

Evolutionary and developmental aspects of phalangeal formula variation in pig-nose and soft-shelled turtles (Carettochelyidae and Trionychidae)

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Abstract In order to examine the evolution of the phalangeal formula in a diverse clade of turtles, including hyperphalangy as a rare condition in this group, we studied 210 specimens representing all extant genera of Trionychidae and their sister taxon, Carettochelyidae. Both groups consist of highly aquatic species with elongated autopods that are either paddle-like (Trionychidae) or transformed to flippers (Carettochelyidae). Phalangeal formulae were obtained mostly by radiographs of alcohol-preserved or dry specimens, as well as by direct counts from skeletons. All trionychids and *Carettochelys* are pentadactylous, but their phalangeal formulae differ. *Carettochelys* exhibits the turtle-plesiomorphic state (manus and pes: 2-3-3-3-3), with no variation in adults. Trionychids exhibit intraspecific variation, ranging from 2-3-3-3-2 to 2-3-3-6-5 for the manus, and from 2-3-3-3-2 to 2-3-3-5-3 for the pes. The extant *Carettochelys* as well as the Middle Eocene *Allaeochelys crassesculpta* are characterized by an elongation of phalanges, whereas trionychids consistently have shorter phalanges. All trionychid genera exhibit some degree of hyperphalangy in digits IV and V, in both the

manus and pes. Phalanges of the clawed digits I–III are very robust compared to phalanges of the non-clawed digits IV and V. The latter contribute significantly to the enlargement of the paddle by their additional phalanges. We hypothesize that this phalangeal pattern is coupled with prolongation of growth processes in the non-clawed digits. The differences in autopod morphology between carettochelyids and trionychids reflect different locomotor patterns related to different natural histories (elongated flippers for high-speed escape in the mainly herbivorous *Carettochelys*; broad paddles for rapid turns during hunting in the mainly carnivorous trionychids). The autopod of *Pelodiscus sinensis* is proposed as an experimental model to examine the developmental basis of adult autopod variation.

Keywords Cryptodira · Growth · Heterochrony · Limb · Skeleton

Introduction

Despite being perceived as structurally constrained by the presence of the shell (Renous et al. 2008), chelonians have successfully diversified to a variety of forms adapted to both aquatic and terrestrial habitats. Such ecological diversity is correlated with different locomotor patterns that are reflected, among others, in different autopod anatomy. A number of studies have focused on the morphology, functional diversity, and evolution of chelonian autopods, but only a few extant species distributed among diverse clades have been studied (Carettochelyidae: Walther 1922; Chelidae: Fabrezi et al. 2009, Sánchez-Villagra et al. 2007a; Cheloniidae: Sánchez-Villagra et al. 2007b; Chelydridae: Rieppel 1993; Emydidae: Rosenberg 1892, Sheil and Portik

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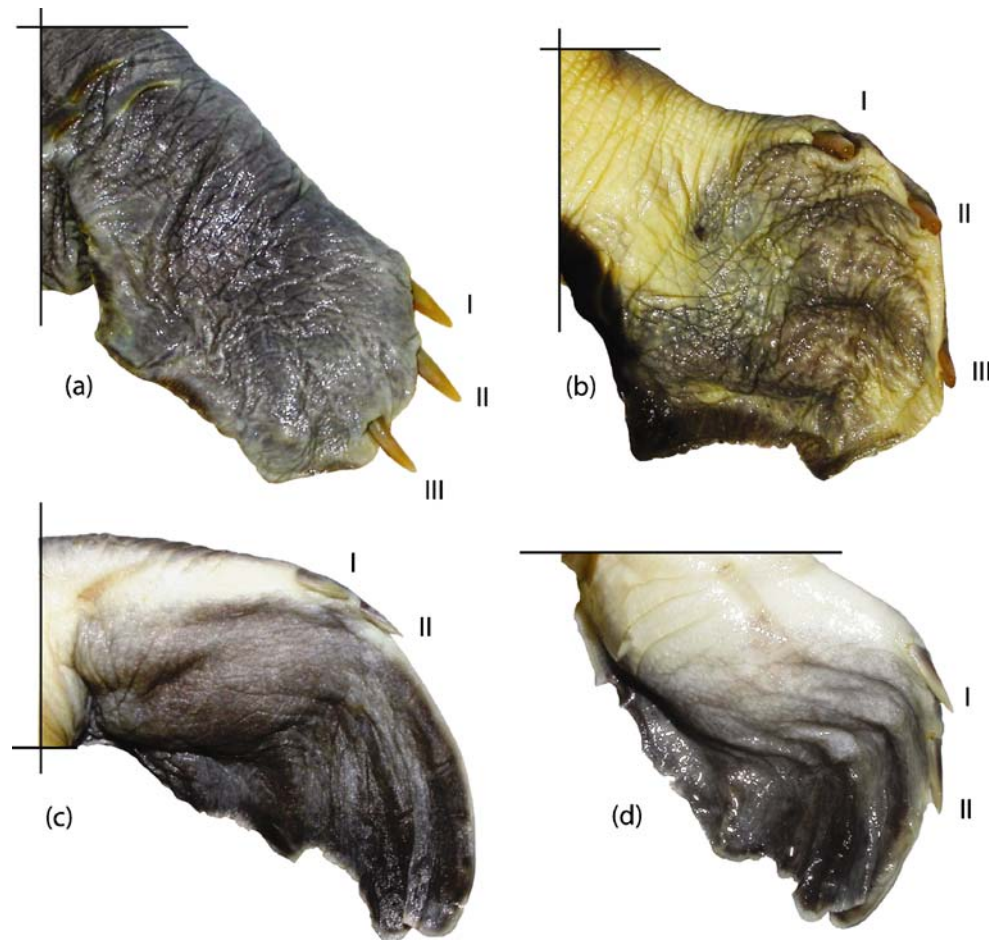
2008; Geoemydidae: Fritz et al. 2006, Ludwig et al. 2007; Pelomedusidae: Sánchez-Villagra et al. 2007a; Podocnemididae: Fabrezi et al. 2009; Testudinidae: Auffenberg 1966, Bramble 1982, Crumly and Sánchez-Villagra 2004, Hitschfeld et al. 2008; Trionychidae: Ogushi 1911, Sheil 2003; general: Baur 1892, Burke and Alberch 1985, Rabl 1910, Walker 1973, Zug 1971). By contrast, much is known about the mechanisms of limb development, including those of the skeletal elements in autopods (Cooper and Dawson 2009; Fröbisch 2008; Gilbert 2006; Richardson et al. 2009; Shapiro et al. 2007; Wagner and Larsson 2007), suggesting autopod evolution as an attractive system for studying the diversification of function and development in the limbs. In this context, examination of variation among taxa and within populations can be useful (Stern 2000). For example, Crumly and Sánchez-Villagra (2004) examined the autopods in a large sample of land tortoises and presented hypotheses about the heterochronic growth processes associated with the observed variation.

The goal of the present study is to comprehensively analyze the phalangeal formula in two closely allied, and morphologically highly derived, clades of extant chelo-

nians, Carettochelyidae and Trionychidae, in order to explore possible evolutionary and developmental aspects related to observed variation. Based on morphological and molecular evidence, Carettochelyidae and Trionychidae constitute sister groups (Fujita et al. 2004; Gaffney and Meylan 1988; Shaffer et al. 1997), yet these clades exhibit contrasting patterns of phalangeal formulae, including one (hyperphalangy) that is unique among turtles and rare among tetrapods.

