The eggshell of the Eocene bird *Lithornis*

GERALD GRELLET-TINNER and GARETH J. DYKE

Grellet-Tinner, G. and Dyke, G.J. 2005. The eggshell of the Eocene bird *Lithornis*. *Acta Palaeontologica Polonica* 50 (4): 831–835.

Although the fossil bird *Lithornis* has been known for more than a century, only in the 1980s were its affinities within the palaeognathous birds (Aves, Palaeognathae) realized and demonstrated by use of osteological characters. Other lines of evidence could, however, be used to test hypotheses of its affinities. To add data to this ongoing investigation, we present the first detailed description of the microscopic morphology of the eggshell of this fossil bird. Our description of eggshells of two species of *Lithornis* is consistent with the placement of this fossil bird within Palaeognathae. Characters that corroborate this position include the presence of three aprismatic structural layers visible by use of scanning electron microscopy (SEM) in the eggshell microstructure. The placement of *Lithornis* phylogenetically close to the extant flighted South American group Tinamidae is supported on the basis of characters present in the structural composition of the eggshell layers of both these taxa.

Key words: Aves, Palaeognathae, *Lithornis*, eggshell ultrastructure, Eocene, London Clay.

Gerald Grellet-Tinner [ggrellet@nhm.org], Department of Earth Science, University of Southern California, Los Angeles CA 90007, USA;
Gareth J. Dyke [gareth.dyke@ucd.ie], School of Biological and Environmental Science, University College Dublin, Belfield Dublin 4, Ireland.

Introduction

Lithornis was one of the earliest described fossil birds. The genus was named in 1840 by Sir Richard Owen (Owen 1840) on the basis of partial fossil remains preserved within a clay nodule (figured for the first time in Owen 1841) collected from the lower Eocene London Clay Formation on the Kent coast, UK (Isle-of-Sheppey) sometime before 1793 (Owen 1846). The original holotype specimen of *Lithornis vulturinus* was housed in the Museum of the Royal College of Surgeons until it was destroyed in the bombing of London during World War II.

Subsequent to this, little more information pertaining to *Lithornis* was published until Houde (1986, 1988) reported the discovery of additional fossil specimens, including both skeletons and fragments of eggshell from the Paleogene of North America, which he considered closely related to *L. vulturinus*. On the basis of this new material, coupled with re-evaluation of existing fossils not previously recognized as “lithornithid” (Harrison and Walker 1977), Houde (1988) designated a neotype specimen for *L. vulturinus*, and named a number of additional species including the North American *Lithornis celetius*. This taxon, from the Paleocene Fort Union Formation of Montana (Houde 1988), was placed within the family Lithornithidae along with two additional genera—*Paracathartes* Harrison, 1979 and *Pseudocrypturus* Houde, 1988. Based on current knowledge of the fossil record, members of Lithornithidae are known from the latest Cretaceous–earliest Eocene of New Jersey (Parris and Hope 2002) through to the upper Eocene of the United Kingdom (Houde 1988; Dyke 2000), spanning some 15 million years (Dyke 2003).

The phylogenetic placement of *Lithornis* (and related taxa) has been questioned over the years since the original discovery of these birds. Owen (1841) considered these birds to be related to extant Cathartidae (New World vultures), whereas Harrison and Walker (1977) placed them closer to Musophagidae (turacos). Houde (1988) was the first to present the hypothesis that *Lithornis* is a basal member of Palaeognathae on the basis of osteological observations and some comparisons of eggshell structure (gross morphology and ground thin sections). This hypothesis was corroborated by the observations of Mikhailov (1997) who reported the eggshell of this genus to be “ratite-like” (Mikhailov 1997: 71). In light of recent advances in the use of ultrastructural features of eggshell anatomy as characters in bird systematics (Grellet-Tinner 2000; Zelenitsky and Modesto 2003), we present description of *Lithornis* eggshell based on two specimens collected in association with skeletons of these birds. We figure in detail the microstructure of *Lithornis* eggs for the first time.

