## Cleidoic eggs: A key to water to land Transition

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## Abstract

The development of the cleidoic egg was a significant adaptation that allowed amniotes to reduce their reliance on aquatic environments for reproduction. Cleidoic eggs are characterised by a rigid, calcified outer shell and additional membranes that aid in the exchange of gases and protect against drying out when on land. The emergence of this intricate egg structure was a crucial advancement in the development of amniotes, enabling them to thrive and adapt in terrestrial habitats. This research examines the evolutionary history and physical structure of cleidoic eggs, investigating their probable emergence during the Carboniferous period and the varied structural adaptations that have developed in various amniote lineages. The current understanding of the evolution and adaptive radiation of amniotes is provided by synthesising fossil evidence, comparative developmental biology, and evolutionary scenarios.

**Key words :** Cleidoic eggs, extra embryonic membranes, oviparous, amniotes, tetrapod.

The development of cleidoic eggs had a vital role in the shift of vertebrate organisms from aquatic to terrestrial habitats in the late Paleozoic epoch, around 300-330 million years ago<sup>1,39</sup>. Cleidoic eggs, exclusive to amniotes, are distinguished from anamniote eggs by their rigid, calcified outer covering and a distinct set of additional membranes outside the embryo, namely the amnion, chorion, and allantois<sup>24,34,47</sup>. The membranes enveloping the embryo create a sac filled with fluid. They facilitate the interchange of gases, storage of water and waste, and provide protection against drying out and physical strain<sup>4,12,14</sup>. The combination of the durable eggshell and additional membranes of the cleidoic egg allowed early amniotes to successfully develop their embryos on land, without the requirement of laying eggs in water. Although the morphology of cleidoic eggs can exhibit considerable variation across various amniote groups, they all share the essential characteristics of a mineralized shell and extraembryonic membranes<sup>33</sup>. The calcitic eggshell serves as a framework and controls the flow of gas and water between the egg and its surroundings

via a system of tiny pores<sup>18,31,46</sup>. Across several groups of organisms, there have been adaptations in the form of extra layers and surface characteristics of eggshells to alter their properties in response to diverse environments and incubation procedures<sup>10,17,54</sup>. The wide range of amniote eggshells, both current and prehistoric, indicates an intricate evolutionary past influenced by the selection for different ecological and biological functions<sup>22</sup>.

This research examines the existing knowledge about the formation and development of the cleidoic egg, utilising evidence from the fossil record, comparative developmental biology, and evolutionary theory. The essay examines the identification of important structural advancements and investigates their functional importance within the framework of the diversification of amniotes in terrestrial habitats. Additionally, this paper also addresses any unanswered problems and suggests potential areas for future research regarding this significant event in the development of vertebrates.

The Cleidoic egg originated from the fossil of *Hylonomus lyelli*, the oldest known amniote, found in the Late Carboniferous lydiite layers of Joggins, Nova Scotia. This fossil is estimated to be around 315-319 million years old. Although there are no preserved eggs found with *Hylonomus* or other early amniote species, the absence of larval fossils and the nature of the environment in which they were deposited clearly indicate that these primitive amniotes had developed a method of reproduction that did not rely on aquatic settings<sup>27</sup>. The oldest known fossil evidence of amniote eggs and embryos is found in the Early Permian

mesosaur Mesosaurus tenuidens from Uruguay and Brazil. This specimen contains well-preserved soft tissues and remnants of eggshells<sup>36,37,50</sup>. The presence of these fossils provides evidence that cleidoic eggs had developed during the Early Permian period, which aligns with the proposed timeline based on skeletal fossils and phylogenetic assumptions. Paleobiological data suggests that the cleidoic egg originated from anamniote predecessors that deposited parchment-shelled eggs in aquatic environments<sup>26</sup>. The process of eggshell calcification and the development of extraembryonic membranes and their associated functions are believed to have occurred gradually, in a sequential manner<sup>2,47</sup>. The presence of a calcified coating encircling a fibrous eggshell in certain modern amphibians may indicate a transitional phase between the flexible eggs of anamniotes and the inflexible eggs of amniotes<sup>30</sup>. The chorioallantoic membrane, responsible for gas exchange in cleidoic eggs, is believed to have originated from the preexisting chorion and allantois in anamniotes. It subsequently underwent modifications to fulfil its specialised respiratory function<sup>25</sup>.

