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Full Length Research Paper

Skeletal ontogeny of Seychelles giant tortoises (*Aldabrachelys/Dipsochelys*)

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The skeletal development of captive bred Seychelles-Aldabra giant tortoises (*Dipsochelys*/*Aldabrachelys*) was studied from late embryos to adults based on 93 specimens (45 embyronic). The pattern of ossification is described and the developments of the characters used to distinguish different morphotypes are investigated. The timing of ossification of different bones in this genus is similar to the patterns reported for other taxa with complete chondrification of the skeleton immediately prior to the onset of ossification. By hatching the main bones of the skull, axial skeleton, limbs and plastron are at least partially ossified. In other turtle carapace, ossificiation has started by hatching but in *Dipsochelys* it does not commence until after hatching. Fusion of carpal and tarsal elements is also delayed. Morphotype differences are apparent in juvenile skeletons in the skull, carapace and fore-limbs. These can be traced to development of structures associated with specific jaw or limb muscles or the effects of muscle action. Three main processes were identified: the progressive development of muscle attachment sites, distortion of cartilage precursors of bones as a result of muscle action, differential growth rates of adjacent bones, differential ossification and differences in the exact form of cartilage bone precursors. These findings shed light on the morphological changes found in different tortoise morphotypes.

Key words: *Aldabrachelys, Dipsochelys,* ontogeny, skeleton growth.

INTRODUCTION

In the 18th, 19th and much of the 20th century taxonomy of tortoises and turtles was based on characteristics of the external appearance, principally the carapace and plastron. Skeletal characters were considered by the main 19th century comparative anatomists, most notably Gray (1831, 1869), Cuvier (1825, 1829), Duméril and Bibron (1834-1836), Günther (1875, 1877) and Boulenger (1889). Modern descriptions of new chelonians include skeletal as well as external characters (e.g. Lapparent de Broin et al., 2006; Praschag et al., 2006; Thomson et al., 2006). These characters are increasingly being combined with molecular data to provide a comprehensive evaluation of taxonomic position of populations of interest. Although molecular data received increasingly more weight in these evaluations, morphological characters will always remain important for recognising and, in most cases, diagnosing taxa.

Morphological studies have relied most on adults showing the typical morphology and very few have attempted to evaluate individual variation (Crumly, 1984), or ontogeny (Bever, 2009). There are three problems inherent in this; in some cases few fully adult specimens may be available and by necessity juveniles may have to be considered, morphology may vary geographically due to genetic drift or local environmental factors, and deciding on how to determine the 'typical' morphology inevitably involves some degree of subjectivity. An ideal study would cover a large sample of animals including all developmental stages and a wide range of populations, including some subject to ecophenotypic variation and some subject to selection or drift. From such studies it may be possible to determine a statistically meaningful typical form, and would also evaluate the significance of deviations from this form. The logistical difficulties of such a study are further complicated in turtles by most species being either highly reduced in range and population or by having been moved between localities by human agency, affecting sample sizes and complicating genetic and

ecological issues. Resolution of historical confusions in taxonomy may in some cases require the synthesis of information on morphology, genetics and adaptation.

In the case of the giant tortoises of the genus *Aldabrachelys/Dipsochelys* (the name of the type species, and hence the generic name, is under petition to the International Commission of Zoological Nomenclature [ICZN] - Frazier, 2009; Bour et al., 2009) of the Seychelles islands and historically Madagascar, the taxonomy has been highly confused due to historical descriptions relying largely on the external morphology of single specimens or unknown origin. Günther (1877) attempted to clarify the situation with a systematic review of all skeletal morphology but this was confounded by the paucity of good material available to him. Shortly afterwards Rothschild (1915) confused the situation still further by returning to consideration of external morphology alone. 79 years later Bour (1994) undertook a careful study of skeletal characters of the extinct Madagascar species and included some data on Aldabran tortoises. In the interim a detailed study of the external morphology of the only surviving wild population of giant tortoises had been undertaken; Grubb (1971) studied the giant tortoise population on Aldabra, providing a clear evaluation of the natural morphology in that locality. Gerlach and Canning (1998) attempted to synthesize all the available information based on a study of skeletal material. This provided diagnoses of six taxa, two ancient extinctions from Madagascar (*D. grandidieri* and *D. abrupta*), one extinct species from Seychelles (*D. daudini*) and three living Seychelles species (the Aldabran tortoise and *D. hololissa* and *D. arnoldi*). Further osteological details were added by Gerlach (1999). These taxa have not been distinguished by genetic studies (Austin et al., 2003; Palkovacs et al., 2003; Le et al., 2006; reviewed in Gerlach, 2004, 2005) although captive bred animals show diagnostic characters in external morphology (Gerlach and Bour, 2003; Gerlach, 2011) suggesting at least some inheritance of characteristics through genetics or maternal effects. Here these forms are considered morphotypes without reference to taxonomic ranking. Captive breeding since 2002 provides the possibility of investigating the development of the morphotypes, making it possible to distinguish inherited characteristics from environmentally influenced development. A study of external development (Gerlach, 2011) has been completed and this is complemented here with an analysis of the skeletal development.

MATERIALS AND METHODS

The nomenclature of this genus (referred to as either *Aldabrachelys* or *Dipsochelys*) and the taxa within it is highly contentious. In order to avoid these contentious aspects the genus name is avoided here and the forms are referred to as morphotypes. The most common form is the Aldabran giant tortoise for which the specific epithet *gigantea* or *dussumieri* has been applied (under review by ICZN: Frazier, 2009; Bour et al., 2009), the author's preference for *dussumieri* is followed here pending the ICZN ruling.

As many skeletons of a wide a range of embryos and juveniles were examined as could be located using animals captive bred by Nature Protection Trust of Seychelles on Silhouette island in Seychelles (embryos and hatchlings of the *arnoldi*, *hololissa* and *dussumieri* morphotypes, and a juvenile *arnoldi*) and from skeletons from wild animals examined on the Seychelles islands of Aldabra and Curieuse (*dussumieri*). This comprised 93 specimens, of which 57 were immature. Material studied is summarised in Table 1. Comparisons were made between different sized individuals and particular attention was paid to characters previously considered taxonomically diagnostic (Gerlach and Canning, 1998). Osteological features were labelled after Gaffney (1972, 1979).

The material examined was not obtained specifically for this study, but rather represent opportunistic examination of specimens arising from natural mortality; no animals were sacrificed for the study. Although this means that no ethical issues are raised the material sampled is not ideal for all aspects of an ontogenetic study in that embryos had not been preserved for staining for detailed microscopical studies and were not uniformly spread across all age groups. A particular limitation is the lack of accurate ageing of embryos; all embryos were dead-in-shell embryos obtained from eggs that had passed beyond the expected hatching date. These covered the full range of developmental stages but their chronological age at death could not be determined. Although the use of standard stages in embryology has been criticized (Werneburg, 2009) uncertainty over the ages of the embryos available for the present study means that the standard event system approach is not practical.

Embryos were dissected under a stereo dissection microscope. Ossification was detectable in embryos equivalent to Yntema's (1968) stage 17 at which point all limb elements are present in cartilaginous form at least. The pattern of ossification is described relative to this reference point. Ossification was identified as the presence of hardened, rugose structures indicative of osteogensis (Rieppel, 1993) as has been used in recent studies where early stages of ossification have been found to be inadequately stained with standard alizarin red stain (Sheil, 2003; Sheil and Greenbaum, 2005; Sánchez-Villagra et al., 2009; Lima et al., 2011). Surface texture and tissue density provide poor contrast in photographs, consequently only drawing of embryonic structures is provided here.

The embryos with some ossification were divided into three categories which could be related to approximate development stages: small embryos with very limited ossification (stages 17-19), half-size to full size embryos with extensive but incomplete ossification (stages 20-21) and almost fully developed embryos with ossification comparable to that of hatchlings (stages 22-26). In the results and discussion developmental stages are referred to the size categories in Table 1.

RESULTS

The first embryo showing ossification is referable to Yntema stage 17 (here assigned to category E1). The degree of ossification and its development are summarised as follows:

Chondrocranium

At E1 the chondrocranium is fully formed. The nasal capsules form approximately 15% of the total neurochranium length. They are rounded with a small

Table 1. Skeletal material examined in the present study.

Age group	Stage	*Dussumieri*		*Arnoldi*	*Hololissa*	*Daudini*	Total
		captive	wild				
Adult		19	9	1	6	1*	36
Juvenile	J4	0	1	0	0	0	1
	J3	0	2	1	0	0	3
	J2	1	0	0	0	0	1
	J1	1	0	1	0	0	2
Hatchling		2	0	2	1	0	5
Embryo	E3	5	0	9	10	0	24
	E2	1	0	5	4	0	10
	E1	2	0	6	3	0	11
Total		31	12	25	24	1	93

*postcranial material only Developmental categories: Adult > 51 cm; J4 = 41-50 cm; J3 = 31-40 cm; J2 = 21-30 cm; J1 = 11-20 cm; hatchling = < 11 cm; E3 = maximum ossification prior to hatching; E2 = extensive but incomplete ossification; E1 = very limited ossification.

