preservation of the 50 µm thick calcium carbonate, which differs significantly from the soft shell of Chinese pterosaur eggs. Estimate of the eggshell conductance implies that the nest had a minimum moisture content of 75%. This moisture estimate, combined with geological and taphonomical data, suggests that *P. guinazui* may have adopted a nesting strategy similar to those of grebes and flamingos rather than being buried on land, as previously hypothesized. Moreover, our results demonstrate that the nesting paleoenvironment of this pterosaur species was closely linked to a mesohaline lacustrine ecosystem in a basin governed by regional tectonic subsidence, a setting characteristic for the feeding and reproduction of modern flamingos.

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1. Introduction

The structure and properties of eggshells are tightly adapted to a narrow range of species-specific incubation environments, and

hatching success is determined by the functional characteristics of the eggshell structure (Board, 1982). Furthermore, as archosaurs lay amniotic eggs in nests rather than bearing live offspring, their reproduction is tightly constrained by environmental factors. Therefore, nest-site selection is critical to developmental rates and hatching success (Göth and Vogel, 1997; Shine, 2005; Grellet-Tinner et al., 2012a). Our knowledge of pterosaur eggs, eggshell morphological structures, and embryos is still limited (Grellet-Tinner et al., 2007; Unwin and Deeming, 2008), although recent discoveries of pterosaur eggs with embryos and hatchlings at various ontogenetic stages in several species of pterosaurs from the lower Cretaceous of Argentina and China (Chiappe et al., 2004; Ji et al., 2004) provide some direct evidence of their reproduction (Grellet-Tinner et al., 2007; Unwin and Deeming, 2008). Here we describe the first partial three-dimensional pterosaur egg most likely of a *Pterodaustro guinazui*, a colonial and filter-feeding

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pterosaur (Bonaparte, 1970, 1971; Chiappe et al., 2000) endemic to the Albian lacustrine deposits of Loma del Pterodaustro in central Argentina. We use the eggshell morphology of this new fossil to interpret the nesting strategy and paleoenvironment of this very specialized pterosaur, the jaws of which were adapted with baleen filter feeding apparatus (Chiappe et al., 2004; Codorníu and Chiappe, 2004).

2. Materials and methods

2.1. Materials

Specimen MIC-V 778: A partial egg discovered at “Loma del Pterodaustro”. The material is now curated in the Museo Interactivo de Contacto’s (MIC) collection at Universidad Nacional de San Luis (San Luis Province). Specimen MIC-V 246 (Chiappe et al., 2004; Grellet-Tinner et al., 2007) recovered at the same site is used for comparative purposes. MIC-V 246 consists of a two-dimensional (flatten concretion) articulated *P. guinazui* embryo in an oval shaped concretion, in which eggshell remnants delimited the original egg shape.

2.2. Methods

The samples for thin section and scanning electron microscopy (SEM) are from specimen MIC-V 778. Each eggshell fragment was

broken off, immediately mounted on an aluminum stub, and coated with gold or palladium. SEM observations were performed using a LEO 1450VP SEM at 10 kV in the LABMEM. Eggshell elemental chemical spectra were obtained with an Energy Dispersive X-ray Spectrometer (EDS) Genesis 2000 attached to the SEM. Due to the fragility and thinness of the eggshell, only one thin section was prepared at the UNSL with DICAST LY 554 epoxy resin and hardener DICURE HY 554 at 20%. The prepared sample was polished with potassium carbide 220 and 400 and observed with the petrographic microscope NIKON Eclipse E400POL from the Mineralogical Museum of Universidad de Córdoba (Argentina). The pictures were taken with a Canon PhowerShot A530. CorelDraw X5 and Adobe Photoshop 9.0 software were used to display the images captured.

The eggshell conductance of MIC-V 778 was estimated ($\pm 5\%$) by calculating the egg volume from its linear dimensions (60 mm \times 22 mm) using the equation (Hoyt, 1979):

$$V = 0.507 L B^2$$

where V is volume in cm^3 , L is egg length in cm, and B is egg width in cm. The mass was then calculated by assuming a density of 1.05. The calculated egg mass allowed comparison of potential eggshell thickness with those of avian eggs of the same size, from the regression equations for birds (Ar et al., 1974), the only extant vertebrates to lay eggs of this size in open nests. The thickness of the eggshell of MIC-V 778 was measured from scanning electron micrographs.