Extant Trionychidae, or soft-shelled turtles, comprise 13 genera and 30 species from North America, Africa, Asia, and New Guinea (Engstrom et al. 2004; Fritz and Havaš 2007; Meylan 1987; Prasad et al. 2007). There is a rich fossil record dating back to the Lower Cretaceous of Asia (Meylan and Gaffney 1992; Nessov 1995) and providing evidence for the group's former occurrence in Australia (Gaffney and Bartholomai 1979) and South America (Head et al. 2006; Wood and Patterson 1973). Trionychids are characterized by a greatly reduced shell covered by a thick, leathery integument instead of horny scutes (Delfino et al. 2010; Scheyer et al. 2007). Their broad, paddle-like autopods (Fig. 1a, b) are exceptional among turtles in

Fig. 1 External morphology of manus and pes in the trionychid *Pelodiscus sinensis* (a, b) and the carettochelyid *Carettochelys insculpta* (c, d). (a) Dorsal view; (b–d) ventral views. Both trionychids and carettochelyids are pentadactylous, but claws are present only on the first three digits (manus and pes) in trionychids, on the first two digits in *C. insculpta*



exhibiting a large number of phalanges in some digits—a rare case of hyperphalangy (Renous et al. 2008; Richardson and Chipman 2003).

Carettochelyidae are represented by their single extant species, *Carettochelys insculpta* Ramsay, but their fossil record indicates a long evolutionary history dating back to the Upper Jurassic or Lower Cretaceous (de Lapparent de Broin 2001), and many fossil taxa have been described from all continents except Antarctica (Danilov 2005; de Lapparent de Broin 2001). Carettochelyids share with trionychids the absence of horny scutes on the shell, but their anterior limbs are flippers rather than paddle-like as in trionychids (Fig. 1c, d), a unique condition among non-marine turtles (Renous et al. 2008). Trionychids and carettochelyids live in aquatic habitats ranging from river mud to deep waters of large rivers and lakes, and from brackish to sea water (Pritchard 2001; Renous et al. 2008).

Previous examinations of the phalangeal formulae of trionychids and carettochelyids, as for most turtles, have been restricted to anecdotal reports or concern only single species (Table 1). In both groups, a large autopod has evolved, with interdigital webbing in adults. However, the locomotor patterns are highly distinct: trionychids mostly use forelimb rowing, with the powerstroke of one forelimb alternating crosswise with that of the opposite hindlimb, whereas carettochelyids are characterized by bilateral forelimb flapping, i.e. simultaneous up-and-down movements of both forelimbs, as in marine turtles (Blob et al. 2008).

A final important consideration in potentially explaining autopod morphology concerns body size and allometry. The role of size in the evolution of limb morphology has been examined in various vertebrate clades (Alberch and Gale 1983), in some cases finding correlations that suggest the role of heterochrony in growth patterns in evolution (e.g. Crumly and Sánchez-Villagra 2004). The large variation in

adult body size of trionychids, which range from 10 or 12 cm to 129 cm shell length (Ernst and Barbour 1989; Tang 1997), offers the chance to examine the effect of this important variable in the diversity of an easily quantifiable character complex, the phalangeal formula.

Material and methods

Phalangeal formulae were recorded from skeletal preparations or, in most cases, from radiographs of dry- or fluid-preserved specimens housed at the Museum of Zoology, Senckenberg Dresden (MTD), the Natural History Museum in London (BMNH), the Naturhistorisches Museum in Basel (NHMB), or the Forschungsinstitut Senckenberg Frankfurt (SMF). A total of 210 post-hatching specimens were analyzed, representing 21 (of the 30) species of all 13 currently recognized trionychid genera plus the only extant carettochelyid genus and species (Table 2). A number of specimens in suboptimal state of preservation were not included in the analyses, in order to minimize the risk of misinterpreting their autopod morphology.

Digits and phalanges were counted according to the standardized method of Padian (1992); intraspecific variation in the number of phalanges in a certain digit is indicated by numbers separated by a slash. We followed traditional anatomical nomenclature by considering the hooked proximal element of pedal digit V as the hooked metatarsal (see Sánchez-Villagra et al. 2007a, and references therein). However, Sheil and Portik (2008) and Fabrezi et al. (2009) recently suggested naming this element the distal tarsal 5. The nomenclature of taxon names follows Fritz and Havaš (2007), except that the former *Aspideretes gangeticus* (Cuvier) and *A. hurum* (Gray) are referred to under the genus name *Nilssonina* Gray after Praschag et al. (2007).

Table 1 Previously reported phalangeal formulae of carettochelyids and trionychids

Taxon	Manus	Pes	References
Carettochelyidae			
<i>Carettochelys insculpta</i>	2-3-3-3-3	2-3-3-3-3	Walther (1922), Zug (1971)
Trionychidae			
<i>Apalone spinifera</i>	2-3-3-4-3	2-3-3/4-4/5-2	Sheil (2003)
<i>Chitra</i> sp.	2-3-3-6-4		Walker (1973)
<i>Cyclanorbis senegalensis</i>	2-3-3-5-4		Rabl (1910)
<i>Lissemys</i> sp.		2-3-3-4-3	Zug (1971)
<i>Pelochelys</i> sp.		2-3-3-4-3	Zug (1971)
<i>Pelodiscus sinensis</i>	2-3-3-4-4		Rabl (1910)
<i>Pelodiscus sinensis</i>	2-3-3-5-4	2-3-3-4-3	Ogushi (1911)
' <i>Trionyx</i> ' sp.		2-3-3-4-3	Zug (1971)

Table 2 Phalangeal formulae of extant carettochelyids and trionyichids

Taxon	M I–III	M IV	M V	P I–III	P IV	P V	n	Max. SL [cm]
Carettochelyidae								
<i>Carettochelys insculpta</i>	2-3-3	3	3	2-3-3	3	3	20	55
Trionyichidae								
<i>Pelodiscus sinensis</i>	2-3-3	3/4/5/6*	2/3/4/5*	2-3-3	3/4/5	2/3/4	46	25
<i>Lissemys scutata</i>	2-3-3	5	3/4	2-3-3	3/4	3	2	<28
<i>Lissemys punctata</i>	2-3-3	3/4/5/6*	2/3/4/5*	2-3-3	3/4	2/3	22	28
<i>Cyclanorbis senegalensis</i>	2-3-3	4/5/6*	3/4/5*	2-3-3	3/4	3	11	35
<i>Dogania subplana</i>	2-3-3	3/4/5	2/3/4	2-3-3	3/4	2/3	11	35
<i>Apalone mutica</i>	2-3-3	3/4	3	2-3-3	4	3	3	36
<i>Nilssonina formosa</i>	2-3-3	4/5	3/4	2-3-3	4	3	3	40
<i>Rafetus euphraticus</i>	2-3-3	5/6*	4/5	2-3-3	4	2/3	2	40
<i>Palea steindachneri</i>	2-3-3	3/4/5	3/4	2-3-3	3/4	2/3	10	43
<i>Apalone spinifera</i>	2-3-3	3/4/5	2/3/4	2-3-3	3/4/5	2/3	22	50
<i>Cycloderma aubryi</i>	2-3-3	4	3/4	2-3-3	3/4	3	2	55
<i>Cycloderma frenatum</i>	2-?-3	5	4/5*	2-3-3	4	3	1	56
<i>Apalone ferox</i>	2-3-3	5	3	–	–	–	1	60
<i>Cyclanorbis elegans</i>	2-3-3	4	2	2-3-3	3/4	3	1	60
<i>Nilssonina hurum</i>	2-3-3	4/5	3/4	2-3-3	3/4	3	5	60
<i>Amyda cartilaginea</i>	2-3-3	4/5	3/4	2-3-3	3/4	2/3	13	70
<i>Nilssonina gangetica</i>	2-3-3	4/5	3	2-3-3	3/4	2/3	8	70
<i>Trionyx triunguis</i>	2-3-3	3/4/5	3	2-3-3	4	2/3	9	95
<i>Chitra indica</i>	2-3-3	3/4/5	2/3	2-3-3	3/4	2/3	17	115
<i>Pelochelys cantorii</i>	2-3-3	5	3	2-3-3	4	3	1	129

