2 Comparative Ontogenetic and Phylogenetic Aspects of Chelonian Chondro-Osseous Growth and Skeletochronology

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CONTENTS

2.1 INTRODUCTION

Form and function are fundamental interdependent strategies of all life. From studies of skeletal and chondro-osseous structure and development, we can gain insights into phylogenetic differences and taxonomic classifications, and we can also better understand how different species—and individuals within species—grow to maturity and respond to the physiological demands of their particular life strategies. Cortical banding patterns within bones correlate to activity patterns of the individual as well as endogenous rhythms, allowing for inferences not only about age and cyclical growth patterns but also previous growth and circumstances that have influenced growth (Suzuki, 1963; Enlow, 1969; Castanet, 2006). Studies of these banding patterns within cortical bone (skeletochronology) have been applied to numerous species of turtles and have allowed us to understand patterns and rates of growth.

In addition to skeletochronology, detailed studies of the chondro-osseous development of appendicular bones have revealed strong similarities among most living chelonians, but with

striking differences for certain large, fast-growing sea turtles (e.g., the leatherback, *Dermochelys*) that separates them from all other turtles (Rhodin et al., 1980, 1981, 1996; Rhodin, 1985).

In this chapter, we summarize the application of skeletochronology for estimates of age and growth rates in turtles, review the two basic patterns of bone growth that occur in turtles, and correlate these patterns of chondro-osseous development with phylogeny. Finally, we discuss how these factors influence rates of growth to sexual maturity, highlighting how the leatherback stands apart from other turtles.

2.2 SKELETOCHRONOLOGY IN TURTLES

2.2.1 Background

Skeletochronology has been used to estimate age and growth in numerous species of reptiles and amphibians (Castanet, 1994; Smirina, 1994). Bones are good recording structures, as they contain layers that form with a predictable periodicity and the layers are different in morphology and optical density, making them easily discernable (Klevezal, 1996). In histologic cross-sections of bone are concentric thin layers that stain dark with hematoxylin. Alternating with these concentric thin layers are broad homogeneous light-staining layers (Castanet et al., 1993; Klevezal, 1996). Castanet et al. (1977) introduced the term *line of arrested growth* (LAG) to identify the thin dark lines characteristic of skeletal growth marks (Figure 2.1).

In bone morphology, LAGs are in the general class of cement or cementing lines and are common throughout all vertebrate bones. Resorption cement lines are found around Haversian canal systems (secondarily remodeled bone with vascular ingrowth), differentiating them from cortical bone, and in the lamellar periosteal deposition of secondary endosteal bone. Resting cement lines (the class to which LAGs belong) are found in the layering pattern of periosteal deposition of new cortical bone (Enlow, 1969; Francillon-Vieillot et al., 1990).

Many skeletochronological studies of herpetological species indicate that LAGs are formed as a result of low metabolism and slowed or no growth associated with seasonal climatic changes. This is likely true but serves only as a partial explanation, considering that LAGs also occur in the hard structures of nonhibernating mammalian species (Klevezal, 1996; Castanet 2006). Castanet et al. (1993) extended the terminology of LAGs to both poikilotherms and endotherms as a general description of a resting cement line marking periodicity in growth. Castanet et al. (1993) also proposed that the formation of LAGs is likely to be endogenous while still potentially synchronized to environmental conditions.

Cyclical formation of LAGs appears to be a universal phenomenon in vertebrates (Castanet et al., 1993; Klevezal, 1996; Simmons, 1992), and there is evidence for endogenous control (Schauble, 1972; Castanet et al., 1993; Simmons, 1992; Esteban et al., 1999). Bone formation and remodeling rates are hormonally controlled and synchronized to circadian patterns (Simmons, 1992). Parathyroid hormone (PTH), calcitonin, and vitamins A, C, D, and K have been found to influence rates of bone formation and remodeling (Buchanan & Preece, 1991; Narbaitz et al., 1991). Specifically, PTH—which stimulates bone resorption—is secreted in response to serum calcium levels.

Studies have demonstrated seasonal variability in skeletal growth rates, not just in poikilotherms (Schauble, 1972; Snover & Hohn, 2004) but also in endothermic mammals (Klevezal, 1996; Castanet, 2006). These patterns may potentially be evolutionarily related to an increased availability of vitamins A, C, and D, with the onset of spring in temperate climates or the wet season in tropical climates (Buchanan & Preece, 1991; Simmons, 1992). However, there is substantial evidence that the spring surge in growth rates is also under endogenous control, as animals that are maintained in captivity also demonstrate this pattern. Schauble (1972) amputated limbs from the newt, *Notophthalmus viridescens*, at different times of the year and observed the regeneration rates. She found that regeneration rates were significantly higher in the spring or early summer months, followed by summer, late summer, early fall, and winter, respectively. As temperature, light levels,

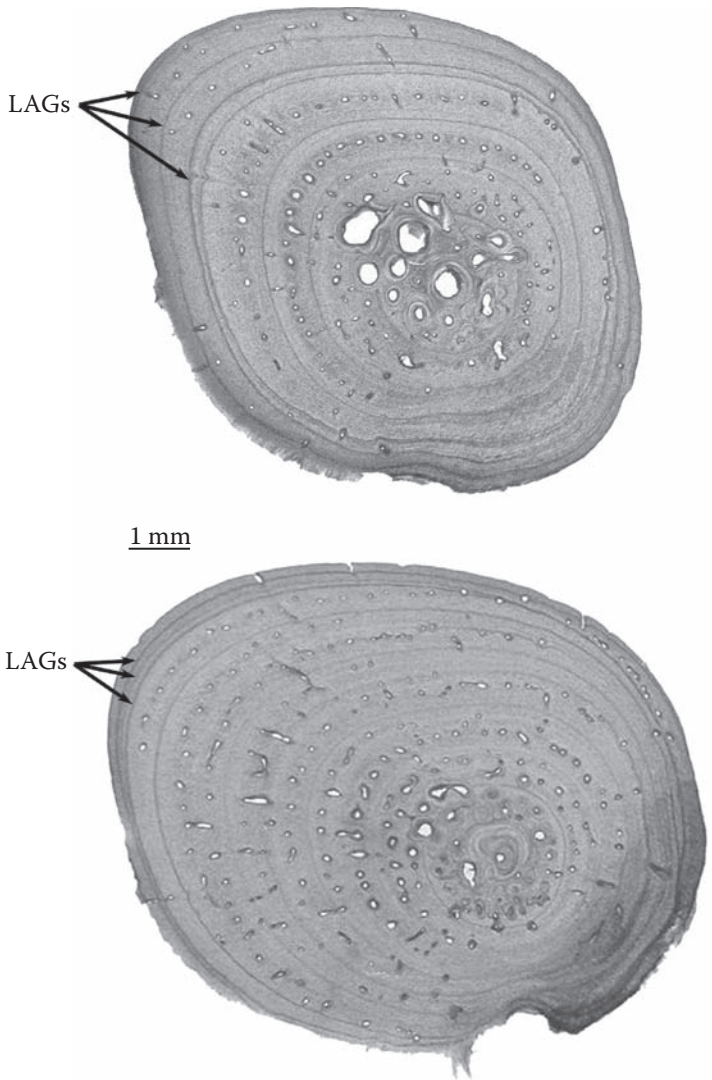

FIGURE 2.1 Cross-sections from humeri of two terrapins (*Malaclemys terrapin*) that have been decalcified and stained with Ehrlich's hematoxylin. Arrows highlight the thin, darkly stained lines of arrested growth (LAGs), and the lightly stained region between LAGs is termed the growth zone and together one LAG and one zone comprise a growth mark. Note how the LAGs are beginning to compress at the outer edge of the lower image. The upper image is from a 15.1-cm straight carapace length (SCL) female, and the lower is from a 16.5-cm SCL female.

and food availability were controlled, these factors could not have played a role in the regeneration rates, suggesting that the results imply the influence of an internal biological rhythm, either endocrine or nonendocrine in nature.

Another line of evidence for seasonal variability in skeletal growth rates is Snover and Hohn's (2004) analysis of bone-growth increments past the last complete LAG in Kemp's ridley humeri relative to stranding date. They found a significant and positive relationship between the amount of new bone deposited after the last LAG and the June–November timeframe. From November to June, the relationship was not significantly different from zero, suggesting that very little new bone

deposition occurs during the winter and that LAGs are deposited in the spring for Kemp's ridleys along the U.S. Atlantic coast.

2.2.1.1 Validating Annual Deposition of LAGs

Three common methods can be employed to directly validate the annual deposition of skeletal growth marks: the study of known-age animals, mark-recapture studies, and mark-recapture studies that incorporate fluorescent marking (Castanet, 1994). All three of these methods have been applied to turtles (Castanet & Cheylan, 1979; Klinger & Musick, 1992; Coles et al., 2001; Snover & Hohn, 2004; Curtin, 2006; Snover et al., 2007b). Snover and Hohn (2004) looked at humeri from known-age Kemp's ridley sea turtles (*Lepidochelys kempii*) that had been tagged as hatchlings and released into the wild. The turtles from their study were subsequently recovered as dead strandings and allowed for validation of annual LAG formation and the recognition of an annulus, or diffuse mark rather than a distinct LAG, that represented an annual growth mark. Curtin (2006) used bones from known-age desert tortoises (*Gopherus agassizii*) from mark-recapture studies to test and validate back-calculation methods to account for LAGs lost to resorption in older animals. Snover (2007a) used humeri from dead stranded loggerhead turtles (*Caretta caretta*) that had been previously captured and tagged to validate that carapace length can be back-calculated from the dimensions of earlier LAGs. Castanet and Cheylan (1979) used fluorescent marking to validate that growth marks were annual in Hermann's tortoises (*Testudo hermanni*) and Greek tortoises (*Testudo graeca*). Klinger and Musick (1992) injected wild loggerheads with oxytetracycline and released them. Bone biopsies were taken from turtles recaptured 1 to 2 years later to validate annual LAG formation. A turtle from that same study was found stranded dead 8 years after injection and presented additional validation (Coles et al., 2001).

2.2.1.2 Resorption of LAGs

As bone increases in size during growth, it is constantly remodeled and reshaped (Enlow, 1969). Hard bone tissues cannot grow through internal expansion, but rather they grow by appositional processes (on periosteally derived cortical bone) with the deposition of new tissue on the surface together with endosteal resorption (Enlow, 1969). This process of resorption results in the loss of the innermost (earliest) growth marks and is a serious limitation in estimating age using skeletochronology. While not a serious issue for shorter-lived amphibians and reptiles, it is especially problematic in long-lived turtles, and the problem is noted to be extreme in age-estimate studies of marine turtles (Klinger & Musick, 1995; Zug et al., 1995, 1997, 2002; Parham & Zug, 1997; Zug & Glor, 1998; Snover & Hohn, 2004; Snover et al., 2007b), resulting in the development of several methods of back-calculation to estimate the number of growth marks lost.

