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Ontogeny of the Paraphalanges and Derived Phalanges of *Hemidactylus Turcicus* (Squamata: Gekkonidae)

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Abstract

Gekkotan lizards of the genus *Hemidactylus* exhibit derived digital morphologies. These include heavily reduced antepenultimate phalanges of digits III and IV of the manus and digits III–V of the pes, as well as enigmatic cartilaginous structures called paraphalanges. Despite this well-known morphological derivation, no studies have investigated the development of these structures. We aimed to determine if heterochrony underlies the derived antepenultimate phalanges of *Hemidactylus*. Furthermore, we aimed to determine if convergently evolved paraphalanges exhibit similar or divergent developmental patterns. Herein we describe embryonic skeletal development in the hands and feet of four gekkonid species, exhibiting a range of digital morphologies. We determined that the derived antepenultimate phalanges of *Hemidactylus* are the products of paedomorphosis. Furthermore, we found divergent developmental patterns between convergently evolved paraphalanges.

1 INTRODUCTION

Gekkotan digits, especially those bearing adhesive pads, are known for their diverse morphologies (Gamble et al., **2012**; Russell, **1972**, **1976**, **1979**; Russell & Bauer, **1988**; Russell & Gamble, **2019**). Derived phalangeal shapes, sizes, and orientations are functionally important for fostering interaction with various substrates and, in some cases, control of adhesive scansors (Bauer et al., **1996**; Russell, **1976**; Russell & Bauer, **1990**; Zhuang et al., **2019**). In some taxa phalangeal formulae depart from the ancestral state (Russell & Bauer, **2008**) through losses or fusions of phalangeal elements (Gamble et al., **2011**; Stephenson, **1960**).

The genus *Hemidactylus* was initially regarded as exhibiting a reduced phalangeal formula (2-3-3-4-3 manus/2-3-3-4-3 pes; Mahendra, **1950**; Stephenson, **1960**), purportedly through loss of a phalanx in each of the third and fourth manual and third, fourth and fifth pedal digits. This was later shown to be erroneous (Russell, **1977**). Instead, all members of *Hemidactylus* exhibit antepenultimate phalanges in the aforementioned digits that are of greatly reduced size and deflected from the proximodistal linear trajectory of the other phalanges (Russell, **1977**: figure 1). Thus, *Hemidactylus* actually exhibits the ancestral phalangeal formula for lizards (2-3-4-5-3 manus and 2-3-4-5-4 pes; Greer, **1992**). Its highly derived antepenultimate phalanges create an inflection in the long axis of digits III and IV of the manus and III, IV, and V of the pes, resulting in a raising of the penultimate and ungual (claw-bearing) phalanges relative to the plane in which the more proximal phalanges lie. This configuration results in the digits having two mechanical units, a proximal region supporting the toe pads and a distal one extending beyond the adhesive region. This contrasts with the incorporation of the distalmost phalanges within the toe pad zone that is the case in many pad-bearing geckos (see Russell, **1976** figure 5 for a depiction of these various configurational patterns). The configuration in *Hemidactylus* allows the toe pads and the claws of these digits to function somewhat independently (Russell, **1972**, **1976**, **1977**).

In addition to the derived condition of its phalanges, *Hemidactylus* also possess paraphalanges (Russell & Bauer, **1988**), paired skeletal elements located medial and lateral to interphalangeal (and sometimes metapodial-phalangeal) joints. These elements may be cartilaginous or osseous, depending upon the taxon in question, and are often embedded within the lateral digital tendons (Russell & Bauer, **1988**; Russell & Gamble, **2019**). Paraphalanges are diverse in shape and size. These morphologies include small, elliptical, pebble-like shapes (e.g., *Lyogdactylus*, *Calodactylodes*; Wellborn, **1933**; Russell & Bauer, **1988**), broad, half-oval shapes (e.g., *Homopholis*, *Blaseodactylus*; Russell & Bauer, **2008**; Gamble et al., **2012**), long, twig-shapes (*Pachydactylus*; Russell & Bauer, **2008**), large, billiard pipe-shapes (*Thecadactylus*; Russell & Bauer, **2008**), and triangular or teardrop-shapes (e.g., *Hemidactylus*, *Gehyra*; Wellborn, **1933**; Russell & Bauer, **2008**). Paraphalanges are hypothesized to aid in control of adhesive scansors and have originated on at least nine occasions within Gekkota (occurring in both the Phyllodactylidae and Gekkonidae) and are present in at least 14 genera (Gamble et al., **2012**, **2015**; Russell & Bauer, **1988**; Supplemental Material 1). Even a fossilized Cretaceous

