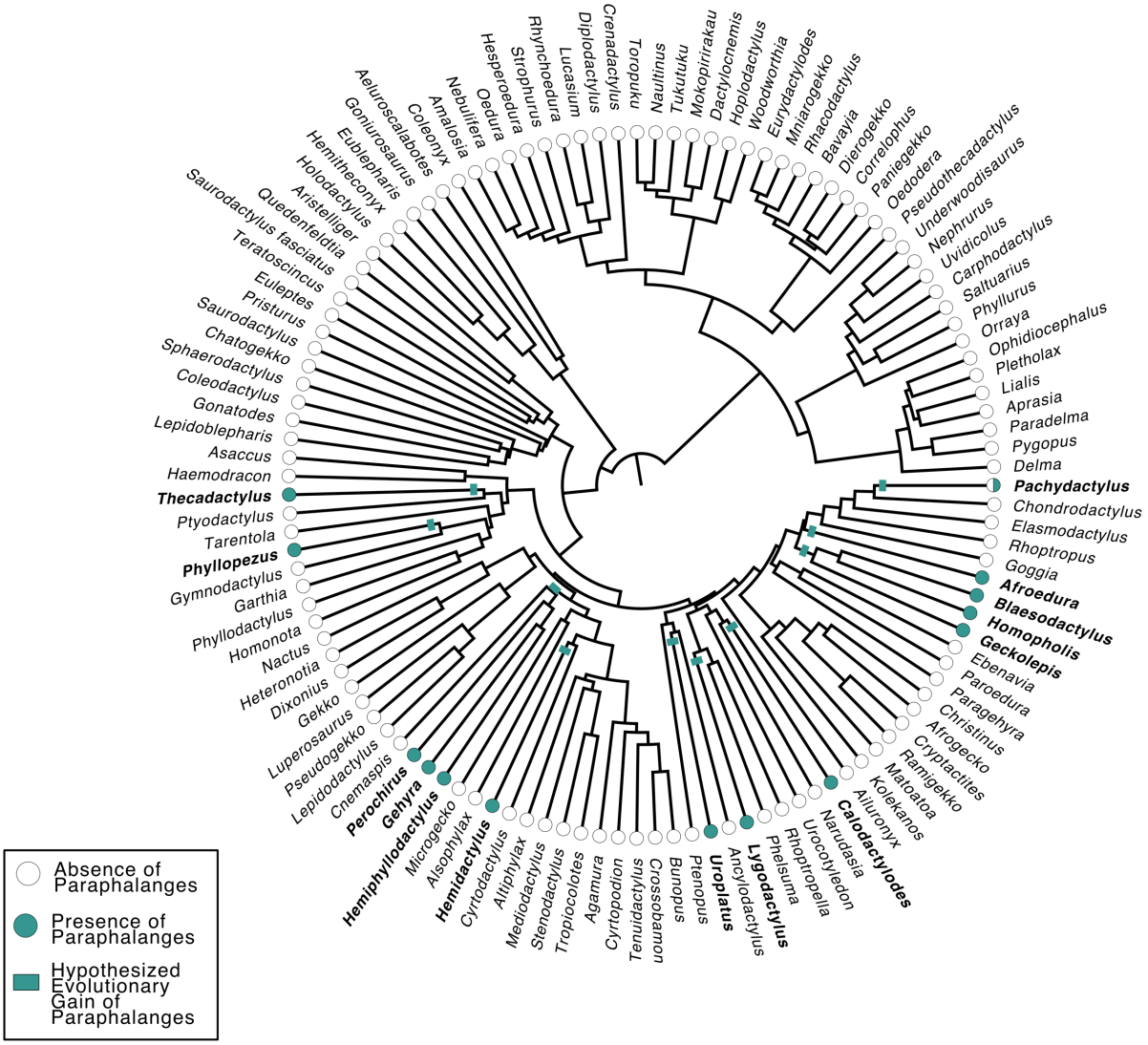
Supplemental Materials

Supplemental Materials 1. Genus-level phylogeny of the Gekkota based on the topology of Gamble et al. (2015). Presence of paraphalangeal elements highlighted in green and is based on Gamble et al. (2012). The half green character state of *Pachydactylus* illustrates that not all species within the genus exhibit paraphalanges. Gamble et al. (2012) hypothesized nine independent origins of paraphalanges in geckos. Following the phylogenetic hypotheses of Gamble et al. (2015), there is potentially an additional origin, as *Afroedura*, *Blaseodactylus*, *Homopholis*, and *Geckolepis* were previously considered a monophyletic group, but now *Afroedura* renders that group polyphyletic.



