




The first report of preovipositional embryonic development in the legless gecko, *Lialis burtonis* (Gekkota: Pygopodidae)

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Funding information

National Science Foundation, Grant/Award Numbers: DBI 2209090, DBI 2109344, DBI 1701714

KEYWORDS: Burton's legless lizard, diceCT, embryo, limb reduced, natural history bycatch

Squamates evolved limb-reduced, elongate body forms, many times (Camaiti et al., 2021; Gans, 1975). Observing the development of these derived morphologies allows us to understand what constrains morphological variation in the evolution of serpentine body plans (e.g., Cohn & Tickle, 1999; Leal & Cohn, 2017). Unfortunately, developmental material of many limb-reduced squamate species remains scarce. Diffusible iodine-based contrast-enhanced computed tomography (diceCT) has revolutionized the acquisition of morphological data without invasive dissection or histological sectioning (Gignac et al., 2016; Blackburn et al., 2024). This and similar contrast-enhancing μ CT methods allow for investigation of both large, adult specimens (Gray et al., 2024) to small, embryonic specimens (Griffing et al., 2019). These methods often provide “natural history bycatch” which can be crucial for understanding the basic biology of understudied species (Callahan et al., 2021; Paluh et al., 2019; Blackburn et al., 2024). Herein, we report a case of “bycatch” in the limb-reduced gecko, *Lialis burtonis*. Members of the genus *Lialis* exhibit a suite of extraordinary morphological derivations, including a highly kinetic and elongate skull, recurved teeth, asymmetrical

viscera, and perhaps most notably, complete reduction of the forelimbs, extreme reduction of the hindlimbs, and body elongation (Kluge, 1974; Patchell & Shine, 1986; Stephenson, 1962; Underwood, 1957). To our knowledge, we provide the first description of embryonic development in the genus and discuss these data in the context of serpentine body plan evolution.

We used diceCT to image the soft-tissue and skeleton of a fluid-preserved, adult *Lialis burtonis* (UF-Herp-43419) as a part of the openVertebrate (oVert) Thematic Collections Network (Blackburn et al., 2024). This specimen was collected from the Port Moresby area of Papua New Guinea on 7 August 1977. The specimen was preserved in formalin and postfixed in 70% ethanol in which it was stored at the Florida Museum of Natural History (Gainesville, FL, USA). We stained the specimen in 1.25% Lugol's iodine for 14 days and then scanned the full body of the adult specimen at 24 μ m voxel resolution, using a GE v|tome|x M 240 at the University of Florida's Nanoscale Research Facility. Upon closer examination of the specimen, we discovered the individual was gravid with two eggs at the time of collection (Figure 1). Both eggs exhibit embryos of similar