Studying the development of living amniotes and anamniotes has given us valuable knowledge on how the cleidoic egg evolved. An analysis of gene expression patterns during the development of the amnion in chickens has uncovered common molecular mechanisms with non-amniote vertebrates. This suggests that the amnion arose by utilising existing developmental pathways. Research on various amniote model species, like mice and lizards, has provided additional insights into the developmental process of extraembryonic membrane creation. It has also uncovered crucial genetic and molecular alterations that were essential for the evolution of the amniotic egg<sup>44,45,51</sup>. Various hypotheses have been suggested to explain the specific stresses that may have influenced the development of the cleidoic egg. An influential hypothesis suggests that cleidoic eggs emerged as a response to the growing dryness and seasonal changes in terrestrial habitats during the late Paleozoic era<sup>11,42</sup>. Cleidoic eggs, by confining amniote embryos within a closed system that is separate from the external environment, offer a mechanism to prevent dehydration and ensure ideal circumstances for development, even in arid settings far away from water<sup>5</sup>. The presence of calcitic eggshell and the microenvironment of the nest provided additional safeguards against UV radiation, severe temperatures, and predators, hence increasing the chances of surviving on land<sup>3,23</sup>.

One alternate theory proposes that cleidoic eggs may have initially developed in aquatic or semiaquatic tetrapods as a strategy to prevent hypoxia<sup>7</sup>. Cleidoic eggs, by confining the embryo and its accompanying membranes under a hard shell that allows the passage of gases, would have enabled embryos to maintain enough oxygen levels for growth, even in sluggish or oxygen-deficient waters. This initial adaptation for aquatic surroundings may have functioned as a preadaptation for later growth into terrestrial habitats. Although the exact factors that drove the selection process are not known, it is certain that the cleidoic egg had a crucial role in overcoming the limitation of aquatic development, enabling amniotes to thrive and adapt in terrestrial habitats. This evolutionary innovation paved the way for the diversification of amniotes during the Mesozoic era and the creation of the prevailing terrestrial vertebrate populations we see today. The diversity and functional morphology of cleidoic eggs have undergone significant evolution since their emergence in the late Paleozoic era. This evolution has resulted in a wide range of egg shapes and structures, which are specifically adapted to various habitats, nesting behaviours, and life cycles. Amniote eggshells exhibit significant variation in their size, shape, and structure between different groups, as well as in the number and placement of pores and extra layers. The presence of this diversity indicates an intricate evolutionary past influenced by the selection for various functional tasks<sup>41,48,54</sup>.

The composition and organisation of the calcitic eggshell is a significant structural characteristic of cleidoic eggs that differs among different amniote groups. Archosaurs, which include crocodilians and birds, have eggshells composed of three layers: a mammillary layer containing calcite crystals that radiate outward, a prismatic layer with vertically growing crystals, and an exterior layer<sup>8,19</sup>. Conversely, the eggshells of the majority of lepidosaurs (including lizards, snakes, and tuatara) consist of a solitary layer of calcite crystals<sup>35</sup>. Turtles, scientifically known as Testudines, display a variety of eggshell architectures that vary in their flexibility and mineralization levels, ranging from easily bendable and low in mineral content to rigid and prone to breaking<sup>28,29</sup>. The variations in eggshell structure have practical consequences for the exchange of gases and water, as well as for providing mechanical protection and creating an optimal environment for incubation. Another important characteristic of cleidoic

egg morphology is the external decoration of the eggshell. Several species have surface features, such as ridges, nodes, spikes, or pore canals, which have an impact on gas exchange and water loss<sup>15,21,52</sup>. Recent studies have demonstrated a correlation between the surface characteristics of eggshells in contemporary avian species and their nesting habitat. Specifically, eggs placed under moist conditions tend to have greater porosity in their shells, which aids in the process of water loss<sup>38</sup>. Certain nonavian dinosaurs, like oviraptorids and troodontids, had eggs with elaborate decorations. This indicates that these surface characteristics served a similar purpose in controlling the nest's microclimate<sup>49,53</sup>.

