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Normal Embryonic Stages of the Chinese Softshelled Turtle *Pelodiscus sinensis* (Trionychidae)

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ABSTRACT—We describe the normal embryonic development of the Chinese soft-shelled turtle, *Pelodiscus sinensis*. We identified 23 stages between the late neurula and hatching, based on chronology and the external morphology of embryos. The anlage of the sensory organs appeared in early stages of the pharyngula, followed by limb buds. The lateral ridge of the carapace, or the carapacial ridge, could be recognized by two weeks of incubation, and the basal structure of the shell was complete by the mid stage (24 days) of development. The hatchling was well pigmented, and the yolk had been entirely absorbed. The developmental stages of *P. sinensis* were comparable with those of other turtle species. The developmental pattern of *P. sinensis* resembled basal taxa rather than more derived groups. Some peculiarities were recognized in the development of *P. sinensis*, including the appearance of cutaneous papillae on the carapace instead of horny scutes. Those features are shared by *Carettochelys*, possibly the species taxonomically closest to the Trionychids.

INTRODUCTION

The embryology of turtles has been studied for over 150 years. Pioneers in this field focused on the early embryonic stages, where gastrulation and neurulation were the main concern, and there has been little work done to establish the normal series of embryonic development. Yntema (1968) was the first to provide a table of normal embryonic stages for the common snapping turtle, *Chelydra serpentina*. Subsequently, emydids (freshwater turtles), cheloniids (marine turtles), dermochelyids (leatherback turtles) and testudinids (land tortoises) were also investigated (Mahmoud *et al.*, 1973; Crasz, 1982; Miller, 1985; Renous *et al.*, 1989; Guyot *et al.*, 1994). A recent rough description of embryonic development of the pig-nosed turtle, possibly the taxonomically closest family to the Trionychidae, was conducted by Beggs *et al.* (2000). We report here normal chelonian development for a trionychid species, *Pelodiscus sinensis*.

The family Trionychidae, which belongs to the suborder Cryptodira and comprises about 250 named species, is one of the most unusual groups among turtles (Prichard, 1979; Ernst and Barbour, 1989). Members of this family are aquatic and widely distributed worldwide. Phylogenetic position of Trionychidae is still a matter of controversy: analyses based on molecular and morphological data are inconsistent (Gaffney, 1984; Shaffer *et al.*, 1997). Recent molecular data,

however, are inclined to conclude that this family belongs to a basal group of the cryptodirans (Shaffer *et al.*, 1997). Trionychids characteristically possess a long and retractile neck as well as three claws on each paddle-like limb, as implied by their family name. They are distinguished from other chelonian families by their unique carapace, which is covered by leathery skin instead of horny scutes. It is generally accepted that Trionychidae are closely related to Carettochelyidae which also lacks horny scutes on the shell. The carapace-plastron complex of one trionychid species, *Trionyx ferox*, has been shown to contain α -keratin. This distinguishes it from many other families, in which only hard β -keratin is detected in the carapace (Alexander, 1969; Baden and Maderson, 1970; Spearman, 1969). Despite these unique features, little is known about trionychid embryonic development, which may differ from other chelonian species.

Over a century ago, Mitsukuri and Ishikawa (1886) investigated the formation of the germinal layer of *Trionyx japonicus*, a synonym for *Pelodiscus sinensis* studied here. Unfortunately, however, they neglected to summarize the embryonic development. Ogushi (1911, 1913) also carried out a detailed anatomy of adult *T. japonicus*, including skeletal, muscular, and nervous systems, but also neglected embryonic development. The present study is intended to fill the gap that spans the late neurula and hatching periods. We describe below 23 embryonic stages of *Pelodiscus sinensis*, a common trionychid of East Asia. We also compare the chronological developmental pattern of this species with those of selected members belonging to the cryptodiran family, including the Chelydridae, Cheloniidae, Emydidae, and especially

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the Pig-nosed turtle, *Carettochelys insculpta*, to elucidate some possible evolutionary trends in turtle development.

MATERIALS AND METHODS

Fertilized eggs of turtles were purchased from a private hatchery in Maisaka, Shizuoka, Japan, during July and August 2000. The eggs were brought to the laboratory and were embedded in styrene foam boxes filled with wood scraps to which moisture was occasionally added. The eggs were incubated at a constant temperature of $30 \pm 2^\circ\text{C}$, reflecting the condition of the hatchery. Embryos usually hatch in 45 to 50 days at this temperature.

Embryos were excised with forceps from the surrounding extra-embryonic membranes in Petri dishes filled with 0.1 M phosphate-buffered saline (pH 7.4). Before fixation, the eggs were weighed and their diameters measured. For all the embryonic stages, fixation of embryos was performed with Bouin's fixative. Ten percent formalin in distilled water was also used for the observation of pigmentation patterns.

Body sizes of 67 fixed specimens were measured. The total body length was measured for the first two stages, and the crown-rump length (CRL) for stages 3 through 17. Thereafter, the anteroposterior length of the carapace was measured, since the actual CRL becomes obscured by retraction of the neck under the carapace.

Photographs of early embryos (stages 5 through 17) and partial morphological characters were recorded with a digital camera (OLYMPUS HC-300Z/OL) mounted on a dissection microscope (OLYMPUS SZX12). For embryonic stages 18 through 27, photographs were taken with a digital still camera (SONY DSC-S70).

RESULTS

We established 23 discrete developmental stages (stages 5 through to 27) in *P. sinensis* specimens, between the late neurula and the hatching. Our staging table begins at stage 5 for two reasons. First, embryonic materials were not available to describe early stages of development, especially from cleavage to gastrulation. Second, our earliest embryo was comparable to the stage 5 *Chelydra serpentina* embryo described by Yntema (1968); Overall morphology of these embryos resembled greatly, and especially their neural folds have developed to the equivalent state of neurulation.

Chronology was primarily used for staging, although external morphological characteristics were also taken into consideration. The times between stages were as follows: one day intervals during the first five stages, two days for stages 6 through 15, and three days for stages 16 to 27, except for the final stage in which the interval was five days. Principal diagnostic features were after Yntema (1968), Mahmoud *et al.* (1973) and Miller (1985), including development of the sensory organs, formation of pharyngeal arches, numbers of pairs of somites, and growth of appendages.

