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Authors: Sheil, Christopher A., and Portik, Daniel

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Formation and Ossification of Limb Elements in *Trachemys scripta* and a Discussion of Autopodial Elements in Turtles

Christopher A. Sheil^{1*} and Daniel Portik^{1, 2}

¹Department of Biology, Dolan Center for Science and Technology, John Carroll University,
20700 North Park Boulevard, University Heights, OH 44118, USA

²College of Liberal Arts and Sciences, Department of Biology, Villanova University,
800 Lancaster Avenue, Villanova, PA 19085, USA

Though sequences of formation and ossification of bony elements have been described for many taxa, controversy surrounds the formation of limb elements in turtles. Three hypotheses for patterns of formation of autopodial elements have been proposed, differing primarily in the origin of Distal Carpal/Tarsal 3, the digital arch, and Centrale 4. Patterns of formation and ossification of limb elements are described for *Trachemys scripta*. These patterns are compared to similar data for representatives of four families of turtles (Cheloniidae, Chelydridae, Emydidae, and Trionychidae). Hypotheses of limb formation are compared in the context of new and published data. Three species (*Trachemys scripta*, *Chrysemys picta*, and *Chelydra serpentina*) suggest that Distal Carpal 3 forms by branching from the ulnare, whereas Distal Carpal 3 may branch from Distal Carpal 4 in *Macrochelys temminckii* and *Chelonia mydas*; data from *Graptemys nigrinoda*, *Apalone spinifera*, and *Eretmochelys imbricata* did not provide evidence for the origin of Distal Carpal 3. Centrale 4 was not observed to branch from the ulnare and apparently arises by de-novo condensation. Distal Carpal 4 did not branch from Centrale 4 in any species. Until the developmental origins of Distal Carpal 3 and Centrale 4 are understood, interspecific variation in the origin of these elements remains, and may explain some of the observed differences. Trends of ossification in the fore- and hind limb autopodium also are summarized. Homology of elements in pedal Digit V is discussed, and we suggest that the hooked proximal element of this digit be recognized as Distal Tarsal 5.

Key words: *Trachemys scripta*, Chelydridae, Trionychidae, Cheloniidae, Emydidae, ossification, developmental sequence, Testudines, Metatarsal V, homology

INTRODUCTION

Sequences of formation and ossification of the cartilaginous and bony skeleton have been studied in numerous vertebrate taxa. Recent examples of general studies of skeletal formation include those for amphibians (e.g., Fabrezi, 1993; Fabrezi and Alberch, 1996; and Sheil, 1999) and reptiles and birds (e.g., Burke and Alberch, 1985; McGowan, 1985; Rieppel, 1992, 1993ab; Maisano, 2002; Sheil, 2003b; Sánchez-Villagra et al., 2008). In addition to providing basic descriptive data for embryonic skeletogenesis, studies such as these also afford insights into the processes involved in the expression of genotypes and the evolutionary origins of complex structures (e.g., Cohn and Tickle, 1999), and in some cases sufficient data have accumulated to make comparisons across major taxonomic groups. Perhaps our clearest understanding of the patterns of ontogenetic change from genotype to phenotype involved in the formation of a complex skeletal structure can be found in the formation of

appendages in tetrapods. Limb development has been studied from many perspectives, including paleontological evidence (Shubin and Marshall, 2000; Berman and Henrici, 2003; O'Keefe et al., 2006), patterns of gross anatomical change through ontogeny (Shubin and Alberch, 1986), and gene expression (Gardiner et al., 1998; Shubin and Marshall, 2000). However, much remains to be understood about limb development (Hinchliffe, 2002), and before a complete picture of the evolutionary development of the limb can emerge for all tetrapods, an accurate understanding of the patterns of formation must be achieved for all known taxa. Given the central importance of basic descriptive studies to our understanding of limb formation, it is surprising that some controversy still exists concerning the basic patterns of development and formation of prominent structural units, particularly in the appendages of some conspicuous amniote groups. A notable example can be seen in the formation of limb elements in turtles, which has been summarized in two seminal papers (Burke and Alberch, 1985; Shubin and Alberch, 1986). An examination of these articles reveals that three very different patterns of limb formation have been described (Fig. 1), particularly relative to the origin and derivatives of the digital arch and central regions of

* Corresponding author. Phone: +1-216-397-3088;
Fax : +1-216-397-4482;
E-mail: csheil@jcu.edu

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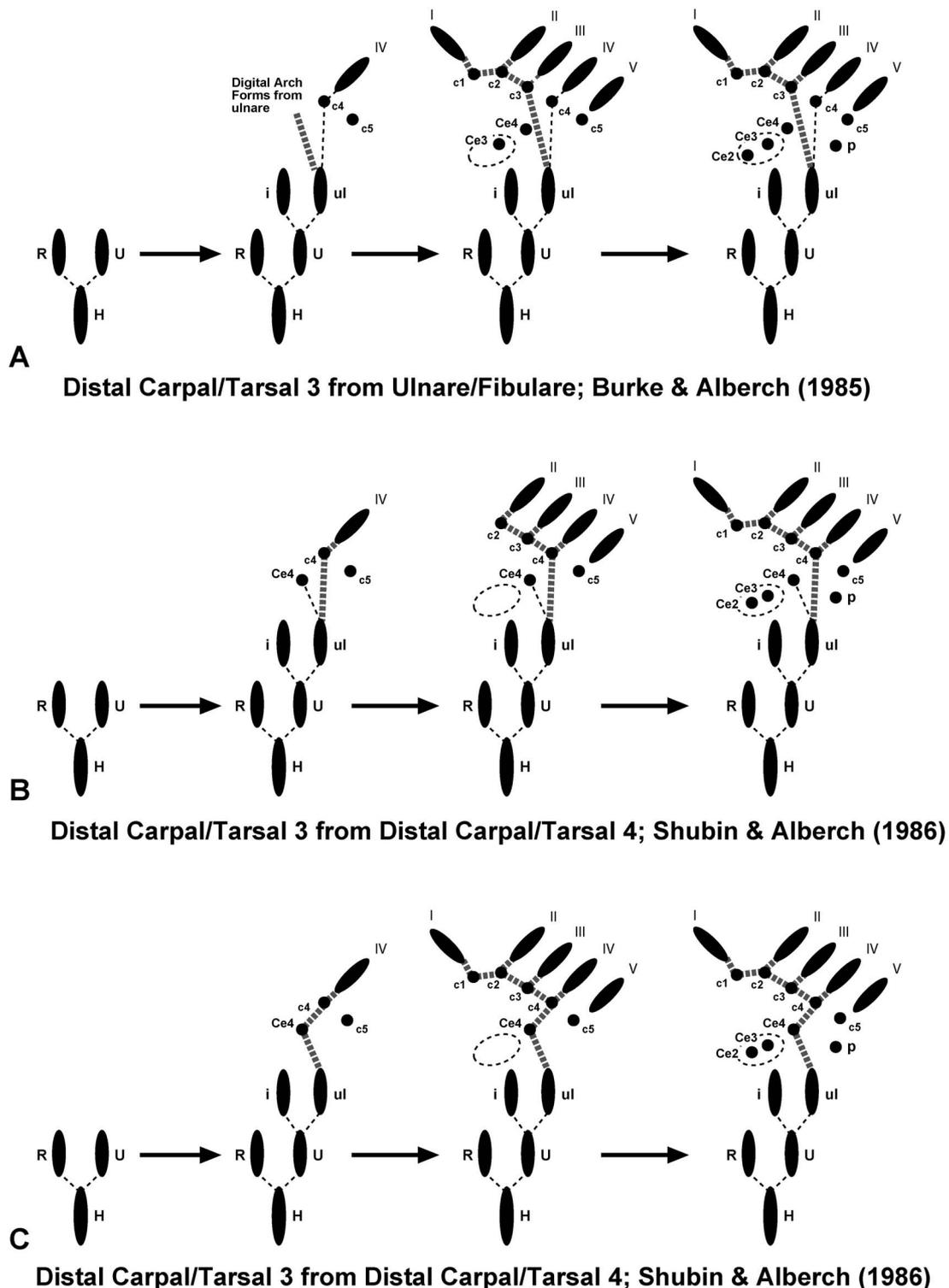


Fig. 1. Alternate hypotheses for the formation of autopodial elements, proposed by (A) Burke and Alberch (1985) and (B and C) Shubin and Alberch (1986). In these scenarios, Distal Carpal/Tarsal 3 and the digital arch branch from the ulnare (A) or Distal Carpal/Tarsal 4 (B and C), and Centrale 4 arises by either condensation (A) or branching from the ulnare (B and C). In *Chrysemys picta*, Distal Carpal 3 was observed directly to be branching from the ulnare by Burke and Alberch (1985: fig. 3), and was observed in close proximity to the ulnare in *Trachemys scripta*. Distal Carpal 3 was observed in close proximity to Distal Carpal 4 in *Macrochelys temminckii* and *Chelonia mydas*, but the origin of this element could not be determined for *Graptemys nigrinoda*, *Apalone spinifer*, or *Eretmochelys imbricata*. Fine dashed lines indicate patterns of connectivity through the developmental history of a particular element by branching and segmentation or by budding and segmentation; thick gray dashed lines emphasize the developmental history of the digital arch, and demonstrate the specific autopodial elements involved in the formation of the digital arch in each hypothesis. Abbreviations: c, distal carpal; Ce, Centrale; H, humerus; i, intermedium; p, pisiform; R, radius; U, ulna; ul, ulnare. Roman numerals indicate digit number.

the autopodium. These hypotheses have not been acknowledged in all subsequent studies, but it seems that of these, the hypothesis of Burke and Alberch (1985) (Fig. 1A) is preferred by most investigators (see also Sánchez-Villagra et al., 2007a). These hypotheses differ primarily in the inferred history and origin of the digital arch and centralia in the manus and pes, as follows.

Digital arch derived from the ulnare independent of Centrale 4, centrale series, and Digit IV (Burke and Alberch, 1985) (Fig. 1A)

According to observations of lab-raised embryos of *Chrysemys picta* and *Chelydra serpentina*, early development of the fore- and hind limb elements is typical of other tetrapods in that the proximal stylopodial elements (humerus and femur) and zeugopodial elements (radius/tibia and ulna/fibula) form in a proximodistal manner of elongation and branching that produces a conspicuous Y-condensation. The ulnare then bifurcates distally to form Distal Carpal 3 (on the preaxial side of the ulnare) and Distal Carpal 4 (distal to the ulnare), thereby establishing the long axis of the appendage. Two branches were observed to originate on the preaxial side of the primary axis of the appendage. The first branch is a derivative of the ulna and forms the intermedium, which is positioned between the ulna, ulnare, and radius; the second branch was observed at the level of the ulnare and was found to extend distally and preaxially from the long axis of the limb (Burke and Alberch, 1985: 124): “At the level of Carpal (Tarsal) 4, this branch turns anteriorly at a right angle to the primary axis. This is the anlage of the digital arch ([their] fig. 6), which will give rise sequentially to Distal Carpals (Tarsals) 3, 2, and 1. Digit IV forms as a continuation of the primary axis. Digits 3, 2, and 1 form by the sequential condensation of distal carpals/tarsals within the loose precondensation of the arch. The metacarpals (-tarsals) form as a continuous branch of the corresponding distal carpal (tarsal).” Their Fig. 5 indicates a weak connection between the ulnare and the precondensation of the digital arch (represented at this stage by Digit III only), which is positioned preaxial to the developing Digit IV. Digit V also was observed to appear by de-novo condensation of cartilage postaxial to Digit IV, simultaneously with Digit III. Their discussion of the formation of the central region of the carpus and tarsus suggests that this region remains undifferentiated (except for the intermedium) during formation of the derivatives of the digital arch (i.e., elements of Digits III, II, and I). In the carpus, Centrale 4 was observed to form in the region between the ulnare, intermedium, and Distal Carpals 3 and 4. Slightly later in development, these authors noted the initial condensation of an elongate central element preaxial to Centrale 4 (suggestive of Centrale 3) and in the position of a presumptive radiale (though, as stated by Burke and Alberch [1985], this is not derived from the radius and therefore cannot be considered a radiale). In the carpus, the pisiform, Centrale 3, and Centrale 2 were the last elements to differentiate in the autopodium. In the tarsus, an amorphous precondensation of cartilage differentiated nearly simultaneously with the distal tarsal and metatarsal elements. In this rectangular precondensation, proximal condensations were observed to occupy the positions of the fibulare (i.e., calcaneum), intermedium, and Centrale 1.

Later in the development of *Chelydra*, the authors note that a conspicuous Centrale 4 was apparent proximal to Distal Tarsal 2. These authors suggest that at least three elements fuse to form the astragalus—the intermedium, Centrale 4, and Centrale 1. In some specimens, these elements were observed to fuse with the fibulare to form the astragalocalcaneum seen in adult specimens of some species. These authors did observe some variation between *Chrysemys* and *Chelydra* in the final shape and configuration of the centrale in the hands and feet (Burke and Alberch, 1985: 128–130).