In addition to the eggshell, the extraembryonic membranes of cleidoic eggs also display variants that correspond to various developmental and ecological requirements. The chorioallantoic membrane in birds and some nonavian dinosaurs is extensively supplied with blood vessels, which is an adaptation that enables efficient exchange of gases in these highly active and metabolically demanding species $^{6,46}$ . On the other hand, numerous lizards and snakes possess a relatively uncomplicated chorioallantois, which indicates their lower metabolic needs<sup>40</sup>. The development of a more intricate and well-supplied chorioallantois, which is a membrane involved in gas exchange during embryonic development, may have been necessary for the rapid growth rates and active behaviours observed in birds and certain extinct taxa such as pterosaurs. Fossilised eggshells offer a comprehensive account of the evolutionary development of different structural adaptations. Fragments of eggshells have been discovered in many prehistoric amniote taxa, such as non-avian dinosaurs, pterosaurs, and early synapsids<sup>20,43</sup>. A significant number of these fossil eggshells retain intricate microstructural characteristics, which provide insights into their composition, biological functions, and nesting conditions<sup>13,16</sup>. Analysis of fossil eggshells has uncovered that certain taxa, such as titanosaur sauropods, deposited numerous eggs in extensive communal nesting grounds, indicating a reproductive approach distinct from that of its contemporary counterparts<sup>30</sup>.

Fossilised embryos and nests, along with eggshells, offer additional understanding of the development and purpose of cleidoic eggs throughout evolution. The discovery of a nesting colony of the Cretaceous theropod Torvosaurus, with more than 500 eggs and many embryos, has provided valuable insights on the various reproductive strategies of extinct amniotes<sup>32</sup>. Comparative analyses of fossilised embryos have also uncovered crucial elements of developmental evolution, such as the lack of an egg tooth in early reptiles and synapsids<sup>9</sup>. The extensive range of cleidoic egg structures observed in both current and extinct amniotes is a result of more than 300 million years of evolutionary adaptation to varying environmental conditions and ecological functions. Continuing research on the growth, functioning, and ancient biology of these features holds the potential to provide more insight into the evolutionary origins of this important adaptation and its contribution to the success of amniotes.

The formation of the cleidoic egg was a crucial advancement in the history of vertebrates, enabling amniotes to overcome the ancient limitation of aquatic growth.

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Cleidoic eggs create a stable microenvironment for growth on land by enclosing the embryo with fluid-filled membranes inside a hard, gaspermeable shell. This evolutionary adaptation allowed amniotes to successfully enter and thrive in terrestrial habitats, paving the way for the emergence of the current major landdwelling vertebrate populations. Since their emergence in the late Paleozoic era, cleidoic eggs have developed a wide range of structural modifications that demonstrate various environmental obstacles and reproductive tactics. Differences in the structure of eggshells, surface patterns, and complexity of extraembryonic membranes are a result of natural selection for various functional purposes, such as controlling the interchange of gases and moisture during embryonic development, as well as adapting to specific nesting environments. The fossil record of eggshells and embryos offers a comprehensive account of the evolutionary history, supplementing findings from comparative developmental studies of existing species.

Ongoing research is shedding light on the developmental foundation and ecological implications of cleidoic eggs, while many concerns concerning their origin and evolution still remain. The unearthing of novel fossil specimens, the utilisation of sophisticated imaging and geochemical methodologies, and the amalgamation of data from molecular developmental investigations all hold the potential to provide additional insights on this crucial occurrence in the evolution of vertebrates. The cleidoic egg, which is a crucial adaption facilitating the diversification of amniotes, continues to be a captivating topic of research for evolutionary biologists and palaeontologists. References :