Institutional abbreviations.—AMNH, American Museum of Natural History, Division of Vertebrate Zoology (Ornithology), New York, USA; BMNH, Natural History Museum (Palaeontology Department collections), London, UK; NMING F, National Museum of Ireland, Division of Natural History (Geology collections), Dublin, Ireland; YPM, Peabody Museum of Natural History, Yale University, New Haven, USA.

Material and methods

Eggshell fragments collected in association with skeletal specimens of *Lithornis vulturinus* and *Lithornis celetius* were used in this study. One fragment was donated to G.J. Dyke by a private collector working in the lower Eocene London Clay Formation at the locality of Walton-on-the-Naze, Essex, UK and was found in association with a humerus and sternum referrable on the basis of osteological characters to *L. vulturinus* (GJD personal observation). Eggshell fragments referrable to *L. celetius* were made available to GGT by the Yale Peabody Museum (YPM). These latter pieces were collected from the Bagtail Quarry in the Paleocene Fort Union Formation (Gallatin National Forest, Montana) in 1942 and include pieces already thin sectioned by Houde (1988). Entire skeletons of *L. celetius* are known from this quarry (Houde 1988). We note that both in the UK and North America, specimens of *Lithornis* are commonly found by collectors disarticulated inside calcareous Paleogene clay nodules. This style of preservation renders it unlikely that individual specimens of these birds will be found directly associated with eggshell material (i.e., preserved sitting on a nest). Samples were broken into smaller fragments, washed and dried for SEM and thin section observations, following the method as implemented recently by Grellet-Tinner (2000) and Makovicky and Grellet-Tinner (2000) (see also Mikhailov 1997; Varricchio and Jackson 2004).

When observed in cross section under SEM, the eggshells of most birds display at least 3 structural layers—the "mammilary layer" (layer 1), "squamatic zone" (layer 2) and "external zone" (layer 3) of Mikhailov (1997). To avoid problems with the homology of these layers, our preference is to assign them numbers (see Grellet-Tinner 2000). The only described exceptions to this are the dromornithids (e.g., *Genyornis newtoni*) that seem to have had only two layers along with *Dromaius novaehollandiea* and *Casuarius casuarius* that have four layers (Mikhailov 1997; Grellet-Tinner 2000). All neognathous birds exhibit what has been referred as a prismatic condition between their layers whereas palaeognathous taxa are characterized by an aprismatic condition, defined by the way the shell layers are delimited (Grellet-Tinner 2000; Grellet-Tinner and Norell 2002; Grellet-Tinner and Chiappe 2004; Buffetaut et al. 2005; Grellet-Tinner in press; Grellet-Tinner et al. in press). Aprismatic layers have sharp and well-defined delimitations between adjacent structural layers whereas the delimitation of prismatic layers is somewhat indistinct and not easily noticeable (Mikhailov 1997; Grellet-Tinner 2000; Grellet-Tinner and Norell 2002).

Description

Houde (1988) presented brief macroscopic and microscopic descriptions of the morphology of eggs referred to *Lithornis* from the Eocene of North America. Mikhailov (1997) noted that eggs referrable to this genus are "ratite-like" in their structure. On the basis of our analyses, we corroborate the fact that the eggs of *L. celetius* have a smooth external surface (as seen in *Struthio*, *Rhea*, and Tinamidae; Mikhailov 1997). Large and widely spaced pore apertures on the eggshell surface are commonly visible to the naked eye. These pores are conical in radial thin section (Fig. 1; Houde 1988: fig. 31). Houde (1988) also noted the presence of a "cuticular layer" of outer "true shell" in these birds that is also similar to that seen in extant Tinamidae (Tyler and Simkiss 1959; Houde 1988: 118).