Digital arch derived from the ulnare, independent of Centrale 4 and the centrale series (Shubin and Alberch, 1986: 346–348) (Fig. 1B)

Written descriptions for the formation of limb elements in *Chelydra serpentina* are consistent with their Fig. 12 and suggest that the digital arch is a derivative of the ulnare, independent of all centralia elements. The first scenario of limb development presented by Shubin and Alberch (1986: 346–348) is assumed to be their preferred hypothesis for limb formation, as it is also summarized in their Fig. 12. According to this scenario, the stylopodium (humerus/femur) gives rise to the zeugopodial elements (radius and ulna/tibia and fibula), and in both the fore- and hind limb the preaxial zeugopodial element does not give rise to a radiale (forelimb) or tibiale (hindlimb). Specifically, the ulna (fibula) forms the intermedium preaxially and the ulnare (fibulare) axially (Shubin and Alberch, 1986: fig. 12b, *C. serpentina*); development of the intermedium was observed to lag behind that of the ulnare (fibulare). According to their Fig. 12b (Shubin and Alberch, 1986: 347), the ulnare then yields two other elements, Centrale 4 (their C4) preaxially and Distal Carpal 4 (their c4), which was also shown to give rise to Metacarpal IV. Elaboration of the axial side occurs relatively early in development of the limb as the intermedium and ulnare branch off of the distal terminus of the ulna. The ulnare (fibulare) then bifurcates to form Centrale 4 (in a preaxial position) and Distal Carpal 4 (distal to the ulnare). Distal Carpal 4 then segments distally to form Metacarpal 4, thereby establishing the primary axis through Digit V. Subsequent development and elaboration of the autopodium of *C. serpentina* is shown in their Fig. 12c, which shows a diffuse field of cartilage distal and preaxial to the intermedium (ultimately this field will give rise to Centrale 3 and 2). Additionally, this illustration depicts the digital arch forming as subsequent derivatives of Distal Carpal (Distal Tarsal) 4. The pattern implied by this illustration is that Distal Carpal (Distal Tarsal) 4 gives rise to Distal Carpal (Distal Tarsal) 3 by anterior budding and segmentation, which gives rise to Distal Carpal (Distal Tarsal) 2, which gives rise to Distal Carpal (Distal Tarsal) 1; additionally, all metacarpals (metatarsals) are shown to be derivatives of their corresponding distal carpal (distal tarsal elements). The final portion of their Fig. 12 clearly shows that: 1) Centrale 3 and 2 branch from, or are associated with, the intermedium; 2) Centrale 4 represents a preaxial branch of the ulnare; 3) Distal Carpal (Distal Tarsal) 4 represents an axial branch of the ulnare; 4) Distal Carpal (Distal Tarsal) 4 gives rise to the digital arch, which is composed of the distal carpals (distal tarsals), metacarpals (metatarsals), and phalanges of Digits IV, III, II, and I;

and V) the pisiform and elements of Digit V form by de-novo condensation of cartilage.

Digital arch derived from Centrale 4 (Shubin and Alberch, 1986) (Fig. 1C)

Though a preferred scenario for limb development appears to be summarized in their Fig. 12 (and described in text of pp 346–348), a second scenario is presented. According to Shubin and Alberch (1986: 348), “The chelonian (and reptilian) digital arch arises from the centrale four ([their] Fig. 12, *Chelydra*, Stage C). The arch produces digits one, two, three, and four as well as their corresponding distal carpalia (tarsalia). This process occurs as follows. The centrale four segments a distal carpal (tarsal) four, the distal carpal (tarsal) four branches, yielding the corresponding digit four and the next-most-anterior distal carpal (tarsal) distal carpal (tarsal) three, Distal carpal (tarsal) three, by a similar process of branching, produces metacarpal (metatarsal) three and a distal carpal (tarsal) two.” This statement contradicts their earlier descriptions of the pattern of formation and branching of Distal Carpal 4 and Centrale 4 from the ulnare. Their earlier scenario suggests that Distal Carpal 4 arises directly from the ulnare, whereas this latter scenario states that Distal Carpal 4 arises from Centrale 4 (their C4). It should be noted that both scenarios describe the same pattern of formation of elements associated with Digits I–IV, including all distal carpals, metacarpals, and phalanges.

Though it is possible that some of the disparity among these three scenarios of limb formation reflects actual interspecific differences among the examined species, some differences certainly can be attributed to alternate interpretations of available data, particularly when incomplete specimens are examined or too few developmental stages are available. This is particularly true given that high degrees of subjectivity have been reported in interpretations of patterns of connectivity based on specimens stained with Alcian blue (Sánchez-Villagra et al., 2007a). An accurate reconstruction of the developmental and evolutionary origin of limb elements in turtles cannot be made until these differences are understood and explained.

Herein, the patterns of formation and ossification of the cartilaginous and bony skeleton are described for the forelimb and manus, and hind limb and pes, of the Red-eared Slider (*Trachemys scripta*). These data are compared with similar information for several other species of turtles: *Apalone spinifera* (Sheil, 2003a); *Caretta caretta* and *Chelonia mydas* (Sánchez-Villagra et al., 2007a); *Chelydra serpentina* (Rieppel, 1993b; Sheil and Greenbaum, 2005); *Chrysemys picta* (Burke and Alberch, 1985); *Eretmochelys imbricata* (Sheil, 2003b); *Graptemys nigrinoda* (Sánchez-Villagra et al., 2008); and *Macrochelys temminckii* (Sheil, 2005). All patterns of element formation are compared and discussed in the context of the formation hypotheses (Fig. 1). These data also allow for comparisons among members of four families of cryptodiran turtles (Cheloniidae [*Caretta*, *Chelonia*, and *Eretmochelys*], Chelydridae [*Chelydra* and *Macrochelys*], Emydidae [*Chrysemys*, *Graptemys*, and *Trachemys*], and Trionychidae [*Apalone*]), and provide insights into our understanding of limb development and ossification in this unique group of amniotes. Finally, a discussion of the patterns of formation and ossification of skel-

etal elements among these turtles is presented and affords a unique opportunity to address issues of homology among bony elements of pedal Digit V, particularly the hooked proximal element of this digit. Conclusions about the identity and homology of centralial elements are beyond the scope of this study.

MATERIALS AND METHODS

Forty cleared and double-stained embryos of *Trachemys scripta* were examined for this study (Appendix 1). Detailed methods for collecting eggs, incubation, and sampling, euthanizing, and preparing embryos are described in Sheil (2003b). The relative developmental stage was assigned to each embryo according to the external morphological criteria of Greenbaum (2002). Observations and illustrations of specimens were made with a Leica MZ12S stereo dissection microscope equipped with a camera lucida. Techniques for clearing and double-staining with Alcian Blue and Alizarine Red follow Wassersug (1976) and Taylor and Van Dyke (1985). The onset of ossification was considered to be the earliest developmental stage at which calcification was apparent in a particular element, and was recognized by the retention of Alizarine Red stain. Terminology of limb orientation is that of Romer (1956: 334), and refers to an anatomical position with the limb extended horizontally and laterally from the long axis of the body, with the plantar surface of the manus or pes directed ventrally and the primary axis of the fore- and hind limb extends through the humerus/femur, ulna/fibula, and Digit IV. The terminology of fore- and hind limb elements (including centralia) is that of Burke and Alberch (1985).

Though the recognition of identical developmental stages facilitates comparative embryonic studies within species, typical indices and metrics of development (such as measures of body size or mass, age, and reference to normal tables that describe sequential anatomical change through ontogeny) are notoriously unreliable (see Ewert, 1985: 93–94, 99–103). Additionally, criteria for recognizing discrete developmental stages by reference to standard developmental tables can be subjective and typically apply only to specific taxonomic groups. For example, standard developmental tables for chickens (Hamburger and Hamilton, 1951) rely extensively on the anatomy of the wings and beak and therefore cannot be used to assess developmental progress in turtles (e.g., Yntema, 1968), which have limb and head anatomies that are radically different from those of a chick. Therefore, all comparisons are made between and among taxa in terms of the sequence and timing of formation and ossification, rather than in reference to precise timing, metrics, or standard stages of development.

RESULTS

Formation and ossification of limb elements in *Trachemys scripta*

Forelimb and manus (Figs. 2, 4)

One Stage-15 specimen (KU 291408; Fig. 2A) bears the cartilaginous buds of the ulnare and intermedium on the distal and preaxial margins of the ulna, respectively. Additionally, the ulna and radius each exhibit only weak separation from the humerus and there is a weak but conspicuous indication of separation between the humerus and pectoral girdle (not illustrated). By mid-Stage 15 (Fig. 2B), the humerus, radius, and ulna are separated, the humerus is more than twice as long as wide, and the radius and ulna are nearly equal in length. The ulnare is spheroid in shape and positioned at the distal terminus of the ulna. The intermedium is distinctly separate from the ulna and is positioned between the distal termini of the radius and ulna. The long axis of the forelimb is conspicuous and extends through Distal Carpal

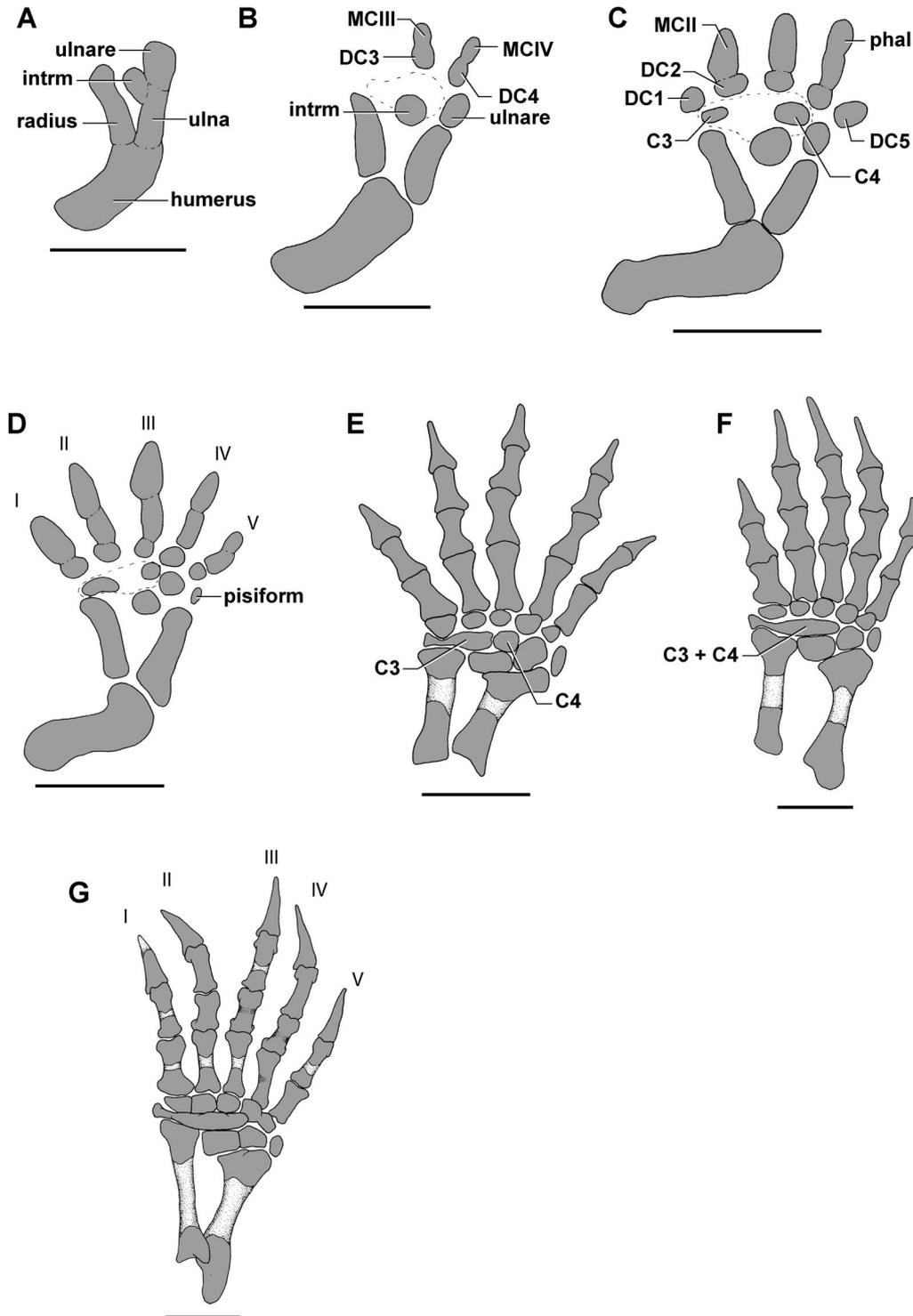


Fig. 2. Development of the right manus of *Trachemys scripta* in dorsal view, illustrating some information on the sequence of chondrification and ossification of the forelimb. **(A)** Early Stage 15 (KU 291408) showing budding of the radius and ulna from the humerus, and the ulnare and intermedium from the ulna. **(B)** Mid-Stage 15 (KU 291420) showing prominent formation of intermedium and ulnare; the primary axis has formed through Metacarpal IV. **(C)** Late Stage 15 (KU 291415); Distal Carpals 1–5 have formed and Metacarpal II–IV are present; Centrale 4 and Centrale 3 also are present. **(C)** Stage 16 (KU 291425) showing Metacarpals I–V and the recently formed pisiform. **(E)** Stage 18 (KU 291431) showing an elongated Centrale 3, the presence of all phalangeal elements, and the fusion of Centrale 3 and Centrale 4. **(F)** Stage 19 (KU 291437); note the fusion of all centralial elements. **(G)** Stage 20 (KU 291448), indicating ossification beginning in the phalangeal elements; Distal Carpals 4 and 5 have fused. Dashed lines indicate regions that have retained weak but conspicuous Alcian Blue stain; the developmental origin (by budding, splitting, or de-novo condensation) is not discernable (see descriptions). Abbreviations: C, centrale; DC, distal tarsal; intrm, intermedium; MC, metacarpal; phal, phalangeal element. Roman numerals indicate digit numbers; stippling denotes bone; grey denotes cartilage. Scale bar=1 mm.