Back-calculation techniques in sea turtles rely on the concept that the spatial pattern of the LAGs is representative of the growth of the animal, and to confirm this assumption a correlation must be established between bone dimensions and body size (Hutton, 1986; Klinger & Musick, 1992; Leclair & Laurin, 1996; Snover, 2002; Snover & Hohn, 2004). Using loggerhead turtles, Snover (2007a) demonstrated that the relationship between carapace length and humerus diameter can be used to accurately estimate carapace length at the time of earlier LAG deposition.

Most back-calculation procedures applied to turtles have not been validated and make assumptions about early growth rates (Klinger & Musick, 1995; Zug et al., 1995, 1997, 2002; Parham & Zug, 1997; Zug & Glor, 1998). Curtin (2006) was able to test and validate back-calculation procedures for the desert tortoise using humeri from known-age animals. She tested two methods presented by Parham and Zug (1997), the ranking protocol, and the correction factor methods and found that the correction factor method provided the most accurate age estimates for juveniles and subadults; however, it underestimated adult ages. For adult tortoises, the ranking protocol provided the most accurate estimates.

2.2.1.3 Skeletochronology and Growth Lines on Scutes

For most species of freshwater and terrestrial turtles, age is most commonly estimated from counts of growth lines on the scutes of either the carapace or the plastron (Germano & Bury, 1998; Wilson et al., 2003). This is a powerful technique as, unlike skeletochronology in turtles, it can be applied to living animals and used to understand the age structure of populations. However, many studies that apply this technique do not provide any validation (Castanet & Cheylan, 1979; Wilson et al., 2003) and in a literature review, Wilson et al. (2003) found that of the studies that did attempt validation, 37% were unable to do so. Similarly, Berry (2002) found that even in juvenile desert tortoises, age could not be accurately determined through scute counts alone. Hence, it appears that whereas counting scute growth lines may be a viable method of age estimation in some turtles (i.e., Stone & Babb, 2005), it is not accurate for all turtles and assumptions should not be made that the method is applicable to a given species without validation. While not strictly valid when used in conjunction with each other, skeletochronology and scute growth line counts from dead turtles can serve as supporting evidence of the annual nature of the two methods (Castanet & Cheylan, 1979; Hart & Snover, unpublished data).

Even when scute growth line counts accurately estimate age, an advantage of skeletochronology over scute growth line counts appears with older adult animals. As growth slows to nearly immeasurable rates in older animals, growth lines can no longer be differentiated on scutes (see Wilson et al., 2003, for review), hence only minimum ages can be estimated. However, in histological preparations of bones LAGs can be generally differentiated even in older animals with near cessation of growth (Snover & Hohn, 2004), allowing for estimates of adult growth rates and longevity (Figure 2.1) (Snover, 2002; Snover & Hohn, 2004; Snover et al., 2007b).

2.2.2 Application of Skeletochronology to Turtles

2.2.2.1 Freshwater Turtles

Freshwater turtles were the first turtles to have skeletal growth marks recognized in their long bones. Mattox (1936) noted skeletal growth marks in the long bones of painted turtles, *Chrysemys picta marginata*, and found a correlation between counts of the marks and turtle size. Peabody (1961) and Hammer (1969) documented periosteal cyclical rings in snapping turtles, *Chelydra serpentina*. Suzuki (1963) and Enlow (1969) found them in the slider, *Trachemys scripta*. Hart and Snover (unpublished data) compared skeletochronology preparations of humeri with plastron scute growth line counts to demonstrate the strong comparison of the two techniques in the brackish-water diamondback terrapin (*Malaclemys terrapin*). Counting of growth lines on plastron or carapace scutes remains the primary means of estimating age for freshwater turtles.

2.2.2.2 Terrestrial Turtles

The first study to validate the annual nature of skeletal growth marks was conducted with two species of tortoises. Castanet and Cheylan (1979) used fluorescent marking to validate annual growth marks in Hermann's (*Testudo hermanni*) and Greek (*Testudo graeca*) tortoises. Recently, skeletochronology has been applied to desert tortoises (*Gopherus agassizii*): Curtin (2006) validated the annual nature of the LAGs in humeri from known-age animals and developed correction techniques to estimate the number of LAGs lost to resorption. Similar to the freshwater turtles, growth lines on scutes continue to be a primary means of estimating age in this group of turtles.

2.2.2.3 Marine Turtles

Of all of the turtle groups, skeletochronology has been applied most frequently to marine turtles. The scutes of the plastron and carapace do not retain growth lines like the freshwater and terrestrial

turtles (however, see Tucker et al., 2001). Hence, skeletochronology has been the primary means of estimating age and inferring growth rates in these turtles.

To date, skeletochronology has been applied to five of the seven species of marine turtles, the loggerhead (*Caretta caretta*: Zug et al., 1986, 1995; Klinger & Musick, 1992, 1995; Parham & Zug, 1997; Coles et al., 2001; Snover, 2002; Bjorndal et al., 2003; Snover & Hohn, 2004), the leatherback (*Dermochelys coriacea*: Zug & Parham, 1996), the Kemp's ridley (*Lepidochelys kempii*: Zug et al., 1997; Snover & Hohn, 2004; Snover et al., 2007b), the green (*Chelonia mydas*: Bjorndal et al., 1998; Zug & Glor, 1998; Zug et al., 2002), and the olive ridley (*Lepidochelys olivacea*: Zug et al., 2006). The annual deposition of LAGs has been validated for loggerheads (Klinger & Musick, 1992; Coles et al., 2001; Snover & Hohn, 2004) and Kemp's ridleys (Snover & Hohn, 2004).

With the exception of leatherbacks, all of these studies used the humerus bone. Generally, LAGs are most clearly visible in the long bones, and the humerus is ideal as it is easily removed from dead animals and it has muscle insertion scars that create landmarks that allow for the identification of sectioning sites that are consistent (Snover & Hohn, 2004). Humeri of leatherbacks are morphologically different from the hard-shelled turtles, and a high level of vascularization and bone remodeling is characteristic of the leatherback skeleton (Rhodin, 1985). This high level of vascularization may limit the usefulness of long bones to skeletochronology studies. However, Rhodin (1985) documented two wide cyclical growth zones in the periosteal bone of the humerus of an adult female leatherback turtle that suggested the possibility of growth cycles related to migration or nesting patterns (Figure 10 in Rhodin, 1985). Zug and Parham (1996) predicted age at sexual maturity of leatherbacks by skeletochronology based on LAGs found in scleral ossicles; skeletochronology of leatherbacks has also been conducted by Avens and Goshe (unpublished data). However, the possible annual nature of these marks has not been validated, and they may instead simply represent the cyclical result of varying rates of bone deposition and growth related to feeding or migration cycles in this high-metabolism species.

2.3 COMPARATIVE CHONDRO-OSSEOUS DEVELOPMENT IN TURTLES

Form and function are indeed fundamental interdependent strategies of all life. This is especially apparent in the patterns of skeletal growth in turtles as seen in the chondro-osseous development of their appendicular bones, particularly in the patterns of endochondral bone growth. In this section, we review and summarize the two basic patterns of bone growth that occur in turtles and correlate these patterns of skeletal morphology with phylogeny as well as the rate of growth to sexual maturity.

We intend to concentrate this review primarily on the leatherback (*Dermochelys coriacea*), focusing on the morphology and growth of its bones and cartilage. We provide additional detail on its unique vascular cartilage canals that apparently help the leatherback to grow its skeleton rapidly to a large body size. Though related to the hard-shelled chelonioid sea turtles in a number of primitive plesiomorphic features, the leatherback has developed an array of unique derived features that doubtlessly render it the most remarkably specialized turtle in the world.

Unique among living sea turtles in its nearly exclusively pelagic habitat, the leatherback regularly migrates into frigid oceanic waters where it feeds almost exclusively on jellies, diving to incredible depths unequalled by other sea turtles or marine mammals (Eckert & Luginbuhl, 1988; Eckert, 1992; James & Herman, 2001; James et al., 2006). It is well adapted for deep dives, with its hemoglobin, myoglobin, and blood oxygen carrying capacity all greater than in other sea turtles—and more similar to marine mammals (Ascenzi et al., 1984; Lutcavage et al., 1990, 1992). It has a higher metabolic activity than other sea turtles and maintains its body temperature well above surrounding water temperatures, a result of gigantothermy, the ability to use large body size, heightened metabolism, and physiological adaptations to avoid heat loss (Frair et al., 1972; Lutcavage & Lutz, 1986; Paladino et al., 1990; Lutcavage et al., 1992; Penick et al., 1998; James & Mrosovsky, 2004).

Like marine mammals, the leatherback has developed heat retention mechanisms of thickened subcutaneous fibro-adipose tissue, combined with countercurrent heat exchangers in intertwined

multiple arterial and venous vascular bundles in its flippers, so as to avoid heat loss in cold waters (Greer et al., 1973). Its body is covered with a corselet of dramatically and uniquely reduced carapacial and plastral shell bones that are reinforced instead with a layer of small irregular intercalated dermal bones (Gervais, 1872). The leatherback skeleton is also unique in having an unusually high degree of neotenic retention of thick cartilages, which are further uniquely specialized through the ingrowth of vascular cartilage canals, a condition totally unlike all other living turtles studied to date (Rhodin et al., 1980, 1981, 1996; Rhodin 1985).

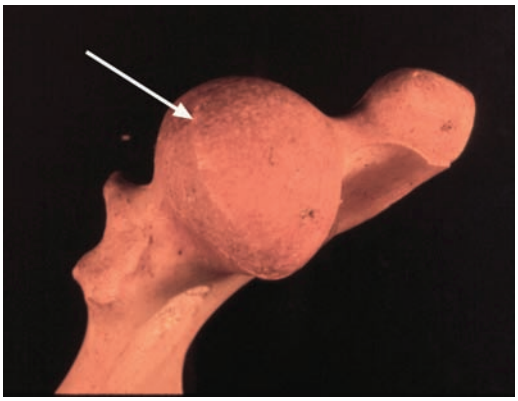

FIGURE 2.2 Proximal humerus, adult *Chelonia mydas*, dry bone preparation, showing smooth articular subchondral surface at the arrow.

Starting out as tiny hatchlings weighing only 30 g and measuring 6 cm in carapace length (CL) (Van Buskirk & Crowder, 1994), leatherbacks grow into the world's largest turtles, with some enormous animals having been recorded at more than 900 kg in weight (Eckert & Luginbuhl, 1988). Leatherbacks reach sexual maturity at about 250 kg with a minimum CL of 120 to 140 cm, about an 8000-fold increase in mass to reach maturity (Márquez, 1990; Van Buskirk & Crowder, 1994). The rate at which that growth is achieved is extremely rapid—much faster than any other reptile (Andrews, 1982)—and similar to the growth rates of some marine mammals.

Based on captive growth studies and patterns of bone growth, Rhodin (1985) previously hypothesized that leatherbacks might reach sexual maturity in as little as 3 to 6 years. More recent skeletochronology work by Zug and Parham (1996) has partially validated that hypothesis and demonstrated that the minimum size at maturity can possibly be obtained as early as 5 to 6 years, with 9 years interpreted as an average minimum age of maturity, and 13 to 14 years considered the average age at maturity. For a turtle of this size, that is phenomenally rapid growth.