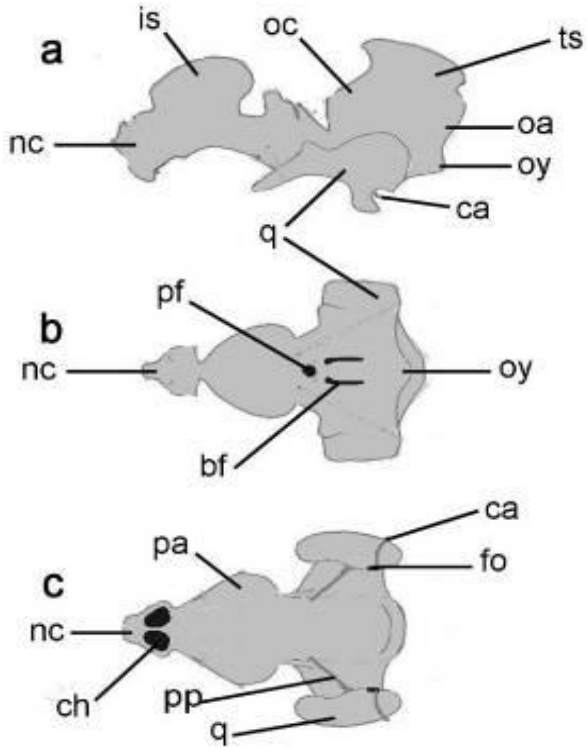

Figure 1. Chondrocranium at stage E1. Cartilage shaded, fenestrae shown in black.
a: lateral; b: dorsal; c: ventral. Bf: basicaranial fenestra; ca: columella auris; ch: choana; fo: fenestra ovalis; is: interobital septum; nc: nasal capsule; oa: occipital arch; oc: otic capsule; oy: occipital condyle; pf: pituitary fenestra; pp: pterygoid process; q: quadrate cartilage; ts: tectum synoticum.

anterior process ventral to the fenestra narina. The lateral walls of the capsules were formed by the antorbital plate. The capsules have wide orbitonasal fenestrae opening to the orbital region. Large choanae open on the ventral surface of the capsules. The pituitary fenestra opens on the dorsal surface of the orbitotemporal region, the fenestra is pentagonal in outline. This is separated from the larger basicranial fenestra by the crista sellaris. The interorbital septum is well ossified, anterior to the foramen for the optic nerve. The otic capsules are well developed and form 30% of the neurocranium length. The fenestrae ovale open lateroventrally. The columella auris is present as a slender rod traversing the space between the fensestra ovalis and the lateral opening of the cavum tympani. The tectum synoticum extends from both otic capsules, meeting dorsally on the midline and projecting forwards and backwards (Figure 1).

Maeckel's cartilage and hyoid apparatus

At E1 both Maeckel's and hyoid cartilages are well chondrified.

Skull

In the earliest ossified embryos (E1) centres of ossification can be identified in membranes surrounding the chondrocranium. These are identifiable as the maxillae, prefontals, parietals, squamosals, jugals, palatines, pterygoids, frontals, premaxillae, postorbitals and vomer. Initially the maxilla forms an elongate plate below and anterior to the eye, the squamosals as triangular plates overlying the otic capsule, the frontals as narrow bars forming the antero-dorsal margins of the orbits (Figure 2). The initial phase of ossification is

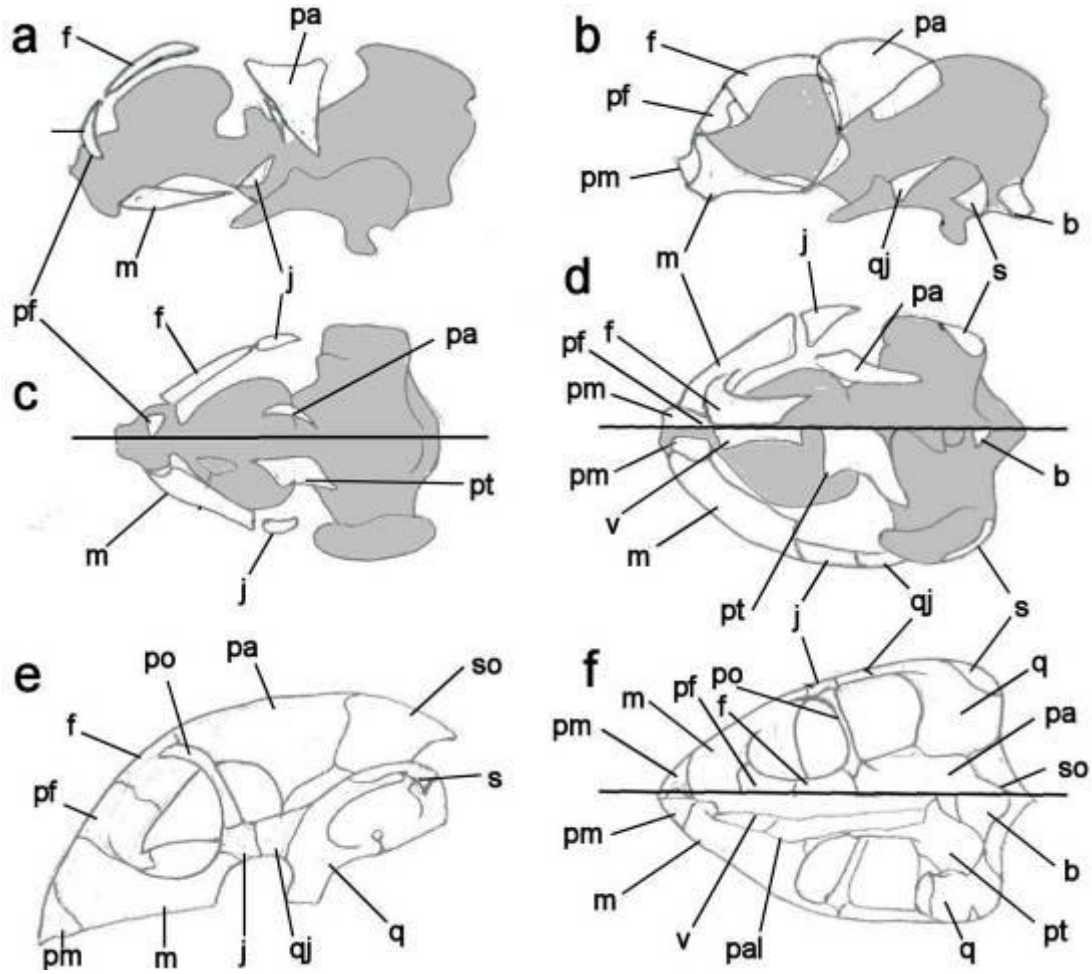

Figure 2. Skull development in embryos showing cartilage (shaded) and bone (white) areas in embryonic stages E1 (a and c), E2 (b and d) and E3 (e and f) in lateral (a, b, e), dorsal (top half of c, d and f) and ventral (bottom half of c, d, and f) views. **b**: basioccipital; **f:** frontal; **j:** jugal; **m:** maxilla; **pa:** palatine; **pal:** palatine; **pf:** prefrontal; **pm:** premaxilla; **po:** postorbital; **pt:** pterygoid; **q:** quadrate; **qj:** quadratojugal; **s:** squamosal; **so:** supraoccipital; **v:** vomer.

followed in E2 first by expansion of the prefrontals dorsally to form the anterior margin of the orbit, the expansion of the jugal to its definitive form and the start of ossification of the quadratojugal and the chondrocranium. The cartilaginous elements that ossify first are the quadratojugal, basisphenoid, squamosals, exocciptials and the stapes (columellae). Subsequently the basioccipital, prootics and opisthotics ossify, the maxilla expands to its definitive shape and size, the prefrontals separates the orbit and the nasal capsule, the palatines expanding. Later the pterygoids expanding to form the main roof of the mouth, the supraoccipital ossifies as a curved plate over the chondrocranium, along with the epipterygoid. The last skull bones to ossify are the quadrates (in E3). These form around the anterior margin of the incisura columella auris, initially forming an even semicircle.