4 and Metacarpal IV, which have not separated yet; Distal Carpal 4 and Metacarpal IV are nearly equal in size. Additionally, Distal Carpal 3 is present and clearly separated from the preaxial side of Distal Carpal 4; Metacarpal III is present but not separated from Distal Carpal 3. A large, amorphous field of cartilage (indicated by dashed lines, Fig. 2) is positioned between the distal ends of the radius and ulna and the proximal margins of Distal Carpals 3 and 4. In a late Stage-15 specimen (KU 291415; Fig. 2C) Distal Carpals 1–5 are present and the digital arch is nearly complete. Conspicuous metacarpals are present on Digits II–IV; however, there is only weak indication of separation of these cartilaginous structures from their corresponding distal carpal elements. Distal Carpals 1 and 5 are spherical and lack metacarpals at this stage. Distal Carpal 5 is positioned postaxial to Distal Carpal 4 and the ulnare. A conspicuous, amorphous field of cartilage occupies the space between the distal terminus of the radius and the distal margins of the intermedium and ulnare. Within this field of cartilage, Centrale 4 has condensed within mesenchyme and is located between the proximal margins of Distal Carpals 3 and 4 and the distal margins of the intermedium and ulnare. Centrale 4 is slightly smaller than the intermedium and nearly equal in size to the ulnare. A very faint condensation (labeled as “C3” in Fig. 2C) is apparent near the distal terminus of the radius; Centrale 3 is smaller than Centrale 4. By Stage 16 (Fig. 2D), all metacarpals are present, and Metacarpals IV and V are separated from their respective distal carpal elements, whereas Metacarpals I–III are not. The proximal phalanx is present on Digits II–IV. In at least one Stage-16 specimen (KU 291425), a small pisiform is apparent on the postaxial side of the ulnare. Centrale 4 is very conspicuous, whereas Centrale 3 is only poorly coalesced and stains faintly blue. Additionally, the space between Centrale 3 and Centrale 4 stains faintly blue, indicating some retention of Alcian Blue and the presence of some cartilage. Proximal phalangeal elements are present but only weakly separated from their metacarpals on Digits II–IV; the phalangeal formula at Stage 16 is 0:1:1:1:1. By Stage 17, all distal carpals and metacarpals are well formed, highly chondrified, and clearly separated from their neighboring elements. On each digit, the proximal phalanx is present and well separated from each corresponding metacarpal, and the second phalanx is present and weakly separated on Metacarpals II–IV; the phalangeal formula is 1:1/2:2:1/2:1. Centrale 3 retains relatively less Alcian Blue than does Centrale 4. In one Stage-17 specimen (KU 291417), a much smaller condensation appears in the field labeled as Centrale 3, indicating the possible, but not definitive, presence of Centrale 2. By Stage 18 (Fig. 2E), all phalanges are highly chondrified and clearly separated from their neighboring elements; the phalangeal formula is 2:3:3:3:2. The pisiform is clearly condensed and about twice as long as wide. The ulnare is slightly larger than each distal carpal. The distal end of the ulna is considerably wider than the mid-shaft of this element, and there is a thin band of weak ossification at the mid-shaft; the radius is ossified at mid-shaft. In KU 291431, Centrale 3 is relatively thin and occupies much of the space between Distal Carpals 1–3, Centrale 4, intermedium, and radius; Centrale 3 (which possibly fused with Centrale 2) and Centrale 4 are not fused in any Stage-18 specimens. By Stage

19 (Fig. 2F), all elements of the manus are present and the forelimb appears like that of an adult specimen, though ossification is lacking in all autopodial elements. The phalangeal formula in all Stage-19 specimens is 2:3:3:3:2. Centrale 3 and Centrale 4 have fused synchondrally to form a broad, highly chondrified Centrale 3+Centrale 4.

By Stage 18, conspicuous but weak ossification is present in the forelimb. In a single specimen (KU 291432) there is very weak indication of ossification without retention of Alizarine Red stain in the humerus, radius, and ulna; in an additional specimen (KU 291431), retention of Alizarine Red stain is apparent but weak in these elements. In general, the degree of ossification appears to be greater in the zeugopodium than in the stylopodium; however, this may be an artifact of the process of staining these bones. Ossification of the carpal elements progresses relatively rapidly, and by early Stage 20 the stylo- and zeugopodial elements are heavily ossified. Considerable ossification of the forelimb is apparent by late Stage 20, and it is possible to discern the general sequence of ossification of the carpus by the end of this developmental stage. In KU 291448 (early Stage 20; Fig. 4A), ossification is apparent in Metacarpals I–IV, and the degree of ossification varies among these elements in the following pattern: IV>I>II>III. Additionally, in KU 291443 ossification is apparent in Metacarpals II–IV, and is most conspicuous in Metacarpals III and IV. By late Stage 20 (KU 291447), ossification is apparent, and the degree of ossification is generally equal in the proximal phalangeal elements of Digits I and III. Weak ossification is apparent in the proximal phalanx of Digit V, and none of the phalanges of Digit II are ossified in this specimen. The general trend to ossification of the phalangeal elements seems to be proximal–distal, and the overall pattern of ossification generally follows that of the initial ossification of the corresponding metacarpals; elements of Digits III and IV are more ossified than those of other digits. By Stage 21 (KU 291441), all metacarpal and phalangeal elements exhibit some degree of ossification, except for the unguis phalanx and metacarpal of Digit V. Ossification of Metacarpal V lags behind that of other metacarpal and phalangeal elements, and this element does not retain Alizarine Red stain until late Stage 22 (KU 291453). By late Stage 23 (KU 291460), all metacarpal and phalangeal elements exhibit a high degree of ossification; however, the proximal and distal autopodial elements lack ossification in all specimens examined, and it is presumed that these elements do not exhibit ossification until after hatching. The general trend to ossification of the phalangeal elements is proximal–distal. In Digits I–IV, phalangeal elements ossify after the corresponding metacarpals; in Digit V, Metacarpal V ossifies only after the corresponding phalangeal elements. By Stage 25, all metacarpal and phalangeal elements are ossified; however, the distal carpals, centralia, intermedium, ulnare, and pisiform were not observed to ossify in any prehatching specimens.

Hind limb and pes (Figs. 3, 5)

An early Stage-15 specimen (KU 291408; Fig. 3A) displays weak separation between the femur, tibia, and fibula. The tibia and fibula are approximately three times longer than wide. At the distal terminus of the fibula, the intermedium and fibulare appear as preaxial and postaxial buds,

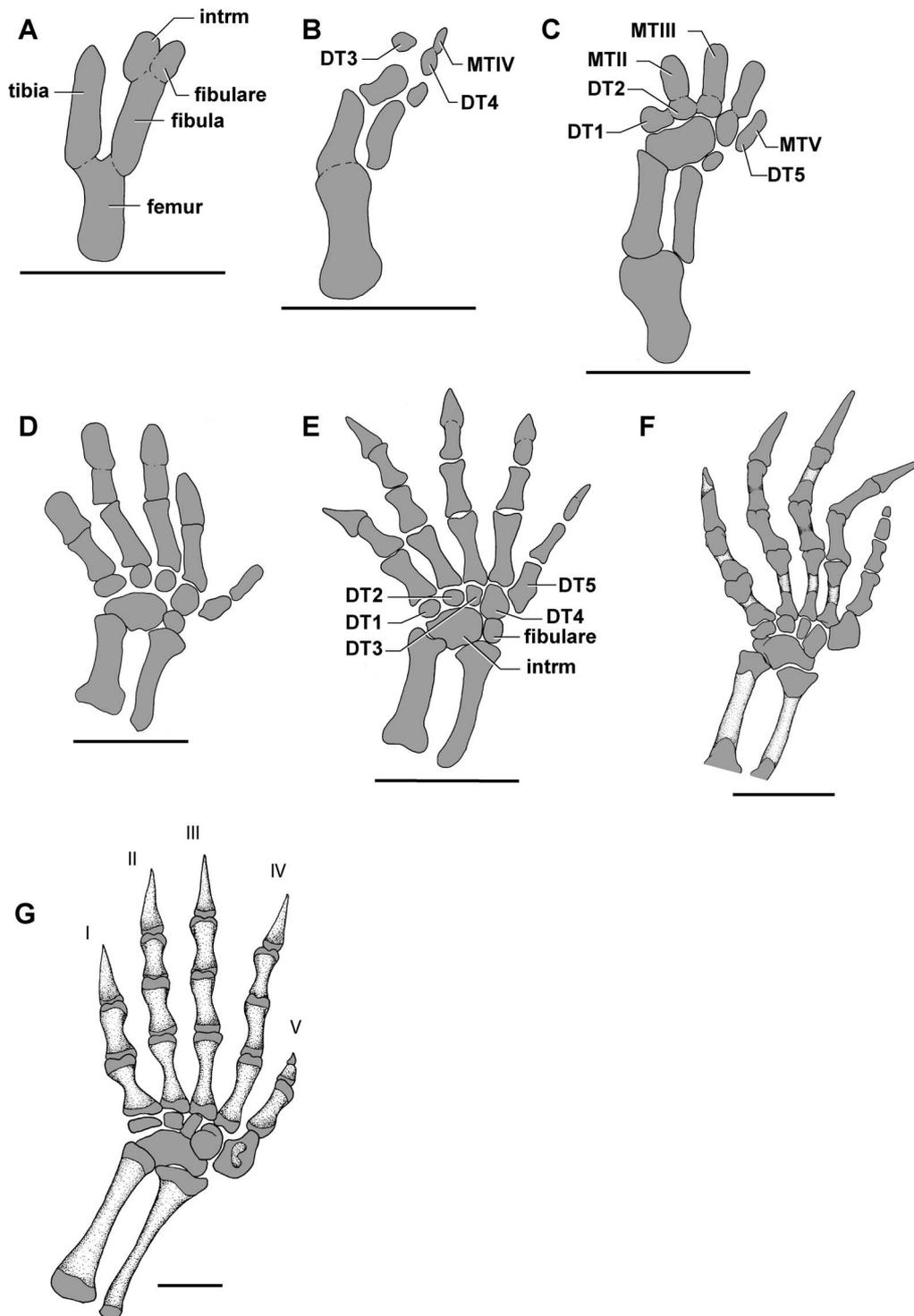


Fig. 3. Development of the right pes of *Trachemys scripta* in dorsal view, illustrating some information on the sequence of chondrification and ossification of the hind limb. **(A)** Early Stage 15 (KU 291408) showing the budding of the tibia and fibula from the femur; the intermedium and fibulare can be seen branching from the fibula. **(B)** Middle-Stage 15 (KU 291420) illustrating the primary axis formation through Distal Tarsal 4 and Metatarsal IV; the intermedium and fibulare are well formed, and Distal Tarsal 3 is apparent. **(C)** Late-Stage 15 (KU 291415); the distal tarsals and Metatarsals II–IV are present; Distal Tarsals 1–3 are fused, and the intermedium has increased in size. **(D)** Stage 17 (KU 291429), showing well-formed Metatarsals I–V and formation of many phalangeal elements in Digits I–IV. **(E)** Stage 18 (KU 291431); nearly all elements present in adult specimens are well formed and present. **(F)** Stage 20 (KU 291448); ossification has begun in the tibia, fibula, Metatarsals I–IV, and several phalangeal elements; the fibulare and intermedium have fused. **(G)** Stage 25 (KU 291494), illustrating nearly complete ossification of the manus. Dashed lines indicate regions that have retained weak but conspicuous Alcian Blue stain; the developmental origin (by budding, splitting, or de-novo condensation) is not discernable (see descriptions). Abbreviations: DT, distal tarsal; intrm, intermedium; MT, metatarsal. Roman numerals indicate digit numbers; stippling denotes bone; grey denotes cartilage. Scale bar=1 mm.

