Developmental basis of limb homology in Pleurodiran turtles, and the identity of the hooked element in the chelonian tarsus

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Although Pleurodiran turtles represent an important component of extant turtle radiation, our knowledge of the development and homology of limb bones in turtles rests mostly upon observations made on derived members of the Cryptodiran clade. Herein, we describe limb development in three pleurodirans: *Podocnemis unifilis* Troschel, 1848, *Podocnemis sextuberculata* Cornalia, 1849 and *Phrynops hilarii* (Dumeril and Bibron, 1835), in an effort to contribute to filling this anatomical gap. For earlier stages of limb development, we described the Y-shaped condensation that gave rise to the zeugopodial cartilages, and differentiation of the primary axis/digital arch that reveals the invariant pattern common to tetrapods. There are up to four central cartilaginous foci in the carpus, and the proximal tarsale is formed by the fusion of the fibulare, intermedium, and centrale 4. Digital development is similar for the five digits. Changes in toe V occur predominantly in the distal tarsale 5. Ontogenetic reduction of phalanges is observed in toe V of *Podocnemis*. Based on these results, we suggest that the hooked element present in the chelonian tarsus, and traditionally recognized as a modified fifth metatarsale, is actually the fifth distal tarsale. Additionally, our data on limb development of pleurodiran turtles supply more taxonomically comprehensive information to interpret limb configuration within the chelonian clade. © 2009 The Linnean Society of London, *Zoological Journal of the Linnean Society*, 2009, **155**, 845–866.

ADDITIONAL KEYWORDS: carpus – chondrogenesis – hooked fifth metatarsale – morphogenesis – ossification – side-necked turtles – tarsus.

INTRODUCTION

A reanalysis of old descriptions associated with new embryological studies have reinforced the evidence of shared patterns of limb development in anurans (Schmalhausen, 1907; Holmgren, 1933; Shubin & Alberch, 1986; Fabrezi & Alberch, 1996; Fabrezi & Barg, 2001), turtles (Holmgren, 1933; Burke & Alberch, 1985; Sheil, 2003, 2005; Sanchez-Villagra *et al.*, 2007a; Sanchez-Villagra, Winkler & Wurst, 2007b; Sanchez-Villagra, Ziermann & Olsson, 2008), lizards (Holmgren, 1933; Mathur & Goel, 1976; Shapiro, 2002; Fabrezi, Abdala & Martínez Oliver, 2007), crocodiles (Shubin & Alberch, 1986; Müller & Alberch, 1990), and birds (Holmgren, 1933; Hinchliffe & Hecht, 1984; Burke & Feduccia, 1997; Feduccia, Lingham-Soliar & Hinchliffe, 2005). Shubin & Alberch (1986) summarized the common traits in the morphogenesis of primary cartilages during early

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limb development in tetrapods, emphasizing shared patterns in the formation of the primary axis and digital arch.

Data on early limb development in the cryptodiran turtles *Chelydra serpentina* (Linnaeus, 1758) and *Chrysemys picta* (Schneider, 1783) were provided by Burke & Alberch (1985). This paper considerably influenced the definition of homologies of limb elements of sauropsids advanced by later authors (Müller, 1991; Burke & Feduccia, 1997; Feduccia *et al.*, 2005). Sheil (2003, 2005) provided additional embryological data for turtles through his studies on limb development in *Apalone spinifera* (de Seuer, 1827) and *Macroclemys temminckii* (Troost, in Harlan, 1835); whereas Sanchez-Villagra *et al.* (2007a) analysed it in *Chelonia mydas* (Linnaeus, 1875) and *Caretta caretta* (Linnaeus, 1758), and Sanchez-Villagra *et al.* (2008) studied limb development in *Graptemys nigrinoda* Cagle, 1954. However, information on early limb development for the basal pleurodiran lineage of turtles (Gaffney, 1975, 1984, 1996) remains limited to some remarks on embryonic condensations (Rabl, 1910). Our present knowledge of limb variation in turtles is thus based mostly on information from Cryptodiran taxa, causing an evident bias on both old and present hypotheses of primary homology (Rabl, 1910; Goodrich, 1916; Schaeffer, 1941; deBraga & Rieppel, 1997; Lee, 1997; Rieppel & Reisz, 1999; Hill, 2005, among others). In order to further illustrate the historical debate that has surrounded limb development in turtles, in Table 1 we present the classical, recapitulative, ontogenetic scheme of chelonian limb development suggested by Holmgren (1933), and compare it with the more recent morphogenetic approach advocated by Burke & Alberch (1985).

Herein, we describe the patterns of primary cartilage morphogenesis and ossification in the pleurodiran *Podocnemis unifilis* Troschel, 1848, *Podocnemis sextuberculata* Cornalia, 1849, and *Phrynops hilarii* (Dumeril and Bibron, 1835). *Podocnemis* belongs to the clade Pelomedusoides, whereas *Phrynops* is included in the sister taxon Chelidae: both genera represent the two major clades of the Pleurodira (Joyce, 2007), and provide a comprehensive sample to describe the limb developmental patterns within Pleurodira. The present study also provides additional developmental evidence to interpret the limb plan in turtles, and to further discuss the available hypotheses of primary homology concerning the tetrapod limb skeleton, in particular the two competing hypotheses of primary homology regarding the hooked element present in the tarsus of turtles (Goodrich, 1916; Carroll & Currie, 1991; deBraga & Rieppel, 1997; Lee, 1997; Rieppel & Reisz, 1999; Hill, 2005).

MATERIAL AND METHODS

The present study describes patterns of early chondrogenesis and subsequent limb development in the pleurodiran species *Po. unifilis*, *Po. sextuberculata*, and *Ph. hilarii*. We examined a total of 16 embryos (stages 14–23) and seven post-hatching specimens of *Po. unifilis*, 12 embryos (stages 12–24) and one post-hatching specimen of *Po. sextuberculata*, and 16 embryos (stages 17–25) and six post-hatching specimens of *Ph. hilarii*. Species, specimen numbers, and collection data (developmental stage and carapace length) are listed in the Appendix. Specimens are deposited in the herpetological collections of Museu de Zoologia da Universidade de São Paulo (Brazil), Centro de Investigaciones Científicas y de Transferencia de Tecnología a la Investigación – CONICET, Diamante, Entre Ríos, (Argentina), and Museo de Ciencias Naturales, Universidad Nacional de Salta (Argentina).

Embryonic specimens were staged following Yntema (1968). We provide detailed descriptions of primary cartilage development and ossification patterns through direct observation of clear and double-stained embryos. Clearing and double staining with Alcian Blue and Alizarin Red whole mounts was performed following the procedure described in Wassersug (1976). Because the Alizarin Red S is a specific dye for the histochemical detection of calcium, the ossification sequence is inferred when positive Alizarin Red S coloration is observed. However, we must note that bone development may start before calcification. Further observations were made in a selected number of histological sectioned embryos from the same embryological series. Histological serial sections (7-μm thick) were stained with hematoxylin-eosin, according to the procedure described by Martoja & Martoja-Pierson (1970).

Anatomical descriptions of muscles and tendons were made in alcohol-preserved specimens of *Ph. hilarii*, *Liolaemus multicolor* Koslowsky, 1898, and *Caiman latirostris* (Daudin, 1801) that were double stained with Alcian Blue and Alizarin Red S, but were not cleared. Temporal staining with iodine solution was performed during dissection in order to get a better contrast between the muscles and tendons (Bock & Shear, 1972). These preparations were conserved in 70% ethanol.

Descriptions, illustrations, and photographs were made with a stereo dissection microscope, Nikon SMZ1000, and a light microscope, Leica DM, equipped with digital camera and camera lucida. Terminology to identify primary cartilage condensations follows the criteria proposed by Burke & Alberch (1985) and Shubin & Alberch (1986).

Table 1. Two major developmental interpretations of the identity of the chelonian limb primary cartilages. Holmgren (1933) observed limb development in *Chrysemys picta* subsp. *marginata* Agassiz, 1857 and one species of *Sternotherus* Gray, 1825, and proposed a scheme based on the concept of the chiropterygium (Gregory, Minner & Noble, 1923). Burke & Alberch (1985) described limb development in *Chelydra serpentina* and *Chrysemys picta*, and emphasized the transient embryonic stages that indicate a common mode of morphogenesis (primary axis and digital arch) among tetrapods

Holmgren (1933)	Burke & Alberch (1985)
Ulnare is distal to the ulna end and originates almost at the same time as distal carpal 4 and metacarpal IV.	Ulnare/fibulare branches off the distal end of the ulna/fibula and forms part of the primary axis.
Distal carpale 4 is distal to the ulnare and forms part of the set of rays.	Distal carpale/tarsale 4 segments distally from the ulnare/fibulare, and forms part of the primary axis. Initially forms an elongated condensation.
Intermedium and centrale 4 are well-defined centres in a prochondrial mass that fills up the carpus between the carpalia, the ulnare, the distal end of the ulna, and the radius.	Intermedium branches off the distal end of the ulna/fibula, merging medially with the distal tip of the radius/tibia.
Rays: the first ray to appear is digit 4. After that, the metacarpale V and carpale 5 follow, and a little later metacarpale III, before any other elements have differentiated in the carpus. Then follows the metacarpale II with carpale 2, and about the same time the intermedium and a small centrale 4 appear, situated proximal to the carpale 4.	Digital arch: at level of distal carpale/tarsale 4, this anlage turns anteriorly at a right angle to the primary axis, and will give rise sequentially to distal carpalia/tarsalia 3–1. Each metacarpal/metartarsal forms as a continuous branch of the corresponding distal carpal.
Digit V is the postaxial ray.	Digit V has no obvious connection with either the primary axis or the digital arch. The initial condensation corresponds to the distal carpale 5 in the manus and to the metatarsale V in the pes.
In the carpus, centrale 4 is a well-defined centre in a prochondrial mass. It is surrounded by the ulnare and intermedium proximally, and distal carpalia 3 and 4 distally.	In the carpus, centrale 4 differentiates next to the intermedium. It is surrounded by the ulnare and intermedium proximally, and distal carpalia 3 and 4 distally.
In the carpus, centralia 3 and 2 originate at the distal border of the rudiment of the central proximale at about the same time as when two weak centres are found, which are not well delimited from the central proximale rudiment. These are the rudiments of two more centrals.	In the carpus, centrale 3 is distal to the radius, and is visible as a faintly stained region medial to the centrale 4. Thereafter it becomes an elongated structure that grows in an anterior direction, to give rise to the centrale 2.
In the carpus, central proximale (1) is placed between the intermedium and the distal end of the radius. It is a big, but only weakly, centred rudiment. It already fuses early with the intermedium.	In the carpus, centrale 2 is distal to the radius end. It lies at the preaxial boundary of the elongated central element (C4 + C3), and is slightly ventral to it. It could arise independently or as a continuation of the centrale 3, separated only by a constriction of the condensation.
In the tarsus, centrale 4 is a slight postaxial condensation in a procartilaginous blastema between the intermedium–centrale 1 and the distal tarsals.	In the tarsus, centrale 4 is distal to the intermedium.
In the tarsus, centrale 1 appears anteriorly of the intermedium, and forms an intermedium–centrale complex.	In the tarsus, centrale 1 is a preaxial condensation, anteriorly of the intermedium, that is incorporated to the intermedium.