M = manus; n = number of specimens; P = pes; Roman numerals refer to digits

Trionyichid species sorted by increasing maximum shell length (Max. SL), the latter rounded to the nearest cm (from Ernst and Barbour 1989)

Phalangeal numbers in boldface exceed plesiomorphic formula for turtles (2-3-3-3-3; Sánchez-Villagra et al. 2007b); numbers with asterisks exceed plesiomorphic condition for all amniotes (2-3-4-5-4; Cooper et al. 2007)

Technical remarks

The highest numbers of phalanges were recorded in trionyichid specimens examined with X-ray photographs and not in skeletonized specimens; this is likely due to the fact that terminal phalanges of non-clawed digits are so minute that they are easily lost during or after preparation. As already suggested by Jacobs (1941), who underlined that radiography is the most promising technique for properly assessing the topography of chelonian autopods, our experience indicates that application of X-raying offers the best results also for the evaluation of phalangeal formulae. However, in a few cases actually mutilated digits, lacking the terminal elements, may have been misinterpreted as being complete. Concerning fossils, it seems reasonable to predict that the highest phalangeal formulae may not be found, and that fully grown specimens of the largest species offer the best opportunity for retrieving the distal supernumerary phalanges.

Results

The examination of 190 trionyichid and 20 carettochelyid specimens confirmed that only the first three digits in the

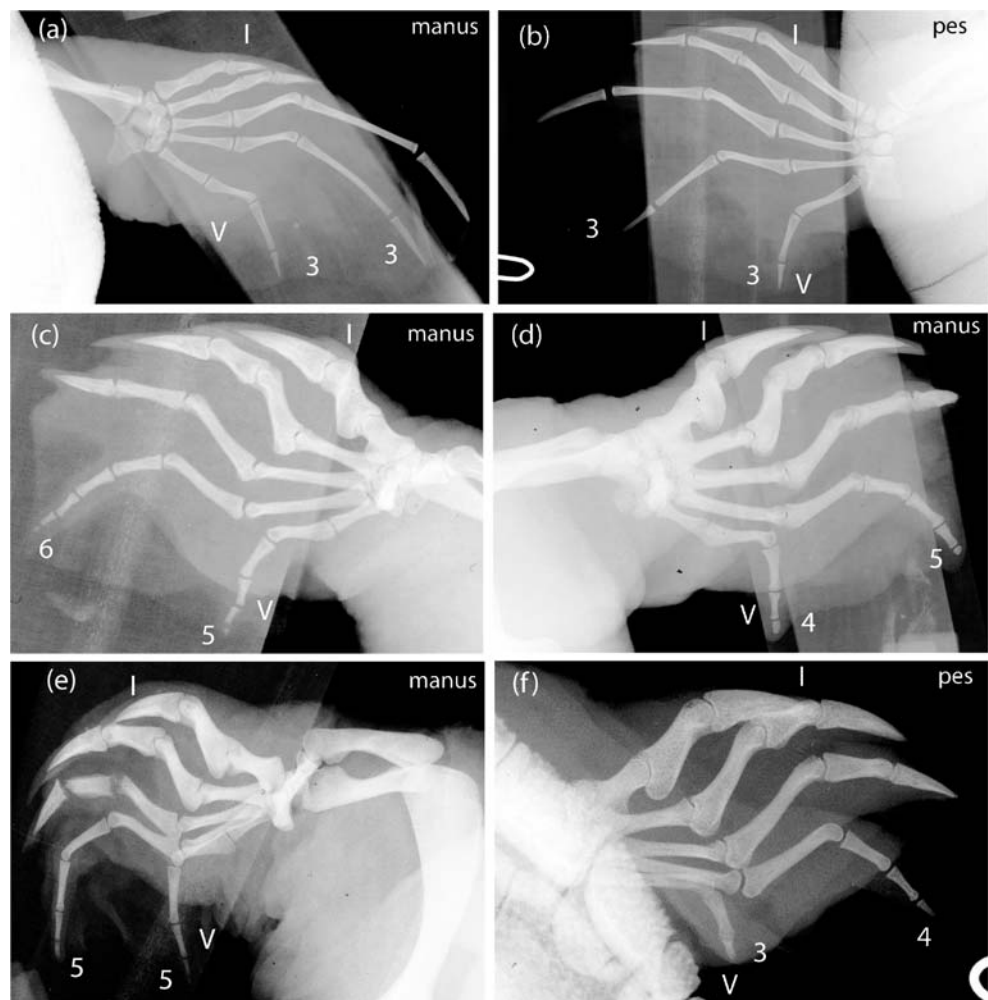
first group, and the first two digits in the second, bear claws on both the manus and pes. The phalanges of clawed digits are considerably thicker than those of non-clawed ones in trionyichids, whereas in carettochelyids no such difference is evident. In both groups and both limbs, the phalangeal formula of the clawed digits is constant: the first clawed digit always has two phalanges only, whereas the other clawed fingers have three phalanges each (however, a fourth phalanx was reported for digit III in *Apalone spinifera* Le Sueur by Sheil 2003; see Table 1). Relevant differences concern the non-clawed digits, so that the corresponding phalangeal formulae clearly differentiate carettochelyids and trionyichids. Carettochelyids have a lower number of phalanges and exhibit no variation in the adult phalangeal formula (manus 2-3-3-3-3, pes 2-3-3-3-3; Figs. 2 and 3), whereas trionyichids have a higher number of phalanges and exhibit intraspecific and interspecific ranges from 2-3-3-3-2 to 2-3-3-6-5 for the manus, and from 2-3-3-3-2 to 2-3-3-5-3 for the pes (Fig. 3; Table 2).

As shown in Table 2, the phalangeal numbers of carettochelyids can be exceeded in all trionyichid genera. Digits IV and V in the manus, and IV in the pes, can have up to 6, 5, and 5 phalanges, respectively; i.e. the range is greater in the manus than in the pes. The digit characterized by the highest number of phalanges is IV for both manus



Fig. 2 Radiograph of *Carettochelys insculpta* (MTD 31365) showing the typical phalangeal formula 2-3-3-3-3 for manus and pes of all 20 specimens studied. In this young turtle (straight shell length = 69 mm) the typical elongation of phalanges is already visible (in particular in digits III and IV)

Fig. 3 Hyperphalangy characterizes trionychid but not carettochelyid turtles. Adult *Carettochelys insculpta* (MTD 30761) show elongated phalanges and the formula 2-3-3-3-3 for both manus (a) and pes (b). In *Cyclanorbis senegalensis* the formula for the manus is 2-3-3-6-5 (c; MTD 44951) or 2-3-3-5-4 (d; MTD 39162). (e) Manus of *Pelodiscus sinensis* (MTD 30740), formula 2-3-3-5-5. The phalangeal formula for the pes is less variable; *Lissemys punctata* (MTD 32372) exhibits the formula most frequent in trionychids, 2-3-3-4-3 (f). Note that the trionychid phalanges of digits I and II are much more robust than those of the other digits, and that the terminal phalanges of digits IV and V can be very small. Roman numerals denote digits, Arabic numerals the number of phalanges of the labelled digit



and pes. In several species, the phalanges of digits IV and V of the manus outnumber those of the corresponding digits of the pes.