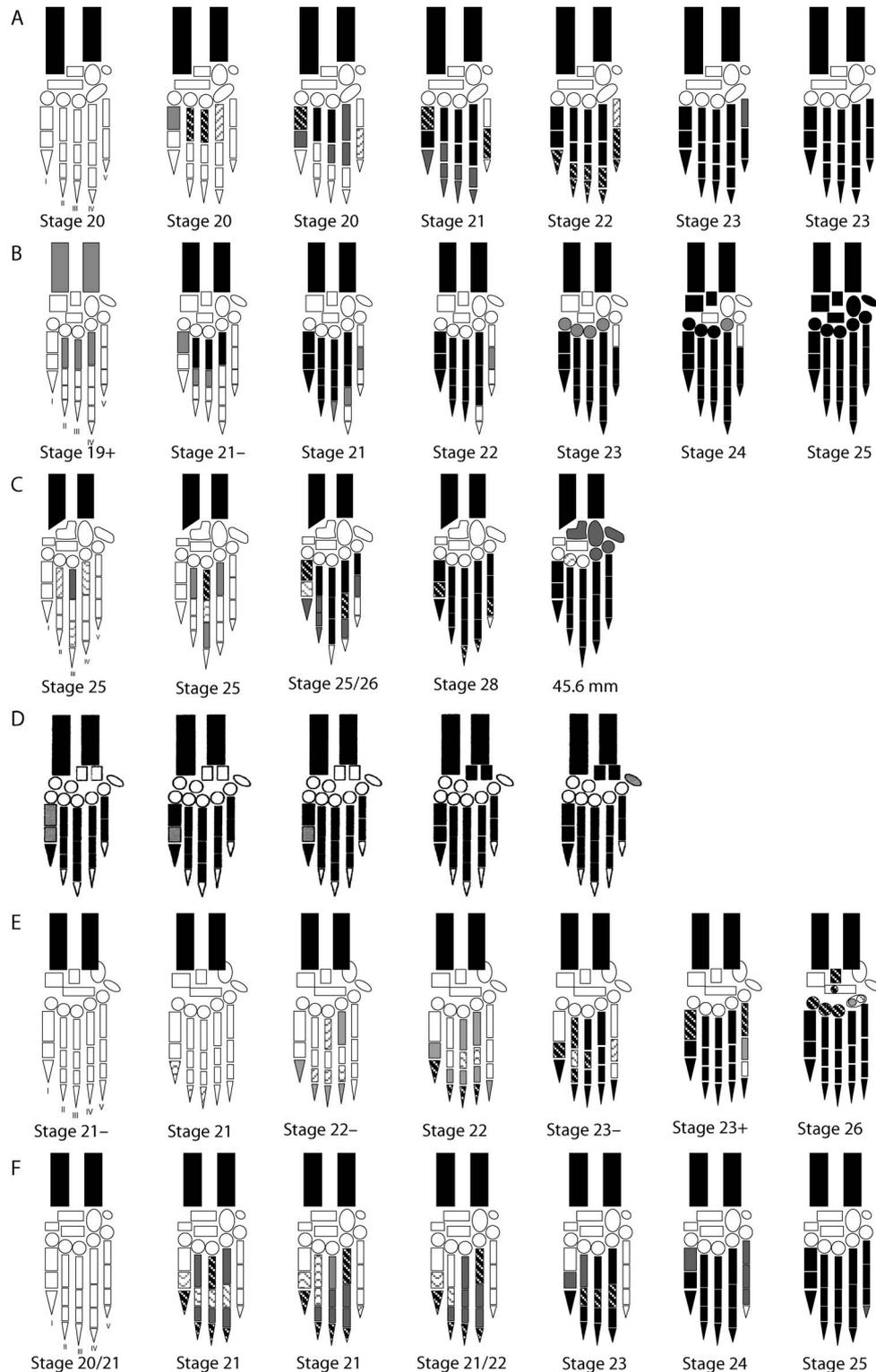


Fig. 4. Schematic diagram comparing the sequences of ossification of manual elements in embryos of *Trachemys scripta* (A) and several other species of turtles (B–E). (B) *Apalone spinifera* (Sheil, 2003a). (C) *Eretmochelys imbricata* (Sheil, 2003b). (D) *Caretta caretta* (Sánchez-Villagra et al., 2007a: fig. 5f–j). (E) *Chelydra serpentina* (Sheil and Greenbaum, 2005). (F) *Macrochelys temminckii* (Sheil, 2005). Observations were based on cleared and double-stained specimens; “+” or “–” indicates specimens that represent relatively late or early phases, respectively, of a particular developmental stage based on the specific morphologies observed for specific criteria for recognizing developmental stages; Roman numerals indicate digit numbers; measurements in millimeters represent crown–rump lengths of post-hatching specimens; degree of ossification (cartilage only with no ossification, weak with initiation of ossification inferred by surface texture and no retention of Alizarine Red stain, weak retention of red stain, strong retention of stain but ossification incomplete, and complete ossification) is indicated by: white<diagonal gray hashing<gray<diagonal white hashing on black background<black.

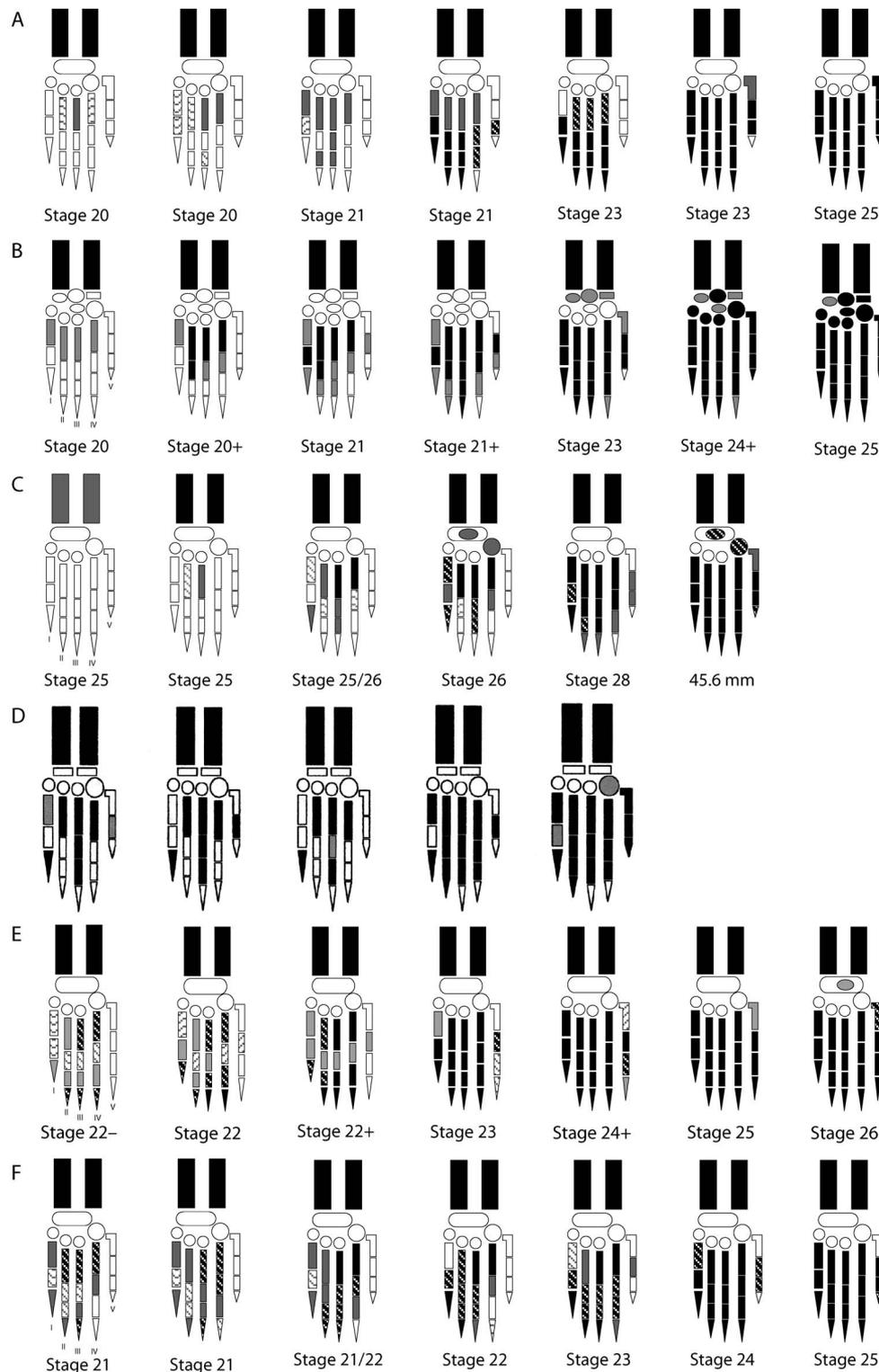


Fig. 5. Schematic diagram comparing the sequences of ossification of pedal elements in embryos of *Trachemys scripta* (A) and several other species of turtles (B–E). (B) *Apalone spinifera* (Sheil, 2003a). (C) *Eretmochelys imbricata* (Sheil, 2003b). (D) *Caretta caretta* (Sánchez-Villagra et al., 2007a: fig. 5f–j). (E) *Chelydra serpentina* (Sheil and Greenbaum, 2005). (F) *Macrochelys temminckii* (Sheil, 2005). Observations were based on cleared and double-stained specimens; “+” or “–” indicates specimens that represent relatively late or early phases, respectively, of a particular developmental stage based on the specific morphologies observed for specific criteria for recognizing developmental stages; Roman numerals indicate digit numbers; measurements in millimeters represent crown–rump lengths of post-hatching specimens; degree of ossification (cartilage only with no ossification, weak with initiation of ossification inferred by surface texture and no retention of Alizarine Red stain, weak retention of red stain, strong retention of stain but ossification incomplete, and complete ossification) is indicated by: white<diagonal gray hashing<gray<diagonal white hashing on black background<black.

respectively. By mid-Stage 15 (Fig. 3B; KU 291420), the femur is more than three times longer than wide and the fibula is clearly separated from the femur. The fibulare is positioned at the distal terminus of the fibula. The intermedium is twice the size of the fibulare and positioned on the preaxial side of the fibulare between the distal ends of the tibia and fibula. By mid-Stage 15, the long axis of the hind limb is conspicuous and extends through Distal Tarsal 4 and Metatarsal IV. Additionally, in KU 291420, Distal Tarsal 3 and Metatarsal IV have formed; Metatarsal IV shows weak separation from, and is slightly smaller in size than, Distal Tarsal 4, whereas Distal Tarsal 3 is positioned preaxially to Distal Tarsal 4, is spherical in shape, and subequal in size to Distal Tarsal 4. One late Stage-15 specimen (KU 291415; Fig. 3C) exhibits a more complete digital arch with the presence of Distal Tarsals 1–5. However, Distal Tarsals 1–3 remain connected and appear as a single cartilaginous structure positioned distal to the large intermedium. Metatarsals II–IV are apparent, with all showing weak signs of separation from their respective distal tarsal elements. Distal Tarsal 5 is slightly thinner and longer than the other distal tarsals, showing elongation and differentiation into the corresponding metatarsal, prior to segmentation. The intermedium is significantly larger than the fibulare and occupies much of the space of the proximal autopodium. By Stage 16, all the metatarsals are present and show at least some indication of separation from their respective distal tarsal elements. The proximal phalanx is apparent on Digits III and IV, with very weak indications of separation between these structures. The distal tarsals are separate and vary in size: Distal Tarsal 5 > 4 > 1 > 2 > 3. The fibulare is approximately subequal in size with Distal Tarsals 2 and 3. The tibia is slightly more than twice as long as wide, whereas the fibula is almost five times as long as wide. The intermedium is slightly smaller than the tibia. By Stage 17 (Fig. 3D), Metatarsals I–V are separated clearly from their respective distal tarsal elements. The proximal phalanx is present and separated on Digits I–IV. The medial phalanx has formed on Digits II and III with weak indications of separation; the phalangeal formula is 1:2:2:1:0. By Stage 18 (KU 291431; Fig. 3E) the distal phalanx is present on Digits I–IV, clearly separated on Digits I and II, and weakly separated on Digits III and IV. Digit V is composed of Distal Tarsal 5, Metatarsal V, the proximal phalanx, and the weakly separated distal phalanx. Distal Tarsal 5 is elongate, rectangular in shape, and has shifted its position postaxially towards the distal end of Distal Tarsal 4. The phalangeal formula is 2:3:3:3:2. By Stage 20 (Fig. 3F), all of the phalangeal elements are well chondrified, and the phalangeal formula is 2:3:3:3:3. Distal Tarsal 5 has enlarged and is conspicuously L-shaped when viewed dorsally, thereby taking on the “hooked” morphology typical of saurians (see discussion below). The fibulare and intermedium have fused to create a large cartilaginous element that occupies all of the proximal autopodium between the zeugopodial and distal tarsal elements. It is unclear whether any centralial elements are incorporated in this structure, since no centralial elements formed as conspicuous and independent elements in the specimens examined, and too few specimens were available at appropriate stages. Ossification has started on the mid-shafts of the tibia, fibula, and Metatarsals II–IV.