- Benton, M. J., and P. C. Donoghue (2007). Molecular Biology and Evolution, 24(1): 26-53.
- Blackburn, D. G. (2015). Journal of Morphology, 276(8): 961-990.
- Brocklehurst, N., M. Ruta, J. Müller, and J. Fröbisch, (2015). *Scientific reports*, 5(1): 1-10.
- 4. Carroll, R. L. (1964). Zoological Journal of the Linnean Society, 45(304): 61-83.
- 5. Carroll, R.L. (1970). *Forma et functio, 3:* 165-178.
- Choi, S., S. Han, N. H. Kim, Y. N. Lee, and T. Choi, (2019). *PloS one, 14*(7): e0219496.
- Collias, N. E. (1997). *The Condor*, 99(2): 253-270.
- D'Alba, L., and M. D. Shawkey, (2015). Journal of Ornithology, 156(1): 399-408.
- 9. Deeming, D. C. (2006). *Palaeontology*, 49(1): 171-185.
- Dennis, J.E., S.Q. Xiao, M. Agarwal, D.J. Fink, A.H. Heuer, and A.I. Caplan (1996). *Journal of morphology*, 228(3): 287-306.
- Dunne, E. M., R. A. Close, D. J. Button, N. Brocklehurst, D. D. Cashmore, G. T. Lloyd and R.J. Butler (2018). *Proceedings* of the Royal Society B: Biological Sciences, 285(1872): 20172730.
- Eckalbar, W. L., E. D. Hutchins, G. J. Markov, A. N. Allen, J.J. Corneveaux, K. Lindblad-Toh, and K. Kusumi, (2013). *BMC Genomics*, 14(1): 49.
- 13. Ensom, P. C. (1997). Proceedings of the Dorset Natural History and Archaeological Society, 118: 79-83.
- 14. Falcon-Lang, H. J., M. J. Benton, and M. Stimson (2007). *Journal of the Geological*

Society, 164(6): 113-118.

- Fernández, M.S., E. Vlachos, M.R. Buono, L. Alzugaray, M. A. Reguero and S. A. Marenssi, (2021). Egg morphology and internal microstructures from a new Antarctic penguin nesting site reveal Pliocene environmental conditions. Palaeontology, 64(5): 725-742.
- Grealy, A., M. Phillips, G. Miller, M. T. P. Gilbert, J. M. Rouillard, D. Lambert, and M. Bunce, (2017). *Molecular phylogenetics and evolution*, 109: 151-163.
- 17. Grellet-Tinner, G. (2006). *Alcheringa*, *30*(1): 141-182.
- Grellet-Tinner, G., L. Chiappe, M. Norell, and D. Bottjer (2006). *Palaeogeography, Palaeoclimatology, Palaeoecology,* 232(2-4): 294-321.
- 19. Grellet-Tinner, G., S. Lindsay, and M. B. Thompson, (2017). *Peer J*, *5*: e3194.
- Hayward, J.L., K.M. Dickson, S.R. Gamble, A. W. Owen, and K. C. Owen, (2011). *Historical Biology*, 23(1): 5-13.
- Huynen, L., B. J. Gill, C. D. Millar, and D. M. Lambert, (2010). Proceedings of the National Academy of Sciences, 107(37): 16201-16206.
- 22. Jackson, F.D. and D. J. Varricchio, (2010). Journal of Vertebrate Paleontology, 30(4): 1142-1156.
- 23. Janis, C. M., and J. C. Keller, (2001). Acta Palaeontologica Polonica, 46(2): 237-261.
- 24. Kohring, R. (1995). *Historical Biology*, *10*(3): 259-275.
- 25. Kin, K., J. Maziarz, and G. P. Wagner, (2014). *Biology of reproduction*, *90*(5): 111-1.
- 26. Laurin, M., and M. Girondot, (1999). Annales des Sciences Naturelles-Zoologie et Biologie Animale, 20(3): 99-104.