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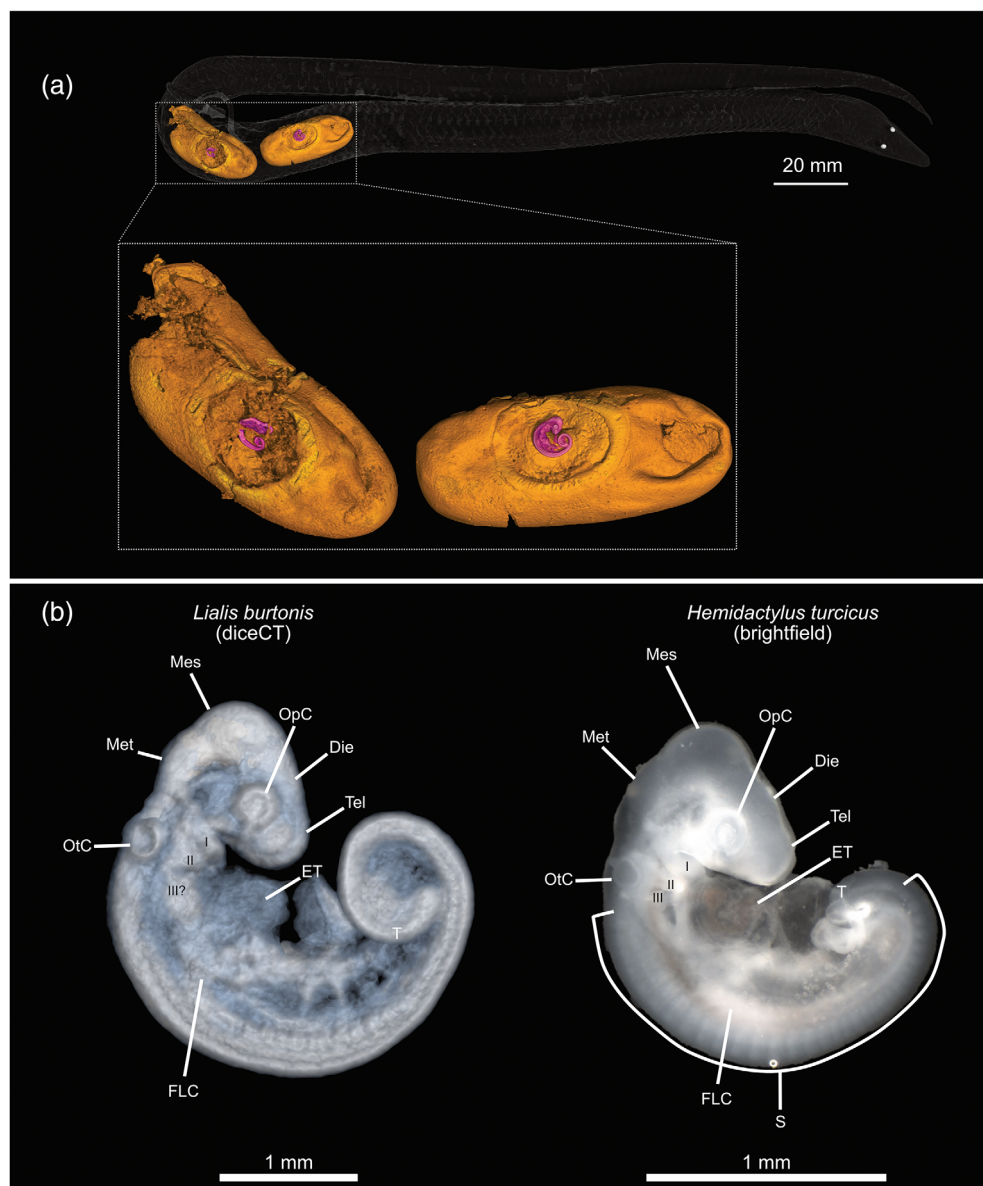
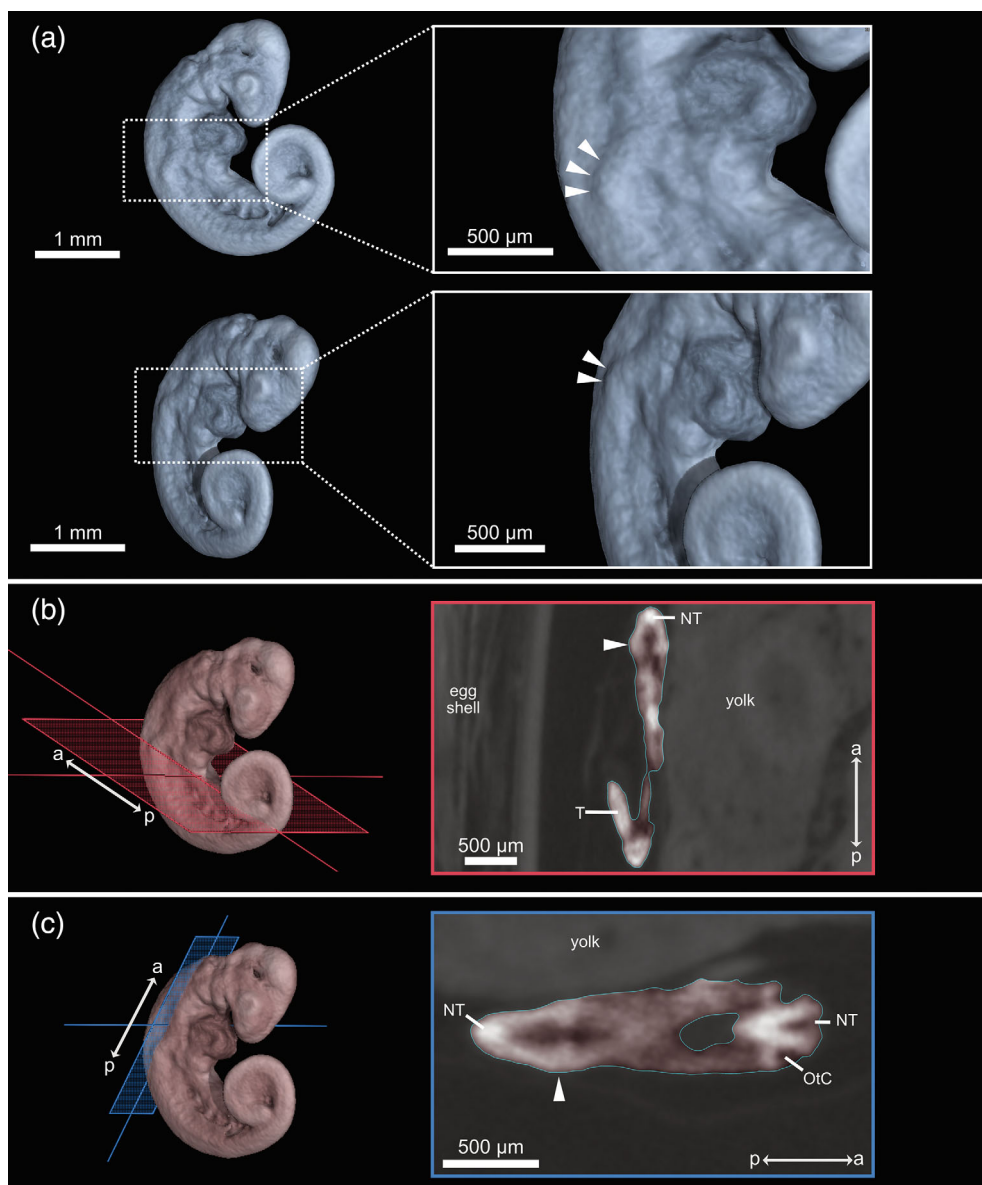