At hatching the premaxillae are small and project downwards slightly on the anterior margin. They develop small denticles and become arched by J3. The maxillae are fully developed at hatching but lack maxillary denticles until J3. The supraoccipital remains small, projecting back beyond the skull margin with a short, pointed spine; it lacks any trace of a supraoccipital crest. By J3 the supraoccipital is developed into a low crest which extends posteriorly beyond the occipital condyle and dorsally to a level equal to the highest point of the parietals. The postorbitals are very narrow and descend at an angle of 45° to the horizontal, the angle becomes closer to horizontal in adults, but in all juveniles examined here it remains close to 45°. The descending process of the parietals of *arnoldi* develops a distinct vertical ridge in juveniles (Figure 3), in the adult this forms a crest unlike in adults. It is not present at any stage in other morphotype. The quadrates are smooth curved bones, the incisura columella auris is fused shortly after hatching

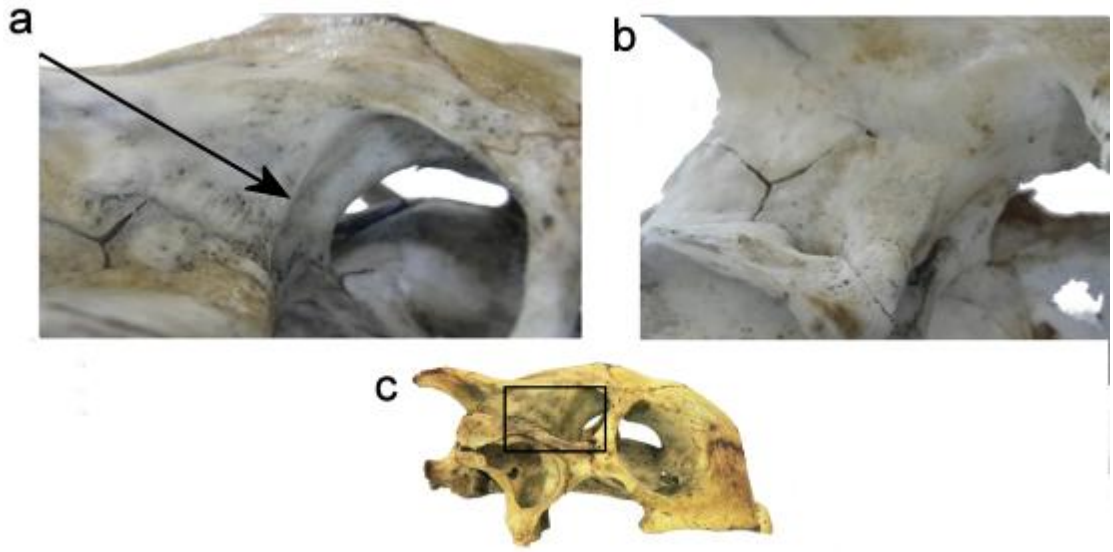

Figure 3. Juvenile (J3) skulls showing the development of the vertical ridge on descending process of the parietals in the *arnoldi* morphotype (a), and its absence in *dussumieri* (b). Location of detailed photographs marked in skull view c.

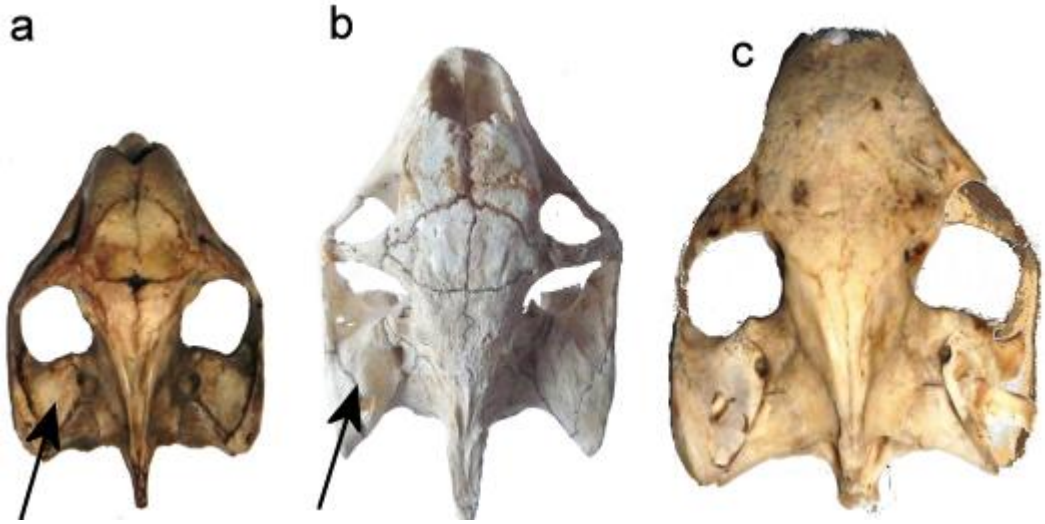

Figure 4. Dorsal view of the tympanic cavity of (a) J3 *arnoldi*, (b) adult *arnoldi* (photo: R. Bour), (c) adult *dussumieri*, showing.

but the upper margin of the quadrate retains an open notch in all juveniles. The dorsal surface of the quadrate is noticeably elevated in the J3 and adult *arnoldi*, associated with an inflation of the postero-dorsal part of the tympanic cavity (Figure 4). At no stage is any inflation of the dorsal surface of the quadrate apparent in *dussumieri*. The squamosals lack any ornamentation and do not reach the back of the otic capsule in embryos and juveniles, at J3 the squamosal approach the posterior margin of the quadrates, full development is reached at J4. The foramen orbito nasale is moderately large (being 3-4% of the width of the skull at that level) at hatching, and remains so in J3, but is reduced in size by J4 (<2%). The processus trochelaris oticum is low in all juveniles; it becomes increasingly prominent in adults, but remains relatively small in *dussumieri*, compared to *hololissa*. On the dorsal surface of the vomer the processus vomerinus dorsalis is not detectable in hatchlings but is fully developed by J3. Ventrally the basisphenoid and the basioccipital are flat in most individuals of all sizes, although some adult of *dussumieri* and *hololissa* have a depression on the basioccipital, it is

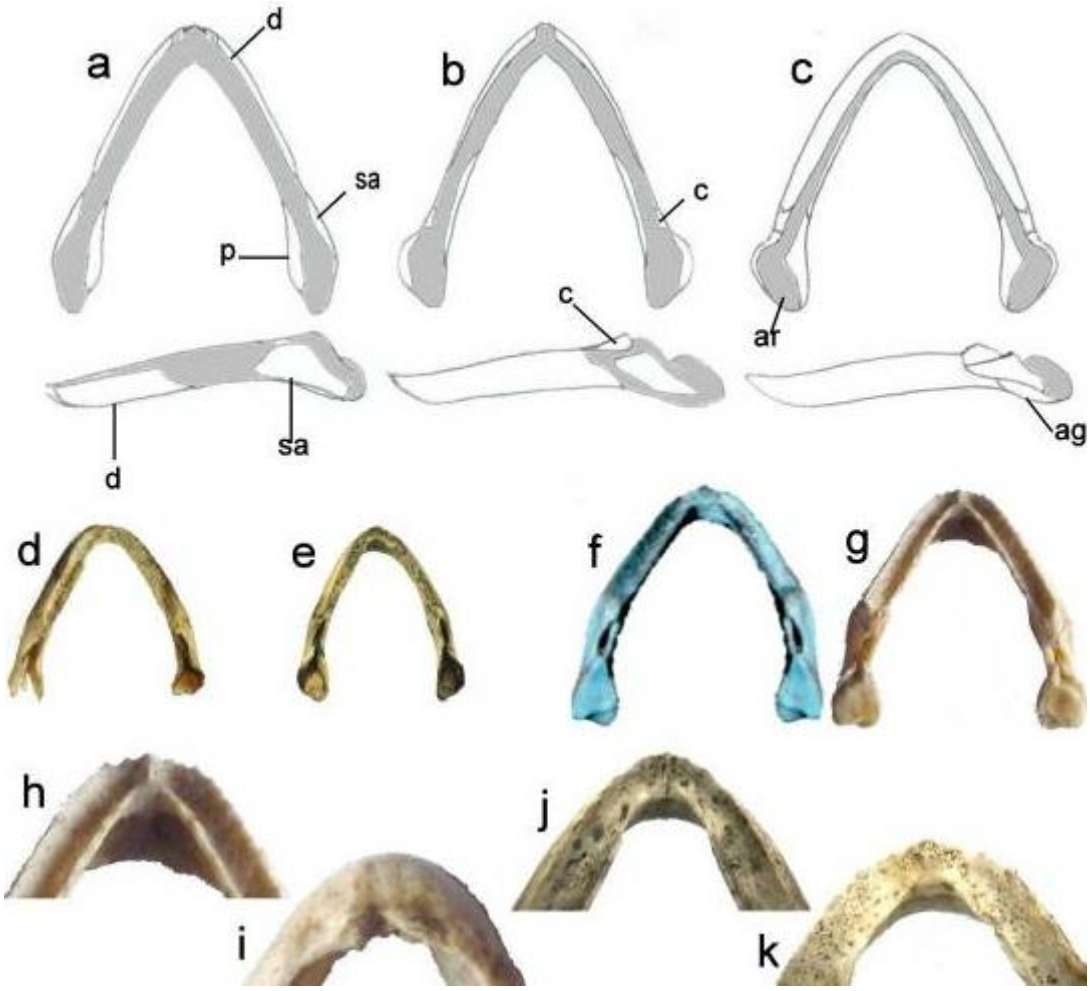

Figure 5. Lower jaws showing ossification patterns (a-c) general outline in dorsal views (d-g) and the detail of the dentary symphysis (h-k). Development is shown in E1 (a), E2 (b) and E3 (c) stages in dorsal (top) and lateral (lower) views, shading – cartilage, white – bone. (d) *dussumieri* J3, (e) *arnoldi* J3, (f) *dussumieri* adult, (g) *arnoldi* adult, (h) arnoldi adult dorsal view showing geniohyoideus ridge, (i) *arnoldi* adult in ventral view showing irregular margin of geniohyoideus ridge due to tendon ossification, (j) *arnoldi* J3 dorsal view showing small ridge, (k) *dussumieri* adult showing lack of ridge. **ag:** angular; **ar:** articular; **c:** coronoid; **d:** dentary; **p:** prearticular; **sa:** surangular.

not known at which stage this develops. The basioccipital of the *arnoldi* J3 is strongly arched. These bones are not fused and only loosely sutured until J4 when fusion occurs. The processus circumolfactorius is large laterally and irregular in shape in hatchlings and juveniles, the medial extension is smaller; a low ridge is present either on the process or posterior to it. This process develops grows more slowly than adjacent bones, resulting in it becoming relatively small in adults.