How does the leatherback achieve such rapid growth? To understand its function and life strategy, we must look at the underlying form and uniquely specialized structure of its skeletal growth patterns. The work we present here is a review of previous work by Rhodin and colleagues (Rhodin et al., 1980, 1981, 1996; Rhodin, 1985) with new material presented on phylogeny and growth comparisons.

All living turtles studied to date*, except for the leatherback, have bones with articular surfaces that have smooth subchondral joint surfaces, covered by thin avascular cartilage (Figure 2.2). The surface of the subchondral bone is smooth in adult turtles but in growing subadults (and in adults or fossils where the superficial smooth subchondral bone has been worn off), multiple uniformly small holes represent the small metaphyseal vascular channels associated with endochondral bone formation (Rhodin, 1985). None of these very small uniform holes represent vascular channels penetrating into the overlying cartilage.

The leatherback has bones with subchondral articular surfaces that have roughened joint surfaces, with several large holes representing blood vessels penetrating into the thick overlying cartilage from the underlying bone (Figure 2.3) and small holes representing the metaphyseal vascular channels associated with endochondral bone formation. In the longitudinal cross-section of the

* Living turtles studied to date include *Dermochelys coriacea*, *Chelonia mydas*, *Caretta caretta*, *Eretmochelys imbricata*, *Lepidochelys kempii*, *L. olivacea*, *Carettochelys insculpta*, *Podocnemis unifilis*, *Geochelone nigra*, *Macrochelys temminckii*, *Dermatemys mawii*, *Platysternon megacephalum*, *Apalone spinifera*, *Sternotherus odoratus*, and *Chelodina parkeri* (Rhodin, 1985), as well as the genera *Trachemys*, *Homopus*, *Testudo*, *Graptemys*, *Pelusios*, *Chrysemys*, *Emys*, and *Terrapene* (Suzuki, 1963; Haines, 1969).

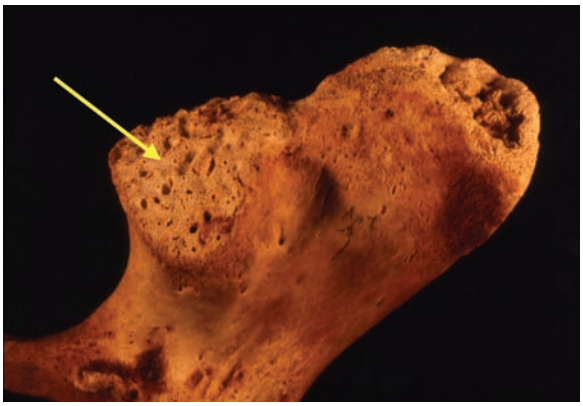

FIGURE 2.3 Proximal humerus, adult *Dermochelys coriacea*, dry bone preparation, showing rough articular subchondral surface at arrow with large holes indicating transphyseal vascular channels penetrating into the cartilage above and small holes indicating small metaphyseal vascular channels associated with subphyseal endochondral bone formation.

proximal humerus joint surface in a fresh bone, regular turtles have thin avascular cartilages (Figure 2.4), whereas leatherbacks have thick and vascularized cartilages with multiple blood vessels coursing through the cartilage (Figure 2.5). Sectioned, preserved adult leatherback bones also show light-colored endochondral bone cones alternating with dark-colored periosteal bone cones in a pattern of minimally remodeled amedullary bone, similar to the pattern seen in marine mammals and unlike other hard-shelled sea turtles (Figure 2.6). The leatherback has many bone growth features that are remarkably similar to marine mammals. Both marine mammals and leatherbacks have epiphyseal cartilaginous vascularization, endochondral and periosteal bone cones, minimally remodeled amedullary bone, and well-vascularized compact bone (Felts & Spurrell, 1965, 1966).

What do we know about actual bone growth patterns in turtles? Prior to the work reviewed here on leatherbacks and other large turtles, studies had only been carried out on small freshwater slider turtles, and their skeletal growth patterns had been assumed to be the pattern typical for all turtles. Work by Suzuki (1963) and Haines (1969) characterized bone growth in small turtles and served as the foundation for work on larger turtles. Rhodin and colleagues (Rhodin, 1985; Rhodin et al., 1996) then investigated the histology of chondro-osseous development in a variety of large turtles, including leatherbacks and giant tortoises and large hard-shelled freshwater and marine turtles, for which details of their bone growth follows.

The appendicular bones of most turtles, including hard-shelled sea turtles, are laid down as cartilaginous anlagen with a diaphyseal periosteal cuff of lamellar cortical bone (Figure 2.7), which is

FIGURE 2.4 Proximal humerus, adult *Caretta caretta*, fresh bone preparation, showing thin avascular joint cartilage at the arrow.

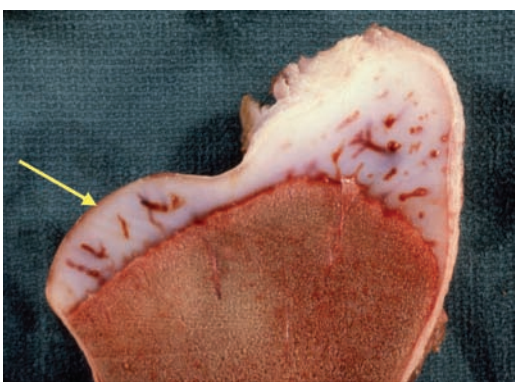

FIGURE 2.5 Proximal humerus, adult *Dermochelys coriacea*, fresh bone preparation, showing thick vascularized joint cartilage at the arrow.

followed rapidly in young post-hatchlings by central diaphyseal cartilaginous cell hypertrophy and calcification. This is followed by vascular ingrowth from the central nutrient artery perforating the mid-diaphyseal periosteal bone cuff (Figure 2.8). This leads to the formation of a central primary ossification center in the mid-diaphysis. In juvenile turtles, this expands toward each metaphysis, while simultaneously cartilage cells in the physeal zone between the epiphysis and metaphysis undergo hypertrophy, calcification, and vascular ingrowth, forming a subphyseal plate that gradually closes, isolating a cone of metaphyseal cartilage that is gradually replaced by bone (Figure 2.9). In subadults, the epiphyseal joint cartilage is relatively thin and avascular with a reasonably smooth underlying subphyseal bone plate (Figure 2.10). Adults have very thin cartilage and very smooth bony subphyseal surfaces.

This pattern of bone growth is typical for all species of living turtles—except the leatherback—such that bone growth proceeds in a unique and specialized pattern. Hatchling leatherback bones are laid down in similar fashion to other hard-shelled turtles (Figure 2.11), and post-hatchlings also undergo initial central diaphyseal vascular ingrowth, leading to a primary diaphyseal ossification center (Figure 2.12). However, at this point further development in the leatherback diverges markedly from other turtles, with rapid ingrowth of vascular canals into the cartilage of the metaphysis, coursing rapidly toward the epiphysis (Figure 2.13). Each of these cartilage canals is associated with a cuff of rapid chondroblastic proliferation and hypertrophied cartilage cells that undergo calcification and rapid ossification.

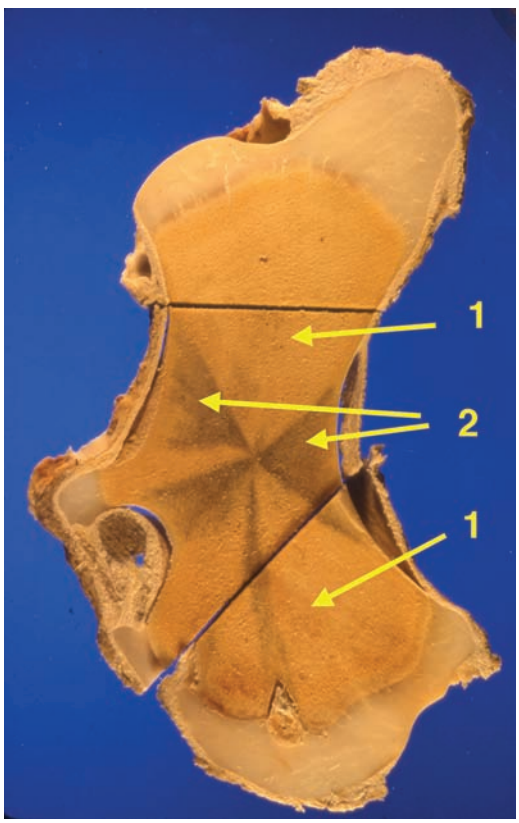

FIGURE 2.6 Humerus, adult *Dermochelys coriacea*, fresh bone preparation, showing (1) light-colored endochondral bone cones and (2) dark-colored periosteal bone cones.

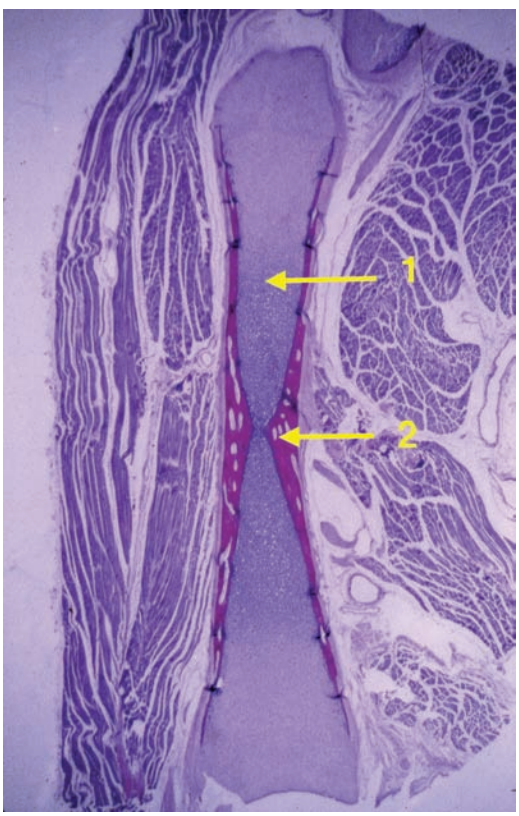

FIGURE 2.7 Ulna of hatchling *Caretta caretta* (SCL = 4.6 cm, H & E stain), showing cartilaginous anlagen and early periosteal cuff of cortical bone at the arrows.