Figures 3 and 4 indicate a trend toward highest frequencies of extra phalanges in the largest X-rayed specimens (regardless of phylogenetic relationships), but at the same time (Figs. 5 and 6; Table 2) the highest numbers of phalanges were not recorded in species with a shell length surpassing 56 cm (but see Discussion). In *Pelodiscus sinensis* (Wiegmann), which in our sample is the species with the largest number of specimens (36), there is no clear variation connected to differences in ontogenetic stage (Fig. 7).

Discussion

The development of elongated digits has evolved differently in two sister clades of aquatic turtles: carettochelyid flippers only have elongated phalanges, whereas the paddle-shaped autopods of trionychids have an increased number of phalanges in some digits. The two groups also

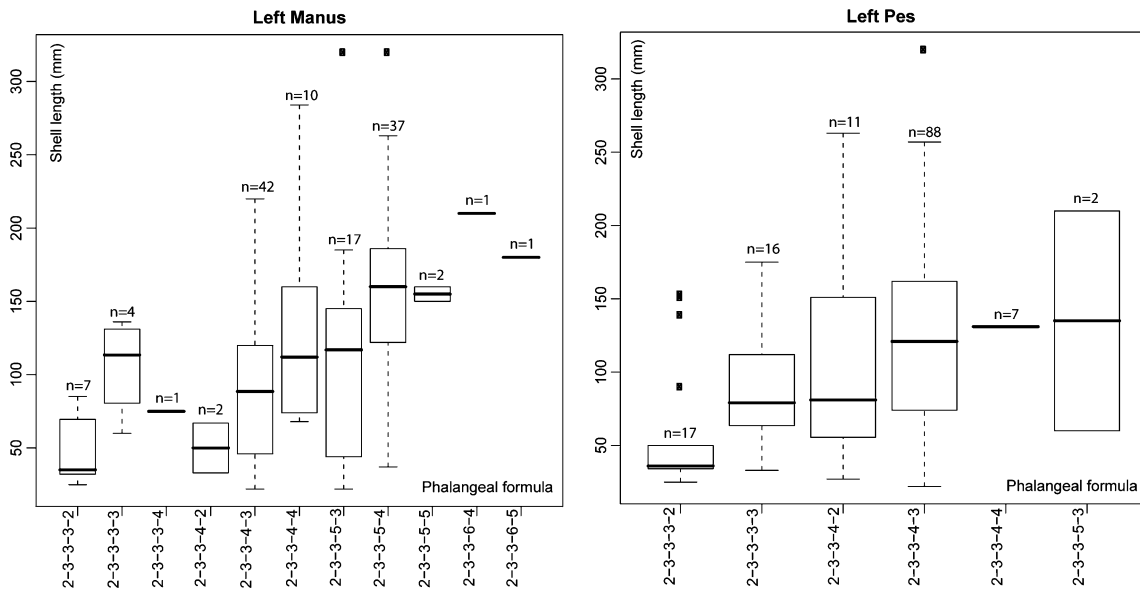


Fig. 4 Phalangeal formula versus straight shell length for trionychid specimens analyzed (all species lumped together; only specimens with known straight shell length considered; data for manus and pes from 120 and 141 specimens, respectively). Horizontal wide lines =

medians; boxes = interquartile ranges; broken lines = ranges; black squares = outliers. Note that formulae with few phalanges in digits IV and V do not occur among large-sized specimens, and the tendency for higher frequencies of extra phalanges at the highest shell lengths

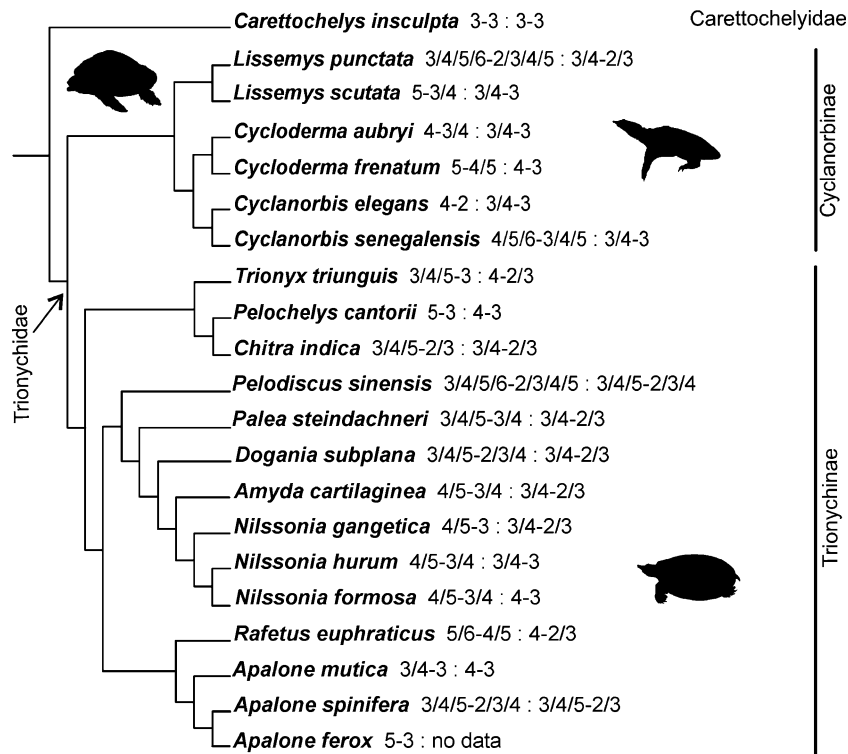


Fig. 5 Phalangeal formulae significantly differ between Carettochelyidae (here represented by the only extant species, *Carettochelys insculpta*) and its sister group, Trionychidae. However, it is impossible to detect clear differences between the two trionychid subclades,

Cyclanorbiinae and Trionychinae, or between individual species. Phalangeal formulae separated by colons (:) refer to the last two digits of the manus (left) and pes (right), respectively. Phylogenetic relationships follow Engstrom et al. (2004)