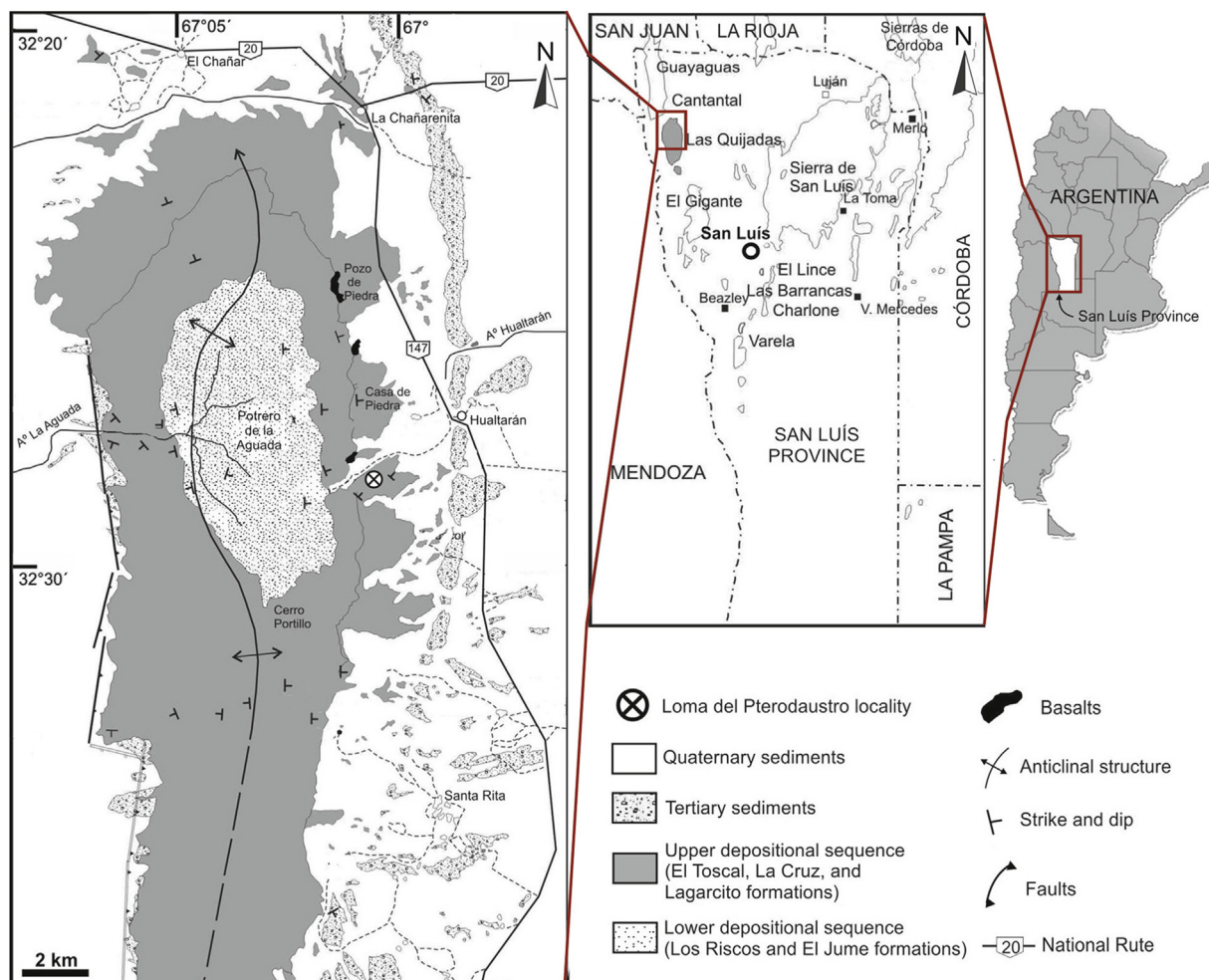


Figure 1. Geographical and geological maps of the Albian lacustrine deposits of Loma del Pterodaustro in the sierra de las Quijadas, San Luis Province, Argentina (Geologic map modified from Rivarola and Spalletti, 2006).