RESULTS

ADULT LIMB PLAN

The carpus has two proximal bones, the ulnare and intermedium, two central bones, and five distal carpalia (Figs 1, 2). Distal carpalia 4 and 5 may appear fused, with two distinct ossification nuclei in both *Podocnemis* species, and only one in some juvenile specimens of *Ph. hilarii*. The medial centrale is a transversely elongated bone distal to the intermedium, whereas the small lateral centrale, which is sometimes cartilaginous, is distal to the radius, and
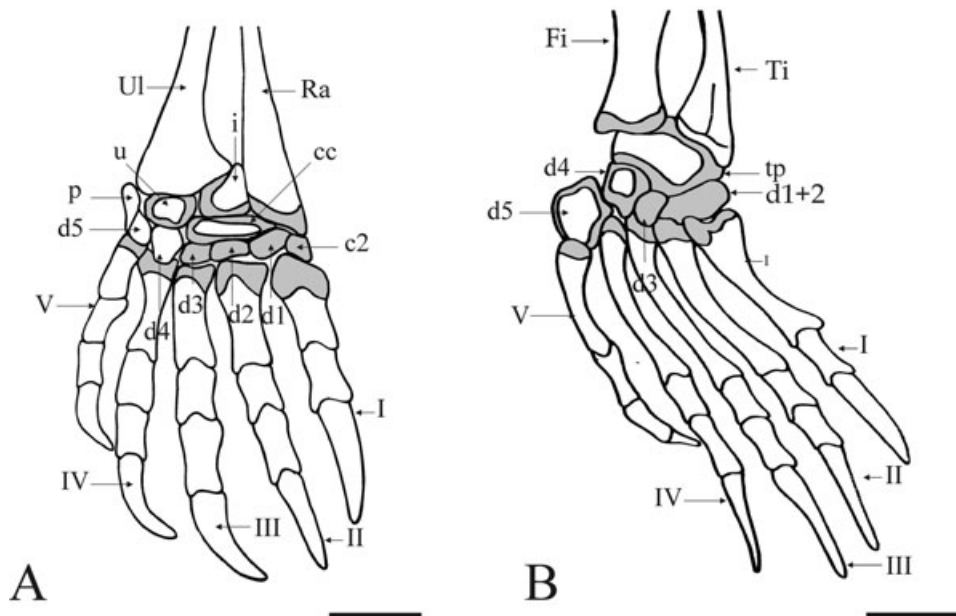
Figure 1. Autopodia of adult *Phrynops hilarii.* Grey areas indicate cartilages. A, dorsal view of right hand. B, dorsal view of right foot. Abbreviations: I–V, digits I–V; c2, lateral centrale or centrale 2; cc, centralia; d1–d5, distal carpalia/tarsalia 1–5; Fi, fibula; i, intermedium; p, pisiform; Ra, radius; Ti, tibia; tp, proximal tarsale; Ul, ulna; u, ulnare. Scale bars: 5 mm.

does not articulate with the intermedium. The pisiform is present. The phalangeal formula in the studied species is 2.3.3.3.3.

The tarsus is formed by the proximal tarsale and five distal tarsalia (Figs 1, 2). Distal tarsale 5 is the largest bone of the series and articulates with a short metatarsal V. In *Ph. hilarii*, distal tarsalia 1 and 2 are fused in a single elongated bone (Fig. 1); whereas in both *Podocnemis* species the five distal tarsalia are present as single bones. The phalangeal formula is 2.3.3.3.3 in *Ph. hilarii*, with reductions in the terminal phalanges of digit V in *Po. sextuberculata* and *Po. unifilis*.

Adult variation of the autopodial skeleton in Pleurodira was analysed in a phylogenetic context by Sanchez-Villagra *et al.* (2007b).

LIMB DEVELOPMENT IN *PODOCNEMIS*

Stage 14 (Figs 3A, 4A)
The first event of bifurcation is still evident in both forelimbs and hindlimbs. The distal end of the humerus/femur remains connected with the ulna/fibula and radius/tibia, respectively, and the primary axis is incomplete because of the incipient differentiation of digit IV. In the forelimb, the ulnare, intermedium, and distal carpale 3 appear as diffuse condensations, whereas distal carpale 4 is already well defined. In the hindlimb, two different and diffuse condensations are visible, distal to the fibula: one corresponds to the intermedium, which is located

between both distal ends of the tibia and fibula, in a proximal and medially oriented position; whereas the other is an elongated element that is postaxially placed, and represents the primary axis condensation of both fibulare and distal tarsale 4, which are not yet segmented.

Stage 15 (Figs 3B, 4B)
The limb paddle reveals the beginning of digital differentiation. In the forelimb, the intermedium, ulnare, and distal carpalia 5–2 already represent well-defined foci. Distal carpalia 3 and 2 appear connected with their metacarpalia. A prechondrogenic area is present between the foci of the intermedium and distal carpalia 4, 3, and 2, where the centralia will develop. In the hindlimb, the cartilaginous foci of the primary axis (fibulare, distal tarsale 4, and metatarsale IV) are segmented. Digits V, III, and II have started their differentiation, and appear as cartilaginous columns with better defined proximal portions. The mass of intermedium is expanded anteriorly and medially, and remains as a diffuse prechondrogenic condensation.

Stage 16 (Figs 3C, 4C)
Two well-defined foci representing centralia appear in the carpus, distal to the intermedium. These foci are identified as centrale 4 and 3. Digit I is differentiated, although distal carpale 1 retains its connectivity with metatarsale I. Proximal phalanges in digits V–II start
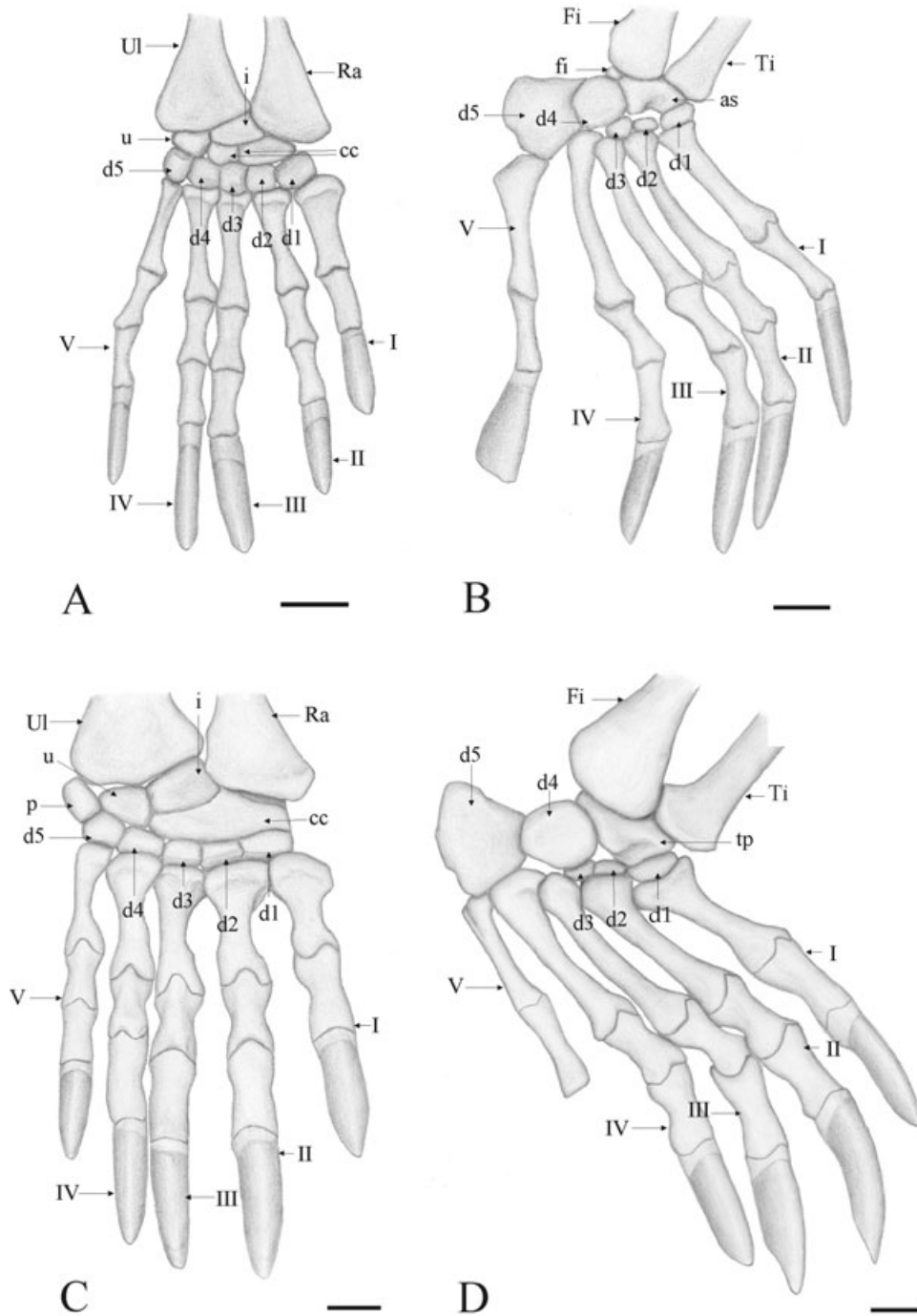
Figure 2. Fully ossified autopodia of adults of *Podocnemis sextuberculata* MZUSP 3218 (A and B), and *Podocnemis unifilis* MZUSP 3209 (C and D). A, dorsal view of right hand. B, dorsal view of right foot. C, dorsal view of right hand. D, dorsal view of rigth foot. Abbreviations: I–V, digits I–V; as, astragalus; c2, lateral centrale or centrale 2; cc, centralia; d1–d5, distal carpalia/tarsalia 1–5; fi, fibulare; Fi, fibula; i, intermedium; p, pisiform; Ra, radius; Ti, tibia; tp, proximal tarsale; Ul, ulna; u, ulnare. Scale bars: 5 mm.