The oval eggs of *Pelodiscus sinensis* weighed between 3.33 and 6.17 g (mean 4.69 g), and measured between 17.6 and 22.15 mm in diameter (mean 20.01 mm), based on 143 eggs. Lengths of embryos during the period of incubation are summarized in Fig. 1. Although the rate of growth was relatively slow for the early stages, or the first 10 days of incubation, it accelerated toward hatching thereafter. As far as the carapace growth was concerned, the rate of growth was some-

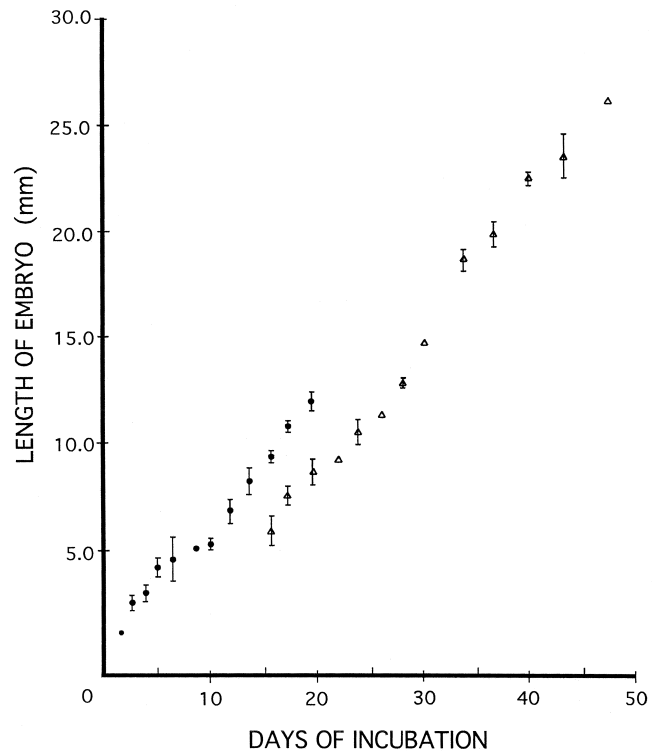


Fig. 1. Lengths of embryos at various stages of incubation. Dots, length of embryos; triangles, lengths of carapace. Each measurement is the mean from at least two different embryos. The standard deviation for each period is also indicated when more than three measurements are available. Note the slow rate of growth at early stages and rapid growth at later stages. As far as the carapace was concerned, the growth rate was slightly sigmoidal.

what sigmoidal (Fig. 1.).

Stage 5 (2 days; 4.2% of incubation period; Fig. 2 A, B)

Approximately three to four pairs of somites are visible. The head fold is conspicuous and covered with the chorio-amniotic membrane. The anterior intestinal portal gapes beneath the head fold.

Stage 6 (3 days; 6.3%; Fig. 2 C, D)

Five pairs of somites are visible. Neural folds are open only at the anterior and posterior ends.

Stage 7 (3.5 days; 7.3%; Fig. 2 E, F, G)

Seven pairs of somites are visible. The optic vesicle is slightly recognized. The heart primordium has appeared as a small bulging on the ventral surface. The anterior neuropore remains. The anterior portion of the head bends ventrally at a right angle due to the cephalic flexure.

Stage 8 (4 days; 8.3%; Fig. 3 A, B)

Ten pairs of somites are visible. The optic vesicle is clearly formed. The otic pit has appeared on the dorsolateral surface of the head. Cervical flexure is apparent. The mandibular arch is evident in the frontal view. The first pharyngeal groove appears faintly. The atrium and ventricle are distinct in the

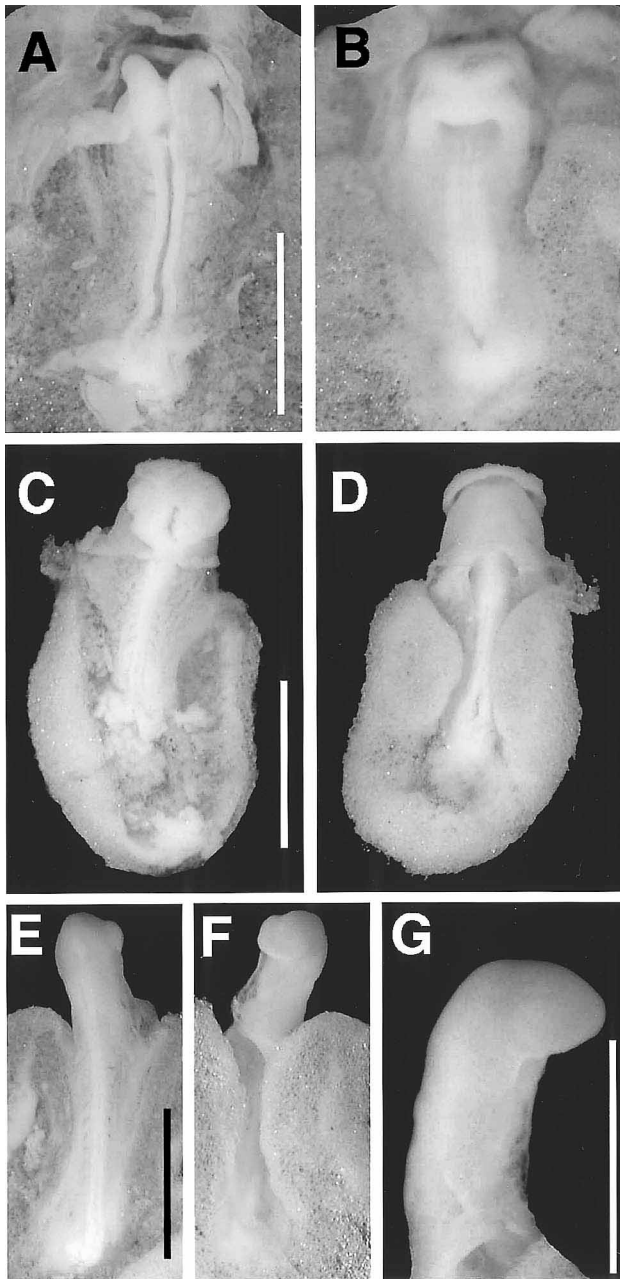


Fig. 2. Photographs of embryonic stages of *Pelodiscus sinensis*: **A** and **B**, dorsal and ventral view of stage 5, respectively; **C** and **D**, stage 6; **E** and **F**, stage 7; **G**, lateral view of head of stage 7. Note the progressive development of the head process. Scale bar=1 mm.

S-shaped heart primordium. The neural tube is closed anteriorly. The lateral body fold is visible.

Stage 9 (5 days; 10.4%; Fig. 3 C, D)

Fourteen pairs of somites are visible. The mandibular arch is obvious on the lateral aspect. The first pharyngeal cleft is open and the second pharyngeal groove is distinct. The lens placode is visible as a small ectodermal pit. A small tail process has formed.