FIGURE 1 (a) μ CT of a gravid *Lialis burtonis* (UF-Herp-43419). Egg yolks isolated in yellow with embryos isolated in magenta. (b) Morphological comparison between the preoviposition *L. burtonis* (UF 43419) embryo (diceCT) and a stage 27 *Hemidactylus turcicus* embryo (brightfield; Griffing et al., 2022). I–III, pharyngeal arches I–III; Die, diencephalon; ET, endocardial tube; FLC, forelimb condensation; Mes, mesencephalon; Met, metencephalon; OpC, optic cup; OtC, otic capsule; S, somites; T, tail; Tel, telencephalon. Question mark assigned to pharyngeal arch III corresponding to uncertain identity of the structure due to the resolution of the scan.

developmental stages; however, one embryo was distorted and in poorer condition than the other. We then reconstructed the raw dataset at $2\times$ resolution ($12\ \mu\text{m}$ voxel resolution) to better visualize the embryo in superior condition. A-180 kV X-ray tube and diamond-tungsten target were used for both scans. The raw X-ray data were processed using the GE datos|x software to produce tomogram and volume files. The volume files were imported into VG StudioMax (Volume Graphics, version 2023.4), and the embryo was segmented using the region-growing, draw, smoothing, and refinement tools. Finally, we collected embryo measurements using Fiji (Schindelin et al., 2012) and compared embryonic morphology with previous embryonic staging series (Dufaure & Hubert, 1961; Griffing et al., 2022; Noro et al., 2009).

The resolution of the isolated embryo does not permit for counting somites to precisely stage it. This is likely exacerbated by distortion of the tissue through the fixation process (Hendrick et al., 2018; Maayan et al., 2022); however, many features are still distinguishable. The embryo exhibits distinct regions of the brain (telencephalon, diencephalon, mesencephalon, and metencephalon), the optic cup with lens and ventral choroid fissure, the otic capsule, and an endocardial tube (Figure 1). Pharyngeal arches I, II, and III (potentially) are distinct. Based on this combination of characters, we assign this embryo as approximately stage 27 (sensu Dufaure & Hubert, 1961). Developmental stage at oviposition is variable among squamates, including within gekkotans (Andrews & Mathies, 2000). Gekkotans that lay rigid-shelled eggs (sphaerodactylids, phyllodactylids, and