Hyoid

The ceratobranchials start to ossify at the same stage as the main skull bones. Only the cornu branchiale ossify and full ossification is apparent by J3.

Lower jaw

Bones of the lower jaw start to ossify at the same time as the first skull bones, starting with the dentary. As this expands to cover Maeckel's cartilage the surangulars, coronoids, angulars, and prearticulars become identifiable as ossified elements (Figure 5). The articulars only starts ossifying after hatching and the anterior portion does not ossify until the late juvenile state.

In juveniles the labial and lingual ridges reach full development by J3, these remain weakly toothed until J4. The internal margin retains the groove for the Maeckel's cartilage throughout life. The articulars become progressively ossified. In the *arnoldi* morphotype the ossified region of the articulars is already longer than wide at J3 (length/width = 1.3), reaching 1.6 in the adult.

Figure 6. Complete skeleton of J3 *arnoldi* showing suturing of all carapacial bones and presence of marginal fontanelles.

In *dussumieri* the ossification is slightly delayed: at J3 L/W = 0.9 and at J4 L/W = 1.4. It has been noted elsewhere that the ossification of the articulars never proceeds beyond L/W = 1.1 in *hololissa* (Gerlach and Canning 1998). Lower jaw bones are well sutured but not fused by J3 in all forms; partial fusion occurs in J4 and is complete in most adults.

In the *arnoldi* J3 the internal surface of the symphysis bears a small ventral lingual ridge corresponding to the exceptional geniohyoideus flange of adult *arnoldi.* This is not apparent in any specimen of any other taxon. The dentary is curved in dorsal view in all stages of all taxa except *arnoldi* in which the dentary is distinctly angled at all stages.

Vertebrae

Vertebral ossification is only apparent in E2, after skull ossification has started. Ossification is initiated in cervical, dorsal, sacral and caudal centra simultaneously. Neural arches begin to ossify shortly afterwards but fusion of centra and neural arches does not occur until well after hatching. In J3 centra and neural arches are still not fused, but fusion is apparent in J4. Ossification in the cervical vertebrae starts from the posterior vertebrae first and in the caudal series it starts in the anteriormost centra. Ossification of the dorsal ribs starts in the middle portions. Once all skull bones are starting to ossify the rib ossification starts to extend medially and laterally and is initiated in the sacral and caudal ribs. Ossification does not extend the full length of the ribs until just before hatching. At hatching the 8th dorsal vertebra is 75% the length of 7th, dorsals 1-7 increase in length faster than 8-9; by J3 the 7th is 61% of the 8th, falling to 35-40% in adults. In *hololissa* the 8th is 80% of the 7th in adults. All vertebrae remain distinct through to J4, although the 8th and 9th are very closely sutured (they are fused in adults). The 1st, 8th, 9th and first sacral are all fused to the overlying neural bones by J3.

Carapace

No evidence of ossification of nuchal or costal bones was found in any embryonic specimens. In other taxa (e.g. Trionychidae) nuchal bones may ossify in the embryo (Sheil, 2005). The periostial collars of the dorsal ribs were expanded in hatchlings. At hatching there is no carapace ossification; only dorsal vertebrae and ribs are ossified (Figures 6 and 7). J1 sees the ossification of the centres of the first neural bones as irregular discs overlying the dorsal vertebrae. By J3 all carapacial bones are at least partially ossified; the neural series and pygal bones are complete but costals still incomplete, leaving all peripheral fontanelles open. In *arnoldi* J3 neurals 1-2 are well sutured, 3-4 are moderately well sutured, 5-7 are very tightly sutured, neurals 3 and 5 reach their maximum size in J3 (52-54 mm compared to 32-55 mm in adults); their shape is: 6-8-4-6-6-6-6. In *dussumieri* the suturing in J4 is more uniform and although the sutures are well developed all are weak and still retain some mobility, at

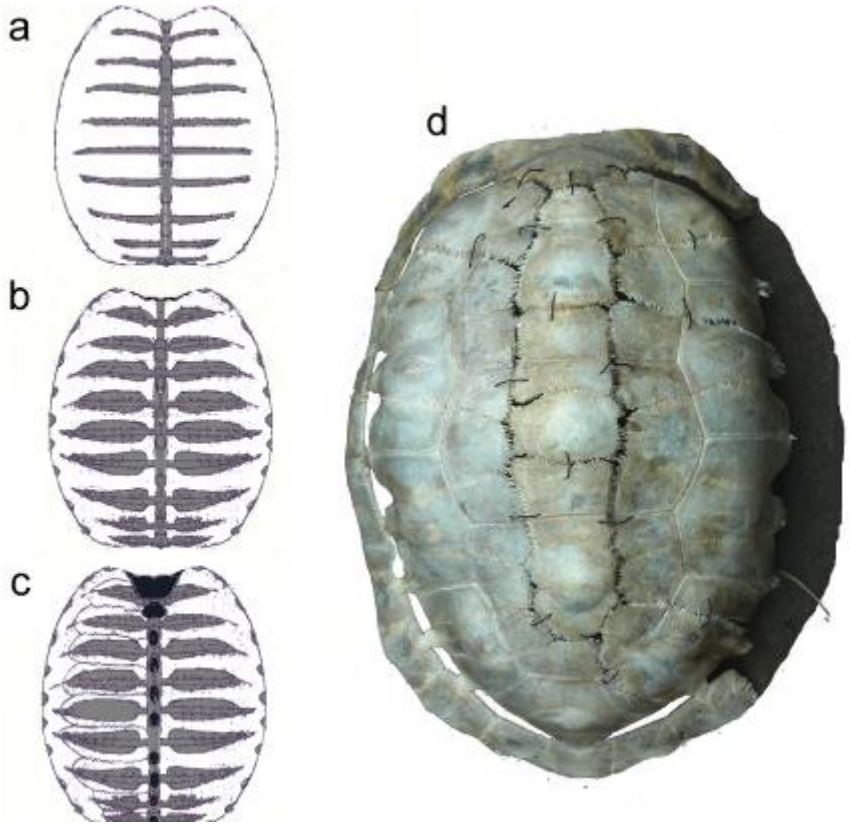

Figure 7. Carapace development in embryo stages E1 (a), E3 (b), hatchlings (c) and J3 juveniles (d). Grey – bone; black - neural bone ossification.

J3 neurals are no more than 50% of adult size; their arrangement is: 4-8-4-8-4-8-6-4. Peripheral bones are all well sutured by J3.

Plastron

The plastral bones start to ossify at the same time as the first vertebrae ossify; in E2 ossification is apparent in the eipplastron, entoplastron, hypoplastron, hyoplastron and xiphiplastron (Figure 8). The plastral bones form as ossification centres in membrane rather than from cartilage precursors. Ossification starts from the centres, spreading outwards. In embryos showing early stages of plastral ossification the epiplastra are crescent-shaped, laterally concave, and the entoplastron is a short, broad structure, curved forwards slightly. The hyoplastra and hypoplastra are thin, strongly curved rods. The posterior portions of the hypoplastra lie close to the anterior extensions of the triradiate xiphiplastra. In late embryos (E3) all these bones have expanded to approximate their adult shapes, although they have only very narrow contacts. In hatchlings the hyohypoplastral and hypoxiphiplastral fontanelles remain very large. At J3 the medial hyohypolastral fontanelle is still large and a small hypoxiphiplastral fontanelle is retained. The epiplastron is still not fully developed posteriorly, with a notch being retained between their posterior margins and the antero-lateral margin of the hyoplastron. All the bones are well sutured. Plastron ossification is almost complete in J4.

Pectoral girdle

At E1 the pectoral girdle has formed as a triradiate cartilaginous structure, with only a slight flattening and distal expansion of the scapular cartilage (Figure 9). The scapula and coracoid first start ossification once the full series of vertebral centra have ossified (E2). Ossification starts in the middle of the scapula and the coracoid cartilaginous precursors. By hatching the cartilage is well ossified.