stem-gekkotan exhibits what appear to be paraphalanges (Fontanarrosa et al., **2018**). Some have suggested these elements are also present in the phyllodactylid genus *Homonota* (Fontanarrosa et al., **2018**); however, data from skeletonized or diaphonized specimens are not included in the literature, and this claim is seemingly erroneous (Fontanarrosa et al., in review). The paraphalanges of *Hemidactylus* are cartilaginous and, in most cases, bilaterally symmetrical (Russell & Bauer, **1988**; figure 1). The most distally situated paraphalanges are often teardrop-shaped and lie adjacent to the penultimate interphalangeal joint of digits II–V (manus and pes; figure 1), supporting the distal portions of the toe pads. In digit IV (manus and pes) there is an intermediate-sized, somewhat lozenge-shaped pair of paraphalanges that lies adjacent to the antepenultimate interphalangeal joint, supporting the proximal part of the toe pad on the longest digit (Figure 1). The more proximally situated paraphalanges are typically small, round and lie adjacent to the metapodial-phalangeal joints (Figure 1). Despite their implied importance to the fascinating adhesive apparatus of geckos, the developmental origins of paraphalangeal elements, regardless of evolutionary origin, are unknown.

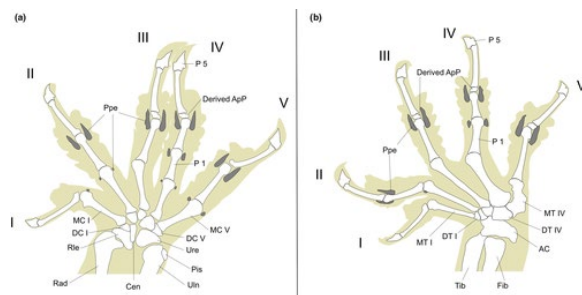


FIGURE 1 Autopodial osteology of adult *Hemidactylus turcicus*, based upon a cleared and stained specimen (TG 3933). (a) Dorsal aspect of the right manus, and (b) dorsal aspect of the right pes. Paraphalanges are dark gray and digits I–V are labeled from left to right. Elements in serial sequence, such as phalanges, are numbered 1–5. Phalanges 2 (digits III) and phalanges 3 (digits IV) of both manus and pes represent derived antepenultimate phalanges (ApP). AC, astragalocalcaneum; Cen, centrale; DC, distal carpals; DT, distal tarsals; fib, fibula; MC, metacarpals; MT, metatarsals; P, phalanges; Pis, pisiform; Ppe, paraphalanx; rad, radius; Rle, radiale; tib, tibia; Uln, ulna; Ure, ulnare.

Shifts in the onset, offset, and rate of developmental events (i.e., heterochrony) are hypothesized to be major drivers of anatomical diversity (e.g., Alberch et al., **1979**; de Beer, **1930, 1951**). Heterochronic shifts result in morphologies which are either less (paedomorphic) or more (peramorphic) developed relative to the adult ancestral condition (McNamara, **1986**). Although peramorphosis has been noted to be an important generator of morphological variation in some gecko lineages (Daza et al., **2015**; Griffing, Daza, et al., **2018**), geckos are considered to be relatively paedomorphic, compared to other lizards, based upon their amphicoelous vertebrate, generally large orbits, derived skull musculature, and unfused parietals (Camp, **1923**; Kluge, **1967**; Rieppel, **1984**; Stephenson, **1960, 1962**; Stephenson & Stephenson, **1956**). Heterochrony has been implicated as a mechanism for reductions in phalangeal elements in taxa such as limb-reduced skinks (Hugi et al., **2012**), tortoises (Crumly & Sánchez-Villagra, **2004**), and salamanders (Alberch & Alberch, **1981**). However, the role of heterochrony in phalangeal reduction found in *Hemidactylus* remains unknown.

With the exception of a single μ CT investigation of the embryonic development of an as-yet undescribed species of African *Hemidactylus* (van der Vos et al., **2018**), no studies have documented the embryonic development of paraphalanges or the derived antepenultimate phalanges of *Hemidactylus*. We herein compare the embryonic development of *Hemidactylus* digits with those of other gekkonids to determine whether: (1) the derived antepenultimate phalanges exhibit developmental heterochrony that results in their reduced structure; and (2) convergently evolved paraphalanges in other gekkotan lineages exhibit similar or divergent developmental patterns.

2 METHODS

We obtained embryos from a captive colony of *Hemidactylus turcicus* established by collecting individuals from non-native populations in Oklahoma, USA (Permit: ODWC-6945). The adults were raised using husbandry methods modified from those of Konečný (2002). We collected 222 embryos of *H. turcicus* following the protocol of Griffing, Sanger, et al. (2018) and fixed them in 4% buffered paraformaldehyde solution. Subsequently, we characterized a complete postovipositional embryonic staging series, following characterizations developed by Dufaure and Hubert (1961) and Griffing et al. (2019). We then subsampled embryos ($N = 15$) from stages 32 (paddle-shaped limb stage) to 43 (immediately prior to hatching), stained cartilage and bone, and cleared remaining tissue (technique modified from Bauer, 1986; Hanken & Wassersug, 1981; Maisano, 2008; Wassersug, 1976; see Supplemental Material 2 and 3 for a detailed protocol and sample sizes for cleared and stained specimens, respectively). Following Bauer (1986), we also cleared and stained a single adult specimen of *H. turcicus* from the captive colony to characterize adult digital morphology. We imaged all cleared and stained specimens using a Nikon® SMZ 74ST stereoscope.