Supplemental Materials 2. Protocol for bone and cartilage staining of embryonic gekkotans. This protocol is modified from those of Wassersug (1976), Hanken & Wassersug (1981), Bauer (1986), and Maisano (2008), and was optimized using small–medium sized gekkotans (i.e. *Gehyra insulensis*, *Hemidactylus turcicus*, *Hemidactylus platyurus*, *Lepidodactylus lugubris*, and *Tarentola mauritanica*). It must be adjusted accordingly for larger species (e.g. *Correlophus ciliatus*, *Eublepharis macularius*).

Specimen should already be fixed in 10% neutral buffered formalin or 4% PFA for at least 24 hours prior. Specimens should be carefully skinned if they are at stage 40 or later (sensu Griffing et al. 2019). To best visualize the cranial bones and cartilage, carefully remove the eyes from preserved specimens before step 3. Place specimens in secure falcon tubes and gently rock tubes for steps 1–11. Each step belongs to a specific process: 1–2, rehydrating from alcohol or removing fixative; 3, cartilage staining; 4–6, destain to remove excess Alcian Blue; 7–12, rehydrate and prepare for digestion; 13, digestion; 14, bone staining; 15–17, additional tissue clearing and Alizarin destaining; 18, permanent storage.

Solutions Needed:

Alcian Blue Solution

70 ml 100% EtOH, 30 ml glacial acetic acid, 20 mg Alcian Blue 8 GX

Trypsin Solution

30 ml saturated aqueous sodium borate, 70ml dH_2O, 1 g Trypsin

Alizarin Stock

70% EtOH saturated with Alizarin red S powder

Alizarin Solution

Use a transfer pipette to add enough Alizarin stock to a 0.5% KOH solution until the solution turns a deep purple color.

[1] Specimen can be left in 75% EtOH for 1–3 days if needed.

[2] Specimen can be left in saturated sodium borate for up to a week if needed.

[3] Check specimens approximately every 5 min — trypsin can digest the embryo to the point of disintegration. Remove the embryo when it is translucent and one can clearly see the vertebral column and the long bones of the limbs.

[4] All St36–37 specimens examined lacked any evidence of ossification, therefore Alizarin step is not necessary. In these earlier stages, soak specimens in 0.5% KOH for step 14.

[5] Failure to clear is rare; however, a small amount of hydrogen peroxide (1–2 drops in 100 mL) can aid the clearing process. Too much hydrogen peroxide exposure can cause tissue to bubble. This can be remedied by placing specimen in 0.5% KOH in a vacuum for 30 min – 2 hours.

[6] Thymol crystals will inhibit fungal growth in glycerin. Changing the glycerin may be required after several months – years. A sign that the glycerin requires changing is if it takes on an orange/pink tint.