However, our observations demonstrate that this interpretation of the eggshell structure based on thin sections is incorrect—the third and outermost layer of *Lithornis* shell is instead aprismatic and composed of calcium carbonate as is characteristic for all palaeognath eggshells (Fig. 1; Mikhailov 1997; Grellet-Tinner 2000). Although a thin "cuticular layer" (Houde 1988) does cover the outer surface of most modern bird eggs after ovideposition and during the early stage of incubation (Deeming and Ferguson 1991), this layer has not often been observed in the fossil record as a result of its chemical and organic composition (GGT personal observation). Preservations of organic structures, including soft tissues, are typical of sedimentation regimes involving stagnation typical of Lagerstätten deposits (Bottjer et al. 2002; Grellet-Tinner 2005). Examples of soft tissue preservation in eggs and eggshells have been reported in few instances (Sochava 1969; Kohring 1999; Grellet-Tinner 2005), mostly restricted to the preservation of the membrana testacea (the protein-rich membrane directly underneath the eggshell), for example in eggs of the non-avian theropod *Troodon formosus* (Zelenitsky et al. 2002; Grellet-Tinner 2005). Recent observation of fossilized testaceaous membrane in titanosaurid eggs from Auca Mahuevo (Argentina) has led to the interpretation that this unique preservation could be bacterially mediated (GGT personal observation).

Our sample of *L. celetius* shell is diagenetically altered, hence internal eggshell structural layers are only visible in small areas whereas little diagenetic alteration is visible in the shell of *L. vulturinus* (Figs. 1, 2). In both taxa, the outer eggshell surface (seen in tangential view; Fig. $1A_3$) is smooth and displays a number of circular pores that range in their diameter from 73 to 80 μm. The presence of these pores is variable between specimens depending on the degree of eggshell re-crystallization. Cross-sectional views reveal the presence of three clear structural and aprismatic layers in these shells, a character unique to palaeognathous birds (Grellet-Tinner 2000). Although the second layer is by far the most conspicuous (Figs. $1A_2$, $2A_2$), all three are clearly visible with the third being thinnest. The overall vertical crystal orientation of this layer differs from that of layer two (Fig. $1A_2$), and averages 25 μm in thickness.

Layer 1 comprises a series of individual shell crystal units fused together and averages 40 μm in thickness. The base of these units are rounded (concave-side down) and comprise