- 27. Laurin, M., and R. R. Reisz, (1995). Zoological Journal of the Linnean Society, 113(2): 165-223.
- 28. Lawver, D. R., and A. M. Debee, (2021). Turtle eggs: *Journal of Morphology*, *282*(10): 1521-1538.
- 29. Lawver, D. R., A. H. Rasoamiaramanana and I. Werneburg, (2021). *Herpetology Notes*, *14*(1): 393-400.
- 30. Lawver, D. R., E. A. E. Smith, and E. R. Schroeter, (2019). Microstructural characteristics of dinosaur eggshells from the Oldman Formation (Campanian), Alberta, Canada. *Cretaceous Research*, *103*: 104171.
- 31. Mikhailov, K. E. (1997). Special Papers in Palaeontology, 56: 1-80.
- 32. Norell, M. A., J. M. Clark, and L. M. Chiappe, (2001). *American Museum Novitates*, 2001(3315), 1-17.
- Osborne, L., and M. B. Thompson (2005). *Copeia*, 2005(3), 683-692.
- 34. Packard, G.C., and R. S. Seymour, (1997). Evolution of the amniote egg. *Amniote* origins: completing the transition to land, 265-290.
- Pike, D. A., R. M. Andrews, and W.G. Du (2012). *Evolutionary Ecology*, 26(4): 847-861.
- Pineiro, G., J. Ferigolo, M. Meneghel, and M. Laurin, (2012). *Historical Biology*, 24(6): 620-630.
- Pineiro, G., J. Ferigolo, A. Ramos, and M. Laurin (2012). *Comptes Rendus Palevol*, 11(5): 379-391.
- Portugal, S. J., G. Maurer, G. H. Thomas, M. E. Hauber, T. Grim, and P. Cassey, (2014). *Journal of Experimental Biology*, 217(18): 3326-3332.
- 39. Sander, P.M. (2012). *Science*, *337*(6096): 806-808.

- Sander, P. M., A. Christian, and C. T. Gee, (2008). Journal of Vertebrate Paleontology, 28(4): 1052-1055.
- 41. Sander, P. M., C. Peitz, F. D. Jackson, and L. M. Chiappe, (2008). *Palaeontographica Abteilung A*, 69-107.
- 42. Sahney, S., M.J. Benton, and H. J. Falcon-Lang, (2010). *Geology*, *38*(12): 1079-1082.
- 43. Schweitzer, M. H., J. L. Wittmeyer, and J. R. Horner, (2005). *Science*, *308*(5727): 1456-1460.
- Shaffer, H.B., P. Minx, D.E. Warren, A.M. Shedlock, R. C. Thomson, N. Valenzuela, and R.K. Wilson (2013). *Genome biology*, *14*(3): 1-23.
- 45. Shedlock, A. M., C. W. Botka, S. Zhao, J. Shetty, T. Zhang, J. S. Liu, and S. V. Edwards, (2007). Proceedings of the National Academy of Sciences, 104(8): 2767-2772.
- Stein, K., E. Prondvai, T. Huang, J. M. Baele, P. M. Sander, and R. Reisz, (2019). Scientific reports, 9(1): 4424.

- 47. Stewart, J. R. (1997). *Amniote origins: completing the transition to land*, 291-326.
- 48. Sues, H. D., and R. R. Reisz, (1998). *Trends in Ecology & Evolution, 13*(4): 141-145.
- 49. Tanaka, K., D. K. Zelenitsky, and F. Therrien, (2015). *PLoS One*, *10*(11): e0142829.
- Villamil, J., P. N. Demarco, M. Meneghel, R. E. Blanco, W. Jones, A. Rinderknecht, and M. Laurin (2015). *Historical Biology*, 28(7): 963-971.
- Wang, Z., J. Pascual-Anaya, A. Zadissa, W. Li, Y. Niimura, Z. Huang and S. Kuraku (2013). *Nature genetics*, 45(6): 701-706.
- Xavier, J. C., P. N. Trathan, F. R. Ceia, G. A. Tarling, S. Adlard, D. Fox and Y. Cherel (2017). *PLoS One*, *12*(3): e0174850.
- 53. Yang, T. R., Y. H. Chen, J. Wiemann, B. Spiering, and P. M. Sander, (2018). *PeerJ*, 6: e5144.
- 54. Zelenitsky, D. K., and F. Therrien, (2008). *Palaeontology, 51*(4): 807-816.