Step	Treatment	St33 (10–12 mm SVL)	St34–35 (12–15 mm SVL)	St36–37 (15–17 mm SVL)	St38–39 (18–20 mm SVL)	St40–41 (20–22 mm SVL)	St42–43 (22–26 mm SVL)
1	dH_2O	1 hr	1 hr	2 hr	2 hr	3 hr	3 hr
2	dH_2O	1 hr – overnight	1 hr – overnight	2 hr – overnight	2 hr – overnight	3 hr – overnight	3 hr – overnight
3	Alcian Solution	2 hr	2.75 hr	4 hr	5.5 hr	6 hr	6.5 hr
4	100% EtOH	15 min	20 min	20 min	30 min	30 min	30 min
5	100% EtOH	15 min	20 min	20 min	30 min	30 min	30 min
6	100% EtOH	15 min	20 min	20 min	30 min	30 min	30 min
7	95% EtOH	15 min	15 min	15 min	15 min	20 min	20 min
8	75% EtOH[1]	15 min – overnight	15 min – overnight	15 min – overnight	15 min – overnight	15 min – overnight	15 min – overnight
9	50% EtOH	15 min	15 min	15 min	15 min	15 min	15 min
10	15% EtOH	15 min	15 min	15 min	15 min	15 min	15 min
11	dH_2O	30 min	30 min	45 min	1 hr	1 hr	1 hr
12	Saturated Sodium Borate[2]	15 min – overnight	15 min – overnight	15 min – overnight	15 min – overnight	15 min – overnight	15 min – overnight
13	Trypsin Solution (37ºC)[3]	15 min	18 min	22 min	25–30 min	30–45 min	50–100 min
14	Alizarin Solution[4]	—	—	—	13 min	16 min	20 min
14	0.5% KOH	10 min	10 min	10 min	—	—	—
15	3:1 0.5% KOH : Glycerin[5]	≥ 4 hr	≥ 4 hr	≥ 4 hr	≥ 5 hr	≥ 6 hr	≥ 6 hr
16	1:1 0.5% KOH : Glycerin	≥ 4 hr	≥ 4 hr	≥ 4 hr	≥ 6 hr	≥ 6 hr	≥ 6 hr

17	1:3 0.5% KOH : Glycerin	≥ 4 hr	≥ 4 hr	≥ 4 hr	≥ 6 hr	≥ 6 hr	≥ 6 hr
18	Glycerin with thymol[6]	Store indefinitely	Store indefinitely	Store indefinitely	Store indefinitely	Store indefinitely	Store indefinitely

Supplemental Material 3. Number of cleared and stained specimens examined in this study per species and embryonic stage (S).

	Hemidactylus turicus	*Hemidactylus platyurus*	*Gehyra insulensis*	*Lepidodactylus lugubris*
S32	0	1	0	0
S33	2	1	1	1
S34	1	2	1	1
S35	1	0	1	1
S36	1	1	1	1
S37	1	1	1	1
S38	2	1	1	1
S39	1	1	2	1
S40	1	1	0	1
S41	2	1	1	1
S42	1	1	1	1
S43	2	1	1	0