Following the same protocols as above, we compared the developing digits of *H. turcicus* to those of *Hemidactylus platyurus* ($N = 12$), *Gehyra insulensis* ($N = 11$), and *Lepidodactylus lugubris* ($N = 10$) using opportunistically sampled embryos. *Hemidactylus platyurus* exhibits both the derived antepenultimate phalanges and paraphalanges exhibited by *H. turcicus* (Russell, 1977; Russell & Bauer, 1988). *Gehyra insulensis* represents another gekkonid lineage (Supplemental Materials 1) that exhibits large paraphalanges, which likely evolved independently of those of *Hemidactylus* (Gamble et al., 2012; Russell & Bauer, 1988), but lacks the derived antepenultimate phalanx morphology exhibited by *Hemidactylus*. *Lepidodactylus lugubris* represents a more distantly related gekkonid lineage (Supplemental Materials 1) that lacks both the derived antepenultimate phalanx morphology exhibited by *Hemidactylus* and paraphalanges (Rieppel, 1994a). We collected embryos of these three species from captive colonies established from the pet trade (*H. platyurus*) or collected from non-native populations in Hawaii, USA (*G. insulensis* and *L. lugubris*; Permit: EX-18-06). Specimens used in this study are housed at Marquette University (TG research laboratory) and can be made available upon request.

3 RESULTS

Postovipositional incubation of *Hemidactylus turcicus* typically takes between 50 and 55 days when incubated at 27°C. We identified 17 postovipositional embryonic stages (Figure 2; see Supplemental Material 4 for detailed description of embryonic development). Embryos generally have reached stage 27 (32 somites) at oviposition; however, one specimen was found to be only at stage 24 (15 somites). No cartilaginous condensations are visible in the digits until stage 33, at which point digital rays are discernible via light microscopy of the untreated embryo. The development of the manus and pes of *H. turcicus*, *H. platyurus*, *Gehyra insulensis*, and *Lepidodactylus lugubris* is presented in Figures 3-6. Simplified schematics of *H. turcicus* autopodial chondrification and ossification sequence are presented in Figures 7, 8.

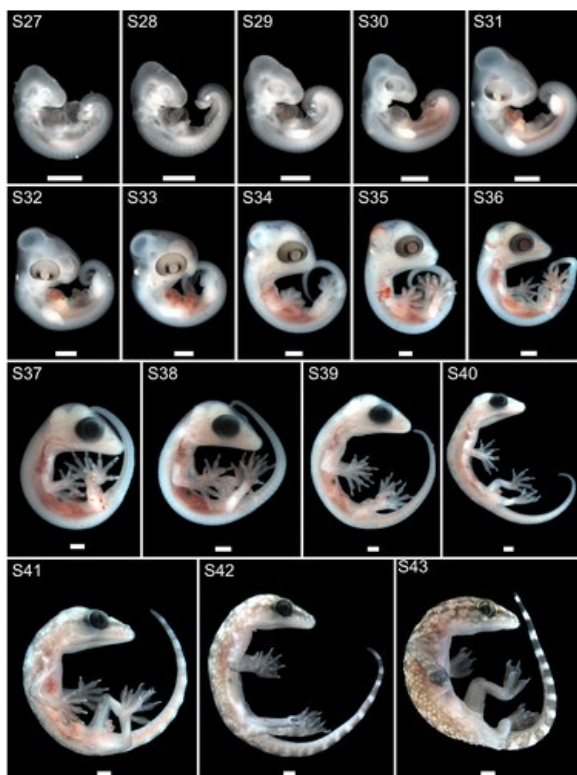


FIGURE 2 Embryonic *in ovo* stages 27–43 of *Hemidactylus turcicus* development. Lateral views of whole embryos. Scale bars = 2 mm. Detailed descriptions of each stage can be found in Supplemental Material 3.

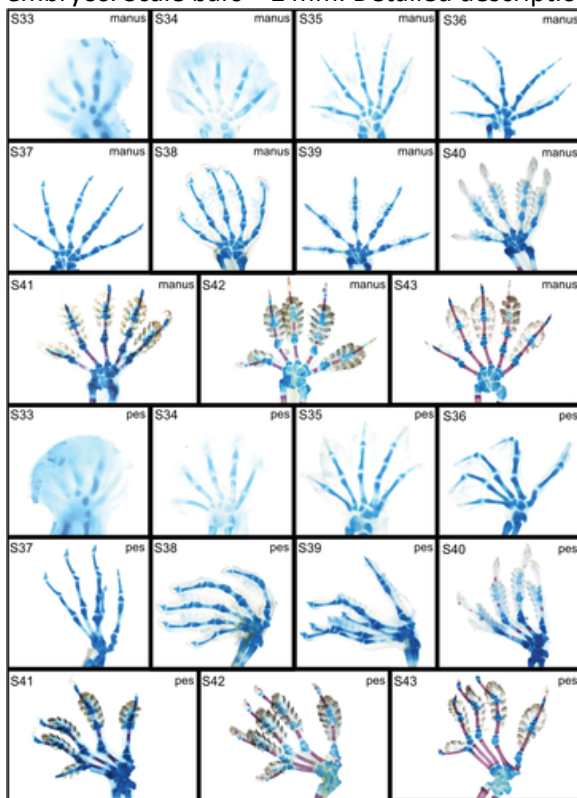


FIGURE 3 Manual and pedal development of *Hemidactylus turcicus*. Dorsal aspect of the right manus and pes of cleared and stained embryos from stage 33 to stage 43. Dense blue stain denotes cartilage and red stain denotes bone.