to segment from metacarpalia. The pisiform is a single, small cartilage located distally to the ulna. In the hindlimb, differentiation of digit I has advanced, and distal tarsale 1 is already differentiated from metatarsale I. Development of proximal phalanges is in progress in digits I–IV. Digit V has two connected condensations: the proximal one is elongated, and seems to be displaced in posterior and proximal
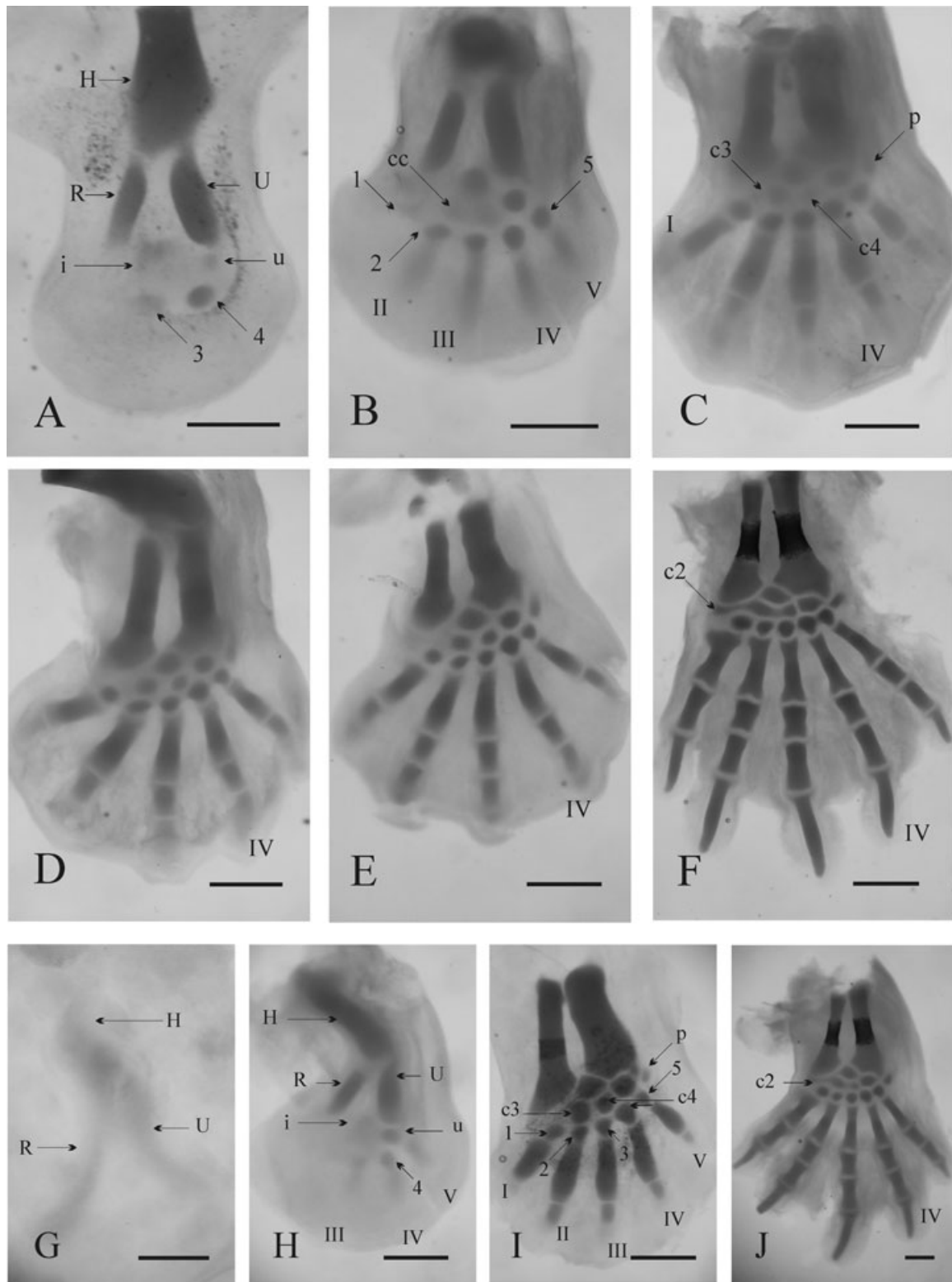
Figure 3. Early forelimb development in *Podocnemis unifilis* (A–F) and *Podocnemis sextuberculata* (G–J). Dorsal views of left limbs. A, stage 14; B, stage 15; C, stage 16; D, stage 17; E, stage 18; F, stage 19; G, stage 12; H, stage 15; I, stage 16; J, stage 19. Abbreviations: 1–5, carpalia 1–5; I–V, digits I–V; c2, centrale 2; c3, centrale 3; c4, centrale 4; cc, centralia; H, humerus; i, intermedium; p, pisiform; R, radius; U, ulna; u, ulnare. Scale bars: 0.5 mm.
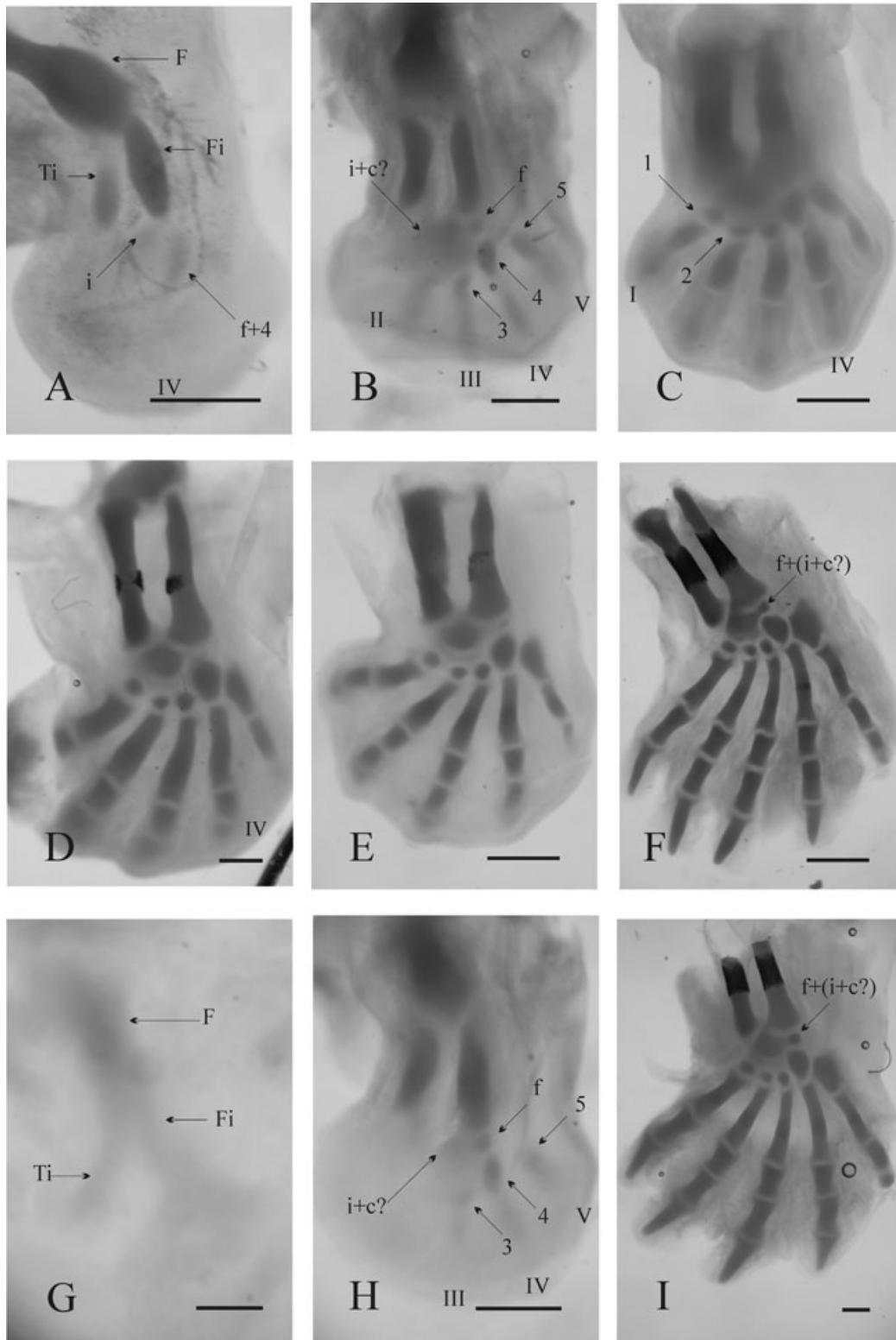
Figure 4. Early hindlimb development in *Podocnemis unifilis* (A–F) and *Podocnemis sextuberculata* (G–I). Dorsal views of left limbs. A, stage 14; B, stage 15; C, stage 16; D, stage 17; E, stage 18; F, stage 19; G, stage 12; H, stage 15; I, stage 19. Abbreviations: 1–5, tarsalia 1–5; I–V, digits I–V; c, centrale; f, fibulare; F, femur; Fi, fibula; i, intermedium; Ti, tibia. Scale bars: 0.5 mm.

directions, and the distal one is shorter with respect to the other metatarsalia. The central portion of the tarsus (that we identified as the intermedium) is at this stage a large cartilaginous element surrounded by the tibia, fibula, and distal tarsalia 4–1. The small cartilage of the fibulare is located ventrally to the large intermedium.

Stage 17 (Figs 3D, 4D)
At this stage, the digital elongation through differentiation and growth of phalanges denotes the end of the paddle-like stage. Vestiges of the interdigital tissues that form the paddle are conserved between digits up to adult stages. In the manus, differentiation of a second phalanx is seen in digits III and IV, which result in the outgrowth of these digits beyond the limits of the paddle. Digits from the pes have an incipient second phalanx, except for digit V that lacks phalanx. Distal tarsalia are completely separated from metatarsalia, and the proximal element of digit V (distal tarsale 5) is the largest. Perichondral ossification appears in the diaphyses of the tibia and fibula.

Stage 18 (Figs 3E, 4E)
Except for digit V, all terminal phalanges have started to develop. In the manus, a prechondrogenic condensation appears anterior to the centrale 3, and extends distal to the radius end.

Stage 19 (Figs 3F, 4F and 5A¹)
Terminal phalanges are well developed and claw-shaped in both fingers and toes I–IV. The phalangeal formula for the manus is 2.3.3.3.3. In the carpus, centrale 2 becomes a distinct cartilage that remains connected to centrale 3. At this stage, centralia 4, 3, and 2 form a transversal cartilage. Distal carpalia 4 and 5 are fused. In the tarsus, the fibulare cartilage fuses with the intermedium. Perichondral ossification in the forelimb occurs at the diaphyses of the radius and ulna, and in the hindlimb has started in the diaphysis of the metacarpale IV.

Stage 20 (Figs 5B, B¹ and 6A)
Forelimb perichondral ossification progresses: among the metacarpalia, the last to ossify is that of the metacarpale I; whereas among the terminal phalanges, the last to ossify is the terminal phalanx of finger V. Intermediate phalanges lack ossification centres. At this stage, the phalangeal formula of the pes is 2.3.3.3.3, and perichondral ossification centres appear in the five metatarsalia, and in the terminal phalanges.

Stage 21 (Fig 5C, D, C¹, D¹)
Perichondral ossification in the penultimate phalanges is observed in fingers I–IV, toes I–III, and in the terminal phalanx of toe IV. At the end of this stage, the terminal phalanx of finger V and the penultimate phalanx of toe IV are ossified.

Stage 22 (Fig 5E, E¹)
Proximal phalanges of fingers II–V, and of toes II–V, still remain cartilaginous.

Stage 23 (Figs 5F, F¹, 6B, C)
Except for the terminal phalanx of toe V, all of the diaphyses of metacarpalia, metatarsalia, and phalanges are ossified. The first centre of endochondral ossification appears in distal tarsale 5. The distal phalanx of toe IV is lost. The phalangeal formula of the pes becomes 2.3.3.3.2.

Stage 24 (Figs 5G, G¹, 6D)
The carpus is still cartilaginous, and a second centre of endochondral ossification appears in the tarsus, which develops into distal tarsale 4. The distal phalanx of toe IV is not pointed, and retains an irregular tip.

Post-hatching (Figs 5H, H¹, 6E)
In the carpus, there are well-developed endochondral centres of ossification in the ulnare, intermedium, the five distal carpalia, centralia 3 and 2, and the pisiform. The only carpal element that remains cartilaginous is the centrale 2. In the tarsus, the intermedium and fibulare correspond to two distinct ossification nuclei, and there is also a single nucleus of ossification in the five distal tarsalia. Toe V has two phalanges, and the distal one is truncated.

The description provided below is based mostly on our embryonic series of *Po. unifilis*. The embryonic series available for *Po. sextuberculata* is identical in most aspects with the former species, and does not provide significant additional information, except for the development of the third distal phalangeal segment of toe IV, which grows distally with a pointed shape, and ossifies before hatching in *Po. sextuberculata* (Fig. 6F). The subsequent development (Figs 3H, J, 4H, I) is similar to that described for *Po. unifilis*. It is worth noting that at stage 12 (Figs 3G, 4G), *Po. sextuberculata* limb buds show the characteristic Y-shaped cartilaginous condensation that corresponds to the branched distal end of the humerus/femur, which gives rise the ulna/fibula and radius/tibia.