3. Results

The Loma del Pterodaustro locality (Lagarcito Formation) in the San Luis Province (Fig. 1) is renown for its Albian monotaxic assemblage of fragmentary and articulated specimens of *P. guinazui*, a pterosaur so far known only from this region. Presently, this material includes several adults, two small juveniles (Bonaparte, 1970, 1971; Chiappe et al., 2000; Codorniú and Chiappe 2004), and an exceptional *P. guinazui* fossil embryo *in ovo* (MIC-V 246) that was preserved in a concretion in two-dimensions with eggshell remains delimiting the egg outline around the embryo (Chiappe et al., 2004; Grellet-Tinner et al., 2007). The new specimen (MIC-V 778) was discovered in 2010 from subfacies 3.1 of the lacustrine lithofacies 3 (Fig. S1), which records offshore lacustrine environments characterized by very fine, laminated siltstones (Chiappe et al., 1998). Subfacies 3.1 has yielded most of the *Pterodaustro* fossils collected from this formation, including MIC-V 246 (Chiappe et al., 2004; Grellet-Tinner et al., 2007). MIC-V 778 was discovered in this subfacies 10 cm below a well-cemented and compressed siltstone layer that yielded baleen-like *P. guinazui* teeth (Fig. S2) during the same fieldwork. The micro-laminated siltstone layer, which yielded MIC-V 778, consists of slightly larger siliclastic grains that are poorly cemented; a partial *P. guinazui* digit was concomitantly recovered from the same layer a few meters from the egg (Fig. S2).

A grey-brown matrix encrusts the egg, but no sediment was present inside the egg when discovered, implying that the egg was complete and not broken at the time of burial. This preservation is most unusual considering the fragility of the specimen and the thinness of its eggshell, so even a short distance of transportation by underwater debris flow (Chiappe et al., 1998) seems unlikely. The preserved section of MIC-V 778 consists of a polar region and adjacent egg sections measuring 25 mm wide and 30 mm long and perfectly matching the narrowest pole of the egg in the MIC-V 246 concretion (Chiappe et al., 2004; Grellet-Tinner et al., 2007), which has an estimated volume and mass of 14.7 cm³ and 15.46 g respectively. In contrast to MIC-V 246, the eggshell of MIC-V 778 is complete (Fig. 3a, c–f) and shows no visible diagenetic alteration. SEM, polarized light microscopy (PLM) and transmittance light microscopy (TLM) microcharacterizations suggest the presence of

three structural layers (if the accessory surficial layer is considered a third structural eggshell layer) in contrast to the single layered eggshell of MIC-V 246 (Chiappe et al., 2004; Grellet-Tinner et al., 2007). It is worth mentioning that MIC-V 246 could be considered bi-layered if its accessory surficial layer is considered a structural layer (Fig. 3b). These eggshell differences are explained in the discussion section. The MIC-V 778 tri-laminated eggshell averages only 50 µm in thickness and is made of calcium carbonate, as determined by EDS analyses (Fig. 3f). The outermost (accessory) layer of the eggshell consists of a 2 µm thin cover of amorphous material (Fig. 3a,d), which is reminiscent of the external covering observed in both modern (Kohring, 1999) and extinct *Mirandornithes* (grebes and flamingos) (Grellet-Tinner et al., 2012a). The middle layer or layer 2 is ubiquitously separated from the accessory layer by a thin groove. Layer 2 is formed by shorter, narrower columns of tabular crystals (Fig. 3c, d) than those of the inner layer (layer 1). In addition, these tabular crystals are vertically or nearly vertically orientated and are perforated by numerous vesicles averaging 2 µm (Fig. 3e), possibly reflecting the occurrence of the organic scaffolding for calcification of the eggshell during its development. The innermost layer, or layer 1, of the eggshell consists of a series of horizontal tabular calcitic crystals (Fig. 3c, e) that originate from a basal layer of thin, small, and elongated vertical crystals without noticeable nucleation centers and radiating spherulites (Fig. 3c). This approximately 30 µm thick layer lacks distinct shell units. As such, it compares best with geckoid eggshells (Schleich and Kästle, 1988; Packard and Hirsch, 1989; Kohring, 1999) and closely resembles the early Cretaceous geckoid eggshell structure from France (Grellet-Tinner et al., 2008). Although the shell of MIC-V 778 is superbly preserved, the scanning electron micrographs do not reveal any pores, for which there could be a number of explanations: (i) pores may not have been evident in a scanning electron micrograph of a radial fracture of the eggshell, which is possible but not likely; (ii) pores were not present or noticeable at the ontogenetic stage at which the egg died; or (iii) all pores were located in the larger pole like in extinct troodontids (Grellet-Tinner and Makovicky, 2006; Grellet-Tinner et al., 2008). It is also worth pointing out that MIC-V 246 microcharacterizations failed to reveal any pores.