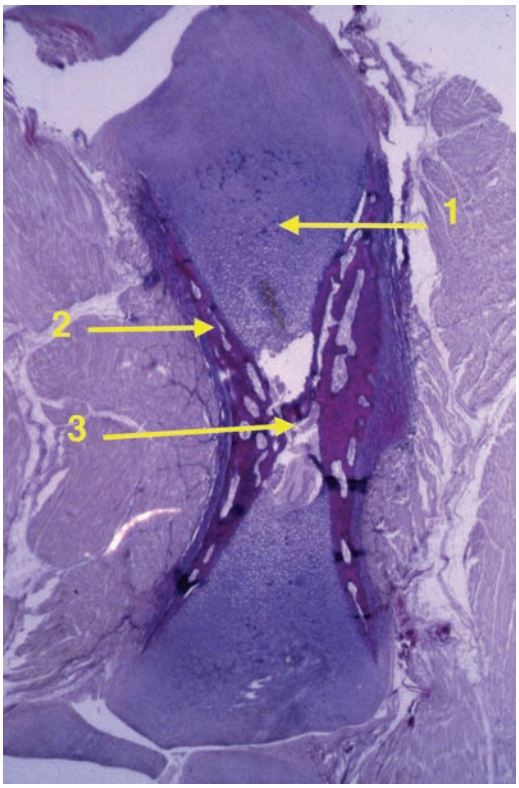

FIGURE 2.8 Radius of hatchling *Caretta caretta* (SCL = 4.4 cm, H & E stain), showing early primary ossification center at the arrow.

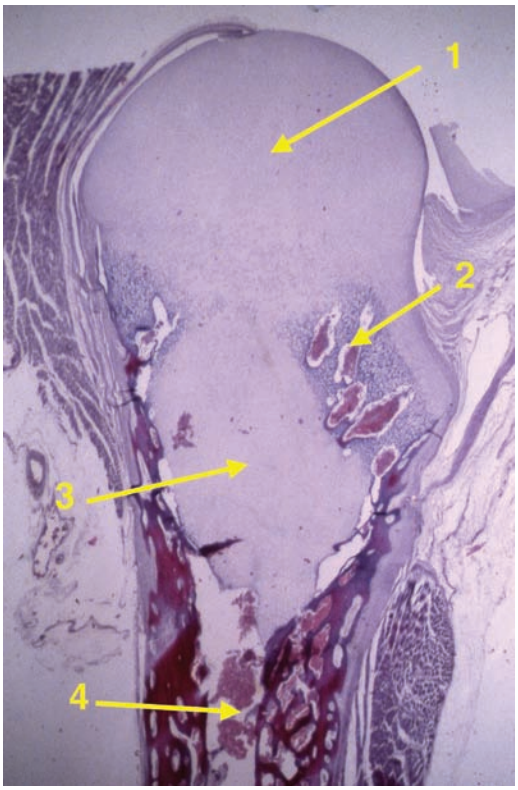

FIGURE 2.9 Proximal femur of juvenile *Caretta caretta* (SCL = 7.0 cm, H & E stain), showing (1) avascular epiphyseal cartilage, (2) vascularized subphyseal ossification plate arising from advancing periosteal ring between metaphysis and epiphysis, (3) avascular metaphyseal cartilage becoming isolated by advancing subphyseal ossification plate, and (4) primary diaphyseal ossification center.

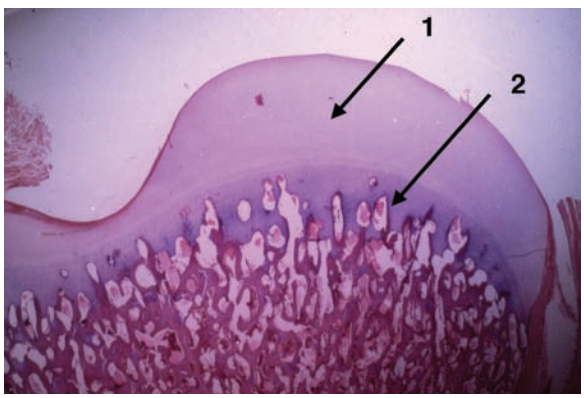

FIGURE 2.10 Proximal humerus of subadult *Lepidochelys kempii* (SCL = 27.5 cm, H & E stain), showing (1) avascular joint cartilage and (2) subphyseal bone plate with advancing calcification and ossification, including small vascular channels associated with subphyseal endochondral ossification.

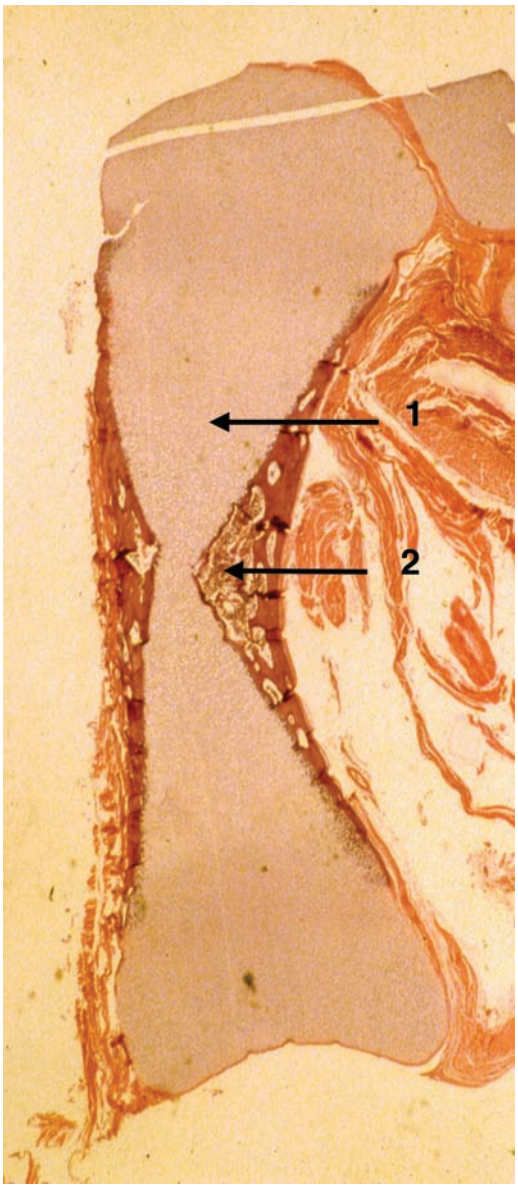

FIGURE 2.11 Metacarpal of hatchling *Dermochelys coriacea* (SCL = 6.5 cm, H & E stain), showing (1) cartilaginous anlagen and (2) early periosteal cuff of cortical bone.

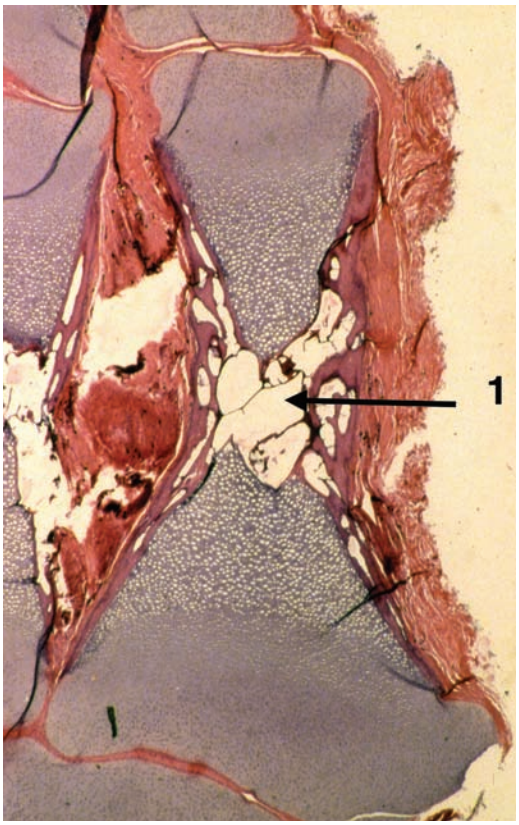

FIGURE 2.12 Metacarpal of juvenile *Dermochelys coriacea* (SCL = 7.0 cm, H & E stain), showing early primary ossification center at the arrow (1).

As ossification proceeds in the metaphysis, vascular cartilage canals penetrate through the subphyseal plate and grow into the thick epiphyseal cartilage under the joint surface (Figure 2.14), leading to the presence of large blood vessels in the joint cartilage (Figure 2.15), which creates the dramatic appearance of blood-filled red vascular canals traversing the bony subphyseal plate and entering deep into the thick white cartilage (Figure 2.5 and Figure 2.16).

The ultrastructural detail of the tips of these vascular canals growing into hatchling cartilage was investigated by Rhodin et al. (1996). Light microscopy of one of those leading vascular buds demonstrates a concentrated active growth cone of specialized chondroclastic tissue boring its way into the cartilage matrix much like a drill with a burr at its tip (Figure 2.17). Below the growth cone tip, the canal is less specialized, filled with vascular channels and hematopoietic cells. A close-up of the growth cone itself located at the tip of the canal shows feeder arterioles that give rise to a cap-like glomerulus of anastomosing large sinusoidal capillaries, drained by venules (Figure 2.18). Between the capillaries and the surrounding cartilage matrix—which shows marginal decreased metachromatic staining indicative of

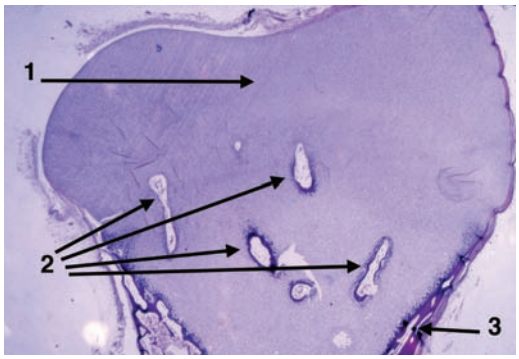

FIGURE 2.13 Proximal humerus of juvenile *Dermochelys coriacea* (SCL = 7.0 cm, H & E stain), showing (1) epiphyseal cartilage that is still avascular, (2) proliferation of vascular canals penetrating the cartilaginous metaphysis, and (3) advancing ring of periosteal bone.

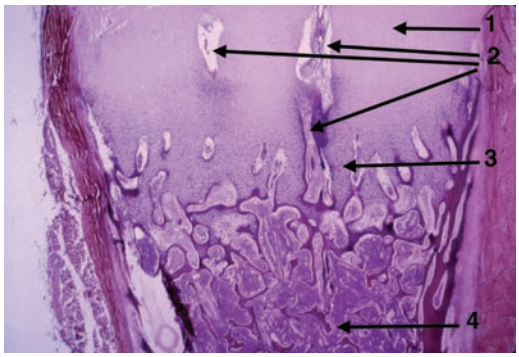

FIGURE 2.14 Metacarpal of larger juvenile *Dermochelys coriacea* (SCL = 40.5 cm, H & E stain), showing (1) epiphyseal joint cartilage, (2) vascular canals penetrating through the (3) subphyseal bone plate into the epipyseal cartilage from the (4) underlying metaphyseal primary ossification center.