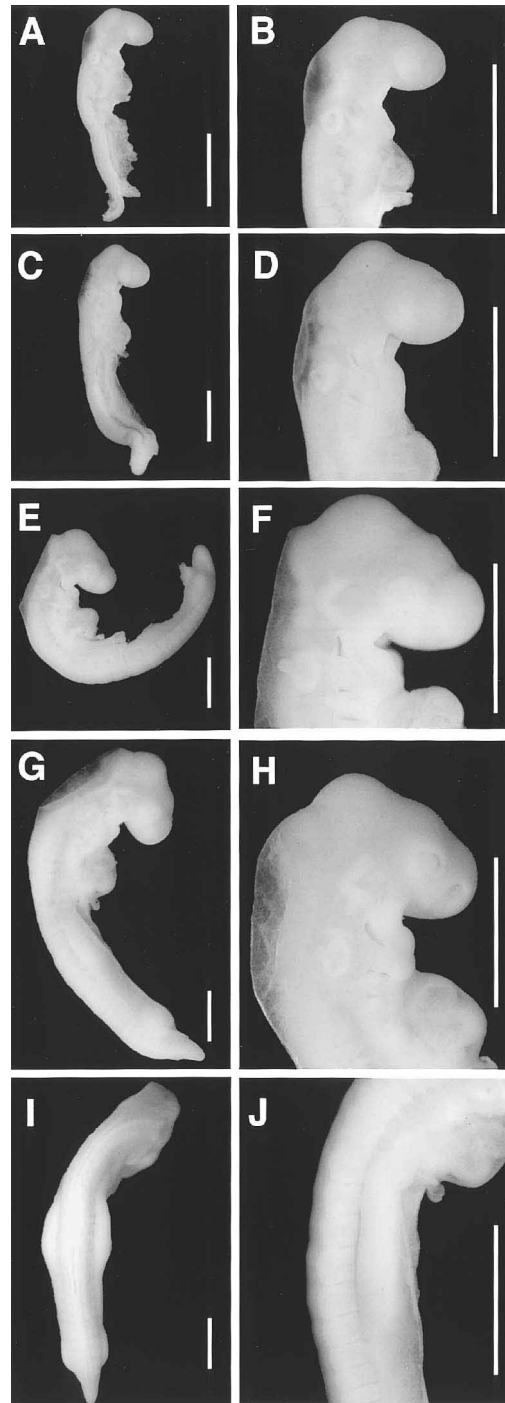


Fig. 3. Photographs of embryonic stages of *Pelodiscus sinensis*: **A** and **B**, lateral view and head region of stage 8, respectively; **C** and **D**, stage 9; **E** and **F**, stage 10; **G** and **H**, stage 11; **I**, dorsal view of stage 11; **J**, lateral view of right forelimb region of stage 11. Note the remarkable development of the sensory organs on the head and pharyngeal arches. Limb buds and a tail bud start to dilate at stage 10 and 9, respectively. Scale bar=1 mm.

Stage 10 (6 days; 12.5%; Fig. 3 E, F)

Twenty-two pairs of somites are visible. The mandibular and hyoid arches are distinct, as well as the first and second pharyngeal slits. The third and fourth pharyngeal grooves have appeared. The otic vesicle is present. The choroid fissure has

become apparent. The dorsal flexure is conspicuous. The contour of the lens is clear. The olfactory pit is faintly seen on the ventrolateral surface of the head. The forelimb bud has appeared as an indistinct bulging lateral to the sixth to thirteenth somites. The posterior intestinal portal is obvious.

Stage 11 (8 days; 18.8%; Fig. 3 G, H, I, J)

Twenty-seven pairs of somites are visible. The fourth pharyngeal arch is distinct. The first to third pharyngeal clefts are open whereas the fourth remains as a groove. The nasal pit has deepened and extends caudally. The anterior edge of the maxillary process has grown to the level of the posterior margin of the eye. The tail is prominent and has three to four somites at its base. The base of the forelimb bud is as long as five to six somites in length. The hindlimb bud dilates laterally as a slight bulge that extends three to four somites wide.

Stage 12 (10 days; 20.8%; Fig. 4 A, B)

Thirty pairs of somites are visible. Curvature of the cervical, dorsal and sacral flexures has increased. First, second and third pharyngeal slits are present. The maxillary process extends rostrally as far as the level of the choroid fissure. The dorsal part of the retina is pigmented. The nasal groove extends posteriorly beyond the anterior margin of the lens. The distance between the intestinal portals is approximately one-third of CRL. The forelimb bud has become thicker proxi-

mally, and is still longer rostrocaudally than proximodistally. The apical ectodermal ridge (AER) is slightly developed on the forelimb bud. Eight to nine somites are visible in the basal part of the tail.

Stage 13 (12 days; 25.0%; Fig. 4 C, D)

Somite numbers are hard to count from this stage onward and will not be specified hereafter. The third pharyngeal arch is covered with the hyoid arch laterally. The dorsal half of the first pharyngeal cleft is closed. The third and fourth slits are open and the fifth groove is first recognized. The maxillary process extends anteriorly beyond the choroid fissure, which is now a distinct streak. The nasal groove invaginates dorsally beyond the lower margin of the lens. The entire retina is pigmented. The limb buds are longer than they are wide, and both the fore and hindlimb buds are nearly equal in length. The digital plate and elbow are just recognizable in the forelimb bud. The AER in the forelimb bud becomes thicker along the margin of the digital plate. The AER of the hindlimb bud has also formed.

Stage 14 (14 days; 29.2%; Fig. 4 E, F)

The body flexure is at maximum. All the pharyngeal slits are covered by the opercular flap of the hyoid arch that extends posteriorly. The mandibular arch also extends rostrally as far as the level of the posterior margin of the lens.