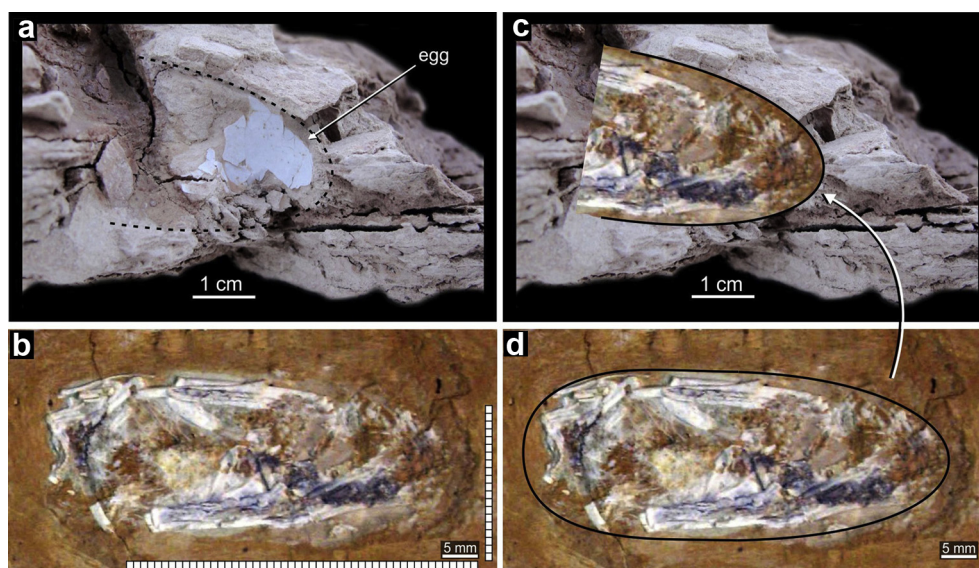


Figure 2. Comparison between MHIN-UNSL-GEO-V 246 and MIC-V 778. (a) Photo of MIC-V 778, the new 3-dimensional egg in the original claystone matrix with its contour outlined. (b) MHIN-UNSL-GEO-V 246 *Pterodaustro guinazui* embryo with the preserved eggshell outlined (Chiappe et al., 2004). (c) The outline of the pointed section of MHIN-UNSL-GEO-V brought to scale and properly orientated matches perfectly the 3-dimensional mold and egg of MIC-V 778. (d) Traced contour of the MHIN-UNSL-GEO-V 246 eggshell to facilitate comparison between the 2 specimens.