Forelimb

By E1 all cartilaginous structures of the forelimbs are formed (Figure 9). The humerus, radius and ulna all start ossifying at the same time as the scapula and coracoid (E2). These are followed by the metacarpals and

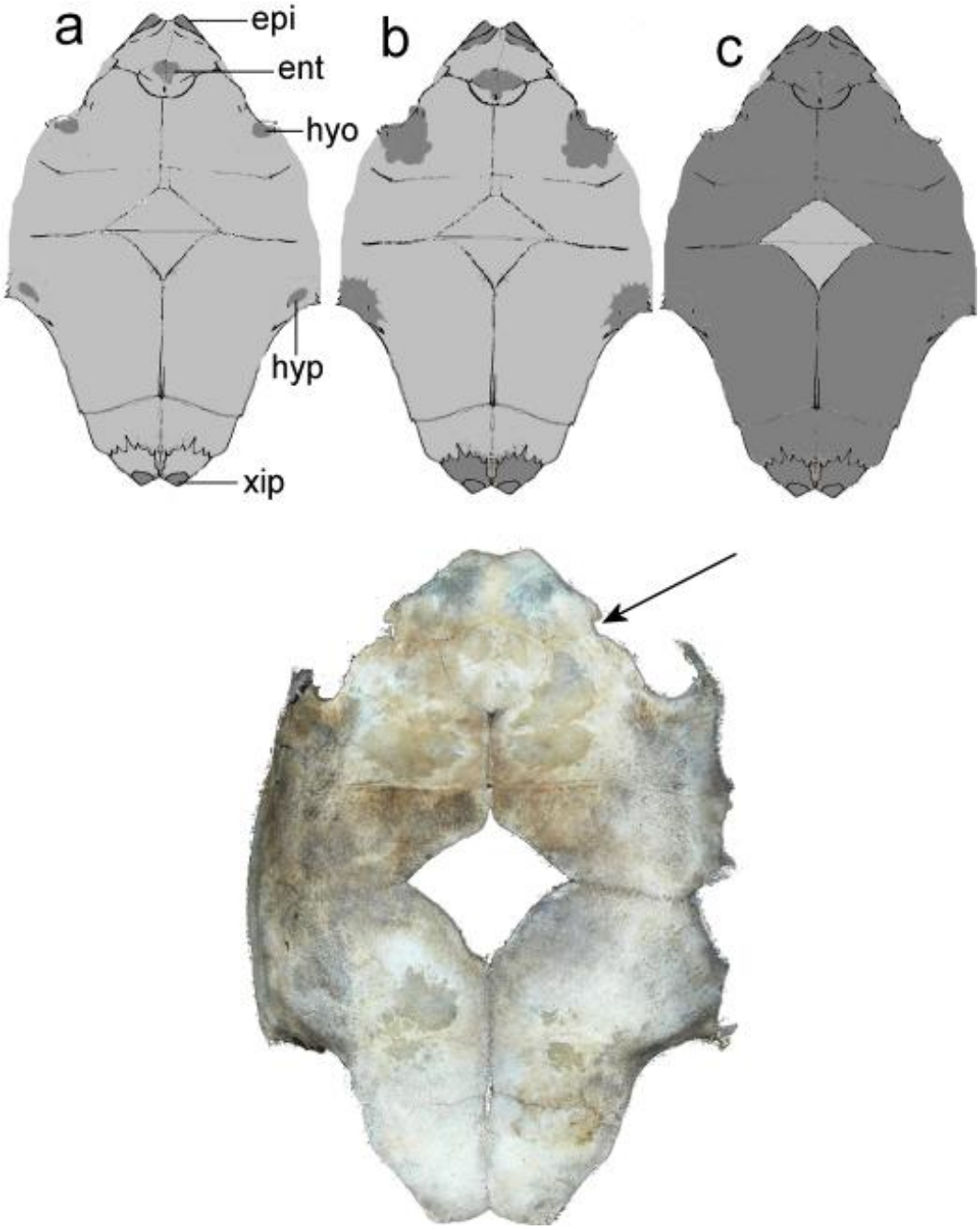

Figure 8. Plastron ossification in embryos (E1 – a, E3 – b), hatchlings (b) and juveniles (J3 - d). The J3 juvenile shows open fontantelles and the notch between the margins of the epiplastron and the hyoplastron (arrowed). **Light grey:** areas ossified in adults; **dark grey:** ossified at each stage; **ent:** entoplastron; **pi:** epiplastron; **hyo:** hyoplastron; **hyp:** hypoplastron; **xip:** xiphiplastron.

phalanges. The ulnare ossifies slightly before the intermedium. The humerus of the J3 *arnoldi* has an indistinct round coranoid fossa, in most others it is oval, but the outline is indistinct in all juveniles. By J3 a slight rugose depression has developed proximal to the humeral head in *arnoldi*, in the adult this forms a distinct pit. This pit is developed as a slight depression or flat facet in large adults of the other morphotypes but is not apparent in juveniles. In the hatchlings the trochanters are poorly developed, but are prominent by J3. They are too poorly developed in hatchlings to determine their relative angles; in juvenile and adult *arnoldi* the major and minor trochanters are parallel. In all other taxa they are always diverging. The ulna very strongly curved, with a

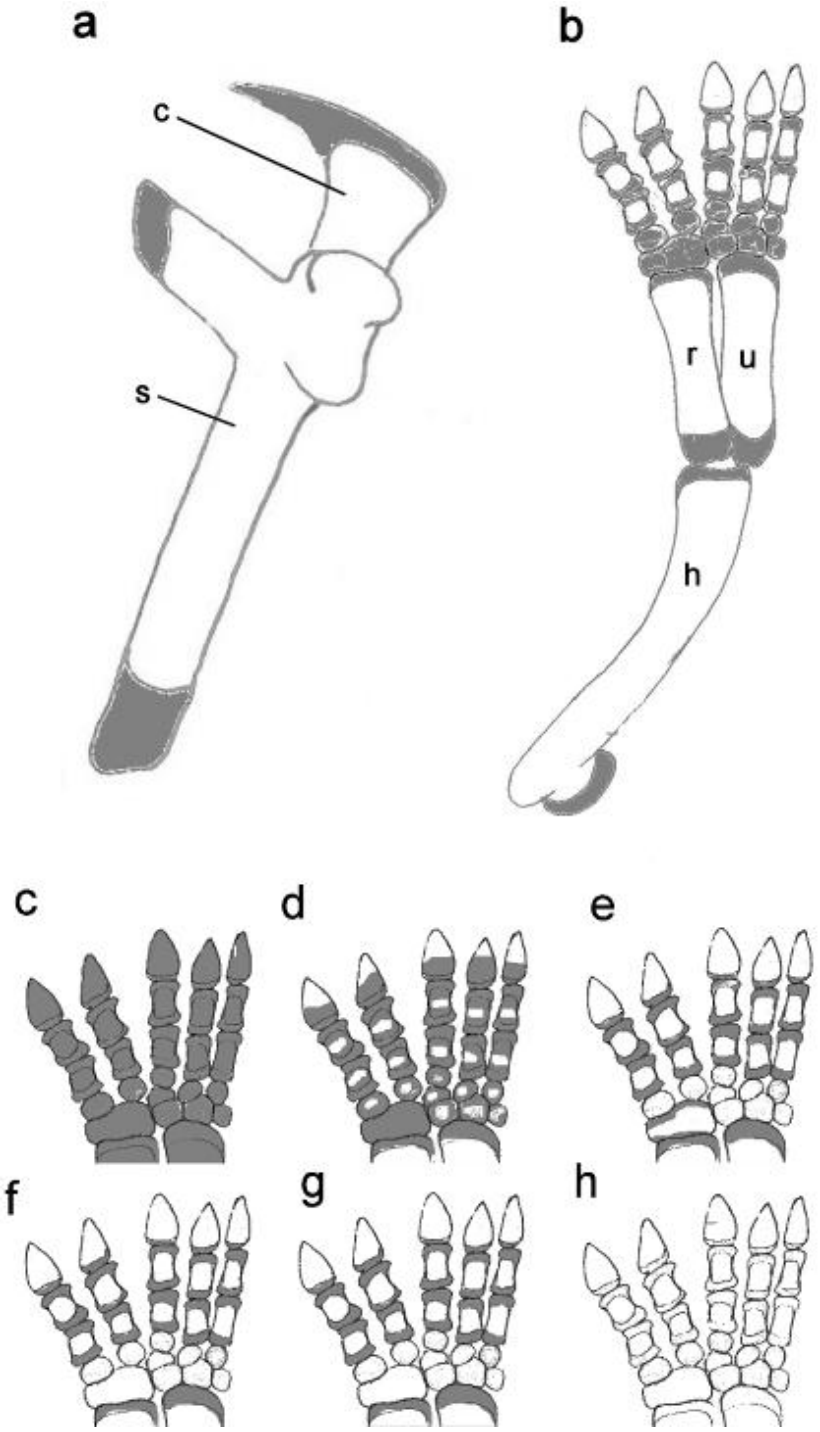

Figure 9. Ossification of pectoral girdle (a), forelimb (b) and carpus (c-h). a-b show areas ossified in embryos in white, ossified by J3 in grey. Carpus development shown in stages E1 (c), E2 (d), E3 (e), hatchling (f), J1 (g) and J3 (h). **white**: ossified; **grey:** cartilage; **c:** coracoid, **h:** humerus, **r:** radius, **s:** scapula, **u:** ulna.

moderately well developed oleocranon process by J3.