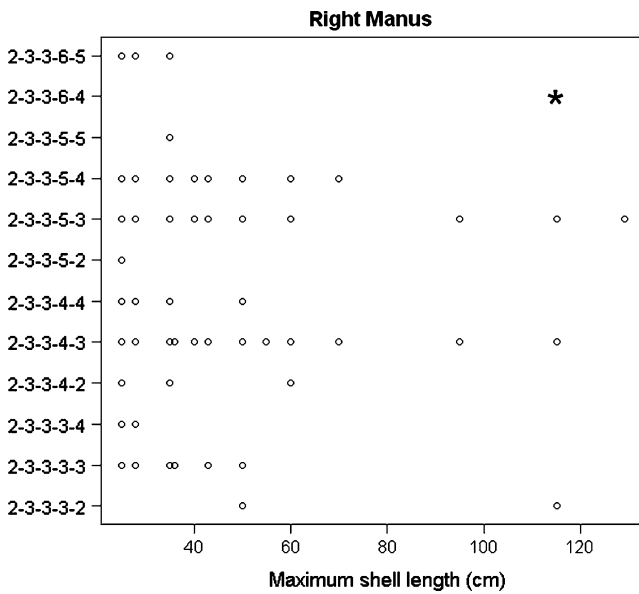


Fig. 6 Phalangeal formula versus maximum straight shell length for trionychid species analyzed. Circles indicate data collected in this study; asterisk, data from Walker (1973). Note that formulae with the highest numbers of phalanges are limited to small-sized species, probably reflecting the absence of fully grown specimens of large-sized species in our sample (see also text)

differ in the degree of intraspecific variation in phalangeal numbers exhibited: non-existent in carettochelyids versus extensive in trionychids. The evolution of hyperphalangy in vertebrates is rare; its occurrence in trionychids can be explored from a functionalist and structuralist perspective. We propose here an experimental model organism to study the mechanism of development in a derived autopod morphology.

Hyperphalangy in trionychids

According to Fedak and Hall (2004: 151), “hyperphalangy is a digit morphology in which increased numbers of phalanges are arranged linearly within a digit beyond the plesiomorphic condition.” This definition should be used with caution, as the ‘plesiomorphic clause’ makes it taxon-dependent. Thus, whereas trionychids do show hyperphalangy relative to turtles, many trionychid species would not show hyperphalangy relative to other trionychids. We do not present an alternative definition here, but would like to emphasize the importance of specifying the phylogenetic context of comparison.

Stem turtles reportedly had phalangeal formulae of 2-3-3-4-3 for the manus and 2-3-4-4-? for the pes in the oldest and most basal species, *Odontochelys semitestacea* Li et al. (Li et al. 2008), and a reduced formula of 2-2-2-2-2 in other Triassic taxa, *Proganochelys* Baur and *Palaeochersis* Rougier et al. (Gaffney 1990). The probably plesiomorphic phalangeal formula for crown turtles is 2-3-3-3-3 for both manus and pes (Sánchez-Villagra et al. 2007a). Therefore, extant

taxa with numbers of phalanges exceeding the formula 2-3-3-3-3 represent clear cases of hyperphalangy. Here, we report the widespread presence of hyperphalangy in Trionychidae. All examined trionychid genera and species show some form of hyperphalangy in both manus and pes (Table 2). The highest number of phalanges occurs in digit IV in both manus and pes, reflecting the lengthening of the autopod, including the skin membrane that follows the clawed digits. None of the trionychid species analyzed has a number of phalanges exceeding the formula 4-6-6-6-6, defined by Fedak and Hall (2004) as the threshold for extreme hyperphalangy, a condition known only among secondarily aquatic vertebrates with flipper limb morphology, such as cetaceans, plesiosaurs, and ichthyosaurs.

Possibly because of limitations in taxon sampling and/or specimen number, the literature reports only two cases of hyperphalangy in non-trionychid extant chelonians. Zug (1971) described the presence of a supplementary phalanx in digit V in the pes of *Dermatemys* Gray (Dermatemyidae; phalangeal formula 2-3-3-3-4; see also Shapiro et al. 2007). In *Pangshura smithii* (Gray) (Geoemydidae), an additional phalanx occurs sporadically in digit V of the pes (2-3-3-3-4; Ludwig et al. 2007). Neither dermatemydids nor geoemydids have any direct phylogenetic relations to carettochelyids and trionychids (Krenz et al. 2005).

Development of hyperphalangy, and the trionychid *Pelodiscus sinensis* as a model

Trionychids differ from other turtles in the way digits have become elongated throughout evolutionary history. In their

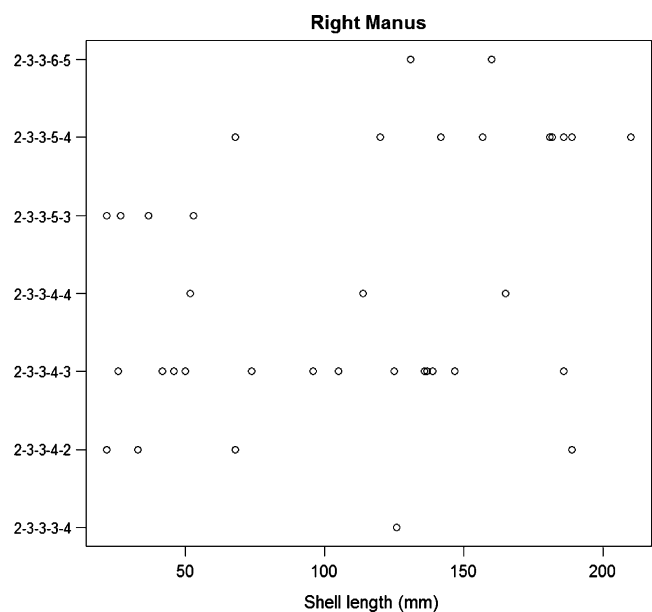


Fig. 7 Individual straight shell length versus phalangeal formula for the studied specimens of *Pelodiscus sinensis*

sister group, the carettochelyids, as well as in marine turtles (Cheloniidae, Dermochelyidae), the phalangeal formula is 2-3-3-3-3 (Bever and Joyce 2005; Wyneken 2001). Both, marine turtles and *Carettochelys insculpta*, have flipper-like limbs without any supernumerary phalanges, but have independently evolved particularly elongated phalanges. The autopods of trionychids are not larger (neither proportionally nor absolutely) than the flippers of marine turtles and *Carettochelys*. Thus, limb size alone cannot be the factor responsible for the evolution of supernumerary phalanges in digits IV and V of trionychids. The latter have just evolved a novel way to have a flipper.

The mechanism by which additional phalanges were gained in trionychids most likely is the prolongation of ancestral growth in digits IV and V, resulting in more phalanges, but not in longer ones. The ontogenetic development of phalanges in the Chinese soft-shelled turtle *Pelodiscus sinensis* provides some clues to understanding the developmental patterns and processes behind the variation in phalangeal formulae in trionychids. Data on autopod development in *P. sinensis* indicate that phalanges were added terminally, not by intercalation of previously formed phalangeal anlagen (Sánchez-Villagra et al. 2009). Hence, the mechanism is the same as in the dolphin flipper (Richardson and Oelschläger 2002).

The autopod anatomy of trionychids is consistent with the developmental model of Richardson et al. (2004) regarding the proximodistal patterning of the limb. In trionychids, as in other groups of vertebrates exhibiting hyperphalangy (cetaceans, ichthyosaurs, plesiosaurs), the distal elements are supernumerary and serially similar, whereas the number of proximal elements (carpals and tarsals, metapodials) remains largely the same due to a compartmentalization of spatial domains (Richardson et al. 2004: Fig. 2).

Bininda-Emonds et al. (2007) and Richardson et al. (2009) found simultaneous chondrogenesis of forelimbs versus hindlimbs in turtles, a pattern also recorded for *P. sinensis* (Sánchez-Villagra et al. 2009). If this is generally true for trionychids, then the higher number of phalanges in the manus relative to the pes must be the result of prolonged or accelerated growth in the former, rather than of an earlier onset of chondrogenesis.