LIMB DEVELOPMENT IN *PHRYNOPS HILARII*

Stage 17 (Figs 7A, 8A)
The primary cartilages of the ulnare, intermedium, centralia 4 and 3, and five distal carpalia appear in the forelimb as single and well-defined condensations. The five metacarpalia and proximal phalanges seem

Figure 5. Sequence of ossification in *Podocnemis unifilis*, dorsal view of left limbs. A–H, forelimbs. A¹–H¹, hindlimbs. A and A¹, stage 18; B and B¹, stage 19; C and C¹, stage 20; D and D¹, stage 21; E and E¹, stage 22; F and F¹, stage 23; G and G¹, stage 24; H and H¹, post-hatched specimen. Scale bars: 1 mm.
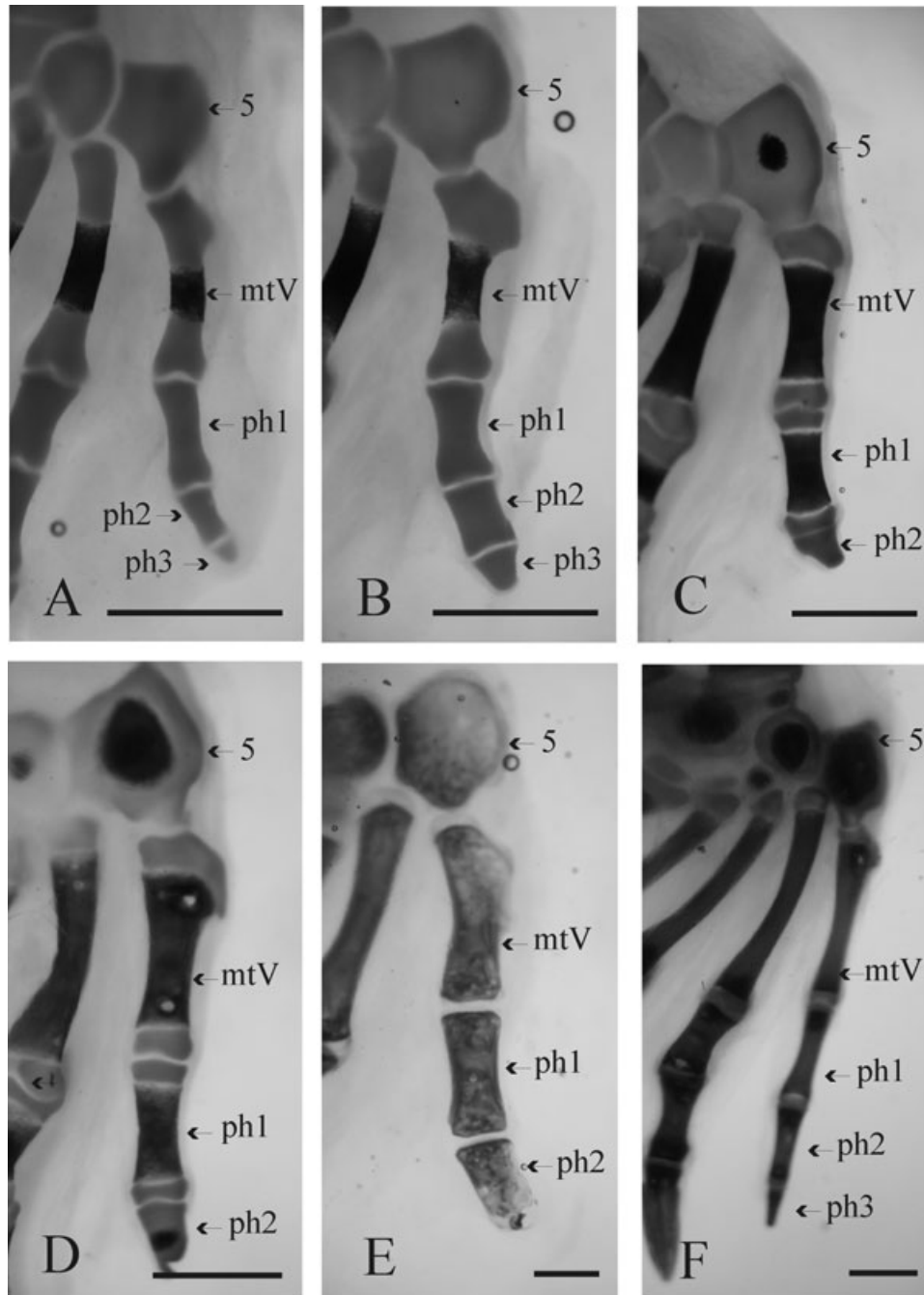
Figure 6. A–E, the sequence of reduction of the distal phalanx in toe V observed in *Podocnemis unifilis* (stages 20, 22, 23, 24, and juvenile). Three cartilaginous phalanges are segmented during digit development, but the distal one is lost before ossification. F, in *Podocnemis sextuberculata*, the third phalanx ossifies before hatching (stage 24). Abbreviations: 5, distal tarsale 5; mt V, metatarsale V; ph1–3, phalanges 1–3. Scale bars: 1 mm.

to be segmented already, except in digit I. The fibulare, intermedium, and the complete series of digital arch cartilages are already differentiated in the hindlimb. Both the intermedium and the fibulare are connected, earlier than those observed in *Podocnemis* spp. Distal tarsale 5 is elongated and displaced dis-

tally. There is a medial prechondrogenic area between the intermedium and distal tarsalia 3–1.

Stage 18 (Figs 7B, 8B)
Centralia 4 and 3 of the carpus start to fuse, whereas condensation of centrale 2 becomes well differentiated
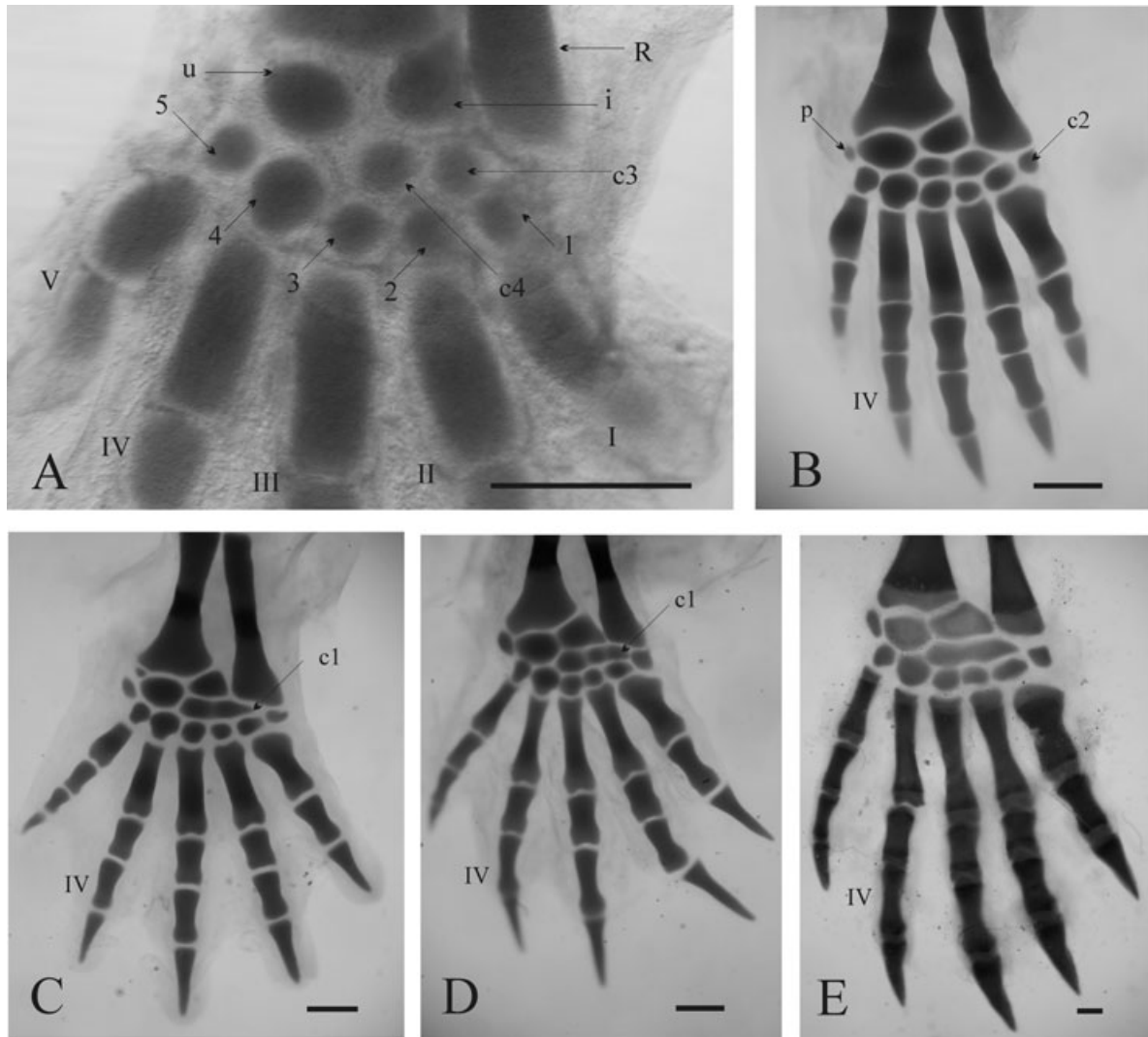
Figure 7. Forelimb development in *Phrynops hilarii*. Dorsal views of left limbs. A, stage 17; B, stage 18; C, stage 19; D, stage 20; E, stage 23. Abbreviations: 1–5, carpalia 1–5; I–V, digits I–V; c1, centrale 1; c2, centrale 2; c3, centrale 3; c4, centrale 4; i, intermedium; p, pisiform; Ra, radius; u, ulnare. Scale bars: 0.5 mm.

distally to the radius. Centrale 2 does not exhibit any connection with other cartilages of the carpus. The cartilaginous pisiform is present. Pointed terminal phalanges are developed in digits I–IV. A transversal condensation occupies the medial area of the tarsus in the hindlimb. This condensation is identified as centrale 4, and shows cartilaginous connectivity with the anterior end of the intermedium. The intermedium and fibulare are fused. Digits I–IV bear pointed terminal phalanges.

Stage 19 (Figs 7C, 8C)
Centralia 4 and 3 fuse to form a transversal cartilage in the carpus, which grows anteriorly into a fourth central condensation that we identify as centrale 1. At this stage, the phalangeal formula is 2.3.3.3.3, and is

completed with the differentiation of the terminal phalanges of digit V. In the hindlimb, the fusion of the intermedium and fibulare is noticeable, but centrale 4 remains separated from the intermedium. Distal tarsale 1 has an anterior expansion. The differentiation of phalanges in toe V is delayed with respect to the other toes.