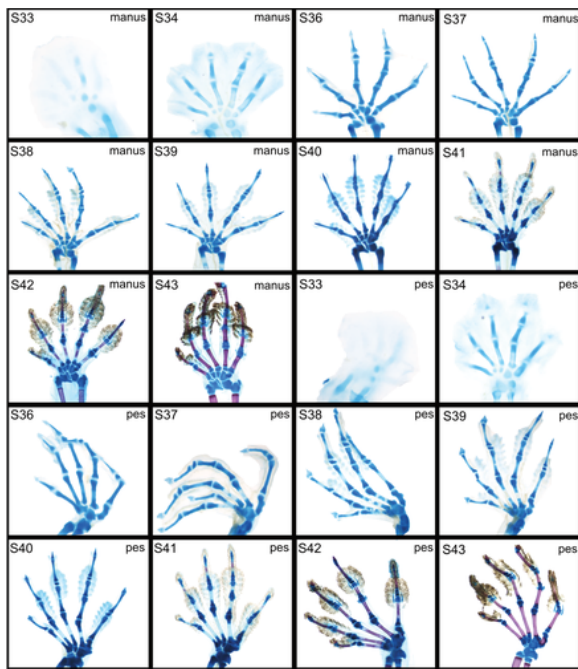


FIGURE 4 Manual and pedal development of opportunistically sampled *Hemidactylus platyurus*. Dorsal aspect of the right manus and pes of cleared and stained embryos from stage 33 to stage 43. Dense blue stain denotes cartilage and red stain denotes bone. Information for stage 35 not present.

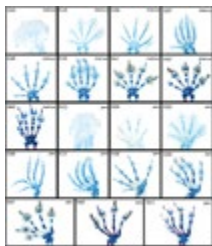
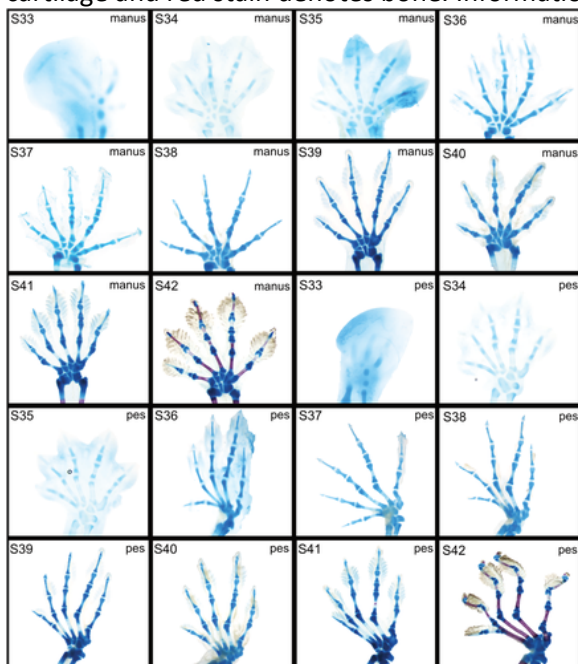


FIGURE 5 Manual and pedal development of opportunistically sampled *Gehyra insulensis*. Dorsal aspect of the right manus and pes of cleared and stained embryos from stage 33 to stage 43. Dense blue stain denotes cartilage and red stain denotes bone. Information for stages 34 (manual) and 40 (manual and pedal) not present.