The primary axis is present in *P. sinensis* development (Sánchez-Villagra et al. 2009). The anlagen of the structures leading to the skeleton of digit IV appear before those of the other digits. After the subsequent development of the digital arch, the digit anlagen are all present at a similar stage of chondrogenesis. Differential growth rate is responsible for the fact that digits I–III are much thicker than digits IV and V in later stages. There is a proximodistal gradient of differentiation and size of cartilaginous elements. As chondrification progresses, this gradient becomes more

visible in the alcian preparations in digits IV and V, whereas the first three digits become less differentiated proximodistally. When the phalanges ossify, the distal phalanx of digit I is the first to start ossification in manus and pes, and the first three digits are the earliest to ossify thereafter (Sánchez-Villagra et al. 2009).

It is a challenge for future studies to examine the molecular basis of phalangeal development in trionychids using *P. sinensis* as model. This species is farm-bred in high numbers for the Asian food market (van Dijk et al. 2000); thus, eggs are easily available. Studies by Nagashima et al. (2005, 2007) provide evidence that experimental manipulation of *P. sinensis* embryos is technically possible, underlining the species' value as a model. Of particular interest in this context are investigations by Nagashima et al. (2007). These authors cauterized the apical ectodermal ridge of the forelimb, resulting in a highly anomalous manus. The interdigital webbing in *P. sinensis* is another attractive object for studying the molecular basis of limb evolution. In bats and ducks, different gene expression mechanisms result in interdigital webbing (Sears 2008). *Pelodiscus sinensis* offers the opportunity to compare the underlying patterns for a third, distantly related clade.

Is there a correlation between phalangeal formulae and adult size?

For another chelonian group, the land tortoises (Testudinidae), Crumly and Sánchez-Villagra (2004) reported a correlation between phalangeal formulae and adult size in that phalangeal and digital loss was associated with small size. At first glance our data suggest a similar trend for trionychids, because formulae with low phalangeal numbers in digits IV and V were not recorded in the largest trionychid specimens (Fig. 4). On the other hand, hyperphalangy exceeding the plesiomorphic amniote condition (2-3-4-5-4; Cooper et al. 2007) seems to be restricted to the smaller species (Table 2). Moreover, phalangeal formulae were found to be more variable in small-sized than in larger species, which suggests ontogenetic variation (Fig. 6). Hence, for trionychids the hypothesis of a correlation between phalangeal numbers and adult size is very tentative. Our data could be biased by the limited number of fully adult specimens of large-sized species. For instance, for the large-bodied *Chitra indica* Gray (maximum shell length 115 cm; Ernst and Barbour 1989) only hatchlings could be studied, whose phalangeal formulae most probably do not represent the adult condition. The suspicion of sampling bias is increased by Walker (1973) having reported six phalanges in digit IV of the manus of *Chitra* sp. (Fig. 6). It seems more likely that maximum body size and phalangeal formula are not correlated in trionychids. This is supported by the lack of correlation between shell length and phalangeal formula in

Pelodiscus sinensis, the best-sampled species in our study (Fig. 7).

Phalangeal formulae in fossil trionychids and carettochelyids

Despite the rich fossil record of trionychids and carettochelyids with many extinct species (de Lapparent de Broin 2001), only limited information is available about their phalangeal formulae. Usually, fossils are preserved as shells or shell fragments. Connected autopod elements, allowing the determination of phalangeal formulae, persist only exceptionally.

The few data available for fossil carettochelyids indicate that the phalangeal formulae of *Allaeochelys crassesculpta* Harrassowitz from the Middle Eocene of Germany closely resembled those of *Carettochelys insculpta*. According to the description of *A. crassesculpta* (Harrassowitz 1922), the two taxa show the same phalangeal formula for the manus. Moreover, both have two clawed digits in the pes (the complete phalangeal formula for the pes of *A. crassesculpta* is unknown due to poor preservation). The phalanges of *A. crassesculpta* are elongated as in extant *C. insculpta* (Figs. 2 and 3a, b; see also Walther 1922: pls. 11, 12). For the fossil trionychid “*Aspideretes*” *singularis* Hay (Middle Palaeocene of New Mexico, USA), the nearly complete forelimb is known (Hay 1908). Its phalangeal formula 2-3-3-?-4? indicates the possible presence of at least one supernumerary phalanx in digit V. These observations provide evidence for the occurrence of elongated phalanges in carettochelyids at least since the Middle Eocene, and of potentially supernumerary phalanges in trionychids at least since the Middle Palaeocene.

Functional considerations

Trionychids, carettochelyids, and marine turtles (Cheloniidae, Dermochelyidae) are the only chelonians in which the manus is larger than the pes (Jacobs 1941)—undoubtedly a character state directly related to aquatic locomotion. While carettochelyids and marine turtles independently developed long flippers with elongated phalanges as underlying bony structures, trionychids used another evolutionary avenue. Their autopods are unique among extant chelonians in having a broad, paddle-like shape, with an enlarged palm and digits connected by extensive webbing. In trionychids there are only three strong claws; elongation of claw-less digits, considerably contributing to the enlargement of the paddle, is achieved not by phalangeal elongation, but by supernumerary phalanges. Cooper et al. (2007; and references therein) suggested that in cetaceans the elongated flippers are associated with high-speed swimming, whereas the larger surface area of broad flippers or paddles is suitable for low-speed turns in shallow water.

However, it is well-known that both trionychids (Meylan 1987; Webb 1962; Zug 1971) and *Carettochelys* (Cann 1998) are powerful swimmers ranking among the fastest freshwater turtles, so that a perfect parallel to marine mammals cannot be drawn. Like in marine turtles, the foreflippers of *Carettochelys* are used for ‘flying’ in the water by simultaneous up-and-down movements (Ernst and Barbour 1989); the hindlegs serve as rudders. By contrast, the paddle-like extremities of trionychids, associated with larger surface relative to *Carettochelys*, are used for alternating, crosswise powerstrokes of forelimb and opposite hind limb (authors’ observ.). We hypothesize that this locomotion type, together with the morphology of trionychid extremities, is well-suited for rapid turns, an ability highly useful when hunting fast prey, including crayfish and fish (Ernst and Barbour 1989). Regarding the mainly herbivorous *Carettochelys* (Cann 1998), it seems reasonable to assume that it uses high-speed swimming to escape predators (Cann 1998). We conclude that the highly different autopod morphologies of carettochelyids and trionychids reflect not only differences in locomotor patterns, but also in natural histories. The evolution of hyperphalangy in digits IV and V in trionychids to achieve paddle-like autopods is novel within turtles and apparently did not take place via simple prolongation of growth associated with an increase in size. However, it remains unclear what factors and pressures have enabled trionychids to override their ancestral developmental programming to evolve the comparatively rare strategy of hyperphalangy to achieve an elongated limb, especially when other solutions to this end exist in other turtle groups.

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References

- Alberch, P., & Gale, E. A. (1983). Size dependence during the development of the amphibian foot. Colchicine-induced digital loss and reduction. *Journal of Embryology and Experimental Morphology*, 76, 177–197.
- Auffenberg, W. (1966). The carpus of land tortoises. *Bulletin of the Florida State Museum*, 10, 159–191.
- Baur, G. (1892). Der Carpus der Schildkröten. *Anatomischer Anzeiger*, 7, 206–211.