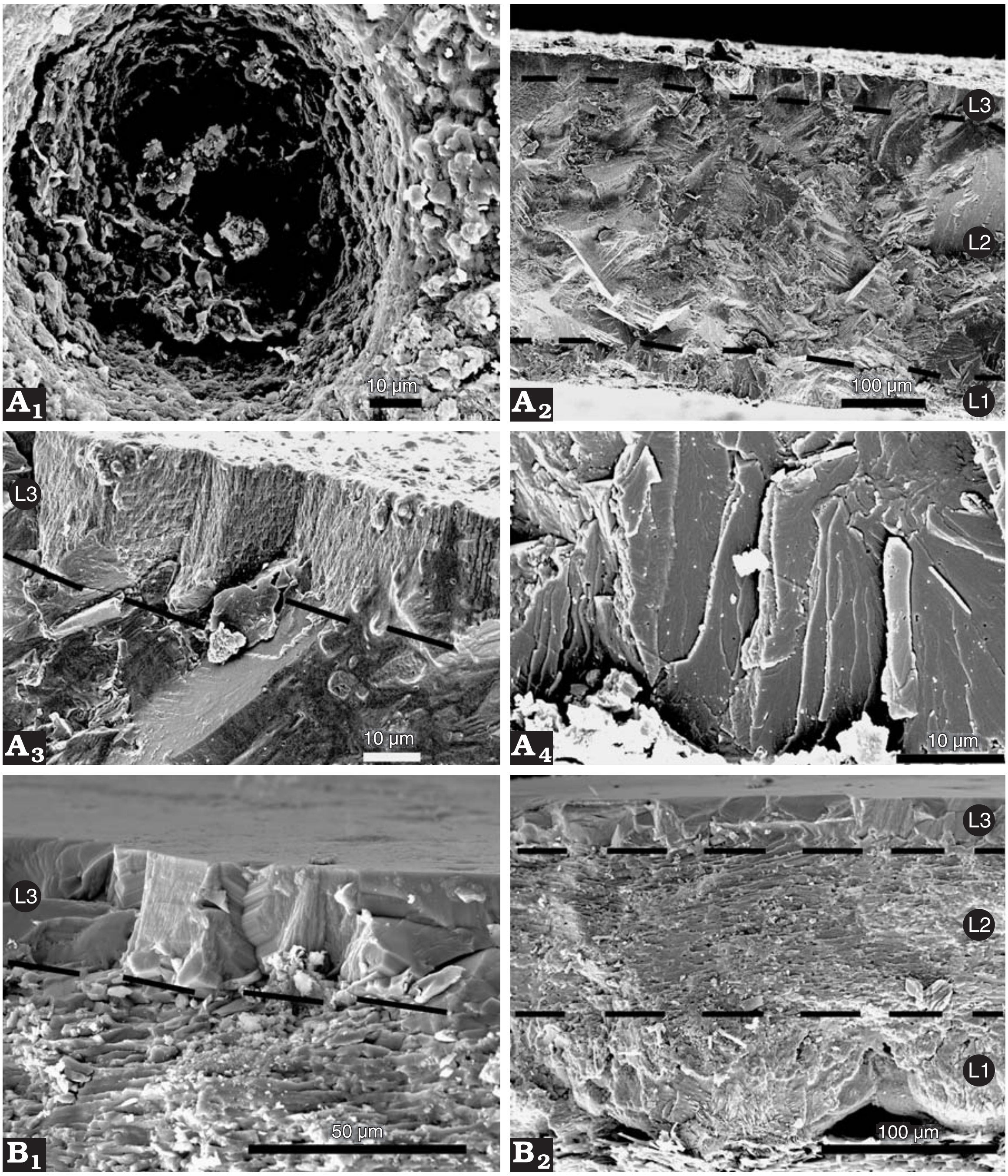

Fig. 1. SEM micrograph images of eggshells. **A**. *Lithornis vulturinus* (Lithornithidae), NMING F 2005-1, Walton-on-the-Naze, Essex, UK, London Clay Formation (lower Eocene). A_1. Circular pore orifice on the eggshell outer surface; A_2. Three aprismatic structural layers (our layers 1–3; see text) compose the entire thickness of the same specimen. Layers, 1 and 3 are relatively thin comparatively to layer 2. A_3. Details of layer 3. A_4. Bladed calcite crystals at the base of eggshell. **B**. *Rhynchotus rufescens* (Tinamidae), AMNH 13376, Recent. B_1. Layer 3 of the eggshell. B_2. Structural layers with bladed calcite crystals at the base of eggshell units.

long, bladed calcite crystals in *L. vulturinus* (Fig. 1A_2) and acicular crystals in *L. celetius* (Fig. 2A_4). In general the size and geometry of layer 1 is reminiscent of that of the tinamou *Rhynchotus*. Of the three layers that comprise the shell units in *Lithornis*, layer 2 is by far the thickest measuring between 307 and 486 µm in our samples. SEM images show a relative difference in the morphology of the calcite crystals located in layer 1, a disparity that is not attributed to diagenetic alteration. Crystals at the base of each eggshell units have their C axes vertically orientated but their aspect ratio (width/length) in *L. celetius* gives them an acicular/tabular morphology. In contrast, the aspect ratio in *L. vulturinus* has produced more bladed crystal shapes. Such a dichotomy is also observable in the structural make up of eggshell of extant Tinamidae (GGT personal observation) but no other known palaeognath group.