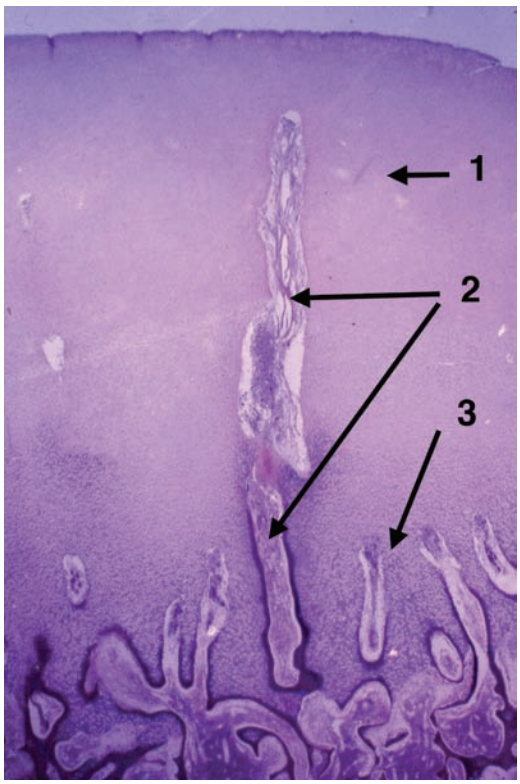

FIGURE 2.15 Proximal humerus of adult *Dermochelys coriacea* (CCL = 135.0 cm, H & E stain), showing (1) epiphyseal cartilage close to the joint surface, a (2) large vascular canal penetrating the (3) subphyseal bone plate into the joint cartilage, surrounded by smaller holes representing metaphyseal vascular channels associated with endochondral bone formation.

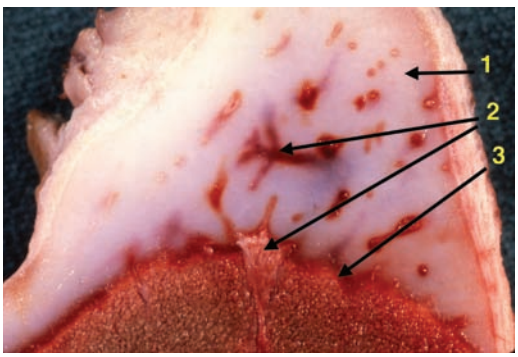

FIGURE 2.16 Humerus of stranded adult *Dermochelys coriacea* (fresh), showing (1) cartilage in the trochanter and (2) large vascular canals penetrating the (3) subphyseal bone plate.

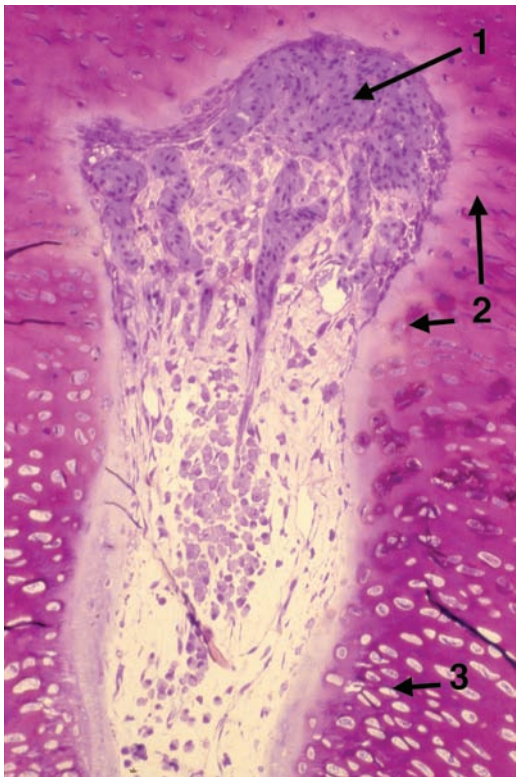

FIGURE 2.17 Proximal humerus of hatchling *Dermochelys coriacea* (SCL = 6.3 cm, H & E stain), showing (1) advancing growth tip of vascular canal bud penetrating into (2) undifferentiated metaphyseal cartilage, causing (3) cartilaginous hypertrophy (see text for detail).

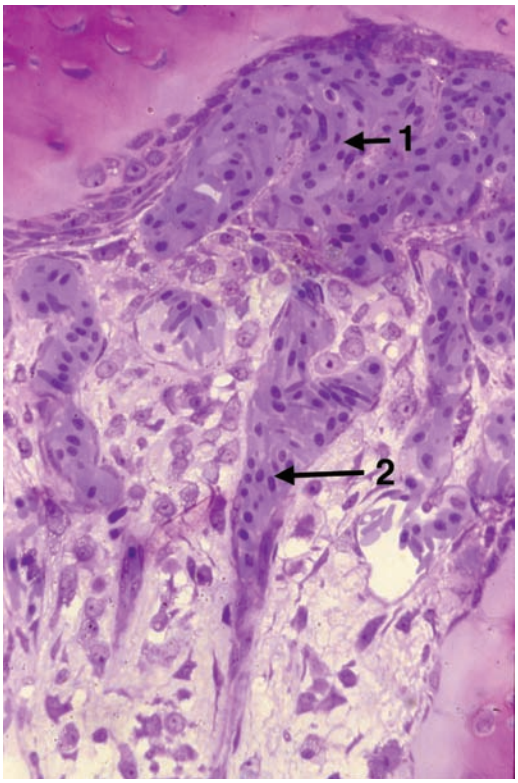

FIGURE 2.18 Humerus of hatchling *Dermochelys coriacea* (H & E stain), detail from Figure 2.17, showing (1) sinusoidal capillaries at the growth tip of the vascular canal and a (2) feeder arteriole (see text for detail).

proteoglycan removal—are layers of small cells that function in the active removal of the cartilage matrix to allow the canal to penetrate into the cartilage.

Using electron microscopy at low magnification, one can further elucidate the ultrastructure of the growth cone tip of the canal (Figure 2.19), which shows the anastomosing sinusoidal capillaries at the tip of the canal penetrating the surrounding cartilage. The cells marked with asterisks along the vascular canal margin are fibroblasts, macrophages, and chondroclasts. Several chondrocytes in the surrounding matrix (marked with dots) demonstrate pyknotic nuclei and signs of cell death.

A close-up view of this area (Figure 2.20) shows a fibroblastic cell within the canal at the very edge of the cartilage, showing a large bulbous cytoplasmic process (at the arrow) penetrating the cartilage matrix. This cell probably actively synthesizes chondrolytic enzymes (Rhodin et al., 1996). The cell next to it is a macrophage with phagolysosomes, active in the removal of cartilage matrix. Also active in the process of cartilage removal are multinucleate chondroclasts (Figure 2.21), where the cell is in such intimate contact with the cartilage matrix that no cell membrane can be discerned at the contact zone marked by red arrows.

Many chondrocytes near the growth cone tip of the canal demonstrate nuclear pyknosis and signs of cell death (Figure 2.22). The cell on the right is a healthy chondrocyte, the one on the left—closer to the vascular canal tip—has died. This cellular death in advance of the leading tip of the canal is probably caused by the release of chondrolytic enzymes by marginal growth cone

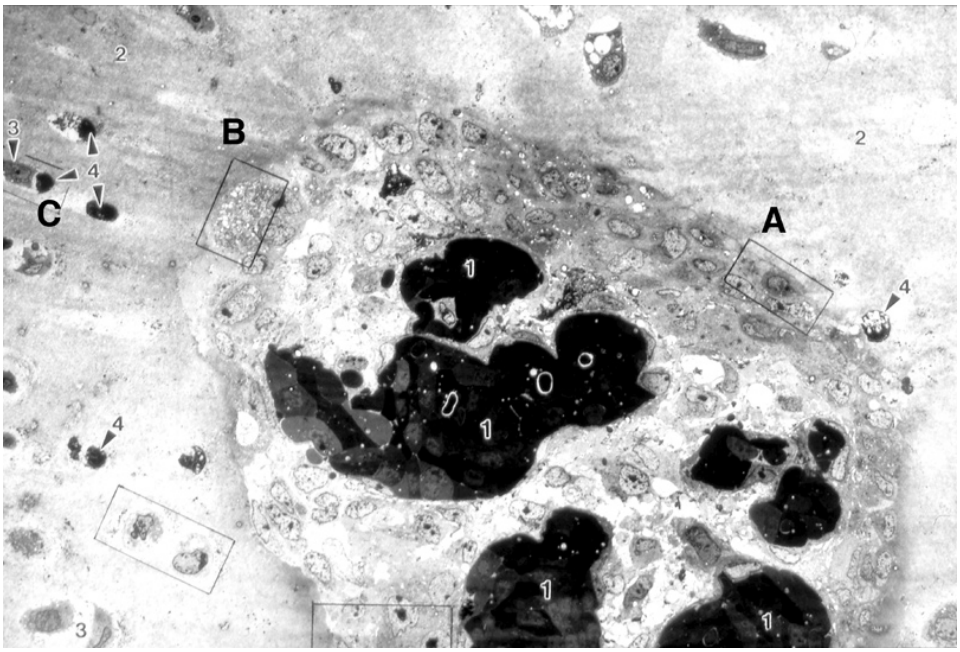

FIGURE 2.19 Humerus of hatchling *Dermochelys coriacea* (electron microscopy), cross-sectional detail of tip of vascular canal bud from Figure 2.17, showing (1) sinusoidal capillaries in tip of canal bud, (2) surrounding cartilaginous matrix, (A, B) rectangles marking chondroclastic cells along the canal edge (see Figure 2.20 and Figure 2.21 for close-ups), (3) hypertrophying chondrocytes, (4) dying chondrocytes, and (C) rectangle marking dying chondrocytes (see Figure 2.22 for close-up) (see text for detail).

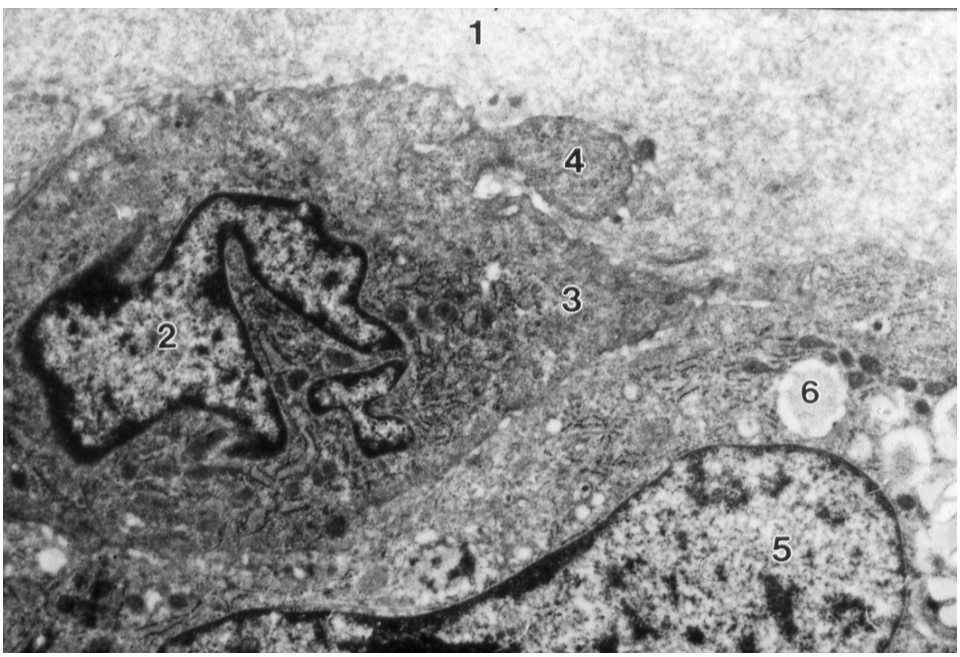

FIGURE 2.20 Humerus of hatchling *Dermochelys coriacea* (electron microscopy), cross-sectional detail of tip of vascular canal bud from Figure 2.17, close-up of rectangle A in Figure 2.19, showing (1) cartilaginous matrix and chondroclastic cells along the canal-cartilage contact zone, including a (2) fibroblast with (3) endoplasmic reticulum and a (4) bulbous cytoplasmic process invading the cartilage, and a (5) macrophage with (6) phagolysosomes.