- Bever, G. S., & Joyce, W. G. (2005). Dermochelyidae—Leder-schildkröten. In U. Fritz (Ed.), *Handbuch der Reptilien und Amphibien Europas. Band 3/IIIB: Schildkröten (Testudines) II* (pp. 235–248). Wiebelsheim: Aula-Verlag.
- Bininda-Emonds, O. R. P., Jeffery, J. E., Sánchez-Villagra, M. R., Hanken, J., Colbert, M., Pieau, C., et al. (2007). Fore limb-hind limb developmental timing across tetrapods. *BMC Evolutionary Biology*, 7/182. doi:10.1186/1471-2148-7-182.
- Blob, R. W., Rivera, A. R. V., & Westnut, M. W. (2008). Hindlimb function in turtle locomotion: Limb movements and muscular activation across taxa, environment, and ontogeny. In J. Wyneken, V. Bels, & M. H. Godfrey (Eds.), *Biology of turtles* (pp. 121–144). Boca Raton: CRC.
- Bramble, D. M. (1982). *Scaptochelys*: generic revision and evolution of gopher tortoises. *Copeia*, 4, 853–866.
- Burke, A. C., & Alberch, P. (1985). The development and homology of the chelonian carpus and tarsus. *Journal of Morphology*, 186, 119–131.
- Cann, J. (1998). *Australian freshwater turtles*. Singapore: John Cann and Beaumont Publishing Pte Ltd.
- Cooper, L. N., & Dawson, S. D. (2009). The trouble with flippers: a report on the prevalence of digital anomalies in Cetacea. *Zoological Journal of the Linnean Society*, 155, 722–735.
- Cooper, L. N., Berta, A., Dawson, S. D., & Reidenberg, J. S. (2007). Evolution of hyperphalangy and digit reduction in the cetacean manus. *Anatomical Record*, 290, 654–672.
- Crumly, C. R., & Sánchez-Villagra, M. R. (2004). Patterns of variation in the phalangeal formulae of land tortoises (Testudinidae): developmental constraint imposed by size and phylogenetic history. *Journal of Experimental Zoology Part B, Molecular and Developmental Evolution*, 302, 134–146.
- Danilov, I. G. (2005). Die fossilen Schildkröten Europas. In U. Fritz (Ed.), *Handbuch der Reptilien und Amphibien Europas. Band 3/IIIB: Schildkröten (Testudines) II* (pp. 329–441). Wiebelsheim: Aula-Verlag.
- de Lapparent de Broin, F. (2001). The European turtle fauna from the Triassic to the present. *Dumerilia*, 4, 155–217.
- Delfino, M., Scheyer, T. M., Fritz, U., & Sánchez-Villagra, M. R. (2010). An integrative approach to examining a homology question: shell structures in soft-shell turtles. *Biological Journal of the Linnean Society*, 55, 462–476.
- Engstrom, T. N., Shaffer, H. B., & McCord, W. P. (2004). Multiple data sets, high homoplasy, and the phylogeny of softshell turtles (Testudines: Trionychidae). *Systematic Biology*, 53, 693–710.
- Ernst, C. H., & Barbour, R. W. (1989). *Turtles of the world*. Washington: Smithsonian Institution Press.
- Fabrezi, M., Manzano, A., Abdala, V., & Zaher, H. (2009). Developmental basis of limb homology in pleurodiran turtles, and the identity of the hooked element in the chelonian tarsus. *Zoological Journal of the Linnean Society*, 155, 845–866.
- Fedak, T. J., & Hall, B. K. (2004). Perspectives on hyperphalangy: patterns and processes. *Journal of Anatomy*, 204, 151–163.
- Fritz, U., & Havaš, P. (2007). Checklist of chelonians of the world. *Vertebrate Zoology*, 57, 149–368.
- Fritz, U., Petzold, A., & Auer, M. (2006). Osteology in the *Cuora galbinifrons* complex suggests conspecificity of *C. bourreti* and *C. galbinifrons*, with notes on shell osteology and phalangeal formulae within the Geoemydidae. *Amphibia-Reptilia*, 27, 195–205.
- Fröbisch, N. B. (2008). Ossification patterns in the tetrapod limb—conservation and divergence from morphogenetic events. *Biological Reviews*, 83, 571–600.
- Fujita, M. K., Engstrom, T. N., Starkey, D. E., & Shaffer, H. B. (2004). Turtle phylogeny: insights from a novel nuclear intron. *Molecular Phylogenetics and Evolution*, 31, 1031–1040.
- Gaffney, E. S. (1990). The comparative osteology of the Triassic turtle *Proganochelys*. *Bulletin of the American Museum of Natural History*, 194, 1–263.
- Gaffney, E. S., & Bartholomai, A. (1979). Fossil trionychids of Australia. *Journal of Paleontology*, 53, 1354–1360.
- Gaffney, E. S., & Meylan, P. A. (1988). A phylogeny of turtles. In M. J. Benton (Ed.), *The phylogeny and classification of the tetrapods, vol. 1* (pp. 157–219). Oxford: Clarendon.
- Gilbert, F. S. (2006). *Developmental biology* (8th ed.). Sunderland: Sinauer Associates.
- Harrassowitz, H. L. P. (1922). Die Schildkrötengattung *Anosteira* von Messel bei Darmstadt und ihre stammesgeschichtliche Bedeutung. *Abhandlungen der Hessischen Geologischen Landesanstalt*, 6, 137–238.
- Hay, O. P. (1908). The fossil turtles of North America. *Carnegie Institution of Washington Publication*, 75, i–iv. + 1–568.
- Head, J. J., Sánchez-Villagra, M. R., & Aguilera, O. (2006). Past colonization of South America by trionychid turtles: fossil evidence from the Neogene of Margarita Island, Venezuela. *Journal of Herpetology*, 40, 378–381.
- Hitschfeld, E., Auer, M., & Fritz, U. (2008). Phalangeal formulae and ontogenetic variation of carpal morphology in *Testudo horsfieldii* and *T. hermanni*. *Amphibia-Reptilia*, 29, 93–99.
- Jacobs, W. (1941). Studien an Wasserschildkröten; Beziehungen zwischen Körperbau und Bewegungsweise. *Zoomorphology*, 38, 118–146.
- Krenz, J. G., Naylor, G. J. P., Shaffer, H. B., & Janzen, F. G. (2005). Molecular phylogenetics and evolution of turtles. *Molecular Phylogenetics and Evolution*, 37, 78–191.
- Li, C., Wu, X. C., Rieppel, O., Wang, L. T., & Zhao, L. J. (2008). An ancestral turtle from the Late Triassic of southwestern China. *Nature*, 456, 497–501.
- Ludwig, M., Auer, M., & Fritz, U. (2007). Phalangeal formulae of geoemydid terrapins (*Batagur*, *Callagur*, *Hardella*, *Heosemys*, *Kachuga*, *Orlitia*, *Pangshura*, *Rhinoclemmys*) reflect distinct modes of life. *Amphibia-Reptilia*, 28, 574–576.
- Meylan, P. A. (1987). The phylogenetic relationships of soft-shelled turtles (Family Trionychidae). *Bulletin of the American Museum of Natural History*, 186, 1–101.
- Meylan, P. A., & Gaffney, E. S. (1992). *Sinaspideretes* is not the oldest trionychid turtle. *Journal of Vertebrate Paleontology*, 12, 257–259.
- Nagashima, H., Uchida, K., Yamamoto, K., Kuraku, S., Usuda, R., & Kuratani, S. (2005). Turtle-chicken chimera: an experimental approach to understanding evolutionary innovation in the turtle. *Developmental Dynamics*, 232, 149–161.
- Nagashima, H., Kuraku, S., Uchida, K., Kawashima, K., Ohya, Y., Narita, Y., et al. (2007). On the carapacial ridge in turtle embryos: its developmental origin, function and the chelonian body plan. *Development*, 134, 2219–2226.
- Nessov, L. A. (1995). On some Mesozoic turtles of the Fergana Depression (Kyrgyzstan) and Dzhungar Alatau Ridge (Kazakhstan). *Russian Journal of Herpetology*, 2, 134–141.
- Ogushi, K. (1911). Anatomische Studien an der japanischen dreikralligen Lippenschildkröte (*Trionyx japonicus*). I. Mitteilung. *Morphologisches Jahrbuch*, 43, 1–106.
- Padian, K. (1992). A proposal to standardize tetrapod phalangeal formula designations. *Journal of Vertebrate Paleontology*, 12, 260–262.
- Praschag, P., Hundsdoerfer, A. K., Reza, A. H. M. A., & Fritz, U. (2007). Genetic evidence for wild-living *Aspideretes nigricans* and a molecular phylogeny of South Asian softshell turtles (Reptilia: Trionychidae: *Aspideretes*, *Nilssonina*). *Zoologica Scripta*, 36, 301–310.
- Pritchard, P. C. H. (2001). Observations on body size, sympatry, and niche divergence in softshell turtles (Trionychidae). *Chelonian Conservation and Biology*, 4, 5–27.
- Rabl, C. (1910). *Bausteine zu einer Theorie der Extremitäten der Wirbeltiere*. Leipzig: Engelmann.