By Stage 18, conspicuous but weak indication of ossification is present in the hind limb. By early Stage 20 (KU 291444; Fig. 5A), Metatarsals I–IV exhibit some degree of ossification at mid-shaft, though there is asymmetry in this specimen between the appendages; the degree of ossification is greatest in Metacarpal III, and the degree of ossification is nearly equal in Metacarpals II and IV. In another Stage-20 specimen (KU 291448), the degree of ossification is greatest in Metacarpals III–IV and relatively weak in Metacarpals I–II. Among the phalangeal elements of this specimen, ossification is only apparent in the proximal phalanx of Digit I and the medial phalanx of Digit III. By early Stage 21 (KU 291446), the following phalangeal elements are ossified: the proximal and medial phalanges of Digit III, the medial phalanx of Digit II, and the proximal phalanx of Digit I. Ossification of phalangeal elements of Digits IV and V lags behind that of Digits I–III, and it is not until Stage 24 that the phalangeal elements of Digit V appear ossified. Relative to other metatarsals, Metatarsal V indicates ossification relatively later in development, and is not conspicuously ossified in all specimens until Stage 24. In Digits II–IV, the general trend to ossification of phalangeal elements is from the medial phalanx to the proximal phalanx, followed by ossification of the distal phalanx. Digits I and V have only two phalangeal elements, and the trend of ossification is proximal–distal; however, both elements exhibit ossification by late Stage 21 in Digit I, but not until Stage 24 in Digit V. The hooked Distal Tarsal 5 does exhibit ossification at mid-body prior to hatching; Distal Tarsal 5 is the only distal tarsal element to exhibit ossification prior to hatching in any specimen examined for this species. With the exception of Distal Tarsal 5, none of the other proximal autopodial elements of the pes exhibit ossification prior to hatching (Fig. 3G).

DISCUSSION

Patterns of element formation in the manus of turtles

In all species examined, the forelimb bud appears early in development, and most cartilaginous elements of this structure arise in the general pattern and sequence described by Burke and Alberch (1985) and Shubin and Alberch (1986). The humerus elongates and splits distally to form the ulna and radius, and the primary axis of this appendage ultimately extends through the ulna, ulnare, and elements of Digit IV. The ulna typically is more robust than the radius, and the intermedium was observed to bud and branch preaxially from the ulna. Based on patterns of connectivity observed in each species, it is clear that Distal Carpal 3 gives rise to Distal Carpal 2, which gives rise to Distal Carpal 1, and that upon branching and splitting, each distal carpal also gives rise to its corresponding metacarpal; the phalanges of each digit arise by elongation and segmentation of their proximal counterparts. Additionally, Distal Carpal 5 was inferred to form by de-novo condensation of cartilage postaxial to Digit IV and the ulnare, and in no study was this element observed to be connected to, or branch from, any element of Digit IV or the ulnare (however, see Sánchez-Villagra et al. [2007a] below for a discussion of *Chelonia mydas*). Shortly after its appearance, Distal Carpal 5 undergoes elongation and segmentation to form Metacarpal V and corresponding phalanges. In all cases, the pisiform appeared relatively early in development as a weakly

stained structure positioned postaxial to Digit V. Differences among taxa were observed primarily in the formation of the digital arch (specifically, in the origin of Distal Carpal 3) and the centralial region (specifically Centrale 4).

The appearance and formation of the digital arch seem to occur quickly, and the precise origin of Distal Carpal 3 is shrouded somewhat by uncertainty. In all previous studies (including ours) this uncertainty likely is a result of relatively low sample sizes for the short period of development over which Distal Carpal 3 forms. Burke and Alberch (1985: fig. 5) presented the most explicit observation of the origin of the digital arch in the form of an illustration that shows the digital arch, which begins with the formation of Distal Carpal 3, arising as a preaxial bud from the anterior margin of the ulnare, not from Centrale 4 or Distal Carpal 4 (sensu either hypothesis in Shubin and Alberch, 1986) (Fig. 1B, C). However, neither Burke and Alberch (1985) nor Shubin and Alberch (1986) presented photographs or specimen numbers to document or allow confirmation of their observations for the origin of the digital arch—this statement is not offered as a criticism to invalidate their work, but serves only to emphasize the point that few direct observations have been presented in any study to confirm the explicit origin of the digital arch in turtles. For all species reviewed and examined in our study, only a handful of specimens offer some indication of the origin of the digital arch, and specifically whether Distal Carpal 3 arises from the ulnare (Fig. 1A) or Distal Carpal 4 (Fig. 1B, C). It should be noted that Burke and Alberch (1985: fig. 5) made the only direct observations of Distal Carpal 3 connected to any previously formed autopodial element.

An early Stage-15 specimen of *Trachemys scripta* (KU 291420) shows a well-formed Distal Carpal 3 that lacks connectivity to Distal Carpal 4, but that is in close proximity to a loosely-stained field of cartilage that occupies the space between this element and the ulnare; this observation may support Burke and Alberch's origin for Distal Carpal 3 from the ulnare (Fig. 1A). According to Burke and Alberch (1985: 124–125), formation of the digital arch and Distal Carpal 3 of *Chrysemys picta* occurs by preaxial branching from the ulnare (Fig. 1A). In *Chelydra serpentina* (KU 290232 and 290183; Sheil and Greenbaum, 2005), Distal Carpal 2 is seen branching from Distal Carpal 3, and there is a clear separation between Distal Carpals 3 and 4, as well as Distal Carpal 3 and the centralial region. The close proximity of Distal Carpal 3 to the ulnare may suggest that Distal Carpal 3 does arise via branching from the ulnare (consistent with Burke and Alberch' (1985) hypothesis; see Fig. 1A); however, explicit connectivity between Distal Carpal 3 and the ulnare was not observed. Too few specimens were available for *Eretmochelys imbricata* (Sheil, 2003b) and *Apalone spinifera* (Sheil, 2003a) to make direct observations on the specific origin of the digital arch in the manus. Sánchez-Villagra et al. (2007a) and Sánchez-Villagra et al. (2008) provide brief discussions of the formation of the manus in *Chelonia mydas* and *Graptemys nigrinoda* (respectively), but do not offer explicit descriptions of the origin of the digital arch. However, lines of connectivity among elements in their summary of digit formation in the manus and pes in *C. mydas* (Sánchez-Villagra et al., 2007a: fig. 3) imply that Distal Carpal 3 arises via preaxial budding from Distal Carpal 4

(sensu Shubin and Alberch, 1986) (Fig. 1C). *Macrochelys temminckii* (KU 290329; Sheil, 2005) shows a weakly-stained Distal Carpal 3 within a field of cartilage on the preaxial side of Distal Carpal 4, suggesting that Distal Carpal 4 may give rise to Distal Carpal 3 (sensu Shubin and Alberch, 1986) (Fig. 1B, C). Observations from *Macrochelys* and *Chelonia* may support an origin of the digital arch via branching from Centrale 4. The origin of Distal Carpal 3 is unknown for *Eretmochelys*, *Apalone*, *Graptemys* and *Caretta*, likely because of a lack of appropriate specimens. Observations made from *Chelydra*, *Trachemys*, and *Chrysemys* suggest that Distal Carpal 3 originates by branching from the ulnare. Until future studies of autopodial development in turtles explicitly demonstrate Distal Carpal 3 originating by means of branching from Distal Carpal 4 (sensu Shubin and Alberch, 1986) (Fig. 1C) or Centrale 4 (sensu Shubin and Alberch, 1986) (Fig. 1B), we suggest that the preferred hypothesis for the origin of this element and the digital arch should be that of Burke and Alberch (1985: Fig. 1A). Additionally, it remains possible that interspecific variation exists in the formation of the digital arch of turtles.

Other major differences in the formation of manus were observed primarily in the origin and modification of centralial elements, which differ considerably across taxa and, as with the digital arch, it seems that some uncertainty surrounds this region of the forelimb. Burke and Alberch (1985: Fig. 1A) suggested that Centrale 4 arises via condensation within the centralial region of the manus, whereas Shubin and Alberch (1986) (Fig. 1B, C) suggested that Centrale 4 arises via branching from the ulnare. Again, direct observations, photographs, and explicit illustrations of this event are uncommon, and attempts to discern the origin of centralial elements lead one to evaluate how observations of chondrogenic events that are made from cleared and stained specimens may be interpreted. For example, it is known that chondral primordia of bony elements arise by several methods (Shubin and Alberch, 1986: fig. 5): (Option 1) de-novo condensation of chondrocytes forming a single new primordium; (Option 2) elongation and eventual segmentation of an existing structure, forming two new primordia; and (Option 3) terminal bifurcation and segmentation of an existing structure, forming three new primordia. If an investigator is examining cleared and double-stained specimens to infer the identity of elements that lie adjacent to one another, what means are available to determine which method(s) contributed to the formation of each structure? If relatively younger specimens show connections and/or weak separation between elements, it can be inferred from direct observations that either Option 2 (elongation and separation) or Option 3 (bifurcation) was responsible for their formation, as is the case for formation of stylo- and zeugopodial elements and phalanges. However, if structures are absent in younger specimens and present in older specimens, without observable connectivity of chondral elements between stages, it is not proper to assume that the new structure(s) were formed by Option 1 (de-novo condensation). In the latter scenario, it is possible that these structures formed and separated quickly between available specimens, especially if these developmental processes occur quickly. Though this may be the case for the formation of Centrale 4, which does arise quickly in the manus of turtles, we suggest that the scenario

that Centrale 4 arose by budding and branching is unlikely, because explicit, strong connectivity or branching has not been observed in the manus of any turtle species studied, despite the broad taxonomic and stage-specific coverage provided in numerous studies.

In *Trachemys scripta*, a well-defined Centrale 4 appears by late Stage 15 within a broad field of stained tissue in the centralial region, and a second field of cartilage that is presumed herein to represent Centrale 3 (Fig. 2C) is observed distal to the radius and articulates with Distal Carpals 1 and 2. Centrale 3 progressively expands through ontogeny, and by Stage 17 (KU 291429) two dense centers of chondrification are found within this broad field of cartilage. By Stage 19 (KU 291437) Centrale 3 and Centrale 4 were observed to fuse and form a broad Centrale 3+Centrale 4. This pattern of fusion of centralial elements is similar to that reported for *Chrysemys picta* (Burke and Alberch, 1985). In *Apalone spinifera* (Sheil, 2003a) a weakly-stained, amorphous field of cartilage appeared between the radius, intermedium, ulnare, and Distal Carpals 1–3. Though it was not possible to infer the exact origin of the first centralial element (by branching or simple condensation), two diffusely connected regions within this field were observed to retain considerable quantities of Alcian Blue stain. Later in development (by Stage 17), this field splits to form separate Centrale 4 and Centrale 3, which were mislabeled as “Centrale” and “radiale” by Sheil (2003a: fig. 7b, c, e). Centrale 4 was observed to articulate with the ulnare, intermedium, and Distal Carpal 3, whereas Centrale 3 articulated broadly with the radius, distal margin of the intermedium, and Distal Carpals 1 and 2. Additionally, there was no indication of a separate field of cartilage in the autopodium that might be considered Centrale 2, though it should be noted that Centrale 3 is considerably larger than Centrale 4 and may serve a functional role otherwise taken by Centrale 2 or a larger Centrale 2+Centrale 3. In *Eretmochelys imbricata* (Sheil, 2003b), connectivity between Centrale 4 and neighboring elements was not observed, and too few specimens were available to infer the precise origin of any centralial elements. However, an amorphous, weakly-stained field of cartilage considered to represent the primordium of Centrale 4 (incorrectly labeled simply “Centrale” by Sheil [2003b: fig. 47b]) was observed between the intermedium and Distal Carpals 1 and 2 relatively early in development. Additionally, Centrale 3 (incorrectly labeled “Centrale 2” by Sheil [2003b: fig. 47c, e]) was observed distal to the radius and proximal to Distal Carpal 1 in at least one Stage-25 specimen, well after the formation of the digital arch and nearly all phalangeal elements. In both *Chelydra serpentina* (Sheil and Greenbaum, 2005) and *Macrochelys temminckii* (Sheil, 2005), Centrale 4 (which was misidentified in *M. temminckii* as “Centrale 3” or “Centrale 2” by Sheil [2005: 92]) appeared as a strongly chondrified structure between the distal carpals, intermedium, and ulnare well after the digital arch had been established. It should be noted that there is no apparent connectivity between this first centralial element and any neighboring elements and, as with Burke and Alberch’s (1985) study of *C. serpentina* and *C. picta*, this may imply that at least Centrale 4 forms by condensation, not by branching from the ulnare (sensu Shubin and Alberch, 1986) (Fig. 1B, C). Shortly after formation of Centrale 4, an oblong

cartilaginous body (tentatively referred to here as the presumptive Centrale 2+Centrale 3) appears preaxial to Centrale 4 and between the radius, intermedium, and Distal Carpals 1 and 2 in *C. serpentina* and *M. temminckii*. Between Stages 17 and 21, this slender structure was inferred to split, thereby forming discrete Centrale 2 and Centrale 3. By Stage 22, Centrale 3 fused with Centrale 4, resulting in the formation of Centrale 3+Centrale 4 (which articulates broadly with Distal Carpals 1–4) and the isolation of a relatively small Centrale 2 that articulates with Distal Carpal 1 and the radius. In *Chelonia mydas* (Sánchez-Villagra et al., 2007a), two centralial elements were observed in the manus well after formation and elaboration of the digital arch; however, written discussions of these elements were limited only to observations of their relative sizes. In the legend for their Fig. 3, Sánchez-Villagra et al. (2007a: 259) state, “A faint condensation distal to the intermedium and ulnare is present in the manus of the smaller specimen (A), but it is not illustrated at this stage because it is too faint to identify with any certainty.” It may be reasonable to assume that this structure is Centrale 4, based on timing of formation and spacial location. Identification of this structure as Centrale 4 would be consistent with the pattern of formation and location of this element in other turtles (Shubin and Alberch, 1986). Additionally, lines of connection drawn between elements in the manus (Sánchez-Villagra et al., 2007a: fig. 3a, b) imply patterns of branching and connectivity observed among these elements, and we assume that these authors are suggesting that Centrale 4 (inferred by relative size and location) forms by branching from the ulnare, whereas the next-preaxial element (Centrale 3) forms by de-novo condensation within the centralial region (i.e., it lacks connectivity with neighboring elements). It also is worth noting that lines of connectivity in their Fig. 3 suggest that Digit V forms from the digital arch by branching from Distal Carpal IV, not by de-novo condensation of Distal Carpal 5 and elongation and segmentation as seen in all other turtles. Their text makes no specific mention of how elements of Digit V form in *C. mydas*. In *G. nigrinoda* (Sánchez-Villagra et al. 2008), two centralial elements were observed but explicit identities were not assigned. The largest and oldest specimens of *Graptemys* demonstrated a single broad central element that articulates with Distal Carpals 3, 2 and 1. In all species examined, Centrale 4 was observed as a chondrified structure within the centralial region and without obvious connections to neighboring elements. Though it remains a possibility that Centrale 4 may arise via branching from the ulnare, it is our opinion that Centrale 4 arises by de-novo condensation within the field, sensu Burke and Alberch (1985; Fig. 1A). Detailed studies focusing specifically on the origin of centralial elements will be required to resolve this issue, as the possibility of inter-specific variation exists in the formation of the digital arch.