Developing manus of *H. turcicus*

Stage 33: Cartilaginous condensations of the ulnare, distal carpals II–V, and metacarpals I–V are visible. Additionally, the faint outlines of phalanx 1 (digits II–V) and phalanx 2 (digit IV) are evident. Proximal to the manus the cartilaginous radius and ulna are evident. **Stage 34:** The previously noted elements display more distinct shapes and boundaries. The first cartilaginous appearance of the radiale, phalanx 1 (digit I), phalanx 2 (digit III), and phalanx 3 (digit IV) is evident. **Stage 35:** The metacarpal and phalangeal elements present in the previous stage are relatively slimmer and more elongate in appearance. The antepenultimate phalanges of digits III and IV are evident as small condensations and exhibit the miniscule proportions that are retained into adulthood and skeletal maturity. Cartilaginous condensations of distal carpal I, the centrale, phalanx 2 (digits I, II, V), phalanx 3 (digits II, III, V), phalanx 4 (digits III, IV), and phalanx 5 (digit IV) are now visible. **Stage 36:** Epiphyses of the metacarpals and proximal phalanges are evident as the widest parts of these elements. Cartilaginous condensation of the pisiform is now visible. **Stage 37:** The proximal portions of the ungual phalanges are wider than the distal epiphyses of the penultimate phalanges and their distal portions are tapered to points. Cartilage staining is partially dissipated within the diaphysis of the metacarpals and proximal phalanges, signaling the onset of endochondral bone development. **Stage 38–39:** The epiphyses of the metacarpals and proximal phalanges are wider than their diaphyses, creating a dumbbell-like shape. Similar patterns of reduced intensity of blue staining occur in the diaphyses of the phalanges. **Stage 40:** Cartilage staining is largely eliminated from the metacarpals and phalanges, with the exception of their epiphyses and the miniscule antepenultimate phalanges of digits III and IV. The first faint appearance of the distalmost paraphalanges is visible adjacent to the penultimate interphalangeal joints of digits II–V, specifically situated adjacent to the distal epiphyses of phalanx 1 (digits II, III, V) and phalanx 2 (digit IV). Even fainter cartilaginous condensations of the intermediate paraphalanges of digit IV and the proximal paraphalanges of digit V are visible adjacent to the distal epiphysis of phalanx 1 and the metapodial-phalangeal joint, respectively. These early paraphalangeal condensations are thin, lozenge-shapes. **Stage 41:** Ossification is underway in the metacarpals, phalanx 1 and 2 of all digits, phalanx 3 of digits II and V, and phalanx 4 of digits III and IV, but the small antepenultimate phalanges of digits III (= phalanx 2) and IV (= phalanx 3) remain cartilaginous. The developing paraphalanges adjacent to the penultimate interphalangeal joints of digits II–V are more distinct. The proximal paraphalanges of digits I, III, and IV are now faintly visible adjacent to the metapodial-phalangeal joint. **Stage 42:** Ossification is underway in all phalanges with the exception of the antepenultimate phalanges of digits III and IV. A new ossification center is visible in the ungual phalanx of digit IV. **Stage 43:** Ossification is complete in all phalanges with the exception of the antepenultimate phalanges of digits III and IV, the distal carpals, radiale, ulnare, pisiform, and centrale, all of which remain cartilaginous and exhibit no centers of ossification. The paraphalanges also remain cartilaginous.

Developing pes of *H. turcicus*

Stage 33: Cartilaginous condensations of metatarsal IV and distal tarsals (II–IV) are visible. Additionally, the faint outlines of the remaining metatarsals and phalanx 1 (digits III–V) are visible. The cartilaginous tibia and fibula are evident proximal to the pes. **Stage 34:** The aforementioned elements present more distinctive shapes and more clearly defined boundaries. The first faint cartilaginous appearance of the astragalocalcaneum, phalanx 1 (digits I–V), and phalanx 2 (digits III, IV) is evident. **Stage 35:** Cartilaginous condensations of phalanx 2 (all digits), phalanx 3 (digits II–V), and phalanx 4 (digit IV) are visible. **Stage 36:** Cartilaginous condensations of the ungual phalanges of all digits, as well as distal tarsal I, are visible. **Stage 37:** The proximal portions of the ungual phalanges are wider than the distal epiphyses of the penultimate phalanges and their distal portions are tapered to points. Cartilage staining exhibits reduced intensity in the diaphyses of the metatarsals and proximal phalanges, signaling the onset of endochondral bone development. **Stage 38–39:** The epiphyses of the metatarsals and proximal phalanges are wider than their diaphyses, creating a dumbbell-like shape. Similar patterns of reduced intensity of cartilage staining are evident in the diaphyses of the more distal phalanges. **Stage 40:** Cartilage staining is almost absent from the metatarsals and phalanges, with the exception

of metatarsal I, the epiphyses of long bones and the antepenultimate phalanges of digits III–V. Ossification is evident in the diaphyses of metatarsals II–IV, phalanx 1 of digits III–V, and the penultimate phalanges of digits II–IV. The first faint appearance of paraphalangeal condensations is visible adjacent to the penultimate interphalangeal joints of digits III–V, specifically situated adjacent to the distal epiphyses of phalanx 1 (digits II, III, V) and phalanx 2 (digit IV). Even fainter cartilaginous condensations of the intermediate paraphalanges of digit IV are visible adjacent to the distal epiphysis of phalanx 1. Like those of the manus, the early pedal paraphalangeal condensations are thin, lozenge-shapes. **Stage 41:** With the exception of digit I and metatarsal V, ossification has progressed in the metatarsals, in phalanx 1 of all other digits, and the penultimate phalanges of all other digits. The paraphalanges adjacent to the penultimate interphalangeal joints are evident and more distinct in shape in digits II–V. The proximal paraphalanx of digit II is now faintly visible adjacent to the metapodial-phalangeal joint. **Stage 42:** Ossification is advanced in all metatarsals and phalanges with the exception of the antepenultimate phalanges of digits III–V. **Stage 43:** Ossification is complete in all phalanges with the exception of the antepenultimate phalanges of digits III–V, the distal tarsals, and the astragalocalcaneum, all of which remain cartilaginous. The paraphalanges also remain cartilaginous.