By E1 all bones of carpus were chondrified as distinct elements. Ossification was not detected in carpal elements until E3 when all bones of the manus are at least partially ossified but remain separate. By J3 the radial, proximal central and medial 2 are all fused. In

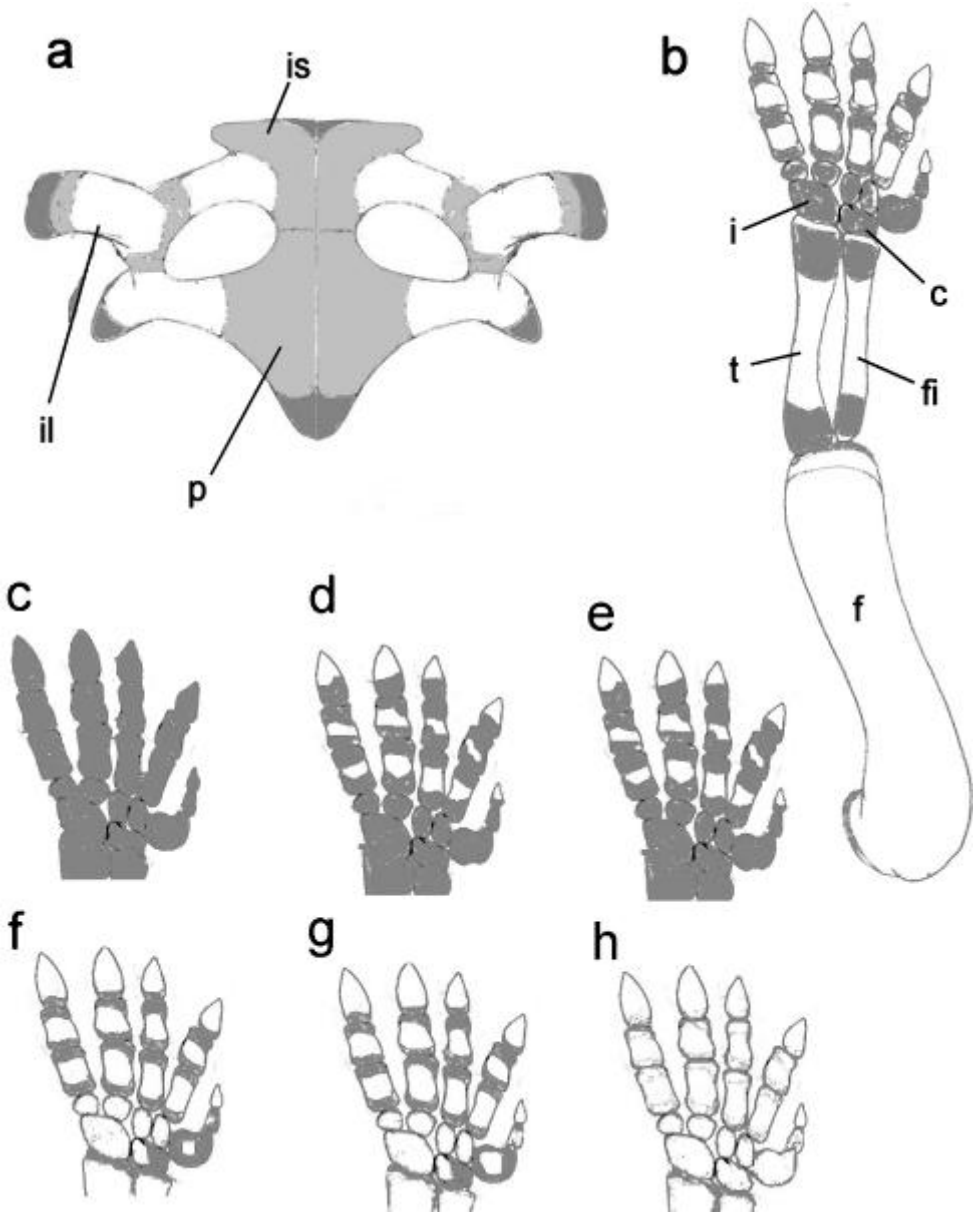

Figure 10. Ossification of pelvic girdle (a) and hind limb (b) and tarsus (c-h). a-b show areas ossified by hatching in white, by J3 in grey, areas of adult pelvic ossification in dark grey. Tarsal ossification shown at developmental stages E1 (c), E2 (d), E3 (e), hatchling (f), J1 (g) and J3 (h). white – ossified; grey - cartilage. **c** – centrale; **f** – fibula; **i** – intermedium; **is** – ischium; **il** – ilium; **p** – pubis; **r** – radius; **u** – ulna.

adults of the genus there is a variable degree of fusion of carpal bones, always including the radial, proximal centrale and medial 2 and sometimes medial 3.

Pelvic girdle

The pelvic girdle is chondrifieds by E1. Ossification of the pelvic bones coincides with the pectoral girdle (E2). All elements appear to ossify at about the same time (Figure 10). A large cartilaginous prepubis remains anterior to the pubic bones in all specimens except for adult females where it is fully ossified. The pubic bones do not fuse until the adult stages; in *hololissa* fusion never occurs (Gerlach and Canning, 1998).

Hind limb

All limb bones are chondrified by E1. The femur starts

Table 2. Developmental differences between *Dipsochelys* morphotypes.

Character	*Dussumieri*	*Arnoldi*	*Hololissa*	Cause of differences
Supraoccipital crest	moderate	small	large	post-hatching muscular stress
Parietal vertical ridge	absent	present	absent	post-hatching muscular stress
Dentary lingual flange	absent	present	absent	post-hatching muscular stress
Inflation of quadrate	absent	present	absent	post-hatching tendon ossification
Processus circumolfactorius restriction	moderate	moderate	notable	post-hatching ontogeny
Dorsal vertebrate 8-9	short	short	long	post-hatching ontogeny
Articulars	normal	normal	truncated	reduced ossification
Processus vomerinus dorsalis	normal	normal	reduced	reduced ossification
Dentary	curved	angled	curved	embryonic cartilage formation
Humeral trochanters	diverging	parallel	diverging	embryonic cartilage formation
Neural bone shape	regular	regular	distinctive	post-hatching ontogenetic fusion

ossification shortly before the pelvis, followed the tibia and fibula in stage E2 and then the metatarsals, tarsals and phalanges (Figure 10). A lateral groove for the tibialis inferior muscle is present in females but was not observed in any juveniles. The tibia shape remains constant in all developmental stages, being a more or less cylindrical shaft. The femur is well ossified by hatching. In the tarsus chondrification is complete by E1 but ossification does not appear until E3 when all elements are ossified. They are separate at the start of E3 but by hatching (J1) the intermedium and centralia are fused but the fibulare remains separate. Fusion of the fibulare with the intermedium and centralia occurs in J4. All other bones of the pes remain separate in adults.

DISCUSSION

The pattern of ossification described above for Seychelles-Aldabra giant tortoises is similar to that reported for other taxa (Sheil, 2005; Sánchez-Villagra et al., 2009) in terms of the timing of ossification. Chondrification is complete by E1 (comparable to Yntema stage 17-19) when ossification starts. Ossification starts in several parts of the skeleton but is initially most rapid in the skull; the plastron starts to ossify after the maxilla and dentary, as is characteristic of cryptodires (the timing being reversed in pleurodires (Werneburg et al., 2009). This onset of plastron ossification is comparable to Yntema stage 19 at which ossification has commenced in many turtles (Sheil, 2003; Bona and Alcalde, 2009; Sanchéz-Villagra et al., 2009), with some species showing ossification slightly later (Rieppel, 1993; Sheil and Greenbaum, 2005). Carapacial ossification is typical of turtles in the costals starting to ossify before the neurals (Scheyer et al., 2008; Lima et al., 2011), although the ossification is relatively late. Post-hatching ossification of the nuchal has been reported for *Emydura subglobosa* (Werneburg et al., 2009), although in that species costal ossification had started in the embryo. In *Dipsochelys* ossification of the periostial collars of the ribs occurs embryonically but rib expansion is limited until hatching, consequently no distinct costal bones are detectable until the first juvenile stages. Until a wider range of tortoises have been studied ontogenetically the significance will remain obscure.