Stages 20–21 (Figs 7D, 8D)
In the forelimbs, perichondral ossification is advanced in the ulna and radius, and begins in the metacarpals. There is an elongated central complex formed by three distinct cartilaginous foci (centralia 4, 3, and 1), and centrale 2 remains separated. Some specimens at this stage show fused distal carpalia 5 and 4. In the hindlimb, centrale 4 starts to be incorporated into the
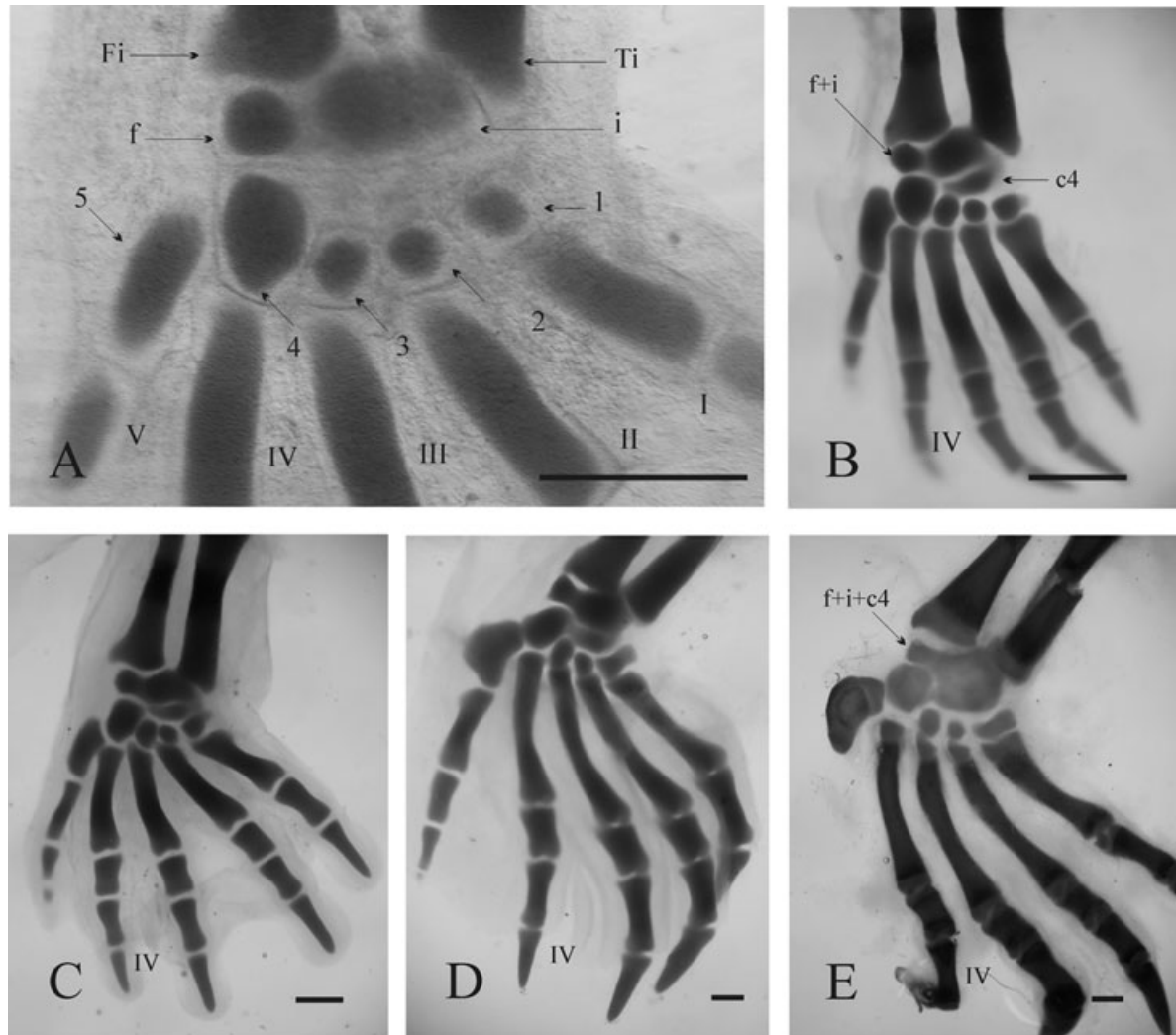
Figure 8. Hindlimb development in *Phrynops hilarii*. Dorsal views of left limbs. A, stage 17; B, stage 18; C, stage 19; D, stage 20; E, stage 23. The centrale 4 is completely integrated with the intermedium mass forming the proximal tarsale. Distal elements of toe V were lost in this specimen. Abbreviations: 1–5, tarsalia 1–5; I–V, digits I–V; c4, centrale 4; f, fibulare; Fi, fibula; i, intermedium; Ti, tibia. Scale bars: 0.5 mm.

cartilaginous mass of the intermedium. Distal tarsale 5 becomes dorsally expanded. The terminal phalanx of toe V is differentiated. Perichondral ossifications occur in the fibula, tibia, metatarsalia, and phalanges of digits II–IV.

Stage 25 (Figs 7E, 8E)
Both carpus and epiphyses of the long elements of the forelimb are still in a cartilaginous condition. Centrale 4 in the hindlimb is fully incorporated into the proximal tarsale. The distal tarsale 5 shows the first signs of the appearance of an endochondral ossification centre. The complete phalangeal formula is 2.3.3.3.3.

Post-hatching
Carpal ossification takes place in a postaxial direction. Distal carpalia 5 and 4 develop into a single ossification centre. Ossification in the central complex appears as two distinct postaxial nuclei that can be described as centralia 4 and 3. The centrale 2 is the last carpal element to ossify. In the hindlimb, both the calcaneum (fibulare) and the astragalus (central 4 + intermedium) ossify in the proximal tarsale after ossification of distal tarsalia 5 and 4. In some specimens, the proximal tarsale shows distinct ossification nuclei that correspond to the fibulare, intermedium, and centrale 4 (Fig. 12B).

DISCUSSION

Data on limb development in pleurodiran turtles were shown to be relevant to a review of aspects related to the definition of primary homologies of limb bones in turtles. More specifically, we raised four issues that deserve a thorough review based on the additional observations on limb development in pleurodiran turtles described here: (1) does digit V belong to the embryonic series of primary cartilages of the digital arch?; (2) is the hooked bone of turtles homologous to the fifth hooked metatarsal of diapsid reptiles?; (3) how many cartilaginous foci of the central series develop in the carpus of turtles?; and (4) how many cartilaginous foci form the proximal tarsale of turtles? We will discuss these four issues in more detail below.

DOES DIGIT V BELONG TO THE EMBRYONIC SERIES OF PRIMARY CARTILAGES OF THE DIGITAL ARCH?

This issue has already been addressed by Burke & Alberch (1985), Shubin & Alberch (1986), Burke & Feduccia (1997), and Sanchez-Villagra et al. (2008). These authors did not find any connectivity between digit V and the primary axis and/or the digital arch in tetrapod limbs, and suggested that digit V is formed as a *de novo* condensation. However, some data in anurans (Fabrezi & Barg, 2001), lizards (Fabrezi et al., 2007), and turtles (Sanchez-Villagra et al., 2007a) suggested that digit V could arise as a condensation of the digital arch and/or the ulnare/fibulare. In turtles, the connectivity between distal carpale/tarsale 5 and distal carpale/tarsale 4 was interpreted at early embryonic stages of development in *Chelcnia mydas* (Sanchez-Villagra et al., 2007a: fig. 3). Despite the fact that we did not observe any cartilaginous connection between ulnare/fibulare and digital arch elements, in the species analysed, digital arch development starts with the differentiation of digit IV, whereas digits III and V differentiate almost simultaneously (Figs 3, 4, 7, 8). The pattern of digit development is similar, with each digit appearing as a cartilaginous column in which the rounded, well-condensed proximal portion corresponds to the distal carpale/tarsale, and the distal, elongated segment represents the metacarpale/metatarsale element.

Thus, homology of all digits, including digit V, is supported by: (1) their similar pattern of development; (2) the timing in which digit V appears, after the primary axis condensations and before the anterior digits (II and I); and (3) the similar relationships between the distal carpalia/tarsalia with the corresponding digit in the digital arch, shown in histological sections (Fig. 9).

IS THE HOOKED BONE OF TURTLES HOMOLOGOUS TO THE FIFTH HOOKED METATARSAL OF DIAPSID REPTILES?

A hooked element in toe V is present in the Lepidosauromorpha and Archosauromorpha, and, although its presence in *Youngina* and other primitive diapsids remains a controversial issue (Goodrich, 1916; Lee, 1997; Rieppel & Reisz, 1999), it is assumed by most authors that it represents at least a synapomorphy of the clade Sauria of diapsid reptiles (Rieppel & deBraga, 1996; deBraga & Rieppel, 1997; Rieppel & Reisz, 1999). Most authors also consider the hooked element as being a modified fifth metatarsale, and regard the fifth distal tarsale as being absent in Sauria and turtles (Goodrich, 1916; deBraga & Rieppel, 1997; Lee, 1997; Rieppel & Reisz, 1999; Hill, 2005).

Robinson (1975) was the first to detail the morphology of the hooked fifth metatarsale in extant lepidosaurians. She described two conditions of the fifth metatarsale that could be found in both fossil and extant reptiles: (1) a straight metatarsale V, similar in shape to the other four metatarsalia, that articulates with the fifth distal tarsale by a terminal proximal facet; (2) a hooked metatarsale V, with a proximal end that expands medially to metatarsale IV, and articulates with the latero-plantar surface of distal tarsale 4. Robinson (1975) also observed that a distal tarsale 5 is always absent when the fifth metatarsale is hooked, highlighting that the hooked metatarsale V plays a double role as part of both distal tarsal and metatarsal rows. Three distinct hypotheses of homology were advanced at that time in order to account for the modified morphology of the fifth toe in reptiles: (1) the hooked bone represents an enlarged distal tarsale 5 in turtles (Sheil, 2005); (2) for some reptiles, the hooked bone is an enlarged metatarsale V, whereas the distal tarsale 5 is lost (Goodrich, 1916; Schaeffer, 1941; Carroll & Currie, 1991); (3) the hooked bone could represent the fusion of the fifth metatarsale with the distal tarsale 5 (Rabl, 1910; Holmgren, 1933; Robinson, 1975).

Recent studies based on an ontogenetic series of embryos have shown that the hooked element of the fifth toe in Lepidosaurian (Fabrezi et al., 2007) and Archosaurian reptiles (Müller & Alberch, 1990) is in fact a modified metatarsale, supporting the previous hypothesis of homology advanced by Goodrich (1916). However, the hooked element of turtles, widely recognized as a fifth metatarsale (Burke & Alberch, 1985; Sheil, 2003; Sanchez-Villagra et al., 2007a, b), seems to be formed by only the fifth distal tarsale (Sheil, 2005; this study; *contra* Rieppel, 1993a).