The developing manus and pes of the other gekkonid taxa examined

Skeletal development of the manus and pes of *Hemidactylus platyurus* is overall similar to that of the congeneric *H. turcicus*. Differences are present in the earliest cartilaginous condensations visible in the Stage 33 manus and pes which, in *H. platyurus* lack cartilaginous condensation in distal carpal II, metacarpals I and II, phalanx 1 (digits II, III, and V), and phalanx 2 (digit IV; Figures 4, 7). *Hemidactylus platyurus* at this stage also lacks cartilaginous condensation in distal tarsals II and III, metatarsals I–III and V, and phalanx 1 (digits III–V; Figures 4, 8). The first signs of paraphalangeal development occur in stage 40 and are overall similar in shape to those of *H. turcicus* (Figure 9). In the manus, cartilaginous paraphalangeal condensations lie adjacent to the penultimate interphalangeal joints of digits II–V, the antepenultimate interphalangeal joint of digit IV, and the metapodial-phalangeal joints of digits I and V (Figures 4, 9). In the pes, cartilaginous paraphalangeal condensations lie adjacent to the penultimate interphalangeal joints of digits II–V, and, in addition, there is an extremely faint outline of the antepenultimate interphalangeal joint of digit IV (Figures 4, 9). The first signs of manual or pedal ossification are visible in stage 41, which, in both cases, are less advanced than that displayed by *H. turcicus* (Figures 3, 4, 7, 8).

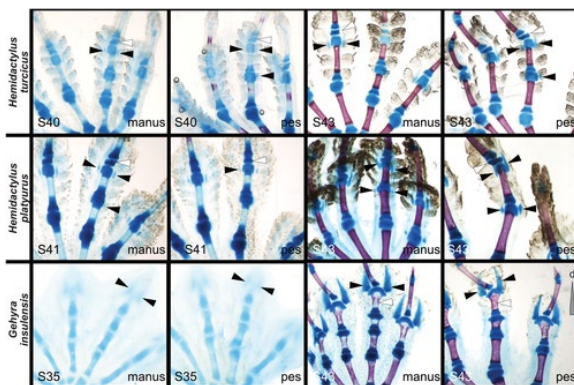


FIGURE 9 Development of paraphalanges and derived phalanges of *Hemidactylus turcicus*, *Hemidactylus platyurus*, and *Gehyra insulensis*. All images illustrate the dorsal aspect of the right manus or pes. Dense blue stain depicts cartilage and red stain indicates bone. Black arrows indicate paraphalanges and white arrows indicate antepenultimate phalanges of either digit IV or III (C). Gray arrow head points in the distal (d) direction.

Chondrogenesis in the manus and pes of *Gehyra insulensis* is first evident in Stage 33, there being cartilaginous condensations present in the ulnare, distal carpals III and IV, metacarpals III and IV, and phalanx I (digit IV) of the manus (Figures 5, 7) and in the astragalocalcaneum, distal tarsals II–IV, metatarsals III–V, and phalanx I (digit IV)

of the pes (Figures 5, 8). Development proceeds in a similar sequence to that observed in *H. turcicus*, with three exceptions. First, the onset of ossification in *G. insulensis* is less advanced than in *H. turcicus*. Ossification is visible at stage 41 in the manus and pes, but only as faint ossification of phalanx 3 (manual digit III) and metatarsals III and IV. At this stage, most of the metacarpal, metatarsal, and manual/pedal phalangeal elements are ossified in *H. turcicus* (Figures 3, 5, 7, 8). Second, in contrast to *H. turcicus*, ossification of all phalanges is complete in the pes of *G. insulensis* prior to hatching (Figures 5, 8). Finally, extremely faint condensations of the paraphalanges are visible at stage 35 in the manus and pes, adjacent to what will become the penultimate interphalangeal joint, prior to cartilaginous condensation in the penultimate and ungual phalanges (Figures 5, 9). Unlike the initial paraphalangeal condensations of *Hemidactylus*, those of *G. insulensis* are teardrop-shaped (Figure 9).

Chondrogenic expression in the manus and pes of *Lepidodactylus lugubris* is first visible in Stage 33 through extremely faint cartilaginous condensations of the ulnare, distal carpals III and IV, metacarpals II–IV, and phalanx 1 (digit IV) of the manus (Figures 6, 7) and distal tarsal IV, metatarsals II–V, and phalanx 1 of digit IV of the pes (Figures 6, 8). At Stage 34, chondrogenic expression is more advanced in *L. lugubris* than *H. turcicus*. Everything but the pisiform, ungual phalanges (digits I–V), and penultimate phalanges of digits II, III, and V are present as cartilaginous condensations in the manus (Figures 6, 7). Similarly, everything but the ungual phalanges (digits I–V), and penultimate phalanges of digits II–V are present as cartilaginous condensations in the pes (Figures 6, 8). Compared to *Hemidactylus turcicus*, the earliest evidence of ossification occurs in stages 42 (vs 41) and 41 (vs 40) for the manus and pes, respectively (Figures 3, 6–8).