Patterns of element formation in pes of turtles

Most cartilaginous elements of the hind limb arise in the general pattern and sequence described by Burke and Alberch (1985) and Shubin and Alberch (1986). Development of the hind limb generally commences after that of the forelimb. The primary axis of this appendage ultimately extends through the fibula, fibulare, and elements of Digit IV.

The intermedium buds and branches preaxially from the fibulare early in development and eventually broadens to occupy much of the proximal portion of the autopodium. After formation of the digital arch, Digit V arises postaxial to Digit IV via de-novo condensation, and subsequent elongation and segmentation of the chondral primordium lead to the formation of the metatarsal and phalanges of this digit (see below). As in the forelimb, direct observations and connectivity between chondral elements indicate that Distal Tarsal 3 branches preaxially to form Distal Tarsal 2, which branches to form Distal Tarsal 1; each of these tarsal elements elongates and splits distally to form the corresponding metatarsals and phalanges. However, as in the forelimb, formation of the digital arch and centralia deserve attention, as few direct observations confirm the precise origin of Distal Tarsal 3. Specifically, it is unclear whether the pedal digital arch forms in all species of turtles by branching from the fibulare (sensu Burke and Alberch, 1985) (Fig. 1A) or from Distal Tarsal 4 (sensu Shubin and Alberch, 1986) (Fig. 1B, C). Burke and Alberch (1985: fig. 6b) presented a compelling photograph that clearly shows Distal Tarsal 3 branching preaxially from the fibulare in *Chelydra serpentina*. However, in *C. serpentina* (Sheil and Greenbaum, 2005), *Macrochelys temminckii* (Sheil, 2005), *Eretmochelys imbricata* (Sheil, 2003a), *Chelonia mydas* (Sánchez-Villagra et al., 2007a), *Graptemys nigrinoda* (Sánchez-Villagra et al., 2008), *Apalone spinifera* (Sheil, 2003b), and *Trachemys scripta*, direct connectivity between Distal Tarsal 3 and the fibulare was not observed. Lack of observations of connectivity between these elements in subsequent studies may reflect inadequate sample sizes for appropriate developmental stages. However, it is apparent from the number of available specimens that the early formation of Distal Tarsal 3 and the digital arch occurs relatively quickly, and direct observations of these developmental events will require examination of many specimens at the appropriate stages of development. In all available specimens, Distal Tarsal 3 appears nearly simultaneously with the de-novo condensation of the proximal-most element of Digit V, and apparently loses connectivity with either the fibulare or Distal Tarsal 4 shortly after its origin. In *Trachemys scripta*, the intermedium branches simultaneously with segmentation of the fibulare from the fibula. By Stage 15 (Fig. 3B; KU 290420), Digit IV is well established and the intermedium and Distal Tarsal 3 are clearly separated from all neighboring elements, and it is not possible to determine the precise origin of Distal Tarsal 3. The digital arch quickly forms Digits I–III, and the intermedium expands to occupy the centralial region. None of the available specimens of *T. scripta* indicate the presence of centralia within this region of the pes.

Early formation and modification of the centralial elements also occur quickly, and the precise order of events and identity of individual elements is shrouded somewhat by uncertainty. The intermedium arises as a preaxial branch from the fibulare and quickly enlarges to occupy most of the proximal autopodium. The centralial region is occupied by elements that ultimately fuse with the intermedium and contribute to the formation of the astragalus. In *Chelydra serpentina*, a single, broad centralial element was observed between the intermedium and Distal Tarsals 1–4 (Sheil and Greenbaum, 2005: fig. 12d). By Stage 21, this centralial ele-

ment fused with the intermedium and fibulare (i.e., calcaneum) to form a large astragalocalcaneum. This was consistent with Burke and Alberch's (1985) observations. However, Burke and Alberch (1985:127) also observed the fusion of the intermedium and Centrale 4 with an anterior centralial element (identified as Centrale 1) to form the astragalus. Sheil and Greenbaum (2005) did not observe an independent center of chondrification consistent with the position of their Centrale 1; however, this may be an artifact of not having a specimen at the appropriate developmental stage to make this observation. Burke and Alberch (1985:127) also noted the fusion of the astragalus and fibulare (i.e., calcaneum) in some specimens, as well as the appearance of a neomorph "pretarsale". Though fusion between the astragalus and fibulare was observed by Sheil and Greenbaum (2005), the "pretarsale" was not seen in any available specimens of *C. serpentina*. In a single Stage-17 specimen of *Macrochelys temminckii* (KU 290329; not illustrated by Sheil, 2005), the other extant chelydrid turtle, there was no indication of connectivity between centralial elements and the fibulare, but an amorphous field of cartilage occupied the centralial region of the pes. In a later Stage-17 specimen (KU 290325; not illustrated by Sheil, 2005) two distinct condensations were observed in the centralial region; their positions indicate the presence of Centrale 4 (articulating with Distal Tarsals 2–4) and Centrale 1 (articulating with Distal Tarsal 1). The fibulare and intermedium fused to form the astragalus by Stage 18, and the centralial region appears as a single, well-chondrified structure occupying the space between the astragalus and Distal Tarsals 1–3. Fusion of the astragalus, fibulare, and centralial elements to form a large astragalocalcaneum occurred by Stage 20.

The few available specimens of *Eretmochelys imbricata* (Sheil, 2003a) did not provide direct evidence for the origin of any centralial elements, and in Stage-24 specimens (e.g., KU 290516) the entire pes is present and lacks any elements in the centralial region. This is consistent with observations made for *Chelonia mydas* (Sánchez-Villagra et al., 2007a: fig. 3) and *Caretta caretta* (Sánchez-Villagra et al., 2007a: fig. 4), which were reported to lack centralia in the pes. In a Stage-17 specimen of *Apalone spinifera* (50A6; Sheil, 2003a), the proximal autopodium is occupied by a large, cartilaginous astragalus that contains several densely stained regions that, based on special or relative position, correspond to the fibulare (i.e., calcaneum), intermedium, Centrale 4 (which articulates with Distal Tarsal 3 and 4), and a centralial element that articulates with Distal Tarsal 1 and intermedium. Formation of the centralial region of the pes is somewhat unclear, as separate centralia were not observed in any specimens. Sánchez-Villagra et al. (2008) reported two separate centrale that they observed to fuse with the intermedium relatively early in development of the pes.

Patterns of ossification in manus and pes of turtles

General trends exist in the relative timing and direction of ossification (proximal–distal or distal–proximal) in regions that include the metapodials, phalanges, and proximal and distal carpal/tarsal elements of the manus (Fig. 4) and pes (Fig. 5). The humerus/femur ossifies before the ulna/fibula and radius/tibia in all taxa, demonstrating a proximal–distal

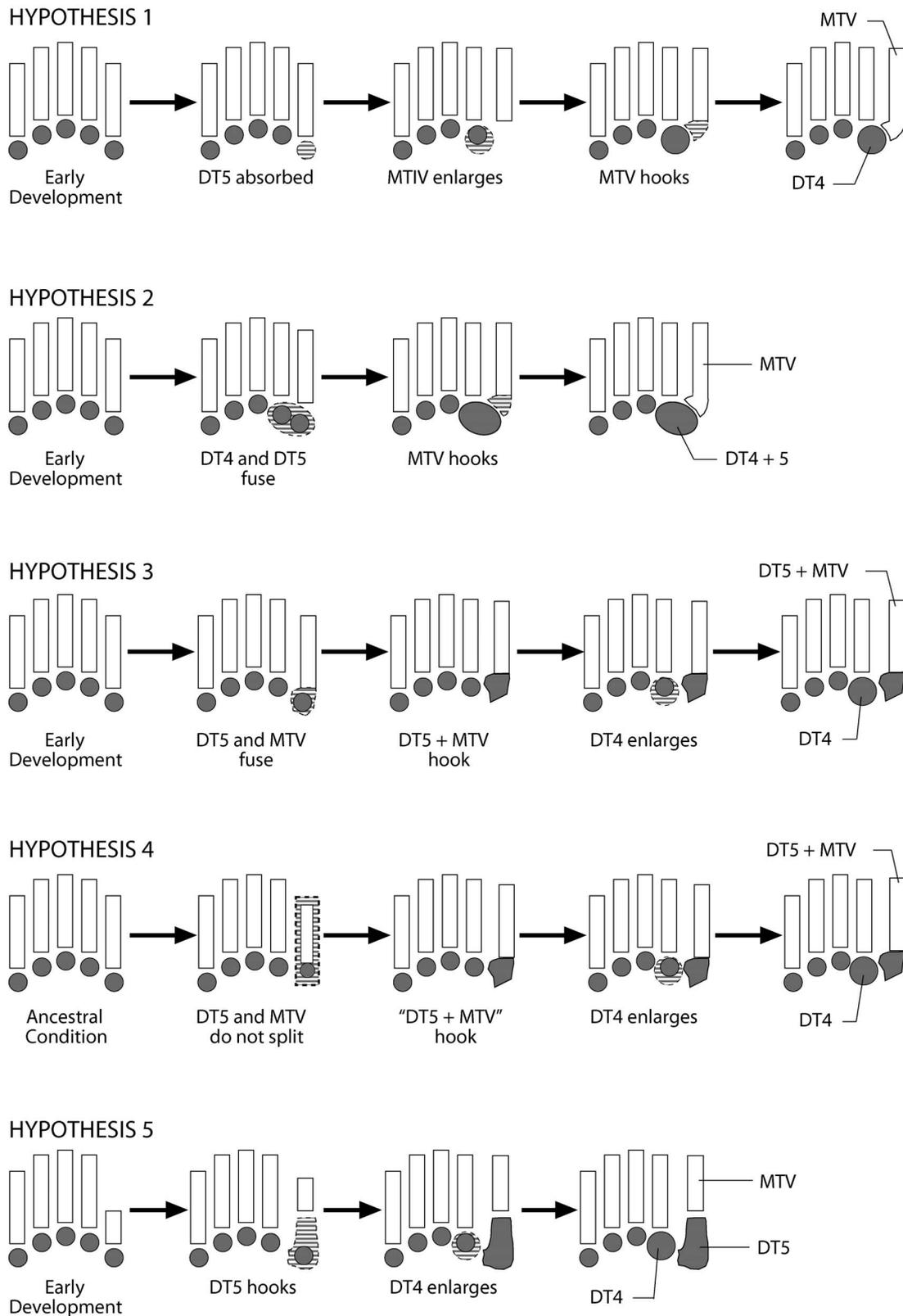


Fig. 6. Schematic diagram of five competing hypotheses to explain the formation and homology of elements in Pedal Digit V. Hypotheses **A–D** assume that the hooked proximal element of Digit V is not Distal Tarsal 5, whereas Hypothesis **E** assumes that it is. Hypotheses **A–C** and **E** assume that in early development, the first element to appear in pedal Digit V is Distal Tarsal 5, which forms by postaxial de-novo condensation of cartilage. Hypothesis **D** assumes that Distal Tarsal 5 and Metatarsal V never split early in development. Solid grey denotes distal tarsal elements; gray hashing indicates enlarging fields of cartilage, or fusion between chondrified elements; white indicates metatarsal elements. Abbreviations: DT, distal tarsal; MT, metatarsal.

pattern of ossification for these bones. Within each species, ossification of metapodials and phalanges commences at the same or a similar stage between the fore- and hind limbs; however, between species ossification of these elements commences at different developmental stages (but see Sánchez-Villagra et al., 2007a).