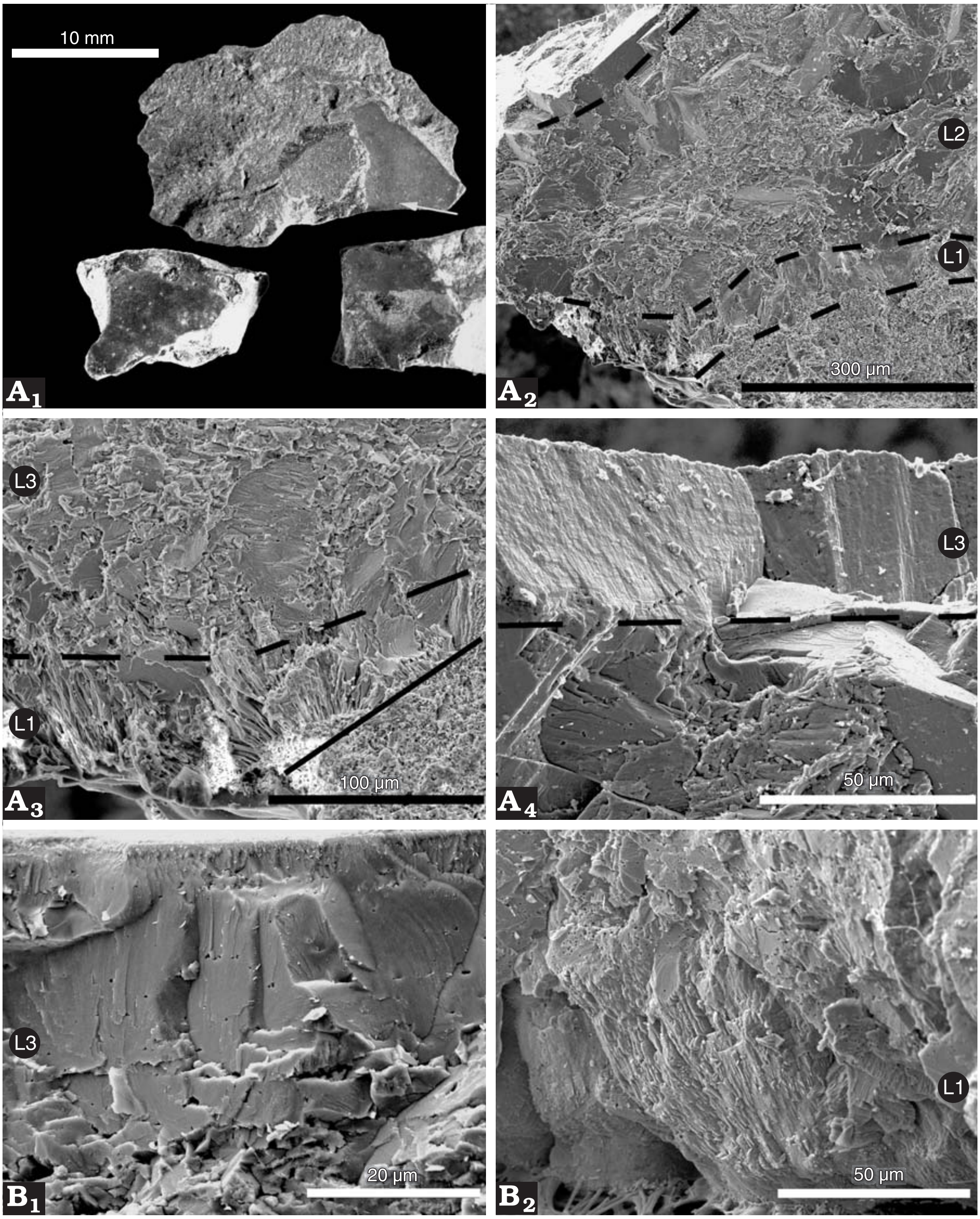

Fig. 2. SEM micrograph images of eggshells. **A**. *Lithornis celetius*, YPM PU 16961, Bagtail Quarry, Montana, Fort Union Formation (Paleocene). A_1. Circular pore orifice on the eggshell outer surface (arrow). A_2. Even though it is hard to see, the entire thickness of the eggshell consists of three aprismatic structural layers (layers, 1 and 3 are also relatively thin comparatively to layer 2; see text). A_3. Detail of layers 1 and 2 (note the acicular crystals at the base of the eggshell units). A_4. Detail of layer 3. **B**. *Tinamus tinamus* (Tinamidae), AMNH 15478, Recent. B_1. Layer 3. B_2. Layer 1 with acicular calcite crystals at the base of eggshell units.

Discussion

Despite some historical debate with regard to the systematic placement of *Lithornis* and other fossil members of Lithornithidae, evidence from comparative skeletal morphology (Houde 1988) and phylogenetic analyses of osteological characters (Dyke 2000, 2003; Clarke and Norell 2002) has converged to the view that not only are these birds primitive palaeognaths but that they may also be closely related to extant Tinamidae (Bertelli et al. unpublished data).