By hatching the main bones of the skull, axial skeleton, limbs and plastron are at least partially ossified, but the carapace is not. The other set of bones that are not fully ossified at hatching are the wrist and ankle bones; ossification of these starts shortly before hatching but the complete ossification and fusion of elements does not occur until late juvenile or even sub-adult stages. This is exemplified by the complete ossification of all elements but the retention of a separate astragalus and calcanaeum in a 7 year old J3 juvenile. Fusion of these elements is probably progressive throughout life, accounting for the variability in the degree of fusion (Auffenberg, 1966). This means that the flexibility of the feet decreases with age; hatchlings may be expected to have mobile carpal and tarsal joints, enabling dynamic foot movement which would be useful in climbing whereas adults have largely immobile feet which are only well adapted to supporting weight in plantigrade walking. This change in structure is in accordance with known age differences in walking behaviour. In at least some aquatic turtles ossification and fusion of carpal and tarsal elements occurs before or at hatching (Sheil and Portik, 2008; Sánchez-Villagra et al., 2009; Vieira et al., 2011), although in some it may be delayed, even into sub-adult stage (Bona and Alcalde, 2009). Variation in timing of fusion in the different turtles probably reflects species-specific aspects of locomotion.

Other ontogenetic changes in the skeleton mainly arise from progressive development of structures associated with muscle attachment. Some of these are associated with the differences between morphotypes (Table 2). The main muscle systems that result in bone stress and modification of the basic skeleton are those associated with head movement, feeding and movement of the fore-

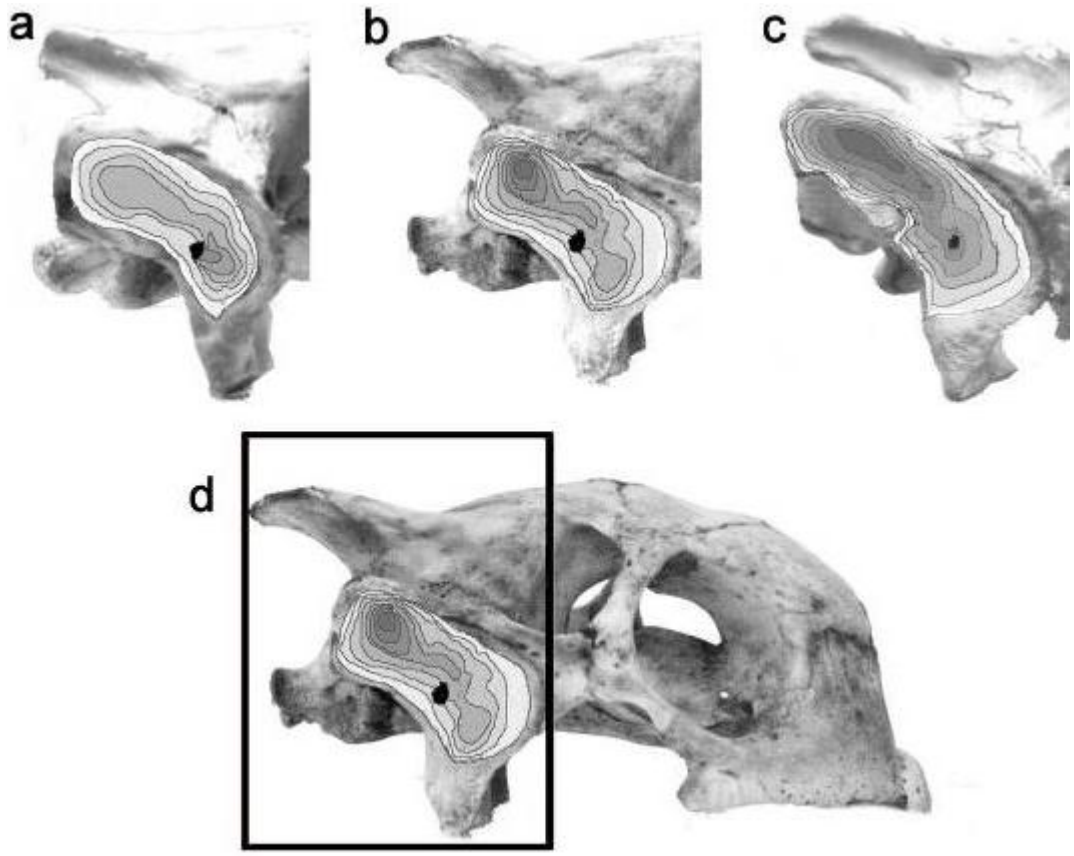

Figure 11. Contours of the inside of the tympanic cavity showing. (a) J3 *dussumieri*; (b) J3 *arnoldi*; (c) adult *arnoldi*; (d) J3 *arnoldi* skull showing area of detail.

limb. The head is protracted by the splenius capitus muscle which attaches to the supraoccipital. Increasing muscular stress in this region through life results in the development of a prominent supraoccipital crest in the relatively large headed *hololissa* morphotype and the largest *dussumieri*. Lower jaw adduction is by the adductor mandibulae which run over the cartilagio transiliens on the processus trochelaris oticum, onto the supraoccipital crest, repeated stress on this process through life results in the increasing development of the crest and of the processus trochelaris oticum. Protraction of the lower jaw is partly effected by contraction of the pterygoideus which runs from the descending process of the parietals to the angular. In the *arnoldi* morphotypes the attachment of the pterygoideus on the parietalis is marked by a vertical ridge which starts to develop by J3. Jaw opening is much weaker than closing in turtles and as a result of this low stress additional ossification is rare. Propylinal jaw action is particularly well developed in the *arnoldi* morphotypes (Gerlach, 1999) and the ossification of the dentary attachment site of the geniohyoideus muscle is a conspicuous dentary lingual flange in the adult. The ossification of the attachment site starts in juveniles through ossification of the tendon of the geniohyoideus (Figure 5) and is not seen in any other morphotypes.

Muscular contraction may also cause skeletal changes in other ways. The *arnoldi* morphotypes differs from other taxa in having a distinctly inflated quadrate. This has been reported to correspond to the attachment site for the adductor mandibularis (Gerlach, 1999) which inserts on the angular and is associated with lower jaw protraction. The raised area of the quadrate is extremely thin bone (broken in the adult) and which may not be suitable for muscle attachment. The geniohyoideus attaches onto the opisthotic and prootic which are very slightly raised in this area, and not onto the inflated part of the quadrate. Further examination of the region reveals that the inflation of the quadrate is the result of the postero-dorsal expansion of the tympanic chamber (Figure 11). This would arise from distortion of the chamber caused by vertical pressure on the medial margin of the articular process of the quadrate resulting from contraction of the pterygoideus muscle. This distortion of the tympanic chamber is also seen in other chelonian species with a well developed propylinal bite (Gerlach, in prep.).

In some cases progressive growth of individual bones

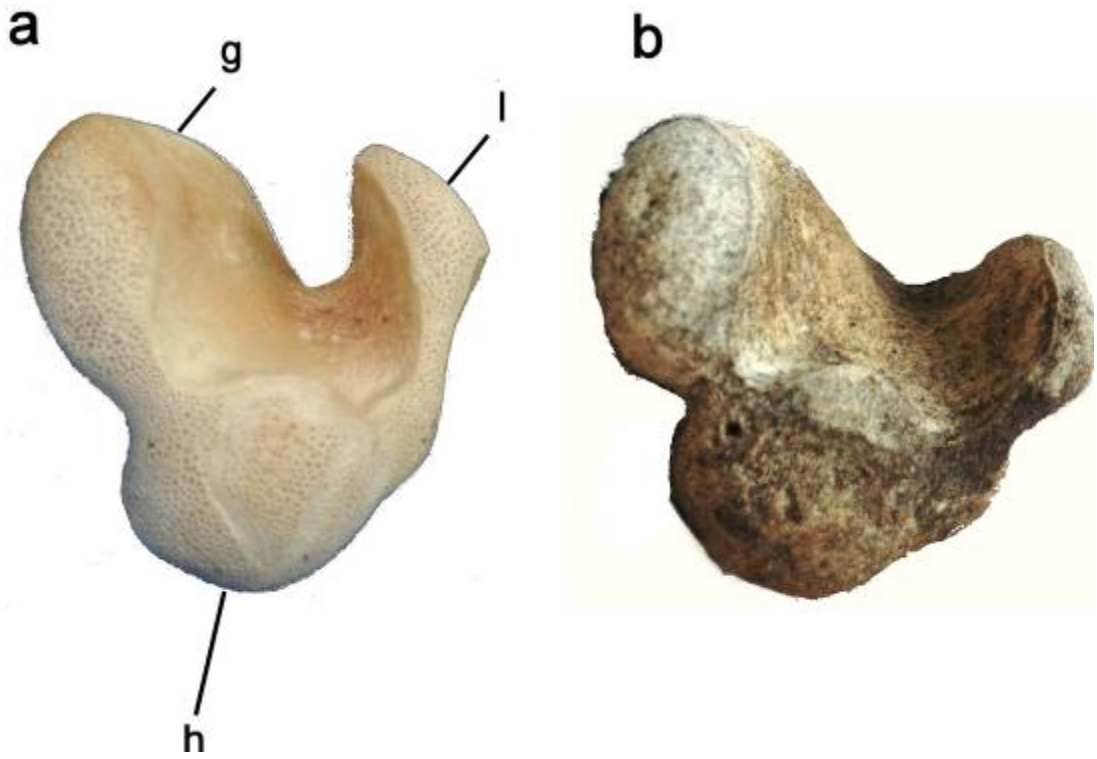

Figure 12. Humeri in proximal view showing orientation of trochanters, a) J3 *arnoldi*; b) J3 *dussumieri.* **g:** greater trochanter, **h:** humerus head, **l:** lesser trochanter.

appears to lead to other ontogenetic changes. An increase in skull width relative to height results in the postorbitals becoming more horizontal in larger individuals; this is due to the relatively fast lateral growth of the quadrates and quadratojugals, and to a lesser extent the postorbitals. This also results in the restriction of the processus circumolfactorius; the parietals that comprise the processus grow more slowly than the adjoining postorbitals. In the axial skeleton differential growth is apparent in the posterior dorsal vertebrae, with reduced growth in dorsals 8-9 of the *arnoldi* and *dussumieri* morphotypes resulting in relatively short posterior dorsals. These may be associated with the relatively small overlying neural bones.