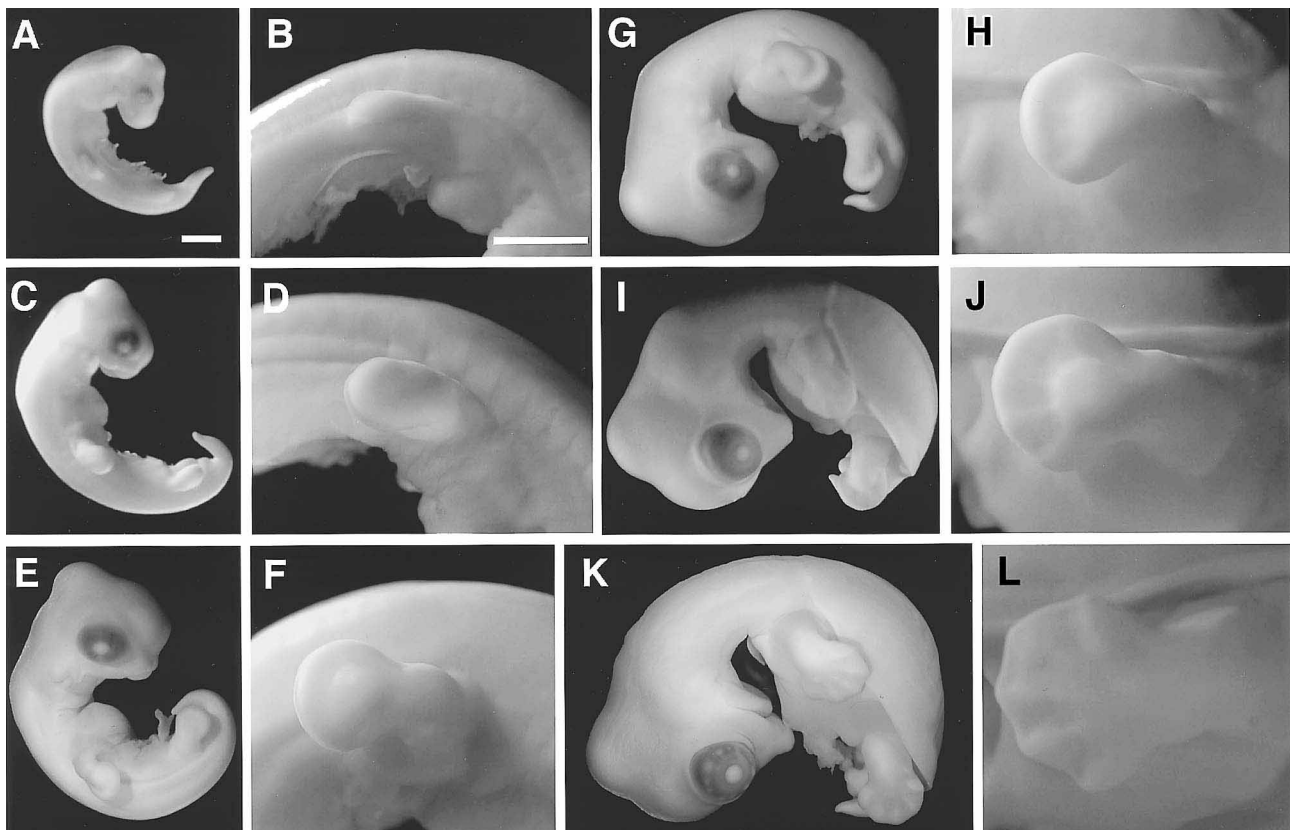


Fig. 4. Photographs of embryonic stages of *Pelodiscus sinensis*: **A** and **B**, lateral view and right forelimb region of stage 12, respectively; **C** and **D**, stage 13; **E** and **F**, stage 14; **G** and **H**, stage 15; **I** and **J**, stage 16; **K** and **L**, stage 17. Note the progressive development of the digital plate and digital ridges in limbs and first appearance of lateral ridge of the carapace at stage 14. Scale bar = 1 mm.

The maxillary process fuses with the lateral nasal process. The iris begins to be pigmented. The primordial carapace margin (carapacial ridge) has appeared as a longitudinal ridge along the trunk. The digital plate is apparent in the hindlimb.

Stage 15 (16 days; 33.3%; Fig. 4 G,H)

The lateral surface of the neck has become flat due to caudal extension of the flap of the hyoid arch. The medial and lateral nasal processes join to create a nostril. The mandibular process on each side has fused with its counterpart medially. The carapacial ridge is distinct.

Stage 16 (18 days; 37.5%; Fig. 4 I, J)

The lower jaw extends anteriorly beyond the choroid fis-

sure. The eyelid has begun to overgrow the eyeball. The external nares have become distinct as round openings. Subtle pigmentation appears in the dermis between the eye and snout. Interdigital grooves and the digital ridge are indistinctly formed on the digital plate. The anterior border of the carapace is obvious. The posterior margin of the plastron and the bridge is clearly discerned between both limbs. Rib primordia are visible through the carapace.

Stage 17 (20 days; 41.7%; Fig. 4 K, L)

Approximately 10–13 scleral papillae are visible in the eye. The iris is distinct. Dorsal extension of eyelid is notable. The choroid fissure has become inconspicuous. The frontonasal process has disappeared. The snout begins to be