The metapodials of all species were observed to ossify before any other elements in both the manus and pes, with the exception of chelydrids (*Chelydra serpentina* and *Macrochelys temminckii*), in which the distal phalanges of several digits ossified before (or perhaps simultaneously with) the metapodials. In *C. serpentina* (Fig. 4E), the ungual phalanges of Digits I–III (manus) and I–IV (pes) were observed to ossify first, whereas relative degrees of ossification of these bones in *M. temminckii* (Fig. 4F) suggest that the ungual phalanges of Digits I–IV (manus) and Digits I–III (pes) ossify nearly simultaneously with the metapodials. Among the metapodials of the manus, those of Digits II–IV typically showed the greatest degrees of ossification, suggesting their early dominance in the trend of ossification seen in all turtles. Based on the observed degrees of ossification, the particular metacarpals that lead this trend are: *Trachemys scripta*, Digits II–III (manus) and Digits III–IV (pes); *Apalone spinifera*, Digits II–III (manus and pes); *Eretmochelys imbricata*, Digit III (manus and pes); *C. serpentina*, Digits III–IV (manus and pes; Sheil and Greenbaum, 2005) and Digits II–IV (manus and pes; Rieppel, 1993b); *M. temminckii*, Digit III (manus) and Digits III–IV (pes); and *Caretta caretta*, Digits II–IV (manus and pes; Sánchez-Villagra et al., 2007a). Though the middle metapodials ossify first, it is worth noting that Digit III frequently shows the earliest or greatest degree of ossification, rather than Digit IV. It is interesting that elements of Digit III ossify before those of Digit IV, despite the fact that Digit IV is the first to form in both the manus and pes (see discussion below). Of the metapodials, Metacarpal/Metatarsal V was the last to exhibit ossification in all species except *C. caretta* (Sánchez-Villagra et al., 2007a) (Fig. 4D), in which Metacarpal I was observed to be the last to ossify.

Based on relative degrees of ossification among phalangeal elements, the overall trend in the ossification of phalanges was led in each species by bones of the following digits: *Trachemys scripta*, Digits III–IV (manus) and Digit III (pes); *Apalone spinifera*, Digits II–III (manus and pes); *Eretmochelys imbricata*, Digit III (manus and pes); *Chelydra serpentina*, Digits III–IV (manus and pes; Sheil and Greenbaum, 2005) and Digits I–III (manus and pes; Rieppel, 1993b); *Macrochelys temminckii*, Digit III (manus and pes); and *Caretta caretta*, Digit III (pes; Sánchez-Villagra et al., 2007a), and unknown for the manus. In the digits of most species, ossification of phalangeal elements occurred in a proximal–distal trend; however, exceptions to this trend were observed as distal–proximal ossification in the following cases: *T. scripta*, Digits II–IV (manus); *E. imbricata*, Digits I and III (manus) and Digit I (pes); *C. serpentina*, Digits I–V (manus; Sheil and Greenbaum, 2005) and Digits I–IV (pes; Sheil and Greenbaum, 2005) and Digits I–V and Digits I–IV (manus and pes, respectively; Rieppel, 1993b); *M. temminckii*, Digits I–IV (manus) and Digits I–III (pes); and *C. caretta*, Digit I (manus and pes). Ossification of phalangeal elements of Digit V lags behind that of all other digits in all

species, with the exception of those of the manus in *E. imbricata* (Sheil, 2003b: figs. 47, 50–51) (Fig. 4C) and *C. caretta* (Sánchez-Villagra et al., 2007a: fig. 5) (Fig. 4D).

Ossification of the proximal autopodium in the manus and pes (including the ulnare/fibulare, intermedium, centralia elements, and distal carpals/distal tarsals) varies considerably among the species examined. In the manus (Fig. 4), ossification of these elements was not observed in any pre-hatching specimens of *Trachemys scripta*, *Eretmochelys imbricata*, or *Macrochelys temminckii* (but see below). The greatest number and degree of ossification were observed in the manus of *Apalone spinifera* (Fig. 4B), in which all elements of the manus showed some degree of ossification prior to hatching. In *A. spinifera*, Distal Carpals 1–4 were the first of these elements to ossify, followed by the intermedium and “proximal centrale” (i.e., Centrale 1). The ulnare, Centrale 4, and pisiform were the last to ossify. Based on degrees of ossification, the manus of *Chelydra serpentina* (Fig. 4E) demonstrated a similar pattern in which the distal carpals exhibited the greatest degree of ossification, followed by the intermedium and Centrale 2. Distal Carpals 4 and 5 were observed to fuse in late prehatching and some post-hatching specimens, and two separate centers of ossification were observed within this fused element (Sheil and Greenbaum, 2005; Rieppel, 1993b). In a 45.6-mm SVL post-hatching specimen of *Eretmochelys imbricata* (Fig. 4C), Distal Carpals 4 and 5 showed the greatest degrees of ossification, whereas Distal Carpal 2 showed only weak ossification. In this specimen, only the intermedium, ulnare, and pisiform were observed to ossify. In *Caretta caretta* (Sánchez-Villagra et al., 2007a: fig. 5a–e) (Fig. 4D), the intermedium and ulnare were the only proximal autopodial elements to exhibit ossification in prehatching specimens; the pisiform was observed to ossify after the intermedium and ulnare in at least one post-hatching specimen. In general, the intermedium, ulnare, centralia, and pisiform were among the last to ossify in all species, and in most cases did not exhibit ossification prior to hatching. Distal carpals typically did not ossify in prehatching specimens, with notable exceptions including *Apalone* and *Chelydra*.

Ossification of proximal autopodial elements of the pes appears to be delayed relative to those of the forelimb, and most of these elements lacked ossification in all prehatching specimens (Fig. 5). *Trachemys scripta* (Fig. 5A) lacked ossification in all proximal autopodial elements except the proximal-most hooked element of Digit V (referred to here as Distal Tarsal 5; see discussion of homology below). This hooked Distal Tarsal 5 also was observed to ossify in *Apalone spinifera* (Sheil, 2003a), *Eretmochelys imbricata* (Sheil, 2003b), and *Chelydra serpentina* (Sheil and Greenbaum, 2005; Rieppel, 1993b). In *A. spinifera* (Fig. 5B) this bone ossified nearly simultaneously with the fibulare, intermedium, and “proximal centrale” (i.e., Centrale 1). In *E. imbricata* (Fig. 5C), Distal Tarsal 5 ossified after the intermedium and Distal Tarsal 4, whereas in *C. serpentina* (Fig. 5E) it ossified before the intermedium. The greatest degree of ossification of pedal autopodials was observed in *A. spinifera*, in which the fibulare, intermedium and “proximal centrale” (i.e., Centrale 1) showed considerable ossification prior to that of Distal Tarsals 1–4. In *C. caretta* (Sánchez-Villagra et al., 2007a: fig. 5f–j) (Fig. 5D), the hooked Distal

Tarsal 5 was the first proximal autopodial element to ossify, followed by the large Distal Tarsal 4. In *Macrochelys* (Fig. 5F), Metatarsals I–IV showed greater degrees of ossification than did the phalanges. Metatarsal V (see identification below) was the last to ossify, only after ossification was observed in phalanges of all digits. Ossification of phalangeal elements was distal–proximal for all digits.

Post-hatching ossification of the astragalus was observed only in *Apalone*, *Eretmochelys*, and *Chelydra*. Distal Tarsal 4 exhibited ossification only in embryos of *Apalone* and both cheloniids (*Eretmochelys* and *Caretta*). The hooked proximal element of Digit V exhibited embryonic ossification in *Trachemys*, *Apalone*, *Chelydra*, and *Macrochelys*, but was not observed in either cheloniid.

As suggested by Rieppel (1993b) and Sánchez-Villagra et al. (2007a), incongruence between patterns of formation and ossification of these elements suggests that these processes are decoupled developmental events. In short, the pattern of ossification of these elements is not identical to their pattern of formation.

The hooked proximal element of Digit V

In all previous studies of autopodial development in turtles (including ours), a conspicuous de-novo condensation is reported in both the manus and pes postaxial to Digit IV, well after the digital arch is established (e.g., for *Trachemys scripta*; see Fig. 3C). This cartilage primordium eventually elongates and segments to form the elements of Digit V. In the forelimb the proximal-most element of Digit V (which is the first to appear in this digit) consistently is referred to as Distal Carpal 5 and, with the exception of cases in which the distal carpal elements fuse, all distal carpals generally are equal in size and shape, and each articulates distally with its corresponding metacarpal. However, in Digits IV and V of the pes, the anatomy of the distal tarsals and metatarsals differs considerably from that of the manus. Among amniote tetrapods and basal amniotes (e.g., capthorhinids and nyctophruretids) all distal tarsals are relatively simple and discoidal, and all corresponding metatarsals are straight (Lee, 1997). Among extant diapsids and turtles, the proximal-most element of Digit V, which is hook- or L-shaped and articulates with a large Distal Tarsal 4, consistently is referred to as Metatarsal V (see also Lee, 1997). Among turtles and extant diapsids, this hook- or L-shaped proximal element typically is accompanied by an enlarged distal tarsal element associated with Digit IV. This hooked Metatarsal V has been considered a synapomorphy for Sauropsida (Goodrich, 1916) or Diapsida (Gaffney, 1980). However, it should be noted that Lee (1997) demonstrated that the hooked proximal element of Digit V has evolved convergently in turtles and diapsid reptiles.

Burke and Alberch's (1985) discussion of the homology of limb elements of turtles primarily focused on the proximal elements and only briefly discussed the condition of the elements in Digit V, and their illustrations (Burke and Alberch, 1985: fig. 2b, d) label this element as Metatarsal V for *Chelydra* and *Chrysemys*, respectively. According to Burke and Alberch (1985: 124–125), "[Digit 5] is initiated, in the manus, with the condensation of carpal 5, which subsequently extends distally and gives rise to the metacarpal and phalanges. In contrast, the metatarsal is the first element to

differentiate in the fifth digit of the pes, these data indicate that distal tarsal 5 does not form at all in the two species examined [*Chelydra serpentina* and *Chrysemys picta*] (Fig. 6)." No justification was offered as to why the first element to coalesce in Digit V of the pes, which ultimately remains the proximal-most element of this digit, is identified as Metatarsal V rather than Distal Tarsal 5. In fact, the hooked proximal element of Digit V of turtles has been identified as Distal Tarsal 5 by only a handful of authors (e.g., Hoffman, 1890; Boulenger, 1889; Case, 1939; also see Gaffney, 1990: 255), presumably based solely on relative geographic position within the pes. Lee (1997) also refers to the hooked proximal element of Digit V as Metatarsal V and presents compelling comparative data for the acquisition of a hooked Metatarsal V in turtles only after the apparent loss of Distal Tarsal 5 and anatomical change in Distal Tarsal 4.

Naming this proximal-most element "Metatarsal V" leads to several obvious questions. Is this element truly Metatarsal V rather than Distal Tarsal 5? What is the ultimate fate of Distal Tarsal 5 and by what mechanism is it lost? If this proximal element of pedal digit V is Metatarsal V, how does it become hook-shaped? The identity and homology of elements of the manus and pes have been argued from several lines of evidence (including adult neontological morphology, paleontological data, and development) and at least five general hypotheses have been proposed to explain the developmental history of elements in pedal Digit V (Fig. 6), particularly pertaining to the hook-shaped proximal element of this digit (Lee 1997; Sheil, 2003b: 150–158; Sánchez-Villagra, 2007b).

Hypothesis 1 (Fig. 6A)

Distal Tarsal 5 elongates and segments to form Metatarsal V and the phalanges of Digit V, and the cartilage primordium of Distal Tarsal 5 subsequently is lost by absorption or some developmental mechanism other than fusion with neighboring elements. This requires that the proximal-most element of Digit V is Metatarsal V alone, and that the proximal hook (which articulates with Distal Tarsal 4) is an elaboration of Metatarsal V formed by a preaxial flexure of this bone. This hypothesis also requires an increase in the size of Distal Tarsal 4 to explain the observed sauropsid anatomy of the pes. This hypothesis was discussed recently by Gauthier et al. (1988: 42), who suggested that the simplest explanation for the observed morphology of the saurian pedal Digit V is to posit the wholesale loss of Distal Tarsal 5 (and subsequent size increase for Distal Tarsal 4). Though Goodrich (1916) and Holmgren (1933) stated that Sewertzoff's (1908) data indicated such a loss of Distal Tarsal 5, Robinson (1975) suggested that this was a misinterpretation of Sewertzoff's (1908) original work, which was incorrectly translated from German. Of all developmental studies that have examined details of anatomy in the pes, none has documented the appearance and loss of the first condensation in Digit V subsequent to elongation and separation of Distal Tarsal 5 and Metatarsal V. Lee (1997) infers that Distal Tarsal 5 is lost by absorption rather than fusion with Distal Tarsal 4.