- Renous, S., de Lapparent de Broin, F., Depecker, M., Davenport, J., & Bels, V. (2008). Evolution of locomotion in aquatic turtles. In J. Wyneken, V. Bels, & M. H. Godfrey (Eds.), *Biology of turtles* (pp. 97–138). Boca Raton: CRC.
- Richardson, M. K., & Chipman, A. D. (2003). Developmental constraints in a comparative framework: a test case using variations in phalanx number during amniote evolution. *Journal of Experimental Zoology Part B, Molecular and Developmental Evolution*, 296, 8–22.
- Richardson, M. K., & Oelschläger, H. A. (2002). Time, pattern and heterochrony: a study of hyperphalangy in the dolphin embryo flipper. *Evolution and Development*, 4, 435–444.
- Richardson, M. K., Jeffery, J. E., & Tabin, C. J. (2004). Proximodistal patterning of the limb: insights from evolutionary morphology. *Evolution and Development*, 6, 1–5.
- Richardson, M. K., Gobes, S., van Leeuwen, A., Poelman, A., Pieau, C., & Sánchez-Villagra, M. R. (2009). Heterochrony in limb evolution: developmental mechanisms and natural selection. *Journal of Experimental Zoology Part B, Molecular and Developmental Evolution*, 312, 639–664.
- Rieppel, O. (1993). Studies on skeleton formation in reptiles: patterns of ossification in the skeleton of *Chelydra serpentina* (Reptilia, Testudines). *Journal of Zoology*, 231, 487–509.
- Rosenberg, E. (1892). Über einige Entwicklungsstadien des Handskeletts der *Emys lutaria* Marsili. *Morphologisches Jahrbuch*, 18, 1–14.
- Sánchez-Villagra, M. R., Mitgutsch, C., Nagashima, H., & Kuratani, S. (2007). Autopodial development in the sea turtles *Chelonia mydas* and *Caretta caretta*. *Zoological Science*, 24, 257–263.
- Sánchez-Villagra, M. R., Winkler, J. D., & Wurst, L. (2007). Autopodial skeleton evolution in side-necked turtles (Pleurodira). *Acta Zoologica*, 88, 199–209.
- Sánchez-Villagra, M. R., Müller, H., Scheyer, T. M., Sheil, C. A., Nagashima, H., & Kuratani, S. (2009). Skeletal Development in the Chinese soft-shelled turtle *Pelodiscus sinensis* (Reptilia: Testudines: Trionychidae). *Journal of Morphology*, 270, 1381–1399.
- Scheyer, T. M., Sander, P. M., Joyce, W. G., Böhme, W., & Witzel, U. (2007). A plywood structure in the shell of fossil and living soft-shelled turtles (Trionychidae) and its evolutionary implications. *Organisms, Diversity and Evolution*, 7, 136–144.
- Sears, K. E. (2008). Molecular determinants of bat wing development. *Cells Tissues Organs*, 187, 6–12.
- Shaffer, H. B., Meylan, P., & McKnight, M. L. (1997). Tests of turtle phylogeny: molecular, morphological and paleontological approaches. *Systematic Biology*, 46, 235–268.
- Shapiro, M. D., Shubin, N. H., & Downs, J. P. (2007). Limb diversity and digit reduction in reptilian evolution. In B. K. Hall (Ed.), *Fins into limbs: evolution, development, and transformation* (pp. 225–245). Chicago: University of Chicago Press.
- Sheil, C. A. (2003). Osteology and skeletal development of *Apalone spinifer* (Reptilia: Testudines: Trionychidae). *Journal of Morphology*, 256, 42–78.
- Sheil, C. A., & Portik, D. (2008). Formation and ossification of limb elements in *Trachemys scripta* and a discussion of autopodial elements in turtles. *Zoological Science*, 25, 622–641.
- Stern, D. L. (2000). Evolutionary developmental biology and the problem of variation. *Evolution*, 54, 1079–1091.
- Tang, Y. (1997). Research on a new species of *Pelodiscus*, Trionychidae in China. *Zoological Research*, 18, 13–17 [in Chinese, with English abstract].
- van Dijk, P. P., Stuart, B. L., & Rhodin, A. G. J. (Eds.) (2000). Asian turtle trade. Proceedings of a workshop on conservation and trade of freshwater turtles and tortoises in Asia. Phnom Penh, Cambodia, 1–4 December 1999. *Chelonian Research Monographs*, 2, 1–64.
- Wagner, G. P., & Larsson, H. C. E. (2007). Fins and limbs in the study of evolutionary novelties. In B. K. Hall (Ed.), *Fins into limbs: Evolution, development, and transformation* (pp. 49–61). Chicago: University of Chicago Press.
- Walker, W. F. (1973). The locomotor apparatus of Testudines. In C. Gans & T. S. Parsons (Eds.), *Biology of the Reptilia*, vol. 3, *Morphology* (pp. 1–99). London: Academic.
- Walther, W. G. (1922). Die Neu-Guinea-Schildkröte *Carettochelys insculpta* Ramsay. *Nova-Guinea*, 13, 607–704.
- Webb, R. G. (1962). North American recent soft-shelled turtles (family Trionychidae). *University of Kansas Publications in Natural History*, 13, 429–611.
- Wood, R. C., & Patterson, P. (1973). A fossil trionychid turtle from South America. *Breviora*, 405, 1–10.
- Wyneken, J. (2001). *The anatomy of sea turtles*. Seattle, WA: U.S. Department of Commerce NOAA Technical Memorandum NMFS-SEFSC-470.
- Zug, G. R. (1971). Buoyancy, locomotion, morphology of the pelvic girdle and hindlimb, and systematics of cryptodiran turtles. *Miscellaneous Publications, Museum of Zoology, University of Michigan*, 142, 1–98.