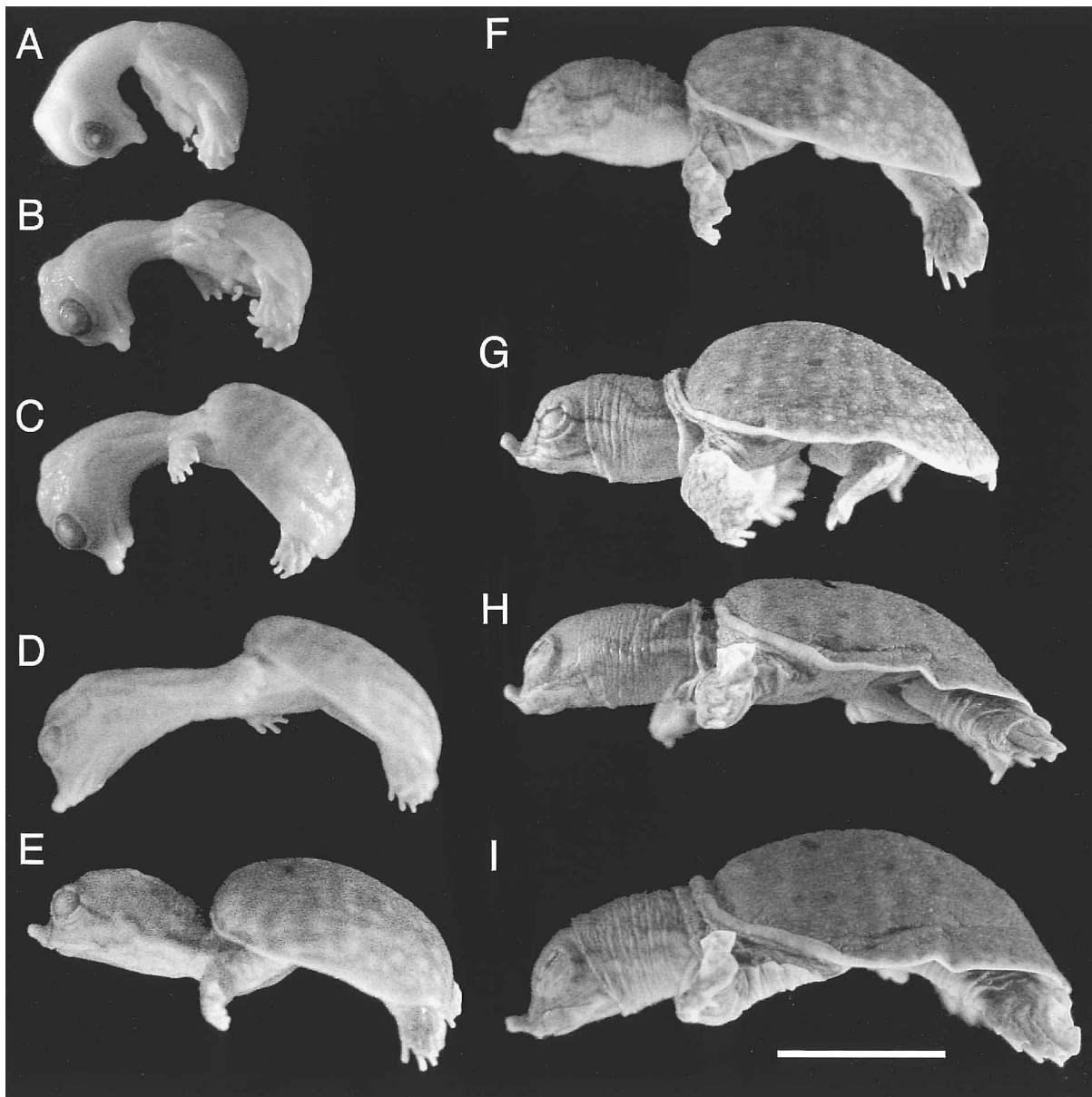


Fig. 5. Photographs of embryonic stages of *Pelodiscus sinensis*: **A**, lateral view of stage 18; **B**, stage 19; **C**, stage 20; **D**, stage 21; **E**, stage 22; **F**, stage 23; **G**, stage 24; **H**, stage 25; **I**, stage 26. Note the pigmentation, which becomes gradually evident on the surface of the body throughout those stages. Scale bar = 10 mm.

prominent. The lower jaw extends rostrally up to the level of the anterior border of the eye. The rudiment of the caruncle appears at the rostral tip of the upper jaw. The anterior boundary of the plastron is clearly recognized. The posterior edge of the carapace protrudes beyond the root of the tail. The periphery of the digital plate is indistinctly serrated. Digital ridges and interdigital grooves are obvious.

Stage 18 (22 days; 45.8%; Fig. 5 A)

The choroid fissure is absent. Facial pigmentation has increased in its intensity. Flecks of pigmentation occur on the carapace, limbs, and lateral surface of the neck and tail. The parietal region of the head has become flat due to the posterodorsal dilation of the cerebral hemisphere. Peripheral serrations of the digital plate have become more pronounced (Fig. 6 A). The plastron margin is clearly formed.

Stage 19 (24 days; 50.0%; Fig. 5 B)

Scleral papillae have become inconspicuous. The maxil-

lary process protrudes ventrally. The lower jaw extends anteriorly beyond the anterior border of the eye. The lower eyelid reaches the lower margin of the iris. The horizontal projection of the snout is prominent in the lateral aspect. The upper and lower lips begin to form. The cerebral hemisphere dilates dorsally so that the parietal region swells relative to the occipital region. A row of pigmentation appears along the mid-dorsal line of the carapace. Minute cutaneous papillae are recognized on the carapace. Preaxial digits become conspicuous, compared with the postaxial digits that have intervening webs (Fig. 6 B).

Stage 20 (26 days; 54.2%; Fig. 5 C)

Scleral papillae have disappeared. Cutaneous papillae are emphasized on the entire surface of the carapace. Black pigmentation is recognized on the carapace in a symmetrical pattern. The bases of the digits are lightly pigmented (Fig. 6 C).

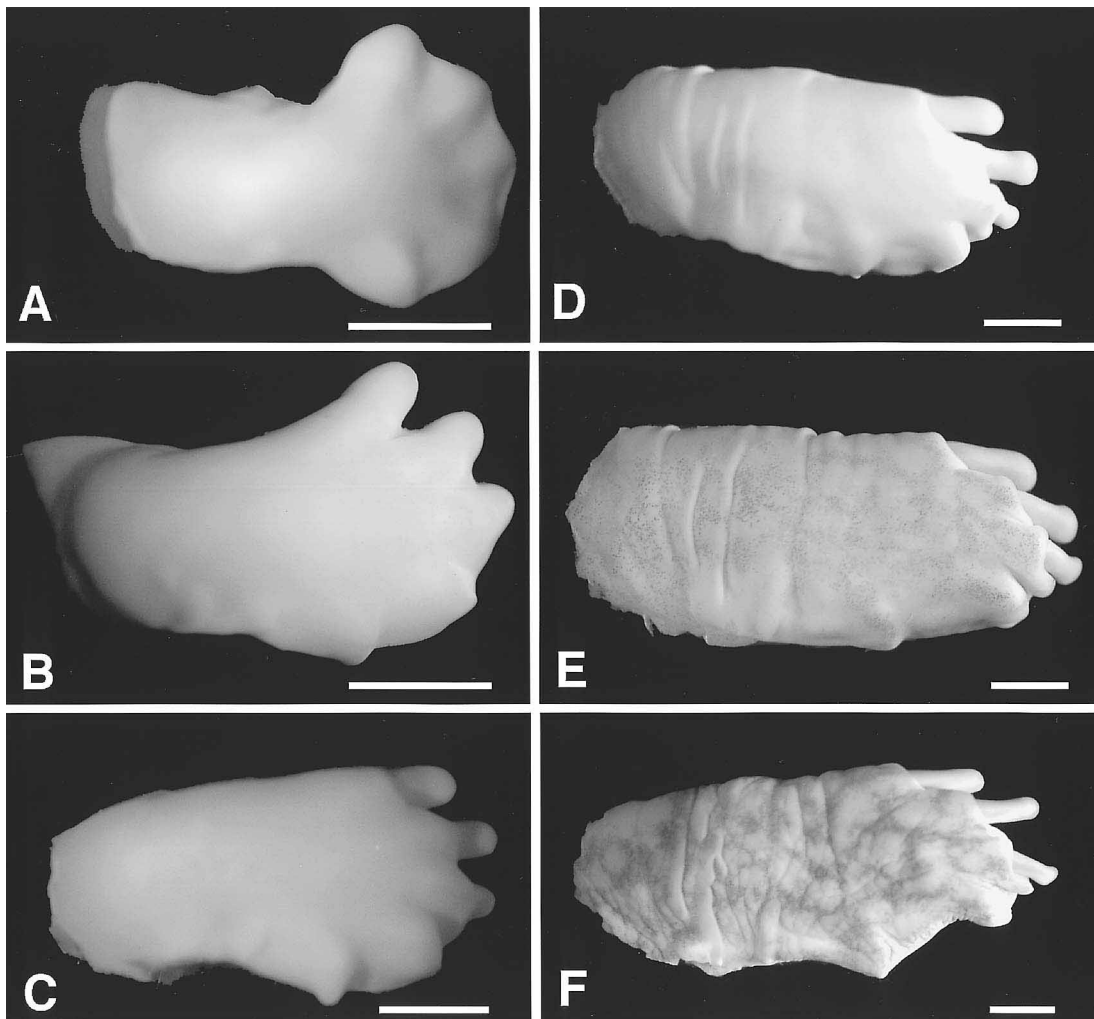


Fig. 6. Photographs of the right forelimb in late embryonic stages of *Pelodiscus sinensis*: **A**, stage 18; **B**, stage 19; **C**, stage 20; **D**, stage 21; **E**, stage 22; **F**, stage 23. Note the remarkable protrusion of preaxial digits (digits I, II, III) and the establishment of claws corresponding to them. Scale bar = 1 mm.