4 DISCUSSION

Development of the autopodia of *H. turcicus*

The early patterning of the tetrapod limb is considered highly conserved (Shubin & Alberch, 1986). In general, lizards exhibit chondrogenic development in the autopodia starting in the proximal elements: the ulnare, distal carpals, and some metacarpals (III–V) in the manus and the distal tarsals and some metatarsals (III–V) in the pes (Diaz Jr. & Trainor, 2015; Fabrezi et al., 2007; Leal et al., 2010; Noro et al., 2009; Sewertzoff, 1908). In the autopodia of geckos we examined, initial chondrogenic expression occurs between stage 32 (paddle-shaped limb stage) and stage 33 (digital condensation stage; Figures 3–8). At this earliest stage, *H. platyurus*, *G. insulensis*, and *L. lugubris* exhibit patterns similar to those reported in previous studies; however, *H. turcicus* exhibits earlier chondrification (Figures 3–8). In the gekkonid gecko *Paroedura picta*, cartilaginous condensations in the autopodia are present approximately at the paddle-shaped limb stage, suggesting it also has an early onset of autopodial patterning similar to *H. turcicus* (Noro et al., 2009). These patterns of earlier chondrification suggest paedomorphy in the early cartilaginous patterning of *H. turcicus*.

All cartilaginous condensations in the manual and pedal digits are present by stage 36 (end of digital webbing reduction stage), which coincides with the onset of toe pad development (Griffing et al., 2022). At this stage, *G. insulensis* and *L. lugubris* have not completed chondrogenesis in the manus (Figures 5–7). Beginning with stage 37, all taxa we examined exhibit all autopodial elements as cartilaginous condensations (Figures 3–8). Ossification is first evident in the manus and pes of *H. turcicus* at stages 41 and 40, respectively (Figures 3–8). Fourteen and nine elements show signs of ossification in the manus and pes respectively at these stages, and in both cases this is more advanced than in the other taxa we examined. Interestingly, the taxa we examined did not exhibit the expected proximal-to-distal ossification sequence typical of most gecko taxa examined (Leal et al., 2010; Rieppel, 1994a; van der Vos et al., 2018). Our sampling demonstrates that several intermediate phalanges, and in some cases metacarpal and metatarsal elements, ossify later than some of the distal phalangeal elements (Figures 3–8). Reptiles do not exhibit ossification sequences that perfectly recapitulate chondrification sequences (Rieppel, 1992a, 1992b, 1993a, 1993b, 1994a, 1994b); however, it is expected that

most metacarpal/metatarsal elements ossify, followed by the proximal phalanges, and then the more distal phalanges (Fröbisch, **2008**; Rieppel, **1994a**). Rieppel's (**1994a**) study of the gekkonid geckos *Gehyra oceanica* and *Lepidodactylus lugubris* also demonstrated this directional sequence of autopodial ossification. However, he also noted that some geckos exhibit advanced ossification in digits III–V compared to other digits, as well as delayed ossification of the phalanges of digits III and IV that show a reduction in size, with particular focus on phalanx 2 (digit IV). This delayed ossification of phalanges of reduced size is also shown in *P. picta* but not in the sphaerodactylid gecko *Gonatodes albogularis* (Leal et al., **2010**; Noro et al., **2009**). Rieppel considered this another example of paedomorphic character states in geckos (Kluge, **1967**; Rieppel, **1984**, **1994a**; Stephenson, **1960**).

The derived antepenultimate phalanges of *Hemidactylus*

The derived antepenultimate phalanges of *H. turcicus* and *H. platyurus* begin to chondrify at approximately the same developmental stage as their non-reduced homologs in *L. lugubris* and *G. insulensis* (Figures **3–8**). However, whereas these non-reduced homologs ossify prior to hatching, the derived antepenultimate phalanges of *Hemidactylus* do not ossify during embryonic development. Phalangeal reduction in some skinks and salamanders is due to paedomorphic truncation of developmental events (i.e., progenesis; Alberch & Alberch, **1981**; Hugi et al., **2012**). The difference in developmental timing observed in *Hemidactylus*, compared to the presumed ancestral condition exhibited by *L. lugubris* and *G. insulensis*, suggests the derived antepenultimate phalanges of *Hemidactylus* are paedomorphic (Alberch et al., **1979**) in their delayed onset of ossification, suggesting that they exhibit post-displacement sensu McNamara (**1986**). However, chondrogenic and osteogenic expression are largely decoupled (Shapiro, **2002**), and the underlying mechanism of how derived antepenultimate phalanges retain their cartilaginous miniscule size compared to other antepenultimate phalanges remains unknown. To address this, future studies could investigate the spatial and temporal expression of important chondrogenic morphogens (e.g., Sox9; Healy et al., **1999**) in the formation of gecko digits.