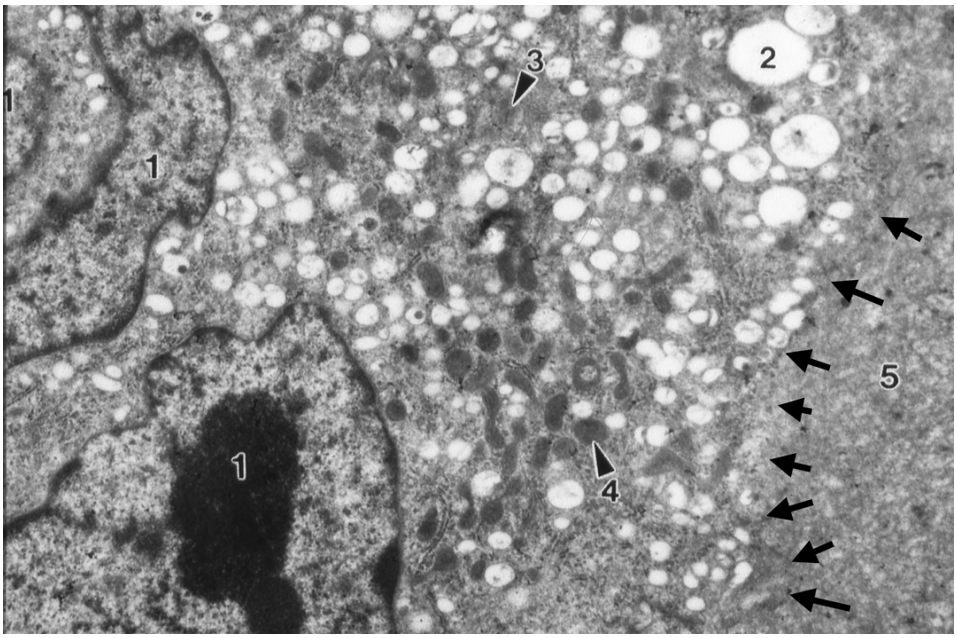

FIGURE 2.21 Humerus of hatchling *Dermochelys coriacea* (electron microscopy), cross-sectional detail of tip of vascular canal bud from Figure 2.17, close-up of rectangle B in Figure 2.19, showing a (1) multinucleate chondroclast with (2) phagolysosomes, (3) primary lysosomes, and (4) mitochondria, in such close contact with the (5) cartilaginous matrix that no cell membrane is visible along the contact zone at the arrows.

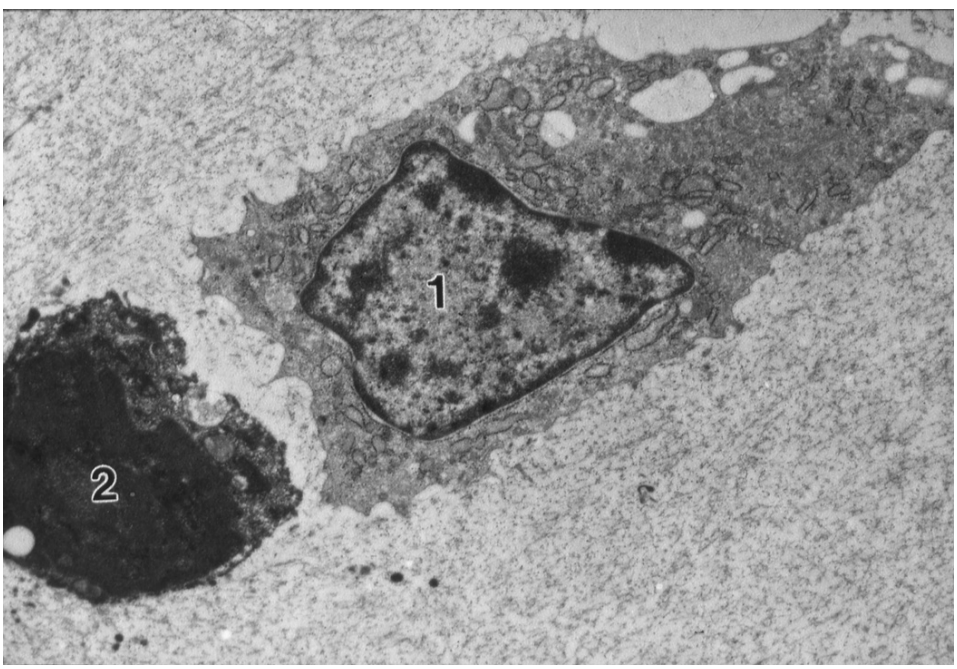

FIGURE 2.22 Humerus of hatchling *Dermochelys coriacea* (electron microscopy), cross-sectional detail of tip of vascular canal bud from Figure 2.17, reversed close-up of rectangle C in Figure 2.19, showing a (1) hypertrophying chondrocyte and a (2) dead chondrocyte closer to the vascular canal (left).

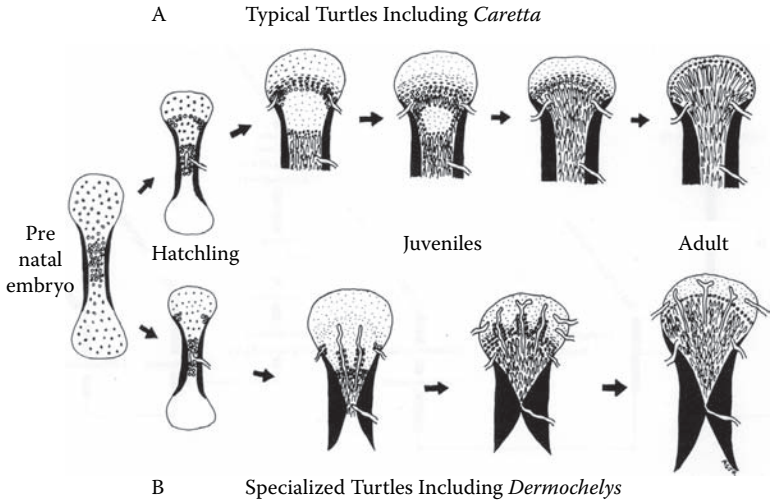

FIGURE 2.23 Schematic representation of the two patterns of skeletal growth that occur in turtles. (From Rhodin, 1985. With permission.) (a) The common pattern in typical turtles is typified by *Caretta*, and the (b) specialized pattern by *Dermochelys*.

fibroblasts (Rhodin et al., 1996). This finding of cellular death near the canal tip had not previously been reported in other vertebrate species with vascular cartilage canals and may be unique to the leatherback (Rhodin et al., 1996). The mechanism probably facilitates more rapid matrix resorption as the vascular canal advances into the cartilage because the dying chondrocytes cannot maintain the proper biochemical environment of the cartilage matrix in advance of the canal tip, which allows for more rapid penetration of the cartilage canal as it grows into the cartilage of the rapidly growing leatherback hatchling. This finding has provided additional support for the hypothesis that leatherbacks have developed cartilaginous vascularization as a specialization related to their rapid growth to a large body size (Rhodin, 1985; Rhodin et al., 1996).

In schematic representation, two patterns of skeletal growth occur in turtles (Figure 2.23, from Rhodin, 1985). The upper pattern, which occurs in hard-shelled sea turtles and all living turtles studied to date (except the leatherback), is characterized by thin avascular cartilage and slow skeletal growth to either small or large body size. The bottom pattern, which occurs only in the leatherback and a few other large extinct marine turtles, is characterized by rapid vascular ingrowth into thick cartilage accompanied by rapid skeletal growth to a large body size.

2.3.1 Implications for Phylogeny

No other living reptile shares the leatherback's pattern of skeletal growth. Though some lizards (notably, large monitor lizards of the family Varanidae) vascularize their cartilages as well, their osteochondral growth mechanisms are different, characterized by perichondral rather than transphyseal ingrowth and the development of secondary ossification centers as opposed to the retention of chondroepiphyses (Haines, 1969). Mammalian and avian patterns of cartilage vascularization are also different from the leatherback and are usually characterized by perichondral rather than transphyseal ingrowth and by the development of secondary ossification centers (Kugler et al., 1979; Moss & Moss-Salentijn, 1983; Kuettner & Pauli, 1983).

Although no living reptiles share the leatherback's specialized chondro-osseous development, what about extinct turtles such as *Stupendemys*, the largest turtle that ever lived? Did its huge body size require vascular cartilages as in the leatherback? Examination of its bones (Figure 2.24) indicates that its joint surfaces had slow-growing avascular smooth cartilaginous epiphyses just like all

FIGURE 2.24 Distal humerus joint surface of adult fossil *Stupendemys geographicus* (SCL midline = 218 cm, straight parasagittal length = 235 cm; Wood, 1976), the largest turtle that ever lived, showing a smooth subchondral surface indicative of overlying thin avascular joint cartilage at the arrow.

other living turtles, and therefore probably reached its huge size slowly.

However, certain extinct sea turtles had skeletal bone structure apparently identical to the leatherback. The giant Cretaceous protostegid turtle *Archelon* had vascular channels penetrating the subphyseal plate from bone into the cartilage above (Figure 2.25), as did the somewhat smaller dermochelyid sea turtle *Corsochelys* (Figure 2.26) and certain other extinct dermochelyid genera, notably *Eosphargis* and *Psephopherus*. Based on these bone structures, these extinct dermochelyid marine turtles would have reached their large size quickly through fast skeletal growth as in the leatherback. However, some dermochelyid turtles did not have vascular cartilages, as in the extinct sea turtle *Desmatochelys*, with typical smooth joint surfaces indicative of thin avascular cartilage (Figure 2.27).

If we look at the phylogenetic distribution of this specialized derived character state of vascularized cartilages on a cladogram of the superfamily Dermochelyoidea (Figure 2.28) as hypothesized by Weems (1988), we see that vascularized cartilage would have had to either evolve four separate times or undergo several reversals. Either scenario does not appear likely. Another phylogenetic view of the same genera as presented by Hirayama (1992) would improve the apparent distribution of this character state (Figure 2.29) but would still have it evolving twice, which may also be unlikely.

Instead, perhaps the unique feature of vascularized cartilage serves as a shared derived character uniting these genera into a monophyletic clade, as hypothesized here (Figure 2.30). Clearly, further work on elucidating overall relationships of multiple character states in these genera will be necessary before any definitive conclusions about phylogeny can be reached, but the unique character state of vascularized cartilages offers a potential key to understanding some of their relationships.