The presence of at least three aprismatic structural layers (seen in microscopic section; Mikhailov 1997) is a synapomorphy of the eggs of Palaeognathae (as opposed to three prismatic layers as seen in all other extant neornithine birds; Neognathae; Grellet-Tinner 2000). Three structural eggshell layers were also observed in an avian egg from the Late Cretaceous Gobi Desert, Mongolia (Grellet-Tinner and Norell 2002), consistent with its interpretation as a neognath. The layers of *Lithornis* eggshell are certainly aprismatic, a condition known to occur only in extinct and extant palaeognaths (Grellet-Tinner 2000).

Although *Lithornis* has been known for over 100 years and was postulated as representing a basal palaeognath since the 1980s, little character data other than osteology have been presented that can be used to corroborate this hypothesis. Further description of its eggshell, however, independently supports its placement within Palaeognathae. However, an additional discovery of eggshells associated with embryonic remains *in ovo* will be required to better assess the significance of *Lithornis* and related birds to the general question of palaeognath and basal neognath evolution by use of oological evidence.

Acknowledgements

We are grateful to Joel Cracraft (American Museum of Natural History, New York) and Luis Chiappe (Natural History Museum of Los Angeles County, Los Angeles) for their support of our work. We also thank Sandra Chapman (Natural History Museum, London), Lyndon Murray (formerly Yale Peabody Museum, New Haven), and Paul Sweet (American Museum of Natural History, New York) for providing access to specimens in their care, Simon Chaplin (Museum of the Royal College of Surgeons, London) for historical information about the Museum of the Royal College of Surgeons (London), and Cyril Walker (Natural History Museum, London) for his continuing advice and support. We are grateful to Sean Modesto (Cape Breton University, Cape Breton, Canada) and an anonymous reviewer for very helpful comments in review; this work was supported by the Frank M. Chapman Fund of the American Museum of Natural History and is dedicated to the memory of Colin J.O. Harrison.

References

Bottjer, D.J., Etter, W., Hagadorn, J. W., and Tang, C.M. 2002. *Exceptional Fossil Preservation: A Unique View on the Evolution of Marine Life*. 424 pp. Columbia University Press, New York.

Buffetaut, E., Grellet-Tinner, G., Suteethorn, V., Cuny, G., Tong, H., Košir, A., Cavin, L., Chitsing, S., Griffiths, P.J., Tabouelle, J., and Le Loeuff, J. 2005. Very small eggs and embryo from the Early Cretaceous of Thailand and the dinosaur–bird transition. *Naturwissenschaften* DOI 10.1007/s00114-005-0022-9.

Clarke, J.A. and Norell, M.A. 2002. The morphology and phylogenetic position of *Apsaravis ukhaana* from the Late Cretaceous of Mongolia. *American Museum Novitates* 3387: 1–24.

Deeming, C.D. and Ferguson, M.W.J. 1991. *Egg Incubation: its Effects on Embryonic Development in Birds and Reptiles*. 462 pp. Cambridge University Press, New York.

Dyke, G.J. 2000. *Taxonomy and Phylogenetics of Fossil Modern Birds: The Early Radiation of Neornithes*. 450 pp. PhD. thesis, University of Bristol, Bristol.

Dyke, G.J. 2003. The fossil record and molecular clocks: basal radiations within Neornithes. *In*: P. Smith and P. Donoghue (eds.), *Molecular Clocks and the Fossil Record*, 263–278. Taylor and Francis, London.

Grellet-Tinner, G. 2000. Phylogenetic interpretation of eggs and eggshells. *In*: A. M. Bravo and T. Reyes (eds.), *First International Symposium on Dinosaur Eggs and Babies*, 61–75. Isona I Conca Dellà, Catalonia.

Grellet-Tinner, G. 2005. The membrana testacea of titanosaurid dinosaur eggs from Auca Mahuevo (Argentina): Implications for the exceptional preservation of soft tissue in Lagerstätten. *Journal of Vertebrate Paleontology* 25: 99–106.