FIGURE 2 (a) μ CT *Lialis burtonis* embryo at two different angles to showcase the bulging forelimb condensation (arrowheads). (b–c) Isolated tomogram sections of the μ CT *L. burtonis* embryo in transverse plane (b) and coronal plane (c) through the forelimb condensation (arrowheads). Anterior (a) and posterior (p) axes illustrated. Additional positional information provided by yolk, egg shell, neural tube (NT), otic capsule (OtC), and tail (T).



gekkonids) generally oviposit eggs at earlier developmental stages than those that lay flexible-shelled eggs (eublepharids, diplodactylids, carphodactylids, and pygopodids; Pike et al., 2012). Indeed, stage 27 is a postovipositional stage known from some gekkonids (Griffing et al., 2022; Noro et al., 2009), while this stage proceeds oviposition in other flexible-shelled laying lineages (Griffing et al., 2021; Wise et al., 2009). The *L. burtonis* embryo fits within this trend.

The preovipositional embryo is 9.4 mm in total length (telencephalon–mesencephalon length = \sim 1.1 mm; mesencephalon–tail tip length = 8.3 mm). The postcranial region of the embryo represents nearly 88% of the embryo length, which is relatively longer than other gecko embryos of similar stages (84%–85%; Griffing et al., 2022; Noro et al., 2009). This relative postcranial length is shorter than other elongate, limb-reduced and limbless squamate

embryos at similar stages (e.g., *Nothobachia*, *Calyptommatu*, *Elaphe*, *Psammophis*; Khannoon & Zahradnick, 2017; Matsubara et al., 2014; Roscito & Trodrigues, 2012).

The embryo appears to exhibit a tissue condensation in the primordial forelimb region (Figure 1). By examining the μ CT reconstruction from different angles, we confirmed that the putative forelimb condensation bulges laterally (Figure 2). Furthermore, by examining individual μ CT tomogram sections through transverse and coronal planes, we found that this bulging condensation is positionally situated near the neural tube such as the developing limb buds of early chick embryos (Bellairs & Osmond, 2005). Although no limb bud is present at this stage in embryonic fully-limbed squamates, such condensation is consistent with a stage 27 embryo (Dufaure & Hubert, 1961; Griffing et al., 2022). Previous work on the limb-reduced lizards *Brachymeles lukbani* (Scincidae) and

Delma molleri (Pygopodidae), both of which lack forelimbs as adults, demonstrate that transient forelimbs are present during embryonic development (Andrews, 2019; Smith-Paredes et al., 2021). Although *Lialis* represents the most extreme limb reduction in pygopodids (Kluge, 1974), our data suggest this lineage is still primed to initiate forelimb development.

Taken together, these data provide rare insight into the development of a derived and embryologically understudied squamate lineage. Although our data do not provide the resolution of traditional histosections, the value of less destructive sampling of rare embryonic specimens cannot be understated. diceCT can provide an avenue to access embryonic data for preovipositional stages or of viviparous and ovoviviparous squamate species. Further opportunistic sampling from μ CT data or traditional embryological work is needed to determine the timing of limb formation and arrest, as well as the mechanisms of body elongation in pygopodids.

AUTHOR CONTRIBUTIONS

Aaron H. Griffing: Conceptualization; writing – original draft; writing – review and editing; investigation; visualization. **Edward Stanley:** Methodology; investigation; writing – original draft; writing – review and editing; visualization. **Daniel Paluh:** Investigation; writing – original draft; writing – review and editing; visualization; methodology.

ACKNOWLEDGMENTS

This work was made possible in part by the National Science Foundation (DBI 2209090 to AHG and DBI 2109344 to DJP). CT scanning was performed as part of the openVertebrate (oVert) Thematic Collections Network (National Science Foundation DBI 1701714 to ELS).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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How to cite this article: Griffing, A. H., Stanley, E. L., & Paluh, D. J. (2024). The first report of preovipositional embryonic development in the legless gecko, *Lialis burtonis* (Gekkota: Pygopodidae). *The Anatomical Record*, 1–5. <https://doi.org/10.1002/ar.25434>