Morphological differences between the proximal elements of toe V in turtles, lizards, and crocodiles are
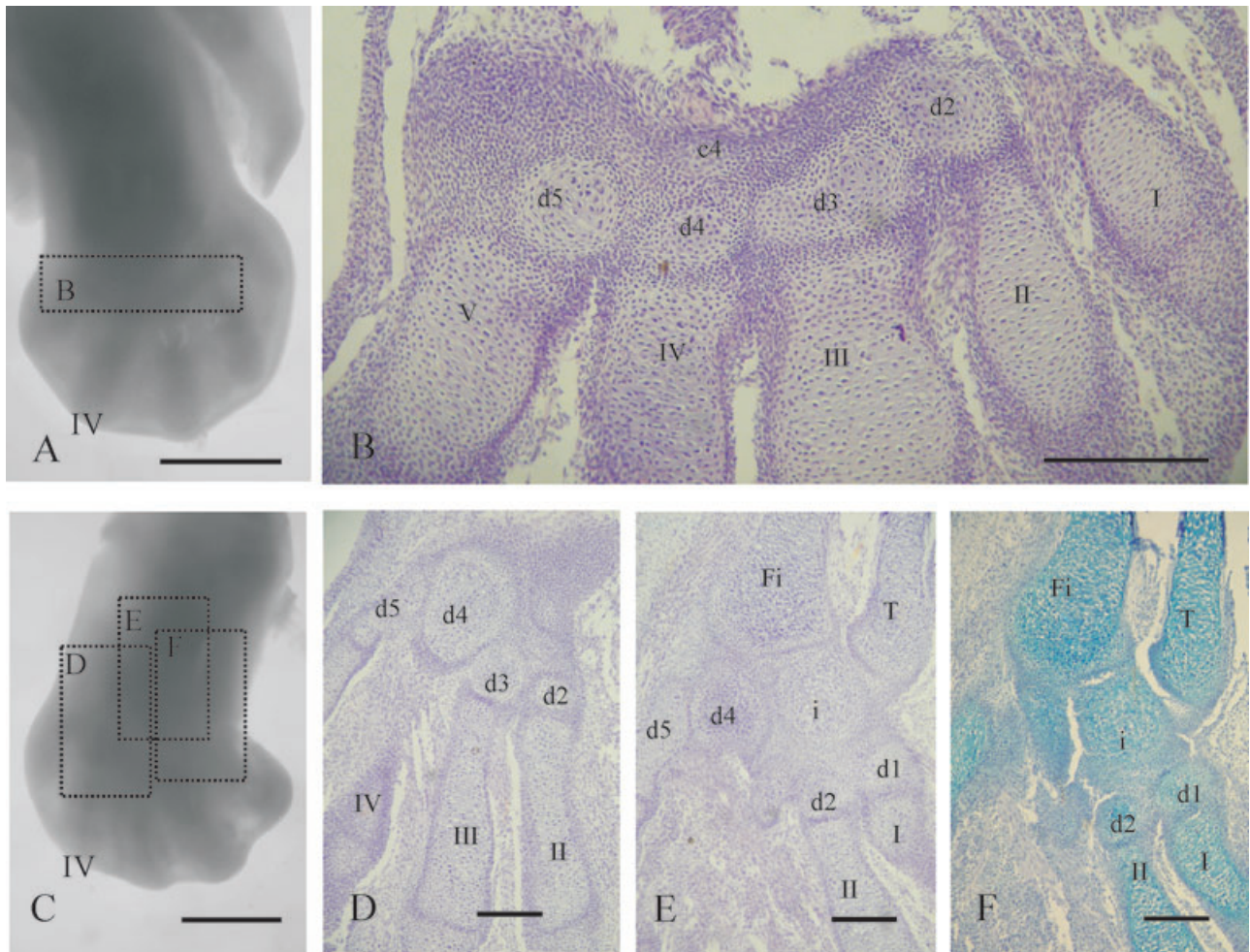
Figure 9. Histological sections of limb buds of *Podocnemis unifilis* at stage 16. A, scale bar: 0.5 mm. The rectangle indicates the area of the carpal section on the right. B, dorsoventral section showing cartilaginous foci of distal carpalia, metacarpalia, and the centrale 4. C, scale bar: 0.5 mm. The rectangles point the areas of the tarsal sections in (D–F). D, the relationships between distal tarsalia 5 and 4 seem to be similar to those of other distal tarsalia. E, the large cartilaginous mass of the intermedium can be seen. F, there are no relationships between the end of the tibia and the cartilaginous condensations of the tarsus. Abbreviations: I–V, digits I–V; c4, centrale 4; d1–d5, distal carpalia/tarsalia 1–5; Fi, fibula; i, intermedium; T, tibia. Scale bars: 0.2 mm in (D–F).

shown in Figures 10 and 11. The proximal bone of toe V in lizards is a hooked element that has a distinctive shape (Robinson, 1975): it is enlarged and expanded proximally, with a well-defined hamate process, and a ventral concave surface that articulates with distal tarsale 4 and metatarsale IV. It bears an outer process dorsally, and two distinct plantar tubercles (lateral and medial). The distal portion of the hooked bone becomes cylindrical, and ends in a distal epiphysis that articulates with the second element of toe V (Fig. 10A, D). In crocodiles, this element is almost vestigial (Fig. 10B; Müller & Alberch, 1990; Rieppel, 1993b). In turtles, the proximal bone of toe V is short, almost polyhedrical, has two lateral concave surfaces to articulate with the distal tarsale 4 and metatarsale

IV, and has a distal small process that supports the second element of toe V (Fig. 10C). This element lacks processes, and its shape does not agree with the features of the hooked element mentioned above. In extant reptiles, the hooked bone extends from both rows of distal tarsalia and metatarsalia, but in turtles the relationship is predominantly with the distal tarsalia, whereas in lizards it is predominantly with the row of metatarsalia (Fig. 11).

Other morphological differences are observed in the patterns of insertion of some muscles (Fig. 10C, D). Comparisons between the hooked bones and associated muscles of turtles and lizards also support the hypothesis that the hooked element of the fifth toe of turtles is in fact the distal tarsale 5, and that the
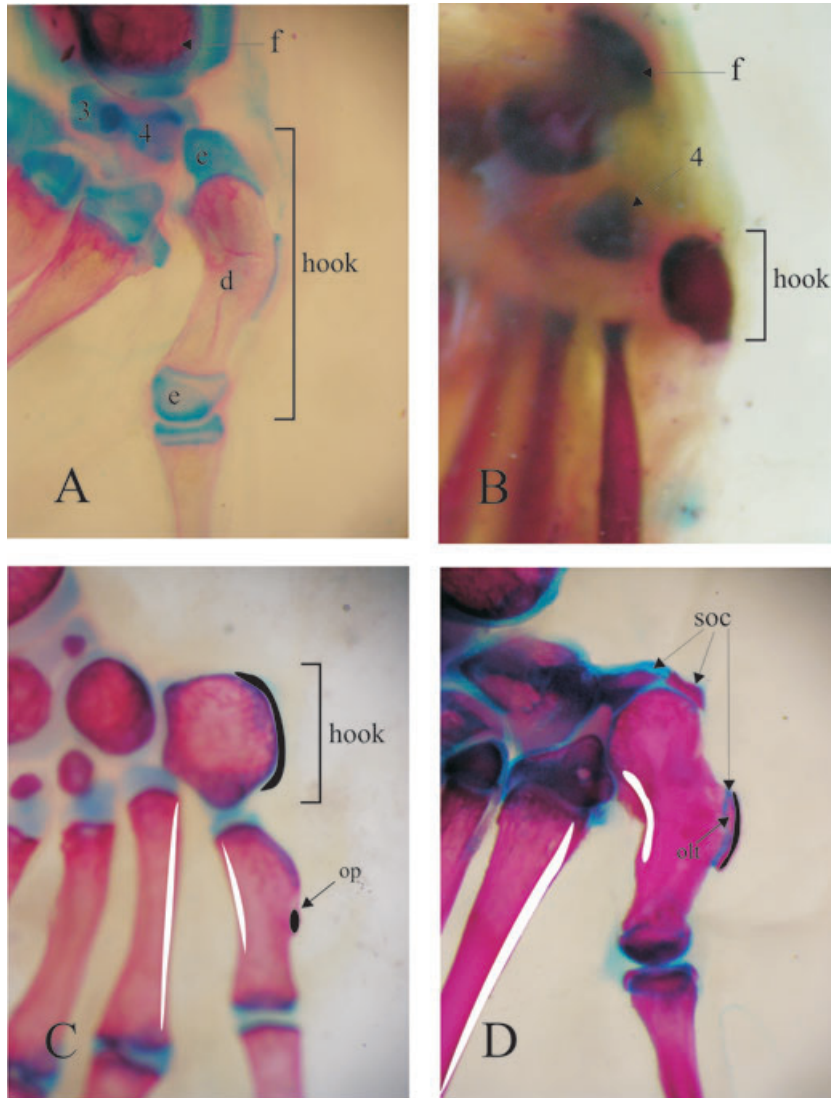
Figure 10. Morphological and structural features of the hook in extant reptiles. A, *Liolaemus multicolor*, juvenile specimen. The hook has cartilaginous distal and proximal epiphyses, and a short ossified diaphysis. This ossification is perichondral. The proximal epiphysis forms a small concave surface to articulate with distal tarsale 4. The distal epiphysis is rounded. B, *Caiman latirostris*, juvenile specimen. The hook is a semispherical ossified element with a short and stout distal process. It is related to distal tarsale 4 and metatarsale IV, but lacks distinct articular surfaces. Toe V in crocodiles is reduced to this element only. C, *Podocnemis unifilis*, juvenile specimen. The hook is a subspherical element with a nucleus of endochondral ossification. It has a wide concave articular surface with the distal tarsale 4, and a distal and convex articular process with metatarsale V. The dorsal and outer surface of distal tarsale 5 bears fibers of two muscles of the crus: peroneus anterior and gastrocnemius (black mark). One of the ligaments of the gastrocnemius muscle is fused to the plantar aponeurosis, and attaches to the outer process of metatarsale V (black mark). The muscles flexor digiti brevis and interosseum insert along the anterior border of metatarsale V, and the posterior border of metatarsale IV (white marks). D, *Liolaemus multicolor*, adult specimen. The hook bears a fully ossified epiphyses and diaphysis. Secondary centres of ossification are visible in the proximal epiphyses and in the lateroposterior medial crest. The peroneus brevis, peroneus longus, and gastrocnemius muscles, as well as the superficial femoral aponeurosis, are attached to the lateral plantar process of the fifth hooked metatarsale. There are also fibers of the deep femoral aponeurosis (formed by tendons of the peroneus and gastrocnemius muscles) attached to the outer lateral plantar tubercle (black mark). Insertions of the interosseum (or intermetatarsale) muscle occur on the hook (white marks). Abbreviations: 3, 4, tarsalia 3 and 4; d, diaphysis; e, epiphysis; f, fibulare; olt, outer lateral plantar tubercle; op, outer process; soc; secondary ossification centre.
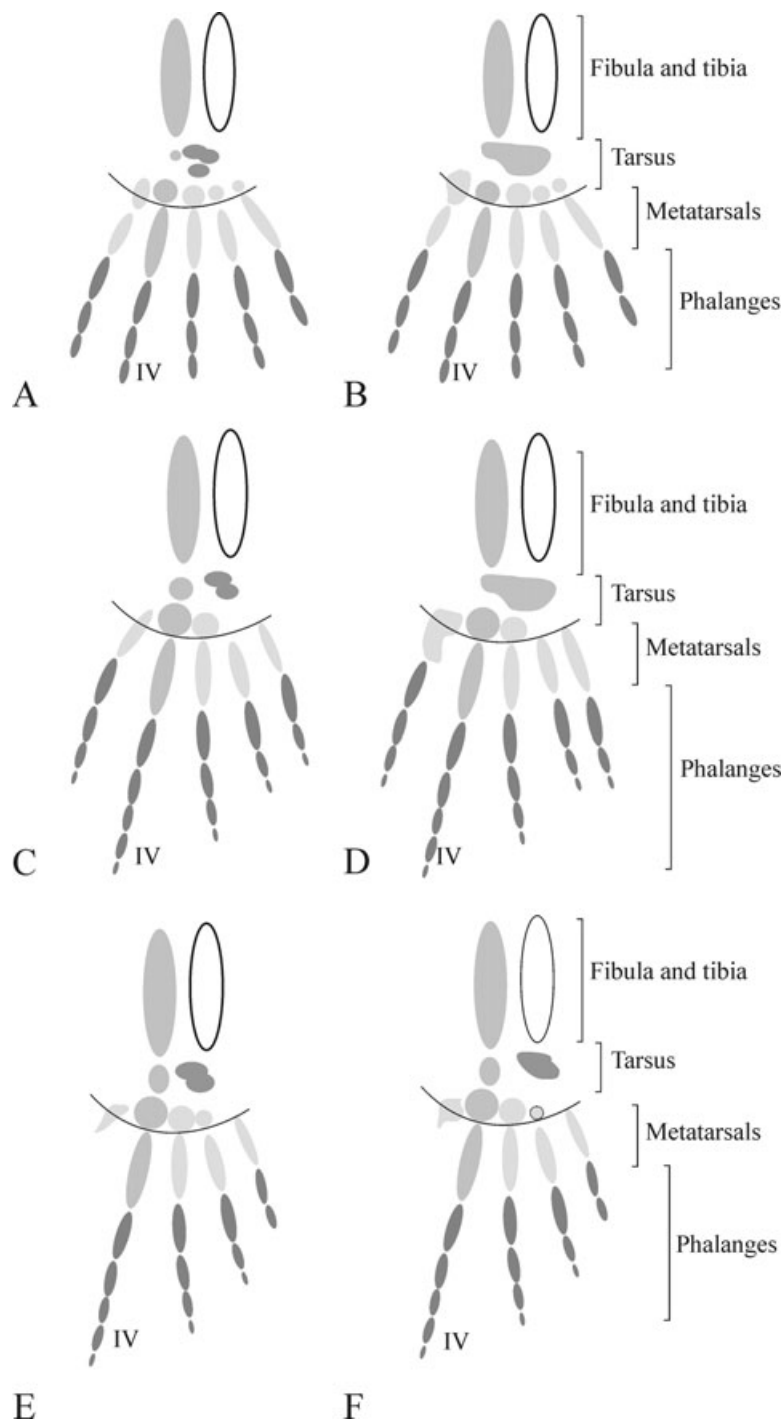
metatarsale V is the second element of toe V. In lizards, insertions of the muscles peroneus brevis, gastrocnemius caput dorsale, gastrocnemius caput ventrale, and lumbricalis are on the lateral plantar tubercle, on the medial portion of the hooked bone, and distal to the distal tarsalia–metatarsalia articulation. The tendons of these muscles in turtles are attached proximally to this articulation, in the poste-

rior outer border of the hooked bone. Only the ligament of the gastrocnemius caput dorsale muscle is inserted distal to this articulation in turtles. Additionally, the interosseoum muscle of lizards inserts along the internal border of the hooked bone, whereas in turtles it inserts along the internal border of the second element of toe V, suggesting that the topographical homology of the fifth hooked metartarsale