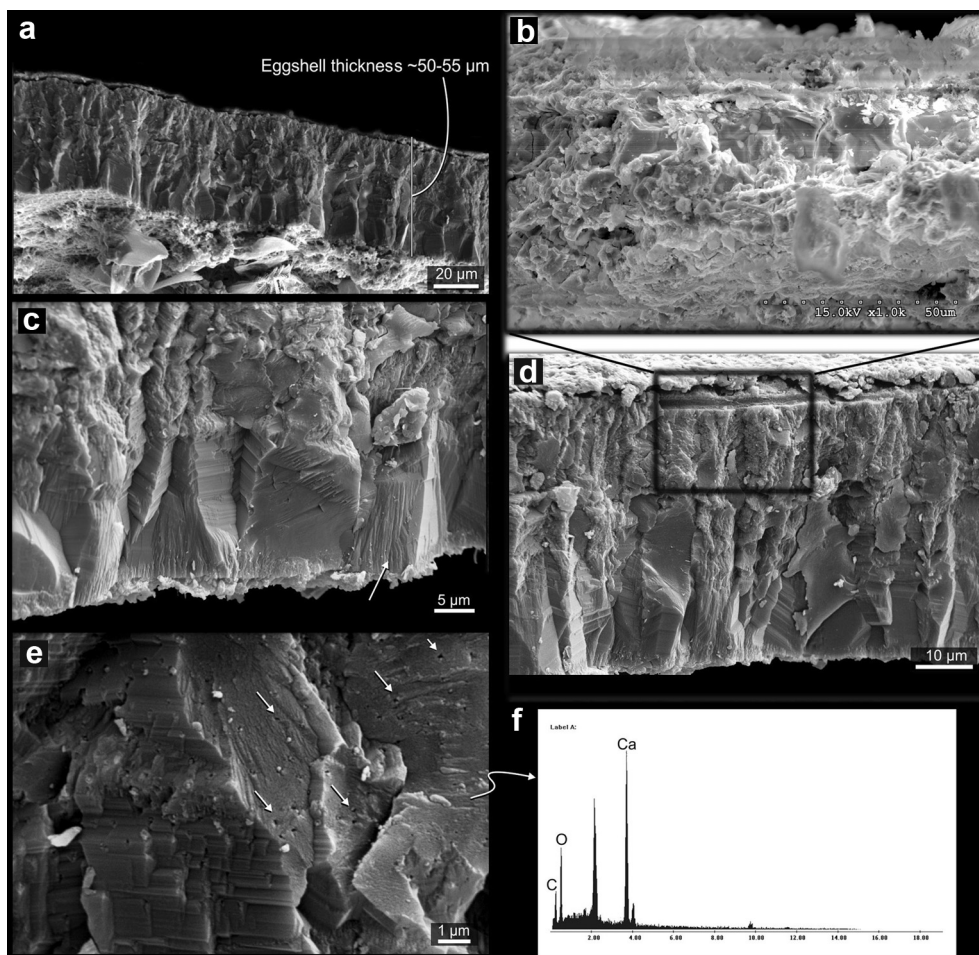


Figure 3. Eggshell comparison between MHIN-UNSL-GEO-V 246 and MIC-V 778. (a, c–f) SEMs and EDS of MIC-V 778. (b) Unpublished SEM (2004) of MHIN-UNSL-GEO-V 246. (a) Entire cross-section of the new eggshell with possible fossilized membrana testacea mixed with clay minerals at its base. The eggshell thickness averages 50 µm. (b) Unpublished 2004 SEM of MHIN-UNSL-GEO-V 246 with a surficial granulated cuticle like outer-layer underlain by a micron-size longitudinal groove that were not recognized in 2004. (c) Magnified SEM view of (a). Note the pristine preservation of the eggshell with three structural calcitic layers (the third cuticular layer is not visible in (c)). The base of layer 1 displays the same structures than the Berriasian geckoid described by Grellet-Tinner et al. (2008). (d) MIC-V 778 SEM transversal view. The outlined square in the MIC-V 778 upper section represents MHIN-UNSL-GEO-V 246 eggshell as observed in 2004. Note that the two specimens share the same outermost cuticular layer underlain by a similar longitudinal groove, which function and origin are still unresolved. MHIN-UNSL-GEO-V 246 missing layer 1 and slightly altered crystallization is easily explained by the diagenetic processes that occurred with the combined oxido-reduction and partial calcium dissolution during the fossilization of MHIN-UNSL-GEO-V 246 embryo and coeval formation of its concretion. (e) Note the tabular crystallization of layer 2 and the vesicles (white arrows) that lodged the eggshell proteins, attesting for a unique Lagerstätte preservation typical of Loma del Pterodaustro. (f) The eggshell EDS shows no ubiquitous alterations in its composition.

4. Discussion

4.1. Taxonomic identification

Although taxonomic identifications of fossil eggs without embryos *in ovo* are always very tentative (Packard and Hirsch, 1989; Grellet-Tinner and Makovicky, 2006; Grellet-Tinner and Fiorelli, 2010), the identity of this new egg is supported by several independent pieces of evidence.

Firstly, the shape and size of this new specimen matches well those of the inconspicuously smaller pole of MIC-V 246, the 2D 60 mm by 22 mm egg, which is positively identified to be a complete embryo *P. guinazui in ovo* (Chiappe et al., 2004; Grellet-Tinner et al., 2007; Unwin and Deeming, 2008). In this regard, the dashed line in Fig. 2 determines the egg outline by tracing the contour of the egg.

Secondly, the vertebrate fauna consists exclusively of several fish taxa and only a single pterosaur taxon, *P. guinazui* (Bonaparte, 1971; Chiappe et al., 2004; Codorníu and Chiappe, 2004; López-Arbarelo and Codorníu, 2007), which independently supports the *P. guinazui* identity of this new fossil. In addition, the new specimen

was recovered a few meters from and in the same microlaminate as, a *Pterodaustro* digit (Fig. S2a, b) and under a block with *Pterodaustro* baleans (Fig. S2a, b).