2.3.2 Implications for Growth

The physiologic role of these vascularized cartilage canals in leatherbacks is clearly to facilitate rapid skeletal growth to a large body size. How fast do leatherbacks grow and, most importantly, how soon do they reach sexual maturity? Bone growth studies have helped us answer these questions. Based on captive growth studies and these patterns of bone growth, Rhodin (1985) previously hypothesized that leatherbacks might be able to reach sexual maturity in as little as 3 to 6 years.

No leatherback has ever been followed from hatchling to adulthood, so we do not yet know exactly how long that growth actually occurs. However,

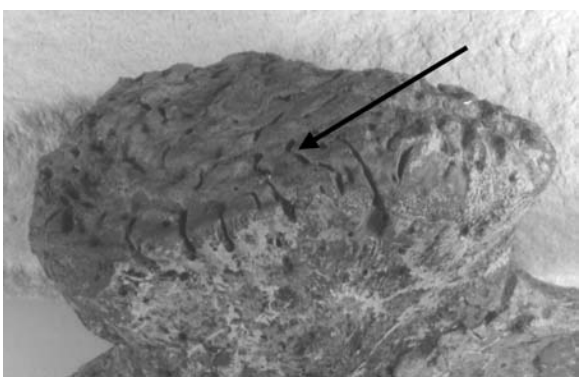

FIGURE 2.25 Proximal carpal bone joint surface of adult fossil *Archelon ischyros*, the largest sea turtle that ever lived (SCL ≈ 190 cm), showing a rough subchondral surface (at the arrow) with large holes indicative of vascular channels extending into overlying thick vascularized joint cartilage.

FIGURE 2.26 Proximal humerus joint surface of fossil *Corsochelys haliniches*, a large sea turtle, showing a rough subchondral surface (at the arrow) with large holes indicative of vascular channels extending into overlying thick vascularized joint cartilage.

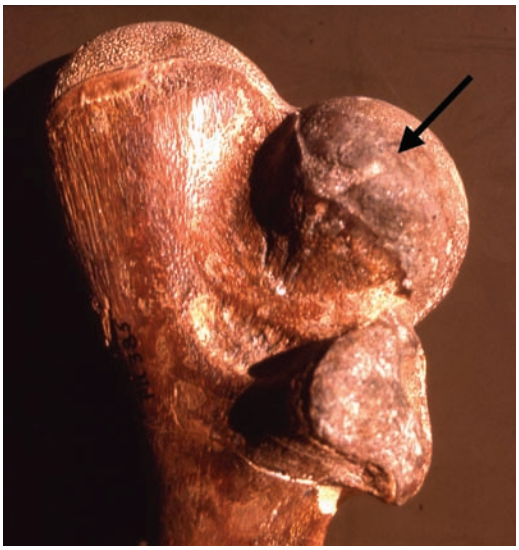

FIGURE 2.27 Proximal humerus joint surface of fossil *Desmatochelys lowi*, a large sea turtle, showing a smooth subchondral surface (at the arrow) indicative of overlying thin avascular joint cartilage.

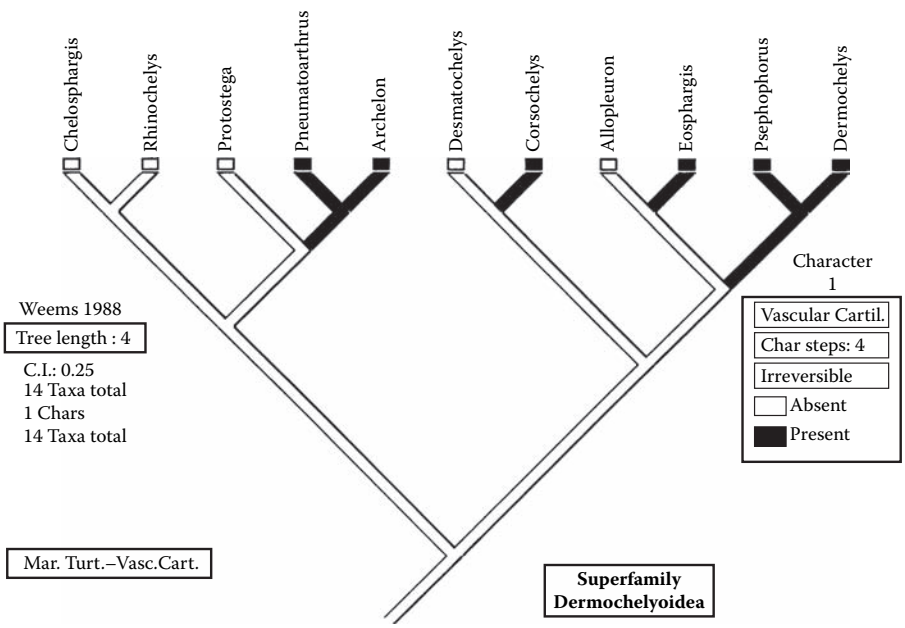

FIGURE 2.28 Cladogram of the superfamily Dermochelyoidea as hypothesized by Weems (1988), showing the distribution of the character state of the presence (black branches and squares) or absence (white branches and squares) of vascularized cartilage.

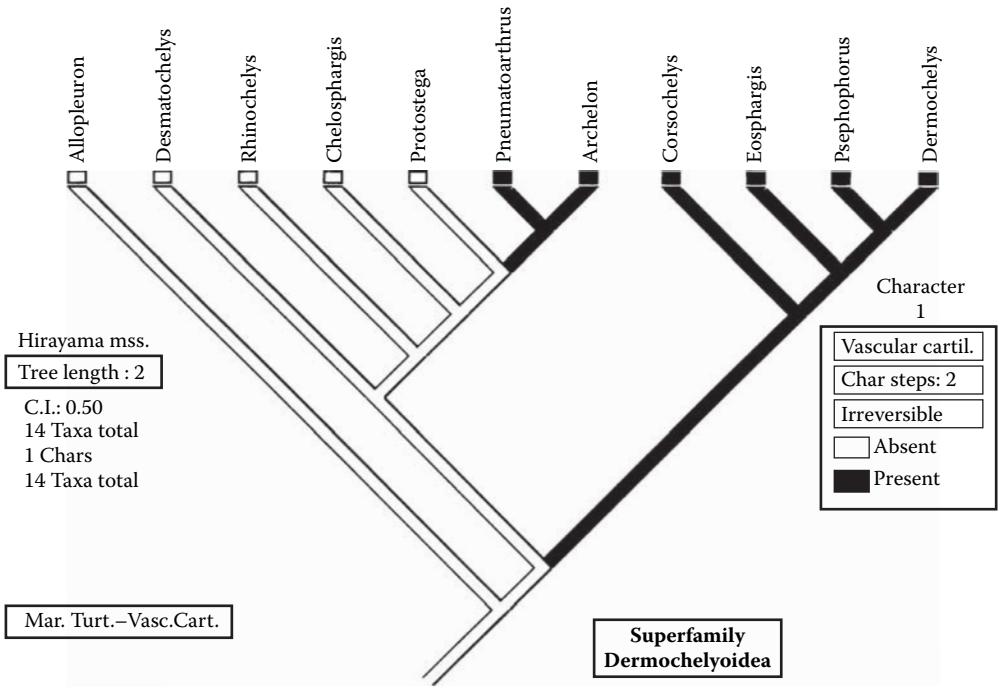

FIGURE 2.29 Cladogram of the superfamily Dermochelyoidea as hypothesized by Hirayama (1992), showing the distribution of the character state of the presence (black branches and squares) or absence (white branches and squares) of vascularized cartilage.

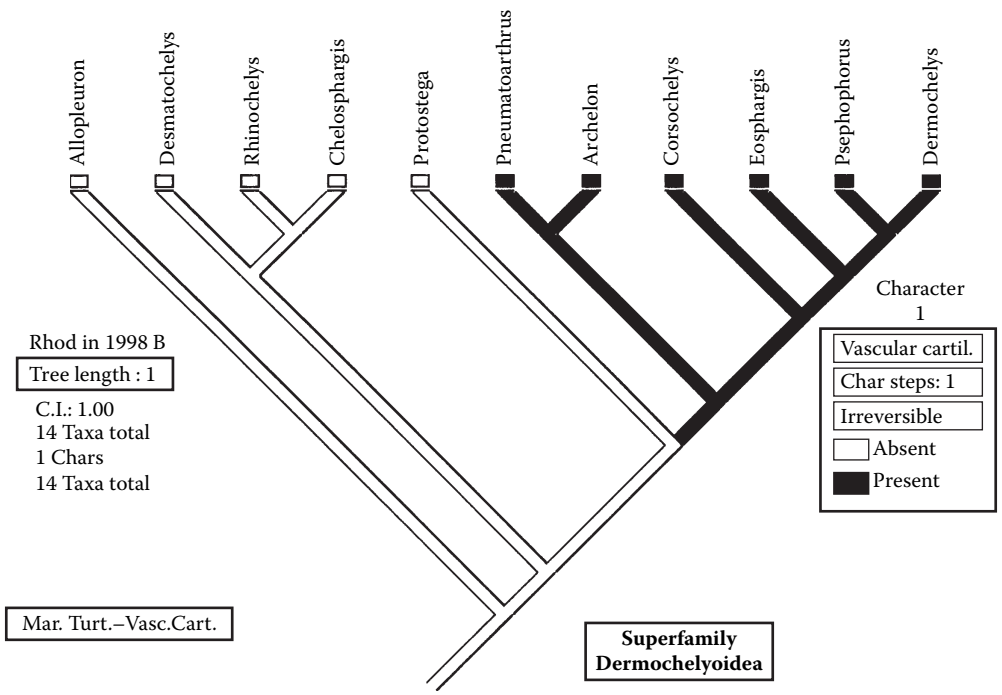

FIGURE 2.30 Cladogram of the superfamily Dermochelyoidea as hypothetically developed by Rhodin for the single character state of the presence (black branches and squares) or absence (white branches and squares) of vascularized cartilage. This is an illustrative example only and not a fully predictive phylogeny.

skeletochronology work on scleral ossicles by Zug and Parham (1996) partially validated the earlier hypothesis and demonstrated that the minimum size at maturity could possibly be reached in as little as 5 to 6 years, and averaging about 9 years to the minimum age at maturity, with 13 to 14 years being an average age of maturity of their studied population of Pacific leatherbacks. New recent studies on captive-reared leatherbacks (Jones, unpublished data) and new skeletochronology work using scleral ossicles from Atlantic leatherbacks (Avens & Goshe, unpublished data) combine to suggest that the age at maturity could be as low as 5 to 10 years but also possibly as late as 25 to 30 years. Based on this uncertainty, for the purposes of our analysis we consider possible ages at maturity for leatherbacks of 5, 10, 15, 20, and 25 years. We follow Zug and Parham (1996) and consider 1445 mm curved carapace length (CCL) as the mean size at sexual maturity. Even with this uncertainty in age to maturity, we can still address the question of how fast leatherbacks grow in comparison to other large sea turtles and marine mammals. Are they more similar to other sea turtles or to marine mammals?