Stage 21 (28 days; 58.3%; Fig. 5 D)

The lower eyelid grows over the lower margin of the iris. Around the orbital region, a radial head stripe becomes apparent. The dorsal margin of the upper lip is clearly formed. The pigmentation of the carapace is uniform and the number of black spots increases. The lightly colored claws are formed (Fig. 6 D).

Stage 22 (31 days; 64.6%; Fig. 5 E)

The snout curves anterodorsally. A few wrinkles are present on the dorsal side of the neck. The pigmentation in neck and limbs rapidly increases (Fig. 6 E). The caruncle is magnified notably and the upper beak has become stiff. The anterior margin of the plastron is pigmented. The dorsal surface of the tail is pigmented.

Stage 23 (34 days; 70.8%; Fig. 5 F)

The lower eyelid approaches the lower margin of the lens. The caruncle starts to fuse with the upper beak, which is as thick as the lower. Head stripe is clear. The upper and lower lips are sparsely pigmented. Cutaneous papillae are distinct on the carapace. Light spots are scattered on the carapace. The plastral plate is partially pigmented. Fore and hindlimbs

are both darkly pigmented (Fig. 6 F).

Stage 24 (37 days; 77.1%; Fig. 5 G)

The pigmentation of the whole body rapidly increases, altering the color from light gray to full gray. Both eyelids are fully formed to cover the entire eye. The light spots on the carapace become distinct. Discrete dark spots on the plastron are arranged symmetrically, especially on those near the bases of the forelegs.

Stage 25 (40 days; 83.3%; Fig. 5 H)

The body color becomes brownish dorsally. White spots appear on the ventral surface of the neck. The anterior marginal line of the carapace becomes thicker and darker. The plastral plate is lightly reddish. The dark spots on the plastron have become larger. The dorsal surface of the tail is darkly pigmented.

Stage 26 (43 days; 89.6%; Fig. 5 I)

On the ventral surface of the neck, white spots have appeared, circumscribed by distinct black lines. The volume of the yolk is reduced relative to that of the embryo; the ratio ranges between 12.8% and 17.6%.

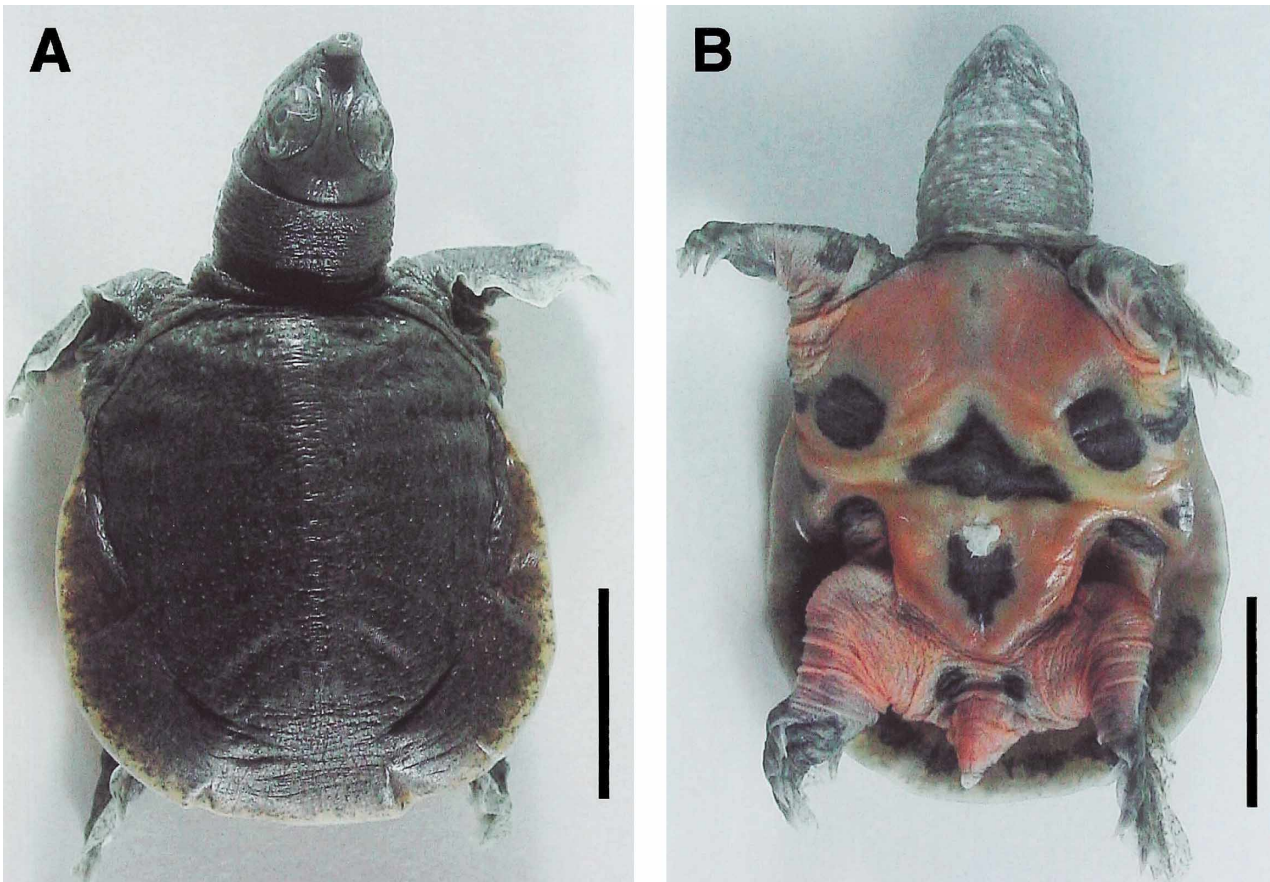


Fig. 7. Photographs of the hatchling of *Pelodiscus sinensis* at stage 27: **A**, dorsal view; **B**, ventral view. Dorsal surface is deep brown, while the ventral surface of the trunk is reddish with symmetrical black spots. The yolk sac is completely withdrawn into the abdomen. Scale bar = 10 mm.

Stage 27 (48 days; hatching period, immediately after leaving the shell; Fig. 7 A, B)

The umbilical hernia has disappeared. The yolk is completely withdrawn into the abdomen and the yolk sac entrance has closed. The coloration of the ventral body is uniformly deep red.