Hypothesis 2 (Fig. 6B)

Distal Tarsal 5 elongates and segments during develop-

ment to form a separate Distal Tarsal 5 and Metatarsal V; subsequently, the cartilaginous primordium of Distal Tarsal 5 is lost by fusion with Distal Tarsal 4, thereby forming a relatively large Distal Tarsal 4+5 that articulates with Metatarsal IV (distally) and Metatarsal V (postaxially). In this hypothesis, the prominent, proximal hook on Metatarsal V would be formed by a medial inflection of Metatarsal V, and the proximal hook of Metatarsal V simply represents an elaboration of this bone. Though distal tarsal elements are known to fuse in some reptiles (Gaffney, 1990; numerous citations in Weishampel et al., 1990), this hypothesis has few apparent proponents among those who study turtles, and there is no direct evidence that Distal Tarsals 4 and 5 do fuse, at least among the species reviewed herein. Interestingly, however, Distal Carpals 4 and 5 have been demonstrated to fuse in the manus of *Chelydra serpentina* (Rieppel, 1993b; Sheil and Greenbaum, 2005) and *Trachemys scripta* (Fig. 2G). In both studies, separate Distal Carpals 4 and 5 were observed to fuse and, upon ossification, two centers of ossification were observed within Distal Carpal 4+5 (summarized in Sheil and Greenbaum, 2005: fig. 13) (Fig. 4D). In none of the reviewed studies of element formation in turtles was similar fusion between distal tarsal elements observed, and two centers of ossification were never observed in the enlarged Distal Tarsal 4, thereby suggesting it is formed by a single element.

Hypothesis 3 (Fig. 6C)

During development, Distal Tarsal 5 elongates and segments to form Metatarsal V; subsequently, the primordium of Distal Tarsal 5 is lost by fusion with Metatarsal V. In this scenario, the proximal hook of Metatarsal V, which articulates with the large Distal Tarsal 4, likely would represent the remnants of Distal Tarsal 5. From a developmental perspective, this hypothesis has received some attention. Holmgren (1933: 269) argued that Distal Tarsal 5 fused to a rudimentary free tarsale, and Robinson (1975: 466) suggested that available data best support the possibility that the hooked bone of Digit V forms by fusion of Distal Tarsal 5 and Metatarsal V. However, Robinson offered only tentative support for this hypothesis, and commented that this embryological evidence needs augmentation and further attention. Likewise, Romer (1956) stated that this hypothesis has been argued unsuccessfully; however, Romer provided no citations to support this statement. Though Harris and Carroll (1977) and Currie (1981) report postnatal fusion of Distal Tarsal 5 and Metatarsal V in tangasaurian younginiforms, their observations are based on incomplete specimens with poorly preserved hind limbs and feet. Smith and Evans (1996) did not report fusion of any distal tarsal elements in any of their juvenile specimens of *Youngina* (a Permian younginiform). Their specimens (Smith and Evans, 1996: fig. 8a) demonstrated a large Distal Tarsal 4, and they reported that the presence of Distal Tarsal 5 could not be confirmed; additionally, though the proximal bone of pedal Digit V essentially is straight, it possesses a prominent lateral process. Goodrich (1942) described the head of this proximal element of pedal Digit V as very broad, suggesting that it was in fact L-shaped. Of all developmental studies that have examined details of anatomy in the pes, none has documented segmentation and subsequent fusion of Distal Tar-

sal 5 and Metatarsal V, and only that of Sánchez-Villagra et al. (2007b: fig. 7) has demonstrated apparent joining of Distal Tarsal 5 and Metatarsal V. Additionally, two centers of ossification have not been reported in this large proximal element, and when ossification commences, it occurs as a single region of ossification in the central mass of this element.

Hypothesis 4 (Fig. 6D)

Distal Tarsal 5 elongates to form the unsegmented primordium of Metatarsal V; however, unlike in all other digits, Distal Tarsal 5 and Metatarsal V never segment to form separate elements, though the distal terminus of this element does elongate and segment to form the phalanges of this digit. Here, the proximal-most element of Digit V would represent a novel digital element different from that seen in the common ancestor of reptiles. Although Sewertzoff (1908) typically is cited as a proponent of the idea that either Distal Tarsal 5 is lost or fused with Metatarsal V, Robinson (1975) argues that a correct translation Sewertzoff (1908: 130) actually states, "...that distale 5 [i.e., Distal Tarsal 5] is fused to the (fifth) metatarsal and no longer detaches itself during ontogenetic development", lending support for the hypothesis presented here. Here too, Romer (1956) commented that this hypothesis has not been argued well, although he does not explicitly describe the logic of his statement, and he failed to provide citations to allow independent interpretation of this statement. Again, it is worth noting that two centers of ossification have not been reported in this large proximal element of pedal Digit V, and when ossification commences in this bone, it occurs as a single region of ossification in the central mass of the element.

Hypothesis 5 (Fig. 6E)

During development, Distal Tarsal 5 elongates and segments to form Metatarsal V, which ultimately forms the phalangeal elements of this digit. Subsequently, Distal Tarsal 5 does not fuse with either of its neighbors (Metatarsal V or Distal Tarsal 4), but enlarges and becomes hook-shaped proximally to articulate with the lateral margin of an enlarged Distal Tarsal 4. Based solely on the examination of the adult morphology, the proximal hooked bone of Digit V has been identified as Distal Tarsal 5 (e.g., Boulenger, 1889; Hoffman, 1890; Case, 1939). Observed patterns of formation seem to support this hypothesis.

Although several authors have discussed the apparent loss of Distal Tarsal 5 (e.g., Rable, 1910; Goodrich, 1916; Schaeffer, 1941; Gaffney, 1990), the precise developmental mechanism by which this element may be lost (via absorption or fusion with a neighboring element) is not always apparent. Possible explanations for the diversity of opinions concerning the homology of elements of pedal Digit V include reliance upon relatively low numbers of specimens of appropriate developmental stages that could provide snapshots of these developmental events, as well as the actual quality of available specimens. Even Holmgren (1933: 255) admitted that the material available for his study was not favorable for examination of tarsal development, particularly in turtles. Additionally, there can be considerable room for misinterpretation of element identity because provided

text descriptions and associated Figs. may not always be congruent. For example, Holmgren's (1933) discussion of the development in the hind limb is relatively comprehensive and discusses Distal Tarsal 5 in some detail, whereas in nearly all of his illustrations, Distal Tarsal 5 is noted with a "?", indicating that the developmental history of this bone is uncertain. Additionally, it might be argued that it is not possible to discern which hypothesis for the origin of bones in Digit V best fits the actual pattern of development, because examining gross morphological change does not provide enough resolution to developmental events that occur very rapidly, or that are determined before chondrification occurs. If this is the case, perhaps other criteria should be used to determine which of Hypotheses 1–5 (Fig. 6) should be preferred. To this end, we consider that each of the required developmental events (loss, fusion, or modification) is an independent assumption of evolutionary change, and we appeal to parsimony to provide an independent criterion for selecting a preferred hypothesis(es) among these options. Hypothesis 1 (Fig. 6A) requires three evolutionary steps and posits the loss of Distal Tarsal 5, modification of Metatarsal V into a hooked element, and enlargement of Distal Tarsal 4. Hypothesis 2 (Fig. 6B) requires two evolutionary steps, which include fusion of Distal Tarsal 5 to Distal Tarsal 4 to form a large tarsal element (Distal Tarsal 4+5), and the modification of Metatarsal V to become hooked. Hypothesis 3 (Fig. 6C), which posits the fusion of Distal Tarsal 5 to Metatarsal V, the change in orientation and shape of Distal Tarsal 5+Metatarsal V to become hooked proximally, and enlargement of Distal Tarsal 4, requires three evolutionary steps. Likewise, Hypothesis 4 (Fig. 6D) requires three evolutionary steps but posits that Distal Tarsal 4 enlarges and that Distal Tarsal 5/Metatarsal V (which do not split), becomes hooked proximally. Hypothesis 5 (Fig. 6E) requires two evolutionary steps that include the modification of Distal Tarsal 5 into a hooked element, and enlargement of Distal Tarsal 4. Based simply on the number of required steps, Hypotheses 1, 3, and 4 are indistinguishable (requiring three steps), as are Hypotheses 2 and 5 (which require only two steps). Although Hypotheses 2 and 5 require the same number of steps, the latter should be preferred, because Hypothesis 2 requires an additional assumption that is not supported observationally—rapid developmental change to Distal Tarsals 4 and 5, and Metatarsal V. However, if rapid developmental change can be shown to operate in Digit V, and to have been responsible for the lack of observations over the past seven decades (Holmgren, 1933–present), then perhaps Hypothesis 2 should be considered. Until empirical evidence is available to this effect, Hypothesis 5 should be the preferred explanation for the developmental history of the elements of Digit V, and the proximal, hooked element of Digit V should be considered Distal Tarsal 5.

Additional evidence for the identity of this hooked proximal element may be derived from patterns of ossification of elements in the pes. Among turtles, the astragalus forms by fusion of the intermedium and centralia elements, whereas the calcaneum is formed only by the fibulare (Peabody, 1951; Rieppel, 1993a; Rieppel and Reisz, 1999). Also, the astragalus and calcaneum may unite, as they do in turtles (Rieppel and Reisz, 1999; see also Gegenbaur, 1964; Sewertzoff, 1908; Rable, 1910). Though the chondral

primordia of these elements fuse early in development, each element begins to ossify as an independent center of ossification within the composite cartilaginous structure, and only later do the individual elements fuse synostotically to their neighbors. Though conspicuous ossification is observed in all distal tarsal and metatarsal elements, multiple centers of ossification (of the type observed in the astragalus and/or calcaneum) were not observed in either the large Distal Tarsal 4 or in the proximal-most element of Digit V (herein considered Distal Tarsal 5) in any specimens. In all specimens of *Chelydra serpentina* in which two centers of ossification were observed in the fused Distal Tarsal 4+5 of the manus, only one center of ossification was observed within the proximal-most element of Digit V (i.e., Distal Tarsal 5), or in the large Distal Tarsal 4 to which it articulates. Based on the assumption that if multiple elements do comprise a larger structure, then multiple centers of ossification will be observed (Peabody, 1951; Rieppel, 1993d), it is therefore unlikely that the autopodium of turtles includes "Distal Tarsal 4+Distal Tarsal 5" or "Distal Tarsal 5+Metatarsal V". Although separate centers of ossification (i.e., epiphyses or apophyses) have been observed in Metatarsal V of some lepidosaurs (Haines, 1969), this is hypothesized to be an autapomorphy for that group (Rieppel and Reisz, 1999), and again, in turtles the hooked element of Digit V should be considered Distal Tarsal 5.

Despite compelling evidence and argumentation to support this alternate hypothesis for the developmental history (and therefore homology and identity) of the hooked proximal bone of pedal Digit V, the terminology of Goodrich (1916) persists, and this element continues to be referred to as Metatarsal V. According to Robinson (1975: 466), "...it is, however, simpler to go on referring to it [Distal Tarsal 5+Metatarsal V] as 'the hooked fifth metatarsal [rather than changing the name of this structure]', as this has become commonly used in the literature..." We do not agree that the terminology of Goodrich (1916) should be maintained simply because it is rooted in common usage. Though it is unfortunate that applying a different term to this element will require considerable care when citing and referring to earlier works to prevent confusion and misinterpretation, the terminology assigned to elements of Digit V should reflect what is understood about their evolutionary and developmental histories. In Digit V, the hooked proximal element that articulates with the postaxial margin of an enlarged Distal Tarsal 4 should be recognized as a hooked Distal Tarsal 5 on the basis of patterns of developmental connectivity through ontogeny, anatomical location, and lack of evidence to the contrary. This hooked Distal Tarsal 5 serves the functional role of connecting Digit V to the rest of the autopodium, and transfers forces of locomotion through the phalanges and metatarsal as the tarsals do in Digits I–IV. Switching to the proposed identity of this proximal element will require reassessing phalangeal formulas in those taxa affected by this change. Additionally, the "hooked Metatarsal V" that defines membership in either Sauropsida (Goodrich, 1916) or Diapsida (Gaffney, 1980) should be considered the "hooked Distal Tarsal 5."

In summary, we consider Distal Tarsals 4 and 5, as well as Metatarsal V to be present, independent elements in the pes of turtles. Digit V is composed of a large, proximally

hooked Distal Tarsal 5 (which articulates with a large Distal Tarsal 4), a relatively short Metatarsal V (which appears much like the phalanges of any pedal digit), and two or more phalanges. The preferred hypothesis for development in Digit V is Hypothesis 5 (Fig. 6E), which best reflects the observed developmental history of the hind limb in turtles, and synthesizes information from formation and ossification.

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Appendix 1. Specimens of *Trachemys scripta* examined for information on particular developmental stages. All specimens are cleared and double-stained embryos; numbers indicated for each stage are KU (The University of Kansas) accession numbers.

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- Stage 14: 291407, 291409–11.
- Stage 15: 291408, 291415, 291419–20.
- Stage 16: 291423, 291425–26.
- Stage 17: 291428–29.
- Stage 18: 291431–32.
- Stage 19: 291434, 291437.
- Stage 20: 291440, 291444, 291447–48.
- Stage 21: 291441, 291443, 291446.
- Stage 22: 291450, 291453, 291456.
- Stage 23: 291454, 291457, 291460–62.
- Stage 24: 291466, 291468, 291470, 291481, 291491–92.
- Stage 25: 291483, 291494.
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