To assess how differently leatherbacks might grow in comparison to other chelonians and marine mammals, we reviewed the literature for information on age and size (length) at sexual maturity for numerous species within these groups, focusing on small cetaceans within the marine mammal group. For turtles, the age at sexual maturity is considered the age when the first clutch is laid. For marine mammals, sexual maturity is when the animal is first fertile, as opposed to age at first reproduction. Table 2.1 details the results of the literature review. With regard to turtles, lengths are typically given as the length of the carapace, either straight length or curved. Lengths of cetaceans are measured from the tip of the jaw to the notch of the tail fluke. Hence, the actual lengths are not necessarily comparable between the two lines, but the general trend in growth rates and where leatherbacks fit are demonstrated.

We ran ordinary least-squares regression on the age at sexual maturity and carapace length for all turtles in Table 2.1, using the values of 5, 10, 15, 20, and 25 years as the age at sexual maturity for leatherbacks (five separate regressions were run). For each regression, we determined Cook's distance values, D_i, for each observation $i = 1 \ldots n$, where n is the number of observations (Cook & Weisberg, 1982). These values measure the effect of deleting the ith observation, and observations with larger D values than the rest of the data have unusual leverage and are likely outliers. Fox (1991) suggests that Cook's distance values greater than $4/(n-p-1)$, where p is the number of parameters, should be considered outliers. In our regressions, this critical value is 0.21, and D_i values for leatherbacks at all the ages to sexual maturity we considered were greater than this value and at least three times higher than the rest of the D_i values. Without leatherbacks, a linear regression through all of the data points for turtles was significant and explained 51% of the variability in length (Figure 2.31). Based on this regression, to have growth rates consistent with the rest of the turtles leatherbacks would have to mature on average at 56 years (95% C.I., 36 to 102 years). The histology of their bone growth, captive growth rates (see references in Rhodin, 1985), and the high rates of recovery recorded on nesting beaches in St. Croix (Boulon et al., 1996; Dutton et al., 2005) do not support such delayed maturity.

In comparison with the relationship between the age at sexual maturity and length for small cetaceans, leatherbacks that mature at 5 to 10 years of age would have growth rates similar to these marine mammals, especially considering that length in leatherbacks is only measuring carapace length and not including the length of the head, as in marine mammals. At ages to sexual maturity of 15 to 25 years, growth rates of leatherbacks would not be similar to marine mammals; however, they would still remain well above those of hard-shelled turtles and fall somewhere between growth rates of chelonids and small cetaceans.

The only living reptiles that approach the leatherback in growth rate on a gram-per-day basis are large crocodilians, with *Alligator mississippiensis* growing at an average rate of about 36 g/d to maturity (Andrews, 1982), and giant Galapagos tortoises, *Geochelone nigra*, growing at rates of as much as 47 g/d in captivity (Case, 1978). The hard-shelled marine turtles grow at average rates of

TABLE 2.1
Estimates of Female Age and Size at Sexual Maturity for Various Turtle Species and Small Marine Mammals*

Species	Age at Maturity (years)	Size (mm)	Source
Marine Turtles			
Kemp's ridley turtle (*Lepidochelys kempii*)	10[a]	600 S	Shaver & Wibbels (2007)
Olive ridley turtle (*Lepidochelys olivacea*)	13[b]	600 S	Zug et al. (2006)
Green turtle (*Chelonia mydas*)	35–40[c]	900 S	Balazs & Chaloupka (2004)
Loggerhead turtle (*Caretta caretta*)	30[b]	900 S	Snover (2002)
Pacific leatherback turtle (*Dermochelys coriacea*)	13–14[b]	1445 C	Zug & Parham (1996)
Freshwater Turtles			
Painted turtle (*Chrysemys picta*)	5–6[d]	160–165 S	Iverson & Smith (1993)
Spotted turtle (*Clemmys guttata*)	12–15[c]	103 S	Litzgus & Brooks (1998)
Snapping turtle (*Chelydra serpentina*)	10–12	280–290 S	Iverson et al. (1997)
Snake-necked turtle (*Chelodina rugosa*)	6.5[e]	210 S	Kennett (1996)
Australian snapping turtle (*Elseya dentata*)	13.5[e]	220 S	Kennett (1996)
Blanding's turtle (*Emydoidea blandingii*)	14–20[f]	192–225 S	Congdon et al. (1993)
Wood turtle (*Glyptemys insculpta*)	17–18[c]	185 S	Brooks et al. (1992)
Mud turtle (*Kinosternon subrubrum*)	5.3–7.3[d]	80-85	Iverson (1979)
Musk turtle (*Sternotherus minor*)	8[c]	80 S	Cox et al. (1991)
Mud turtle (*Kinosternon hirtipes*)	6–8[d]	95–100 S	Iverson et al. (1991)
Terrestrial Turtles			
Texas tortoise (*Gopherus berlandieri*)	4–8 (5)[d]	131 C	Hellgren et al. (2000)
Steppe tortoise (*Testudo horsfieldi*)	9–17 (12.6)[d]	124–177 (148) S	Lagarde et al. (2001)
Box turtle (*Terrapene carolina*)	8[d]	150 C	St. Clair (1998)
Ornate box turtle (*Terrapene ornata*)	8[d]	128 C	St. Clair (1998)
Desert tortoise (*Gopherus agassizii*)	26[b]	190 S	Curtin (2006)
Porpoises			
Harbor porpoise (*Phocoena phocoena*)	3.6	1420	Lockyer et al. (2001)
Dall's porpoise (*Phocoenoides dalli*)	3.8–4.4	1720	Ferrero & Walker (1999)
Finless porpoise (*Neophocaena phocaenoides*)	6–9	1350–1450	Shirakihara et al. (1993)
Dolphins			
Spinner dolphin (*Stenella longirostris*)	3.7–5.0	1650	Perrin et al. (1977)
Common dolphin (*Delphinus delphis*)	8	1707–1728	Ferrero & Walker (1995)
Pacific white-sided dolphin (*Lagenorhynchus obliquidens*)	8.3	1775	Ferrero & Walker (1996)
Northern right whale dolphin (*Lissodelphis borealis*)	9.7–10.4	1998–2011	Ferrero & Walker (1993)

(*continued*)

TABLE 2.1
Estimates of Female Age and Size at Sexual Maturity for Various Turtle Species and Small Marine Mammals (continued)

[*] For turtles, size is measured as carapace length; S indicates straight and C indicates curved. Age in turtles was estimated by either skeletochronology, growth line marks on scutes, mark-recapture direct (animals tracked throughout their life), mark-recapture indirect (growth curves estimated from growth measurements). For cetaceans, size is measured as length from the tip of the jaw to the notch in the rear fluke. All cetacean ages were determined from counts of dentinal growth layer groups. If the authors reported a range of data with a mean, the range is reported here followed by the mean in parentheses.

[a] Age determined by the observation of turtles nesting that had been raised in captivity for the first year of life, marked, and released.

[b] Age determined by skeletochronology.

[c] Age determined indirectly by analysis of growth records from mark-recapture study.

[d] Age determined by counts of growth lines on scutes.

[e] Age determined by a combination of growth line on plastral scutes and a growth model based on recapture data.

[f] Age determined by a combination of scute growth lines and mark-recapture.

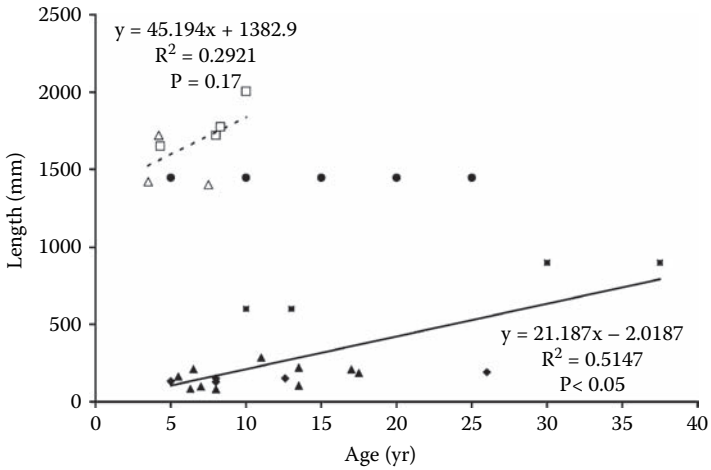

FIGURE 2.31 Plot of age versus size at sexual maturity for turtles and small marine mammals from Table 2.1. Where a range of values was reported, if the authors reported a mean value that value was used in the plot; otherwise, the median value was used. Filled triangles represent freshwater turtles (n = 10), filled diamonds represent terrestrial turtles (n = 5), filled squares represent marine turtles except for leatherbacks (n = 4), and the filled circles represent leatherbacks with the uncertainty in their age at sexual maturity accounted for by considering 5, 10, 15, 20, and 25 years. Based on Cook's distance values, all of these potential ages to sexual maturity for leatherbacks were outliers in the regression of length on age. The solid line represents a linear regression of length on age for the turtles excluding leatherbacks. For marine mammals, the open triangles represent porpoises (n = 3), the open squares represent dolphins (n = 4), and the dotted line is a linear regression between all of the cetacean data.

about 16 to 22 g/d to maturity (Case, 1978; Andrews, 1982), but leatherbacks grow at much higher rates, ranging from a possible 137 g/d if they reach maturity (250 kg) at 5 years of age, 76 g/d if at 9 years, 68 g/d if at 10 years, 46 g/d if at 15 years, 34 g/d if at 20 years, and 27 g/d if at 25 years. Of the smaller marine mammals, fur seals (*Callorhinus ursinus*) grow at about 80 g/d and porpoises (*Phocoena phocoena*) at about 164 g/d, very similar but slightly faster than leatherbacks (Case, 1978).

By comparison, humans grow at the relatively slow rate of about 8 g/dy (Case, 1978). What we have in the leatherback is not only the world's fastest-growing turtle but also its fastest growing reptile. Its bone and cartilage morphology allow that fast growth through its specialized vascularization of rapidly growing cartilage, stimulated no doubt by its heightened metabolism, gigantothermy, and the energetic needs of its pelagic long-distance migratory life.

In summary, the leatherback represents a unique and remarkable life form characterized by specialized and unique morphology. Reptilian in ancestry, testudine in derivation, and chelonioid in affinity, the leatherback has reached a degree of biological specialization unparalleled by other living turtles or reptiles. Its unique specializations make it appear to be converging on the biological regulatory mechanisms evolved by marine mammals; however, given the leatherback's longer evolutionary history, one might more reasonably infer that marine mammals appear to be converging on the leatherback. The leatherback's specialized biology and marvelous adaptations are fertile ground for further studies to increase our understanding of its unique life strategies—providing we can successfully save it from the global threats that are affecting its survival and threatening it with possible extinction, especially in the Pacific (Spotila et al., 1996, 2000; Seminoff et al., 2007). The leatherback represents a rich and unique biological resource whose loss would be both profound and irreplaceable.

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