Supplemental Materials 4. Descriptions of stages 24 and 27–43 of *Hemidactylus turcicus* embryonic development. Descriptions, terminology, and staging convention are based on those of Griffing et al. (2019) for the small gekkonid, *Lepidodactylus lugubris*. When incubated at 27ºC, *H. turcicus* hatched approximately 55 days post-oviposition (dpo). **Stage 24:** One embryo recovered at 0 dpo exhibited 15 somites, as otic capsule, distinct endocardial tube, and the faint outline of the optic cup. Little cephalic swelling is present and any pharyngeal arches or clefts are not clearly identifiable by light microscopy. This embryo's clutch-mate was Stage 27, suggesting there is developmental asynchrony between lizard clutch-mates and that early stages of development progress rapidly. **Stage 27:** Twenty-eight somites are present. The mesencephalon (primordial optic tectum) is bulging but boundaries with other cephalic features are not distinct. The otic capsule is visible and translucent. The optic cup is circular with a distinct choroid fissure present. Pharyngeal arches I–III and pharyngeal clefts 1–2 are distinct. The endocardial tube is prominent and beating. The nephrogenous mesenchyme is visible. No limb buds are visible. **Stage 28:** Thirty-one somites are present. The mesencephalon has distinct boundaries from the diencephalon and metencephalon. **Stage 29:** Thirty-six somites are present. Cephalic boundaries of the metencephalon, mesencephalon, diencephalon, and telencephalon are distinct. The optic cup is ovoid in shape and the retinal pigmented epithelium (RPE) is present, primarily in the posterior of the eye. The nasal pits are present. Pharyngeal arch IV is barely visible. Both fore- and hindlimb buds are present, with the former being larger. **Stage 30:** Forty-two somites are present. The eye is relatively larger with more dense pigment in the RPE. The liver is visible as somewhat opaque condensation posterior to the heart, which now has a distinct atrial and ventricular hemispheres. Both limbs exhibit a distinct apical ectodermal ridge (AER). The autopodium of the forelimb, but not the hindlimb, is distinct. The mesonephric liver is visible. **Stage 31:** Somites are present along the full length of the tail. The optic tectum is bulging dorsally. Fusion of the anterior pharyngeal arches is apparent, with the maxillary arch spanning halfway along the ventral length of the cranium. Frontonasal prominences are distinct. More pigment is present in the anterior of the eye than the previous stage, particularly at the equator. The endolymphatic ducts are opaque. The autopodia are distinct for both the fore- and hindlimbs. **Stage 32:** The pharyngeal arches are fusing to the point of obscuring them. The maxillary and mandibular arches have grown anteriorly. The medial nasal processes (i.e. facial primordia) remain unfused. The eye exhibits dense pigment along the rim of the lens, signaling iris development is underway. The gallbladder is now visible ventral to the liver and posterior to the heart. The autopodia, zeugopodia, and stylopodia are distinct in the forelimbs while only the autopodia is distinct in the hindlimb. Paired genital swellings are visible in the cloacal region. **Stage 33:** The regions of the brain adjacent to the optic tectum are relatively larger, giving the optic tectum a smaller, further posterior appearance — this trend remains until the dorsal aspect of the cranial region is nearly flat. The maxillary arch is adjacent to the fusing facial primordia and the mandibular arch is further anterior than previous. The autopodia, zeugopodia, and stylopodia are now distinct in the hindlimbs. Each autopodium exhibits faint digital condensations. **Stage 34:** The mandibular arch meets the fused facial primordia (i.e. snout). The eye exhibits dense pigment in the RPE. The autopodia have grown in size and exhibit distinct digital condensations and adjacent webbing. Digital webbing recession is beginning; however, the digit tips are not yet free. **Stage 35:** The iris and lens regions have grown in size compared to

the rest of the eye. Digital webbing recession continues and the distal tips of the digits are now free of webbing. The body wall is becoming more opaque, beginning to obscure the viscera from sight. Cloacal swellings, or developing genital buds, of all embryos examined now resemble developing hemipenes more than squamate hemiclitores (Gredler et al. 2015). **Stage 36:** The anterior cranial region is more elongate than the previous stage — this trend continues until stage 41. The nares are faintly visible as well as an external ear. Developing tubercles on the dorsum and tail are faintly visible signaling the onset of scale development. The onset of toe pad development occurs in this stage (Griffing et al. in press). Digital webbing has completely recessed and the digits are free. **Stage 37:** Little to no dorsal bulging of the optic tectum remains. The ribs are faintly visible in the dorsolateral region of the trunk. Opaque white is visible at the distal tips of the digits, signaling the development of keratinized claws. The toe pad is distinct from the two most ungual phalanges. **Stage 38:** The chromatophores of the iris are not yet visible and the eye remains overall very dark. The a pupil is ovoid. More tubercles are visible on the dorsum. Digits appear proportionally longer than the previous stage and scansor ridges of the toe pads are visible by light microscopy. **Stage 39:** The chromatophores of the eye are distinctly visible now. Scales are now visible on the limbs and verntal portion of the tail. **Stage 40:** The brain is barely visible through the skin. Scales are present all over the body and pigment is accumulating, particularly in the dorsum. **Stage 41:** The iris occupies more of the eye than the previous shape, making an almond-shaped pupil. The body wall completely opaque with the exception of the ventral surface of the trunk, limbs, and gular region, which remains faintly translucent. The brain region is fully obscured. **Stage 42:** The body wall is almost completely opaque, scales full developed, and pigment development is complete. Toe pad and tail pad development is complete. Hemipenes remain everted. **Stage 43:** The scales are noticeably hydrophobic when the embryo is submerged in PBS. The hemipenes are inverted. The embryo is ready to hatch.