DISCUSSION

Comparison of developmental patterns among chelonian families.

The normal developmental table in the present study allows us to compare *Pelodiscus sinensis* with other chelonian species. As far as normal embryonic development of turtles is concerned, few attempts have been made to examine interfamilial variance (Ewert, 1985). This is in part due to difficulties associated with comparing normal development among species, i.e., incompatibility of criteria for staging and the available details of observed features. Therefore, we performed simpler and clearer comparisons than the previous description (Ewert, 1985), as discussed below.

Unlike other vertebrate groups such as the Gymnophiona (Dunker *et al.*, 2000), Anura (Chipman *et al.*, 1999) and Squamata (Hubert, 1985), chelonian embryos do not show species-specific features until later stages (Renous, 1989). In considering variation in the development among chelonian species, it is thus reasonable to compare chronological sequences of morphogenesis. Because embryos at oviposition are identical in morphological state across all turtle species, we compared the relative timing of appearance of certain characters, which are comparable across published references, through oviposition (0%) to hatching (100%). To execute this, we selected only those references that describe precise chronological developmental sequences (Yntema, 1968; Mahmoud *et al.*, 1973; Miller, 1985). Unfortunately, other references lacked necessary data, although they expatiated some aspects of specific embryonic development.

In Fig. 8, we summarize interfamilial comparisons of developmental patterns of selected body parts including eyes, limbs and shells. Throughout development, the timings of onset of certain characters were almost constant across the species. An exception was associated with limb development. In *P. sinensis* and *C. serpentina*, appearance of the AER preceded that of the digital plate. In contrast, these two events were reversed in marine turtles and *C. picta*.

As stated above, most interfamilial variation in normal development lies in the relative timing of the appearances of certain characters. In the development of limbs, for example, the tendency in which the differentiation of forelimb precedes that of the hindlimb was shared by all the families except for marine turtles where both fore and hindlimbs develop synchronously (Miller, 1985). In this regard, the developmental pattern of the emydid *C. picta* seemed to be unique among the taxa; a slight delay in the onset of differentiation was shown in several characters. In eye development, the lens and the eyelid of this species appeared later than did those of others.

In limb development, their digital plate and claws also appeared obviously later than in others. Moreover, appearance of the carapace and the plastron of this species are delayed compared with other turtles. On the other hand, there is little variation in relative timing of differentiation across normal development of *P. sinensis* (Trionychidae), *C. serpentina* (Chelydridae), and marine turtles (Cheloniidae). Generally, morphological differentiation in those species tended to appear earlier than that in *C. picta*.

Phylogenetic relationships among the major chelonian lineages remain controversial, despite the number of pioneering studies (Williams, 1950; Bickham and Carr, 1983; Gaffney, 1984; Gaffney and Maylan, 1988; Gaffney *et al.*, 1991; Shaffer *et al.*, 1997). Nevertheless, the phylogenetic position of the family Emydidae is stable as a derived taxon within Cryptodira, no matter what data sets are used for analysis. If phylogenetic hypotheses in which Emydidae is nested within crown groups of the Cryptodira were trustworthy, then the pattern of embryogenesis seen in *C. picta* might be an evolutionarily derived feature in cryptodirans. Although Guyot *et al.* (2000) described a detailed embryonic series of Testudinidae, possibly the closest family to Emydidae, their study lacked the chronological data that we can use for comparison. Thus, to determine whether the tendency seen in the development of *C. picta* may be a synapomorphy for the group Cryptodira, it is important to compare the developmental sequences of testudinids with those of other family members. To understand the evolutionary trends in embryonic development of the order Chelonia, examining pleurodiran development would also be necessary.

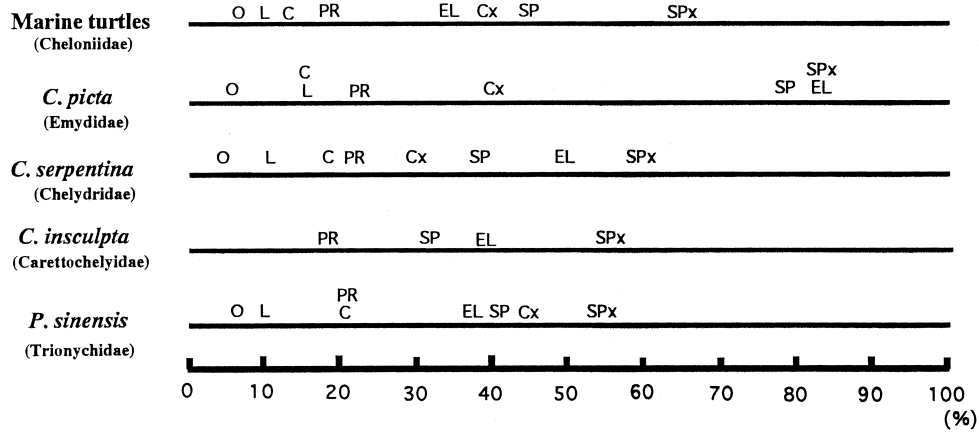
Comparison of the embryonic development between Trionychidae and Carettochelyidae.

Although some aspects of embryonic developmental pattern of *P. sinensis* were similar to other chelonian species, they also show some unique features throughout their normal development. Recently, Beggs *et al.* (2000) calibrated the developmental series for the pig-nosed turtle, *Carettochelys insculpta*, which has been assumed a sister-group of Trionychidae (Gaffney, 1984; Shaffer *et al.*, 1997).

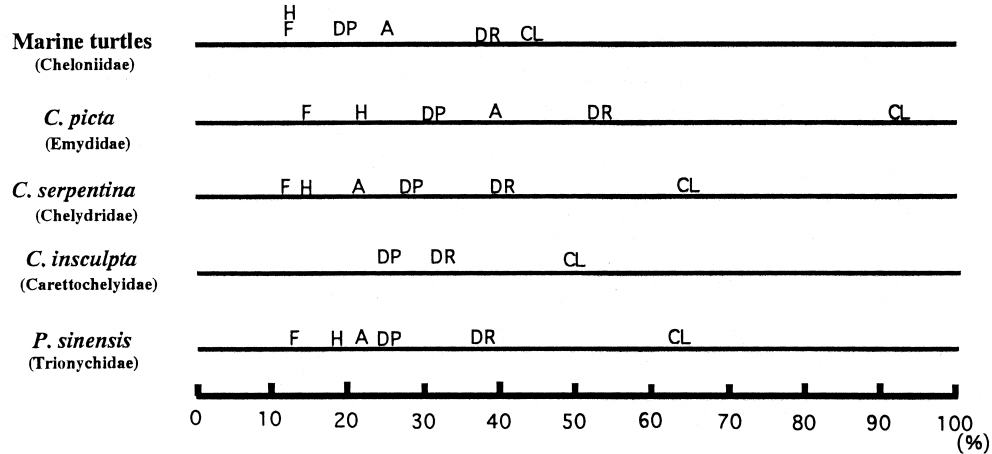
Here we compare the normal development of *P. sinensis* to *C. insculpta*, to find out similarities and differences in developmental pattern between these closely related taxa. To perform this comparison, we aligned developmental events of *C. insculpta* into chronological sequences as well as using the above comparisons. Given that *C. insculpta* hatches 77 days after incubation (Beggs *et al.*, 2000), we established rough alignments of character appearance of *C. insculpta* in comparison with *P. sinensis* (Fig. 8). In eye development, whereas retinal pigmentation and eyelid formation take place at almost the same embryonic period for both species, scleral papillae persist longer in *C. insculpta*. In limbs, while the digital plate forms nearly at the same point, the later events, including the digital ridge and claws, tend to appear earlier in *C. insculpta*. Interestingly, external limb morphology is substantially different in these species. In *C. insculpta*, the first