Figure 11. Simplified scheme of chondrogenic condensations and fusion patterns in the hindlimb ontogeny of turtles, lizards, and crocodiles. The curved line represents the tarsal–metatarsal level of articulation. A, primary cartilaginous condensations of different hindlimb segments are observed in pleurodiran turtles. Toe V is formed by a proximal elliptic condensation (distal tarsale 5) that grows distally beyond the row of distal tarsalia. Metatarsale V is the shortest of the metatarsal series, and bears up to three phalanges. The distal phalanx is not pointed, and may be reduced at older embryonic or post-hatching stages. Embryos of *Podocnemis* and *Phrynops* exhibit the plesiomorphic phalangeal formula proposed for turtles in both autopodia Boulenger (1889), Crumly & Sanchez-Villagra (2004), and Sanchez-Villagra *et al*. (2007b). B, the adult pattern in pleurodiran turtles. Distal tarsale 5 is the largest of the distal tarsal row, and acquires a polyhedrical or subspherical shape, with concave surfaces, to articulate with distal tarsale 4 and metatarsale IV. Digit IV in turtles has a similar length to other digits. C, primary cartilaginous condensations of different hindlimb segments are observed in lizards (Fabrezi *et al*., 2007). Anterior condensations of distal tarsalia 2 and 1 fail to form in lizards. Toe V is formed by a long proximal cartilage that grows distally, far beyond the row of distal tarsalia. This cartilage grows, curves, expands, and develops processes and tubercles. This element has been interpreted as a modified hooked metatarsale V, suggesting that distal tarsale 5 would be absent (Goodrich, 1916; Schaeffer, 1941; Carroll & Currie, 1991; deBraga & Rieppel, 1997; Lee, 1997; Rieppel & Reisz, 1999) or fused with metatarsale V (Holmgren, 1933; Robinson, 1975). Toe V bears four phalanges, the distal one is claw-shaped, and toe IV is the longest of the series. D, the adult pattern of limb elements in lizards. The proximal element of toe V is a hooked element. E, primary cartilaginous condensations of different hindlimb segments are observed in crocodiles (Müller & Alberch, 1990). Toe V is formed only by a single small cartilage, as the phalanges are absent. This element has been interpreted as a modified, hooked metatarsale V (Müller & Alberch, 1990) or as a result of the fusion between distal tarsale 5 and rudiments of digit V (Holmgren, 1933). D, the adult pattern of limb elements in crocodiles. The single element of digit V is a cubical bone with a pointed post-axial process that articulates with distal tarsale 4 and metatarsale IV.

of lizards is actually with the second element of the fifth toe of turtles, and not with the first hooked element, as previously thought.

Another argument relates to the number of phalanges of toe V. The widespread phalangeal formula in living turtles is 2.3.3.3.3 in both autopodia, and is the plesiomorphic formula for pleurodirans (Boulenger, 1889; Crumly & Sanchez-Villagra, 2004; Sanchez-Villagra *et al*., 2007b). Among Pleurodirans, *Pelomedusa* has a reduced formula of 2.2.2.3.2 or 2.2.3.3.2 in the pes (Sanchez-Villagra *et al*., 2007b). At least during the embryonic development, *Phrynops* and *Podocnemis* would have the plesiomorphic formula 2.3.3.3.3 if we consider toe V as being formed by the distal tarsale 5, metatarsale V, and three phalanges. Interpretation of the proximal element of toe V as a modified metatarsale V would imply the assumption that some pleurodiran turtles have four phalanges in toe V (Fig. 11).

There are also developmental differences between the proximal elements of toe V in turtles and lizards that reinforce the hypothesis on the non-homology of their hooked bones (Fig. 11). In turtles, at earlier embryonic stages, the proximal cartilage of toe V appears in the same position as the distal tarsalia, but unlike them, grows distally, and expands into a polyhedrical or subspherical cartilage, extending beyond the line where the distal tarsalia articulate with the metatarsalia (Sheil, 2005; this study). Thus, the articulation of digit V is displaced distally with respect to the other digits. In lizards, the early development of the proximal element of toe V is similar to

the other metatarsals, and grows predominantly in a distal direction, and curves, far beyond the distal tarsale–metatarsale articulation (Fig. 11). After hatching, it expands dorsally, and the hamate process becomes well defined (Fabrezi *et al*., 2007, Figs 10, 11).

The subsequent development of toe V in turtles involves the endochondral ossification of its proximal cartilage, which takes place after the complete perichondral ossification of metatarsalia and phalanges (Fig. 4). In lizards, on the other hand, the proximal cartilage of toe V ossifies perichondrally and earlier than the tarsal elements, as a typical metatarsal bone (Mathur & Goel, 1976; Rieppel, 1992a, b; Arias & Lobo, 2006; Fabrezi *et al*., 2007). Furthermore, its distal and proximal epiphyses develop secondary centres of endochondral ossification (Mathur & Goel, 1976; Rieppel, 1992a, b; Rieppel & Reisz, 1999; Arias & Lobo, 2006; Fabrezi *et al*., 2007). We consider the endochondral ossification of the hooked element of turtles and the timing in which it develops as two strong arguments further supporting its identity as a distal tarsale 5.

HOW MANY CARTILAGINOUS FOCI OF THE CENTRAL SERIES DEVELOP IN THE CARPUS OF TURTLES?

This issue relates to the number of cartilaginous foci of the central series that develop in the carpus of turtles. As the number of individual carpal bones in adult turtles is variable, the identity of medial and proximal elements is not clear. For example, there is
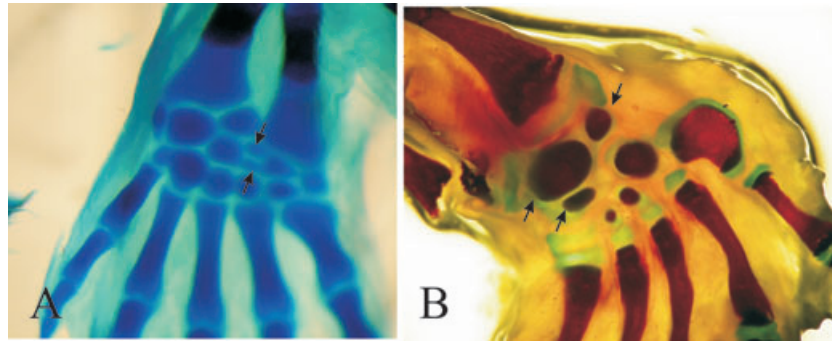
Figure 12. Abnormal morphologies observed during limb development in *Phrynops hilarii*. A, the arrows point to a condensation of centrale 3 that seems to be duplicated. B, the arrows point to three ossification nuclei representing the primary cartilages of the fibulare, intermedium, and centrale 4 in the proximal tarsal.

a proximal and preaxial bone in the carpus of some turtles that has been variably interpreted as a centrale (Holmgren, 1933; Burke & Alberch, 1985), radiale (Sheil, 2005: fig. 7), and 'small accessory element' (Sanchez-Villagra *et al.*, 2007b). A reanalysis of the early stages of development of the carpus in turtles is necessary in order to review the interpretation of the identity of the medial bones (see Table 1).

In the carpus of adults of *Podocnemis* spp., the transverse bone corresponds to a complex of three primary cartilages, which develop preaxially in a prechondrogenic medial blastema (Fig. 3). A similar pattern of development in the adult centrale was described in *Chelydra serpentina* and *Chrysemys picta* (Burke & Alberch, 1985). In *Apalone spinifera*, the primary cartilages of centralia 3 and 4 originate as single focus that divides into three distinctive centralia in the adult (Sheil, 2003), whereas in *Macrocleniys temminckii*, the three primary cartilages originate as a single condensation, and centralia 3 and 4 form a single bone in the adult (Sheil, 2005). Unlike the above condition, four central cartilages are present during the development in *Ph. hilarii* (Fig. 7). Centralia 4 and 3 appear as two continuous foci that remain connected until a third cartilaginous focus appears distal to the radius. The element distal to the radius observed in *Podocnemis* spp. and *Phrynops* would correspond to the definition of centrale 2 (see Table 1; Burke & Alberch, 1985). Sanchez-Villagra *et al.* (2007b) described the 'small accessory element' in the carpus of the pleurodire genera *Hydromedusa*, *Emydura*, *Chelodina*, *Phrynops*, and *Pelomedusa*, and discussed its homology with the mammalian prepollex and sesamoids. Afterwards, Sanchez-Villagra *et al.* (2008) suggested two alternative hypotheses of homology: an atavistic radiale; or a centrale following the criterion proposed by Burke & Alberch (1985). We agree with the interpretation of Burke & Alberch (1985) that the

cartilage that differentiates distal to the radius is actually a centrale. The pattern of connectivity with centrale 3, observed in *Podocnemis* spp., is a clear argument rejecting its identity as a segmented element (radiale or prepollex) of the preaxial axis (radius). The fourth cartilaginous condensation that arises from the anterior border of centrale 3 in *Phrynops* is also a centrale that differentiates later (Fig. 7).

Embryological data reveal that the limb developmental program in turtles may give rise to a maximum of four central foci. Centralia 4 and 3 appear earlier, distal to the ulnare and intermedium, and form a single cartilaginous bar, in which two distinct ossification nuclei will develop. Centrale 2 is distal to the radius, and ossifies later as an independent element (Burke & Alberch, 1985); centrale 1 arises as a continuation of centrale 3. Centrale 2 may be united with centrale 3 only by a cartilaginous constriction. In some species this connectivity is not clear (Burke & Alberch, 1985) or seems to be absent (*Phrynops*; Sheil, 2003, 2005). The fourth centrale element (centrale 1), observed in *Ph. hilarii*, may be interpreted as the last branching event of the central series that is delayed. In *Chelonia mydas* and *Caretta caretta* the adult carpus has only two central bones, one of which is distal to the radius (Sanchez-Villagra *et al.*, 2007a). In *C. mydas* the medial bone is connected with the ulnare, whereas the other bone distal to the radius remains without any connectivity (centrale 2?). The capacity to branch shown by the central condensations is evident in the duplicate centrale 3 observed in a malformed limb bud (Fig. 12A).

HOW MANY CARTILAGINOUS FOCI FORM THE PROXIMAL TARSALE OF TURTLES?