Our data for *H. turcicus* and *H. platyurus* differ from the statements made by van der Vos et al. (**2018**), who describe *Hemidactylus* sp. as exhibiting ossified antepenultimate phalanges prior to hatching. Because the species they investigated remains undescribed there is no information about which clade within *Hemidactylus* it belongs to. To further corroborate paedomorphosis in the derived antepenultimate phalanges of *Hemidactylus*, an embryonic survey of ossification patterns within *Hemidactylus*, spanning the phylogenetic diversity of the group, should be undertaken. Furthermore, including *Dravidogecko* and *Cyrtodactylus* in this potential comparison, which are the sister lineages of *Hemidactylus* and *Dravidogecko* + *Hemidactylus*, respectively (Chaitanaya et al., **2019**; Gamble et al., **2012**), will be informative. All three of these genera exhibit antepenultimate phalanges of reduced size, with those of *Hemidactylus* and *Dravidogecko* showing the greatest degree of diminution and *Cyrtodactylus* exhibiting a more modest expression of reduction (Chaitanaya et al., **2019**; Russell, **1976**). A comparison between these three genera may provide further resolution of our hypothesized developmental pattern.

Paraphalangeal development

The paraphalanges of *Hemidactylus turcicus* and *H. platyurus* are first visible as cartilaginous condensations at stages 40 and 41, respectively (Figure **9**). In both cases, the distalmost paraphalanges which lie adjacent to the penultimate interphalangeal joint are more fully developed than the intermediate and proximal paraphalangeal pairs. Despite developing in the same region of the digit, the distal paraphalanges which lie adjacent to the penultimate interphalangeal joint of *Gehyra insulensis* appear much earlier in development, prior to the recession of interdigital webbing and cartilaginous development of the adjacent phalanx (Figure **9**). The paraphalanges possessed by *Hemidactylus* and *Gehyra* are undoubtedly the products of convergent evolution (Gamble et al., **2012**; Supplemental Material **1**). Those of *Hemidactylus* are embedded within the lateral digital

tendons, while those of *Gehyra* are sheathed in a collagenous connective tissue (Russell & Bauer, **1988**). It is, therefore, not surprising that these analogous structures exhibit different developmental patterns. In well-studied vertebrates, such as chickens (*Gallus gallus*), digital tendonous architecture is established by embryonic day 14 (Edom-Vovard & Duprez, **2004**)—a developmental stage in which the embryo exhibits cartilaginous condensations of all digital elements (Bellairs & Osmond, **2005**; Hamburger & Hamilton, **1951**). In contrast to tendons, less organized collagenous fibrils are present much earlier in the developing chicken autopodium, by embryonic day 7 (Hurle & Fernandez-Teran, **1983**). Therefore, the difference in onset of paraphalangeal development between *Hemidactylus* and *Gehyra* may relate to the temporal differences in differentiation of digital connective tissues. A further developmental histological examination of paraphalanx-bearing taxa may yield insight into the diversity of timing of paraphalangeal appearance, as well as the tissue precursors from which they arise.

Sesamoids, or bones found near joints where they are embedded in tendons are fibro-cartilages (Haines, **1969**), manifesting as small ossifications often found adjacent to the phalanges in many lizard lineages, including *Anolis*, gymnophthalmids, teiids, tropidurids, and *Varanus* (Haines, **1952**; Otero & Hoyos, **2013**). Some have considered the paraphalanges of geckos to be sesamoid elements (Abdala et al., **2019**; Kluge, **1966**); however, no single clear developmental mechanism underlies the evolution of these structures, making tests of homology difficult. Abdala et al. (**2019**) hypothesized a transformative interplay between sesamoid and epiphyseal identity in both developmental and evolutionary diversification of the skeleton. Although our data do not demonstrate that paraphalanges of *Hemidactylus* and *Gehyra* are attached via cartilage to the epiphyses of any digital elements, chondrogenic signals may be sourced from the epiphyses of these structures during paraphalangeal development. Through a combination of histology and exploration of the spatial expression of molecules important for autopodial chondrogenesis (*sox* genes and BMP proteins; Chimal-Monroy et al., **2003**), the source of chondrogenic signal in developing paraphalanges could be identified, thereby providing evidence as to whether or not these structures are sesamoids.

Conclusions

Through our wholemount investigation into phalangeal development, we identified that the reduced antepenultimate phalanges of *Hemidactylus* ossify postnatally, and much later than the presumed ancestral condition we observed in *Gehyra* and *Lepidodactylus*. These structures are paedomorphic through post-displacement. Further work is required, however, for the identification of the mechanisms that allow the antepenultimate phalanges to retain their diminutive size compared to neighboring phalanges. Our investigation demonstrates that the convergently evolved paraphalanges of *Hemidactylus* and *Gehyra* exhibit markedly different developmental patterns. This is likely due to their derivation from different tissue sources that lie adjacent to the phalanges. By investigating the histological profile of these developing regions, in tandem with characterizing the spatial profile of chondrogenic genes, we may begin to understand the origins of these derived structures.

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