COMPARISONS OF DEVELOPMENTAL PATTERN AMONG TURTLE FAMILIES

A. EYE



B. LIMBS



C. SHELL

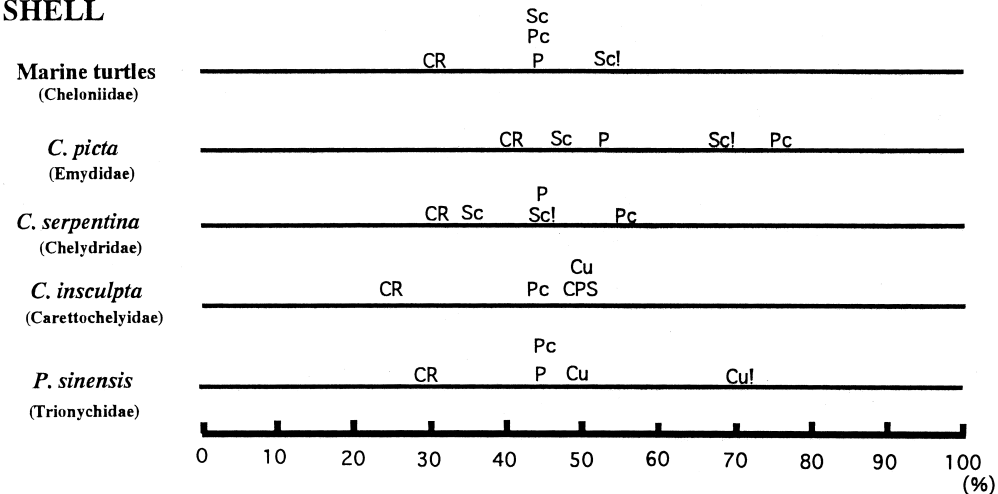


Fig. 8. To compare normal developmental patterns between turtle families, the sequences of normal development of five species, each representing five different families, are aligned through oviposition (0%) to hatching (100%). Some characters of *C. insculpta* could not be added into the comparisons due to lack of information in original description.

Abbreviations are as follows: A, apical ectodermal ridge; C, choroid fissure; CL, claws; CPS, serrations at the carapace periphery; CR, carapace; Cx, disappearance of the choroid fissure; Cu, cutaneous papillae; Cu!, distinct indication of the cutaneous papillae; DP, digital plate; DR, digital ridge; EL, eyelid; F, forelimb; H, hind limb; L, lens; O, optic vesicle; P, plastron; Pc, pigmentation of the carapace; P, plastron; Pc, pigmentation of the carapace; PR, pigmentation of the retina; Sc, horny scutes; Sc!, distinct indication of the horny scutes; SP, scleral papillae; Spx, disappearance of the scleral papillae.

two digits project beyond the web and finally become inconspicuous, unlike the other digits. The posterior three digits are long and covered by well-developed webs to function as a flipper. On the other hand, *P. sinensis* has the first three digits projecting beyond the web (see Fig.6). They are covered by hard claws and efficient in their life.

As seen in marine turtles, limb of *C. insculpta* has become flipper adapted to aquatic environment. To support the flipper, they developed elongated postaxial digits (digits III, IV, V) distally. In contrast, trionychid *P. sinensis*, which is semi aquatic and has a habit of digging in sandy sediment, had to develop the flexible and robust preaxial digits (digits I, II, III) and corresponding claws. At the same time, they also needed to establish postaxial flipper-like structures covered with interdigital webs for swimming. Because such a difference is seen in the early paddle stages of ontogeny, it might be caused by substantial alterations to the developmental program.

As far as shell development is concerned, the two species are almost the same, except that periphery of the carapace of *C. insculpta* becomes serrated at the middle point of their embryogenesis, whereas that of *P. sinensis* does not at any time. It has generally been accepted that, in most chelonian species, both carapace and plastron are covered superficially by a hard horny epidermis, which is made of only β -keratin. In contrast, the shell of soft-shelled turtles appears to be composed of α -keratin, which also covers rest of the body in other species, such as the leatherback turtle, *Dermochelys coreacea* (Alexander 1969; Spearman 1969; Baden and Maderson 1970). Throughout embryogenesis of the trionychid *P. sinensis* and carettochelyid *C. insculpta*, no horny scutes grow on the surface of the shell, as seen in other parts of the body. Unlike the carapace of other three species in which sequential formation of horny scutes is observed, those of both species start to be covered by cutaneous papillae, instead of horny scutes, in the mid stages of development (in *P. sinensis*: stage 19=50% of development; in *C. insculpta*: stage 20=46.8%–53.2%). These papillae become distinct and disperse on the entire surface of the carapace (in *P. sinensis*: st23=70.8%). Overall, the modes of ontogeny in both species resemble each other.

***P. sinensis* embryo for experimental studies.**

Regardless of those unique features of trionychids, including their soft shells, their fundamental morphology does not seem to deviate markedly from the general trend of turtle development. As shown above, the normal chronological developmental pattern of *P. sinensis* does not greatly differ from that of the chelydrid *C. serpentina* that belongs to the basal lineage of Cryptodira. Therefore, as it is bred as a food product, this externally curious but highly accessible animal may prove useful for experimental studies as a representative of non-avian, non-mammalian amniote animals. Especially, evolutionary development of the turtle carapace appears to be one of the most fascinating subject (Burke, 1989; Gilbert *et al.*, 2001) relating to 'heterotopy', the shift of epige-

netic interaction that occurred in the somitic mesodermal derivatives. The present description of normal developmental stages of the soft-shelled turtle will thus help our understanding of the developmental mechanisms involved in such evolutionary novelty.

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