Differential ossification may also account for some of the differences between morphotypes; reduced ossification has been reported for the *hololissa* morphotype (Gerlach and Canning, 1998), which would result in the restricted ossification of the articulars and small processus vomerinus dorsalis. This is also seen in sexual differences of the pelvis where ossification of the epipubis is seen in adult females and not males.

Some notable differences between morphotypes arise from differences in the shape of cartilaginous bone precursors rather than from developmental modification. The dentary of *arnoldi* is straight sided, giving an angled symphysis compared to the curvature of other taxa; this difference is apparent from the earliest full ossification of the dentary and reflects the form of the embryonic Maeckel's cartilage. Similarly the orientation of the humeral trochanters (Figure 12) appears to be set in the early form of the humerus rather than being modified through post-hatching muscle action.

These trochanters are involved in the movement of the humerus, an action which involves a complex of muscles. The greater trochanter supports coracobrachialis magnus which runs onto the coracoid and retracts the humerus. The lesser trochanter supports the supracoracoideus which inserts on the acromion and, the deltoideus which inserts on the scapula, acromion and plastron; both of these rotate, protract and abduct the humerus. In the normal orientation of the trochanters (diverging) the humerus is brought forwards with minimal rotation. In the *arnoldi* form the trochanters are parallel due to medial bending of the lesser trochanter. This results in a lateral shift of the attachment site of the supracoracoideus, resulting in greater rotation of the humerus during protraction. This rotation is also effected by contraction of the subscapularis and the latissimus dorsi. Attachments for the latter are particularly pronounced in arnoldi with a distinct pit proximal to the humeral head its insertion and a depression on the 2nd pleural bone of the carapace for its insertion (Gerlach, 1999).

In addition to the skeletal ontogenetic patterns discussed above there is a notable ontogenetic change in the carapace of some forms of this genus. Neural bone

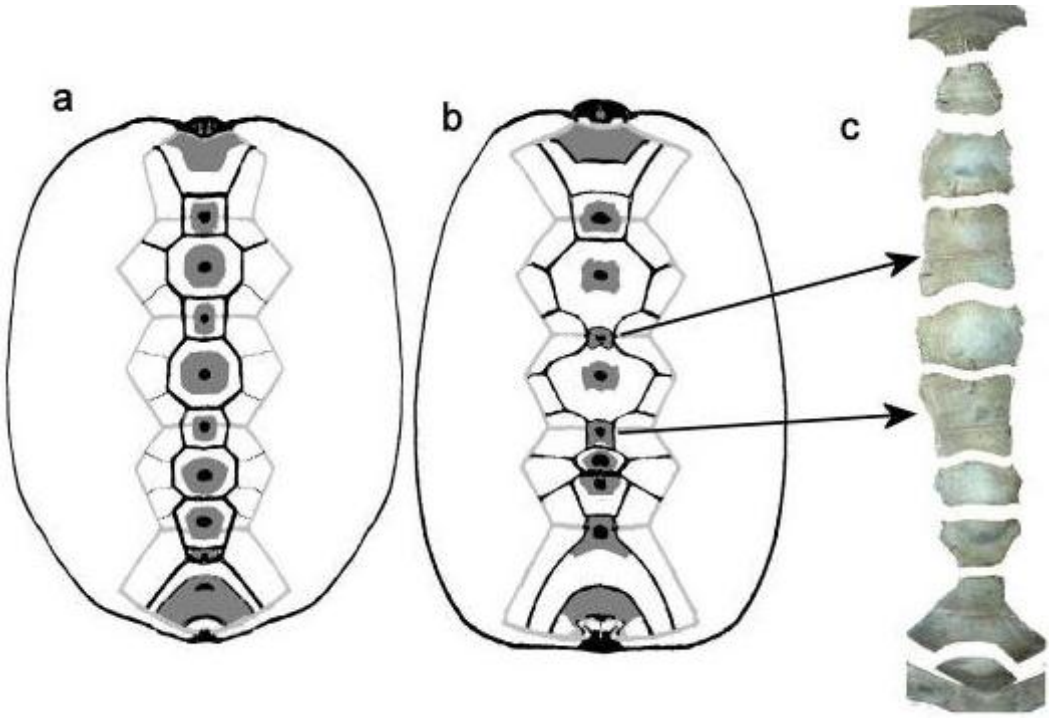

Figure 13. Neural bone development in (a) *dussumieri*, (b) *arnoldi* and (c) *arnoldi* J3 neural series. Black – hatchling or J1 ossification; dark shading - J2-J4 ossification; white – adult ossification. Arrows show *arnoldi* neurals 3 and 5 which reach maximum ossification in J3.

shape differs in adults of the different morphotypes. Anterior and posterior neural bones may vary in shape within a species, but this is rare in the central part of the series (Zangerl, 1969). In adult *Dipsochelys* variation is restricted is to last 2-3 neurals (Gerlach, 1999). Neural 1 is quadrilateral in *dussumieri*, but hexagonal in all other taxa. In the adult *arnoldi* it can be categorised as either quadrilateral or hexagonal; in the juvenile it is clearly hexagonal. Neural 4 is octagonal and neural 5 quadrilateral in all specimens except the J3 *arnoldi* where both are hexagonal. Neural 6 is octagonal in *dussumieri* but hexagonal in all adult and juveniles of other morphotypes. Neurals 3 and 5 are fully developed at this stage and no significant expansion is seen in these bones between J3 and the adult, adults also show partial or complete fusion to the preceding neural (Gerlach, 2004). In the J3 there is strong suturing to the following neural suggesting the onset of this fusion. In all cases the neurals initially ossify as quadrilateral bones, the change to hexagonal or octagonal depends on relative longitudinal or lateral growth rates; bones that grow in all directions evenly or fastest in the longitudinal axis may retain a quadrilateral shape, those that grow faster laterally will tend to become octagonal. Interaction between bones will affect the potential for adoption of these forms, with constrained growth leading to more hexagonal forms. In the case of the *dussumieri* morphotype growth is relatively regular and an alternating pattern of quadrilateral and octagonal bones arises (except posteriorly where fusion of the sacral vertebrae constrains the overlying neural to a hexagonal form. In contrast the *arnoldi* form is constrained by progressive fusing of neurals 1 and 2, 3 and 4, and 5 and 7. This results in constriction of neurals 1, 3 and 5, leading to unusual forms in the juvenile and ultimately into the exceptional adult arrangement of the entire neural series (Figure 13; Gerlach, 1999). It is notable that the incipient fusion apparent in the J3 *arnoldi* and its attaining adult dimensions in neurals 3 and 5 occurs in a juvenile of 38 cm, corresponding to the change in carapace growth pattern reported elsewhere at approximately 30 cm straight length (Gerlach, 2010).

The study presented here demonstrates that in at least some taxa of chelonians skeletal morphotypes may arise from a range of factors, some of which are determined in the embryo by the precise development of cartilaginous and membranous bone precursors, others by progressive post-hatching ossification and bone remodelling through muscular stress and others by differential ossification and growth patterns. As with all vertebrate, the chelonian skeleton is produced by a complex interaction of developmental genes, bone metabolism regulatory genes, behavioural modification, and environmental effects. Thus the precise form of the skeleton is determined by a combination of genetic inheritance and environment. With regard to taxonomically useful skeletal

features it would be desirable to have ontogenetic data for a wide range of genera in order to evaluate the reliability of characters used in taxonomy. This would be particularly valuable when, as in the present analysis, captive bred groups are available where environmental effects can be controlled. The advent of molecular taxonomy may appear obviate the need for such comparative anatomical approaches but these may not be suitable for resolving the relationships between similar or very recent forms. Furthermore the genetic techniques are not applicable to fossil material, other than relatively recent subfossils, and even then sequences may be too fragmented or degraded to be reliable (Pääbo et al., 2004). Detailed morphological and ontogenetic analysis would be particularly desirable in genera containing numerous poorly defined morphotypes of considerable evolutionary interest, such as the Galapagos tortoise complex (*Chelonoidis* spp.) and the *Testudo* genus.

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