Grellet-Tinner, G. (in press). Phylogenetic interpretation of eggs and eggshells: implications for oology and Paleognathae phylogeny. *Alcheringa*.

Grellet-Tinner, G. and Chiappe, L.M. 2004. Dinosaur eggs and nestings: implications for understanding the origin of birds. *In*: J.P. Currie, E.B. Koppelhus, M.A. Shugar, and J.L. Wright (eds.), *Feathered Dragons: Studies on the Transition from Dinosaurs to Birds*, 185–214. Indiana University Press, Bloomington.

Grellet-Tinner, G. and Norell, M.A. 2002. An avian egg from the Campanian of Bayn Dzak, Mongolia. *Journal of Vertebrate Paleontology* 22: 719–721.

Grellet-Tinner, G., Chiappe, L., Bottjer, D., and Norell, M. (in press). Paleobiology of dinosaur eggs and nesting behaviors. *Palaeogeography, Palaeoclimatology, Palaeoecology*.

Harrison, C.J.O. 1979. A new cathartid vulture from the Lower Eocene of Wyoming. *Tertiary Research Special Paper* 5: 29–39.

Harrison, C.J.O and Walker, C.A. 1977. Birds of the British Lower Eocene. *Tertiary Research Special Paper* 3: 1–52.

Houde, P. 1986. Ostrich ancestors found in the Northern Hemisphere suggest new hypothesis for ratite origins. *Nature* 324: 563–565.

Houde, P. 1988. Paleognathous birds from the Tertiary of the northern hemisphere. *Publications of the Nuttall Ornithological Club* 22: 1–148.

Kohring, R.R. 1999. Calcified shell membranes in fossil vertebrates eggshell: evidence for preburial diagenesis. *Journal of Vertebrate Paleontology* 19: 723–727.

Makovicky, P. and Grellet-Tinner, G. 2000. Association between a specimen of *Deinonychus antirrhopus* and theropod eggshell. *In*: A.M. Bravo and T. Reyes (eds.), *First International Symposium on Dinosaur Eggs and Babies*, 123–128. Isona I Conca Dellà, Catalonia.

Mikhailov, K.E. 1997. *Avian Eggshells: An Atlas of Scanning Electron Micrographs*. 88 pp. British Ornithologists' Club Occasional Publications, No. 3. The Nature Conservancy Bureau Limited, Newbury.

Owen, R. 1840. Description of the remains of a mammal, a bird, and a serpent from the London Clay. *Proceedings of the Geological Society of London* 3: 162–166.

Owen, R. 1841. Description of the fossil remains of a mammal (*Hyracotherium leporinum*) and of a bird (*Lithornis vulturinus*) from the London Clay. *Transactions of the Geological Society of London* 6: 203–208.

Owen, R. 1846. *A History of British Fossil Mammals and Birds*. 403 pp. John Van Voorst, London.

Parris, D.C. and Hope, S. 2002. New interpretations of birds from the Navesink and Hornerstown Formations, New Jersey, USA. *In*: Z. Zhou and F. Zhang (eds.), *Proceedings of the 5th Symposium of Avian Paleontology and Evolution, Beijing*, 113–124. China Ocean Press, Beijing.

Sochava, A.V. 1969. Dinosaur eggs from the Upper Cretaceous of the Gobi Desert. *Paleontological Journal* 3: 517–527.

Tyler, C. and Simkiss, K. 1959. A study of the eggshells of ratite birds. *Proceedings of the Zoological Society of London* 133: 201–243.

Varricchio, D.J. and Jackson, F.D. 2004. A phylogenetic assessment of prismatic dinosaur eggs from the Cretaceous Two Medicine Formation of Montana. *Journal of Vertebrate Paleontology* 24: 931–937.

Zelenitsky, D.K. and Modesto, S.P. 2003. New information on the eggshell of ratites (Aves) and its phylogenetic implications. *Canadian Journal of Zoology* 81: 962–970.

Zelenitsky, D.K., Modesto, S.P., and P. J. Currie. 2002. Bird-like characteristics of troodontid theropod eggshell. *Cretaceous Research* 23: 297–305.