The last issue to be addressed concerns the cartilaginous foci that may form the proximal tarsale of turtles. In other extant reptiles, the proximal tarsal is
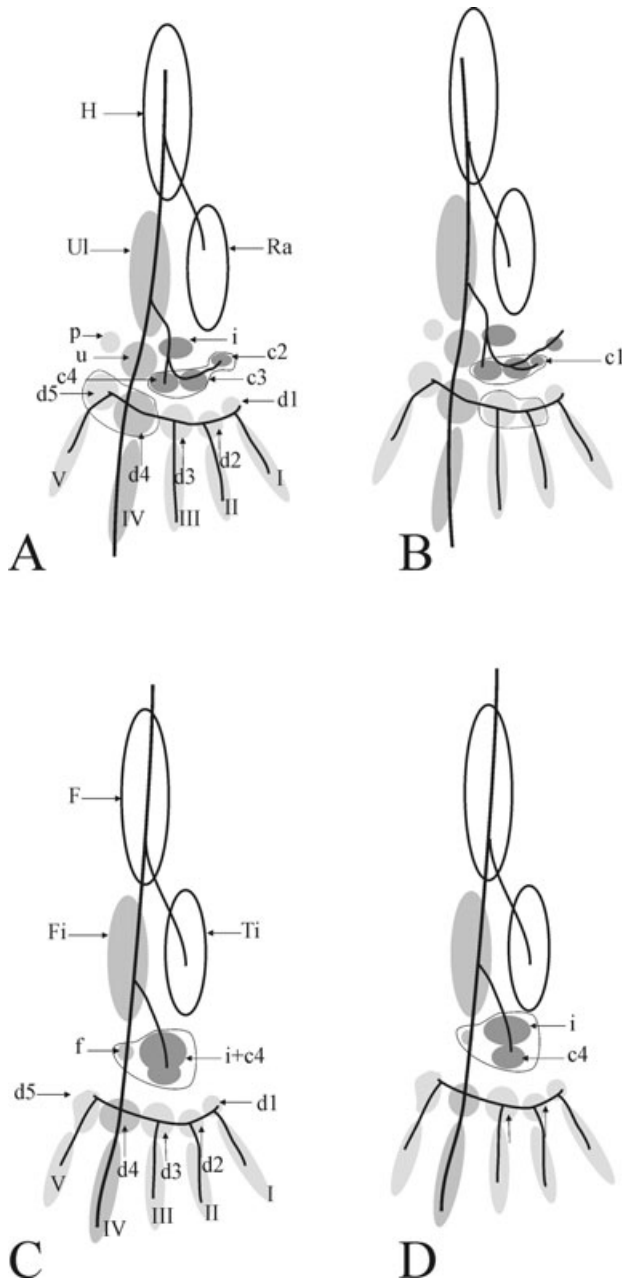
Figure 13. Scheme of limb development in pleurodiran turtles. These patterns were also observed in other turtles. In the forelimbs (A and B), developmental variation involves the number of cartilaginous primordia of the central series. At advanced embryonic stages and after hatching, secondary fusion may occur in the distal carpal series. In the hindlimbs (C and D), centrale 4 may originate as a distinct cartilaginous primordium, or fuse with the intermedium condensation. The astragalus ossification will develop in the intermedium–centrale cartilage. Abbreviations: I–V, digits I–V; c1–c4, centrale 1–4; d1–d5, distal carpalia/tarsalia 1–5; f, fibulare; F, femur; Fi, fibula; H, humerus; i, intermedium; p, pisiform; Ra, radius; Ti, tibia; Ul, ulna.

formed by two cartilaginous condensations: the fibulare and the intermedium–centrale (Müller & Alberch, 1990; Müller, 1991; Fabrezi *et al.*, 2007).The variation in the number of cartilaginous foci incorporated into the proximal tarsale has already been described in turtles (Burke & Alberch, 1985; Sheil, 2003, 2005; Sanchez-Villagra *et al.*, 2007a; Sanchez-Villagra *et al.*, 2008). However, additional developmental information could provide arguments to discuss the origin and evolution of the proximal tarsale and astragalus ossifications in reptiles.

In the adult tarsus of *Ph. hilarii*, there is a proximal tarsale, in which the distinct primary cartilages of the fibulare, intermedium, and centrale 4 are incorporated (Fig. 8). The proximal tarsale of the adult of *Podocnemis* is formed by cartilaginous condensations of the small fibulare and a large intermedium (Fig. 4). In *Podocnemis*, the intermedium differentiates in an amorphous precondensation that occupies the proximal tarsal region. In *Ph. hilarii* (Fig. 8), the condensation of the intermedium grows in an anterior direction, and in its preaxial and distal corners is continuous with a rectangular focus of centrale 4, which extends in a preaxial direction. The intermedium fuses with the fibulare first, and with centrale 4 later. The same pattern of tarsal proximal development was described in *Macrochelys temminckii* (Sheil, 2005), *Chelydra serpentina*, and *Chrysemys picta* (Burke & Alberch, 1985). In *Chelydra serpentina*, Burke & Alberch (1985) described a distinct centrale 1, located anteriorly to the intermedium, that becomes incorporated in the mass of the intermedium–centrale 4 condensation. In *Podocnemis* spp., the condensation of the intermedium remains amorphous and grows towards the medial region of the tarsus, suggesting that the central condensation is incorporated in the single cartilaginous mass of the developing intermedium. This pattern is the same in *Apalone spinifera* (Sheil, 2003) and *Chelonia mydas* (Sanchez-Villagra *et al.*, 2007a). These embryological data suggest the proximal tarsale of turtles may be formed by the fibulare fused with: (1) the cartilage formed by the primary fusion of the intermedium and centrale 4, observed in *Podocnemis*, *Apalone spinifera* (Sheil, 2003), and *Chelonia mydas* (Sanchez-Villagra *et al.*, 2007a); (2) the cartilage formed by the secondary fusion of the intermedium and the centrale 4, as in *Phrynops, Macrochelys temminckii* (Sheil, 2005), and *Chrysemys picta* (Burke & Alberch, 1985); and (3) the secondary fusion of the intermedium, centrale 1, and centrale 4 observed in *Chelydra serpentina* (Burke & Alberch, 1985). Invariably, the endochondral astragalus ossification will develop into the cartilaginous condensation formed predominantly by the intermedium + centrale 4. The astragalus ossification is usually a single nucleus; however, two distinctive

ossification nuclei, representing the intermedium and centrale 4, may appear occasionally in turtles (Fig. 12B).

CONCLUSIONS

Figure 13 summarizes our observations of limb development in the two South American pleurodiran genera *Podocnemis* and *Phrynops* studied (see also Burke & Alberch, 1985; Sheil, 2003, 2005; Sanchez-Villagra *et al.*, 2007a; Sanchez-Villagra *et al.*, 2008). From the present analysis, we conclude that: (1) the developmental program of the carpus involves the differentiation of the ulnare, intermedium, up to four central condensations, and five distal carpalia; (2) the developmental program of the tarsus involves the differentiation of the fibulare, intermedium, up to two central condensations, and five distal tarsalia; (3) the astragalus represents the ossification of the primary cartilages of the intermedium and centrale/centralia; (4) digit V forms part of the digital arch in both limbs; (5) the hooked element in the hindlimb of turtles is a modified distal tarsale 5.

ACKNOWLEDGEMENTS

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APPENDIX

MUSEUM ACRONYMS

DIAM: Centro de Investigaciones Científicas y de Transferencia de Tecnología a la Investigación, Diamante, Entre Ríos, Argentina.

MZUSP: Museu de Zoologia, Universidade de São Paulo, Brazil.

MCN: Museo de Ciencias Naturales, Universidad Nacional de Salta, Argentina.

SPECIMENS EXAMINED

Podocnemis sextuberculata

Embryos: MZUSP 4314, stage 12 (embryo length 7.86 mm); MZUSP 4315, stage 15 (carapace length 5.76 mm); MZUSP 4316, stage 17 (carapace length 5.24 mm); MZUSP 4317, stage 18 (carapace length 6.6 mm); MZUSP 4318, stage 19 (carapace length 14.94 mm); MZUSP 4319, stage 20 (carapace length 16 mm); MZUSP 4320, stage 20 (carapace length 17 mm); MZUSP 4321, stage 20 (carapace length 17.34 mm); MZUSP 4322, stage 21 (carapace length 17 mm); MZUSP 4323, stage 22 (carapace length 26.46 mm); MZUSP 4324, stage 22 (carapace length 27.54 mm); MZUSP 4325, stage 24 (carapace length 32.26 mm).

Adult specimen: MZUSP 3218.

Podocnemis unifilis

Embryos: MZUSP 4339, stage 14 (embryo length 3.54 mm) MZUSP 4338, stage 15 (carapace length 6.84 mm); MZUSP 4337, stage 17 (carapace length 5.24 mm); MZUSP 4336, stage 17 (carapace length 11.50 mm); MZUSP 4340, stage 17 (carapace length 10.74 mm); MZUSP 4335, stage 18 (carapace length 12.46 mm); MZUSP 4334, stage 19 (carapace length 15.66 mm); MZUSP 4333, stage 20 (carapace length 18.50 mm); MZUSP 4332, stage 21 (carapace length 22.46 mm); MZUSP 4331, stage 21 (carapace length 17.64 mm); MZUSP 4330, stage 21 (carapace length 19 mm); MZUSP 4329,

stage 21 (carapace length 24.24 mm); MZUSP 4328, stage 21 (carapace length 26.56 mm); MZUSP 4327, stage 22 (carapace length 21.24 mm); MZUSP 4326, stage 22 (carapace length 29.86 mm); MZUSP 4341, stage 23 (carapace length 33 mm).
Post-hatching specimens: MCN 2202 (six specimens, carapace lengths: 47, 44, 45, 48, 47, and 48 mm, respectively).
Adult specimen: MZUSP 3209 (only dry limb skeletons).

Phrynops hilarii
Embryos: DIAM R-064, stage 14 (carapace length 12.86 mm); DIAM R-011, stage 15–16 (carapace length 13.3 mm); DIAM R-063, stage 16 (only limb buds available, specimen damaged); DIAM R-015, stage 17 (carapace length 17.74 mm); DIAM R-084, stage 18 (carapace length 21.92 mm); DIAM R-012, stage 19 (carapace length 24.3 mm); DIAM R-016, stage 19 (carapace length 20.71); DIAM R-018, stage 19–20 (carapace length 26.89 mm); DIAM R-019, stage 19–20 (carapace length 27 mm); DIAM R-013, stage 20 (carapace length 26.1 mm); DIAM R-020, stage 20–21 (carapace length 29 mm); DIAM R-021, stage 20–21 (carapace length 34 mm); DIAM R-083; stage 20 (carapace length 25.41 mm); DIAM R-024, stage 20–21 (carapace length 39.74 mm); DIAM R-026, stage 25 (carapace length 45.3 mm); DIAM R-010, stage 25 (carapace length 54.1 mm).
Post-hatching specimens: DIAM R-001 (carapace length 62 mm); DIAM R-003 (carapace length 67 mm); DIAM R-002 (carapace length 72 mm); DIAM R-004 (carapace length 140 mm).
Adult specimens: DIAM R-005 (carapace length: 180 mm); DIAM R-006 (carapace length: 240 mm).

Liolaemus multicolor
MCN 2142, post-hatching specimen (29.8 mm snout–vent length, SVL); MCN 2143, juvenile specimen (42.4 mm SVL); MCN 2144, juvenile specimen (40.8 mm SVL); MCN 2141, juvenile specimen (48.4 mm SVL); MCN 2140, adult specimen (78 mm); MCN 2152, embryo at stage 32; MCN 2153, embryo at stage 34; MCN 2154, embryo at stage 37; MCN 2155, embryo at stage 40.

Caiman latirostris
DIAM R-082, juvenile (84 mm SVL); DIAM R-009, juvenile (107 mm SVL).