Thirdly, the fragility of MIC-V 778 and its lack of internal sediment infilling favorably argue for an autochthonic origin, indicating a lack or little transport, which would exclude the *ad hoc* presence by transportation of an allochthonous egg from a different vertebrate not yet reported from this formation and site.

Although the eggshell thickness and structure of MIC-V 778 do not appear, at first, entirely congruent with those of MIC-V 246 (Chiappe et al., 2004; Grellet-Tinner et al., 2007), closer observation reveals their similarities. MIC-V 778 mid- to outer-most shell cross-section is identical to MIC-V 246 (Fig. 3b,d). As such, MIC-V 778 clearly indicates that the innermost layer and a section of the second layer of MIC-V 246 are missing, an artifact that could easily be explained by calcium resorption during embryogenesis (Simkiss, 1961; Dennis et al., 1996; Grellet-Tinner et al., 2004) of the well ossified embryo (Grellet-Tinner et al., 2004, 2007) or/and mobilization of readily available calcium for cement during the formation of the concretion. Considering all the geological and

biological evidence at hand, it is most likely that MIC-V 778 is the first partial *P. guinazui* egg preserved in three-dimensions. Furthermore, this discovery sheds light on the morphology of the previously reported MIC-V 246 eggshell structures (Grellet-Tinner et al., 2004, 2007).

4.2. Eggshell gas diffusion

The nesting behaviors and environments of pterosaurs are still poorly understood (Grellet-Tinner et al., 2007; Lü et al., 2011). Even more confusing, the only pterosaur eggs with embryos obtained from the Yixian Formation in China are soft-shelled (Ji et al., 2004; Unwin and Deeming, 2008), whereas those from Argentina display a well-mineralized calcitic shell. Although the Chinese pterosaur embryos and *P. guinazui* belong to related clades (Lü and Ji, 2006), the presence of soft-shelled and hard-shelled pterosaur eggs in China and Argentina, respectively, is puzzling. However, this conundrum may reflect a similar species-specific reproductive strategy observed in modern Geckonids (Andrews et al., 2013), which lay both hard- and soft-shelled eggs.

Considerable information about potential nest environments can be inferred from the eggshell structure by comparison with the relationships between eggshell morphology and nest environments of extant amniotes (Seymour, 1979; Deeming and Thompson, 1991; Deeming, 2006; Grellet-Tinner et al., 2008, 2012a; Burchard and Deeming, 2009; Grellet-Tinner and Fiorelli, 2010). The calcareous eggshells of amniotes must allow the exchange of respiratory gases to support embryonic metabolism, while simultaneously regulating flux of water vapor. The thickness of the eggshell and the size and number of the pores, which both determine the conductance of the eggshell to respiratory gases and water vapor, are key features that facilitate oxygen supply to the embryo, allow it to excrete carbon dioxide, and prevent excessive water loss (Rahn et al., 1979; Grellet-Tinner et al., 2012b). It is interesting to note that water dynamics of the hard-shelled eggs of the gekkonid *Chondrodactylus turneri* exhibit striking parallels with those of the rigid-shelled eggs of birds (Andrews, 2012). By assuming, based on their polar similarity, that the dimensions of MIC-V 778 were the same as those for MIC-V 246 (60 mm × 22 mm), we estimated the egg mass to be 15.5 g. The thickness of the shell of MIC-V 778 is approximately 50 µm (Fig. 3a), which is less than one third of the thickness expected for the eggshell of an avian egg of similar size (179 µm) (Ar et al., 1974; Ar and Rahn, 1978). As eggshell conductance is inversely proportional to eggshell thickness (Ar et al., 1974), the MIC-V 778 calcareous eggshell would have a conductance of 12.8 mg/d.Torr—more than three times higher than for an avian egg of equivalent size. An avian egg of this mass would have an incubation period of 21.3 days (Ar and Rahn, 1978). Thus, based on modern birds, if we assume that the egg could sustain no more than 14% loss of their fresh mass in water during incubation (Rahn et al., 1979), and that it had an incubation temperature of 30 °C (average incubating temperature for reptiles), we can calculate a minimum vapor pressure in the nest to be 23.9 Torr. However, if the incubation temperature was 36 °C (average incubating temperature for birds), the vapor pressure would be 36.5 Torr. Consequently, if the egg could sustain a mass loss of 14% or less during 21.3 incubation days, the $p(\text{H}_2\text{O})$ of the nest environment would be a minimum of 75%. Moreover, if considering reptilian incubation (hard-shelled eggs of crocodilians, geckos, and turtles generally do not lose water during incubation), and given the relative egg: female mass ratio of pterosaurs (Lü et al., 2011), it is very unlikely that pterosaur eggs would lose water during incubation. Thus, nest humidities would need to be even higher than our calculations suggest. Such a nesting environment is likely to result in the egg being incubated in a nest with a very high

moisture content during some of the incubation time, conditions matching those of grebes (Davis et al., 1984) or primitive flamingos (Grellet-Tinner et al., 2012a) living in similar geological/environmental settings. The eggs of *P. guinazui* and grebes, which incubate their eggs in moist nests (Davis et al., 1984), are of similar size and have similar estimated gas conductances that are about three times higher than in other avian eggs of similar size. The difference between these two species is the way in which the higher conductance is achieved. In *P. guinazui*, high conductance is achieved by having a thin eggshell (short pore length), whereas in grebes high eggshell conductance is achieved through having a larger number of pores through an eggshell that is as thick as that in other birds.

Another curious feature of MIC-V 778 is the ubiquitous 2 µm outer layer of calcium carbonate (Fig. 3a,d), an eggshell feature unknown in reptiles but observed in a few avian taxa, notably Mirandornithes (grebes + flamingos). Termed the “accessory layer or surface crystal layer”, it may function in avian eggs that are incubated in relatively wet or muddy nests to prevent water from occluding the pores through the eggshell and thus prevent a reduction in the eggshell conductance to oxygen (Board, 1982). An analogous structure composed of a waxy substance also occurs in the eggs of Adelie penguins, where it functions to reduce water loss to the very dry Antarctic atmosphere (Thompson and Goldie, 1990). In that case, the outer layer erodes during incubation, which increases the eggshell conductance so that the metabolism of the late term embryo is not compromised. Conversely, there is no indication of erosion of this thin outer layer of the eggshell in MIC-V 778, as this surficial cover is still present in MIC-V 246 that contains a late-stage embryo (Chiappe et al., 2004; Grellet-Tinner et al., 2007). The retention of this accessory layer throughout the incubation is also well substantiated in Mirandornithes, thus adding more biological support to the similarities between *P. guinazui* and Mirandornithes nesting strategies.

4.3. Pterodaustro Mirandornithes-like nesting strategies

The question whether *P. guinazui* brooded its eggs in open or in buried nests has been highly debated (Grellet-Tinner et al., 2007; Unwin and Deeming, 2008). The thin eggshell and potentially high eggshell conductance of MIC-V 778 could still be congruent with the previous conclusion made for MIC-V 246 that the eggs were incubated in covered nests (Grellet-Tinner et al., 2007; Unwin and Deeming, 2008). However, the sum of all the taphonomic and biological evidence gathered for MIC-V 246 and MIC-V 778 allow us now to reconsider this original hypothesis in favor of incubation in nests built in palustrine ecosystems, where the water could have intermittently permeated in the nests under a semi arid climatic regime (Rivarola and Spalletti, 2006). In addition, the two fining upward sequences of Sierra de las Quijadas represent the sedimentary infill of an active margin of a half graben basin. This basin was characterized by an initial synrift stage and a final postrift stage, governed by regional tectonic subsidence (Rivarola and Spalletti, 2006). As such, the geology of Loma del Pterodaustro indicates that the environmental conditions were analogous to those of the Bardenas paleoflamingo with a floating nest and eggs (Grellet-Tinner et al., 2012a). This independent paleoenvironmental comparison is further supported by with a concentration of soluble salts (potassium and sodium) present in the laminated and massive pelites (0.16% and 0.14% respectively) of the alkaline and mesohaline Loma del Pterodaustro lake (Perino et al., 2011) and the prevailing semi-arid conditions at that time (Rivarola and Spalletti, 2006; Perino et al., 2011). Therefore, as previously implied by Chiappe et al. (1998), it appears that the best *P. guinazui* modern ecomorph in terms of habitat (Chiappe et al., 1998) in mesohaline

environments and filter feeding strategies (Chiappe et al., 1998) are Phoenicopteriformes. As such, examination of MIC-V 778 and estimation of its conductance properties, combined with taphonomic, geological, and environmental observations strongly suggest that the *P. guinazui* reproduction mode was most comparable to Mirandornithes, thus further indicating that this pterosaur species was in all aspects an ecomorph of modern flamingos.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.gsf.2014.05.002>.

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