THE APPENDICULAR SKELETON IN THE HATCHING AND IN YOUNG *BUNOPUS TUBERCULATUS* BLANFORD, 1874 (GEKKONIDAE, REPTILIA)

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ABSTRACT

The appendicular skeleton and its calcification are described in *Bunopus tuberculatus* at 3 ages: at hatching, 6-month-old and 12-month-old. Between the 3 ages there are several differences in the osteological pattern. The epiphyses of the long bones are separated from the diaphyses by a hyaline growth zone, while the phalangeal epiphyses are fused with their diaphyses or remain separated. The mature adult limbs have many sesamoids and metaplastic calcifications. This study shows how developmental osteology can help in the identification of gecko bones.

INTRODUCTION

The reptilian appendicular skeletal pattern has long interested comparative anatomists and embryologists because they believed to reveal the phylogenetic relatioships of animals (Sewertzoff, 1908; Holmgren, 1933; Romer, 1956; Stephenson and Stephenson, 1956; Stephenson, 1960; Haines, 1969; Porter, 1972; Mathur and Goel, 1976; Benton, 1985; Burke and Alberch, 1985). However, studies on the distribution and calcification of the accessory calcification centres in gekkonid limbs are relatively few and the results are often conflicting (Heidsieck, 1928; Stephenson and Stephenson, 1956; Stephenson, 1960; Haines, 1969;

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Mohammed, 1986). The objective of this work is to study the osteological characteristics and the calcification pattern in the appendicular skeleton in *Bunopus tuberculatus* at hatching and in 6-month old (6MO) and 12-month old (12MO) individuals in order to determine the relationships among members of gekkonids.

MATERIALS AND METHODS

Twenty gravid females geckos, *Bunopus tuberculatus* were collected from different localities in the State of Qatar and maintained in the laboratory until oviposition. The eggs were incubated at 25-32°C in petri dishes lined with moist cotton-wool beds. After hatching, all geckos were reared in the laboratory at 28°C ± 2 and a mean relative humidty of 48%, and fed on insects in the presence of water and sodium chloride. Samples of 3 geckos were studied at hatching and after 6 months (6MO) and 12 months (12MO) following hatching. The geckos were cleared and stained *in toto* with alcian blue (for cartilaginous muccopolysaccharides) and alizarine red S (for bone) according to Simons and Van Horn (1971). Calcification patterns of bone and cartilage were distinguished by the fact that they posessed a spongy appearance. All drawings were made by aid of an M15 Wild microscope equipped with a drawing-tube. Bone terminology generally followed that of Stephenson (1960), Romer (1956) and Haines (1969).

RESULTS
Pectoral girdle (Fig. 1)

At hatching, the dagger-shaped interclavicle and paired clavicles, scapulae and coracoids are bony. The suprascapulae, epicoracoids and the unfenestrated sternal rhomboidal plate are still cartilaginous. The scapulae and coracoids are jointed at the glenoid cavity by a cartilaginous zone. At 6MO, the anterior epicoracoid parts start to calcify while at 12MO the sternal plate is slightly calcified mesially. At the latter age, the glenoid cavity is lined by a cartilaginous layer. The 2 clavicles are jointed at the midline with a cartilaginous zone. The suprascapulars are calcified proximally while their dorsal portions are largely of uncalcified cartilage.

Fore limbs (Figs. 2 and 3 and Tables 1 and 2)

At hatching, the long and short bones have cartilaginous epiphyses. The radiale, centrale and carpalia-1 to -5 are fully cartilaginous while the ulnare has a median calcified centre. Pisiform and basal sesamoids at the metacarpal distal heads are cartilaginous. The intratendinous metaplastic bone of ulnar epiphysis is present. The palm sesamoids has calcified centres. The digital formula is 2 3 4 5 3.

At the age of 6MO, the humerus proximal epiphysis has 3 metaplastic calcification centres while the distal one has only 2. The radial epiphysis has an irregularly
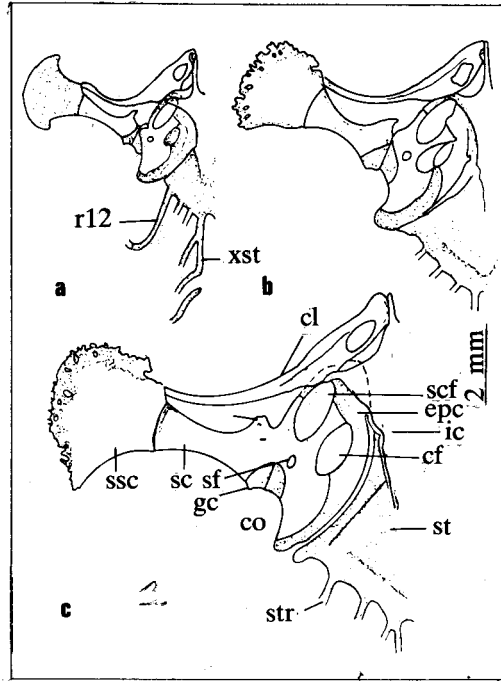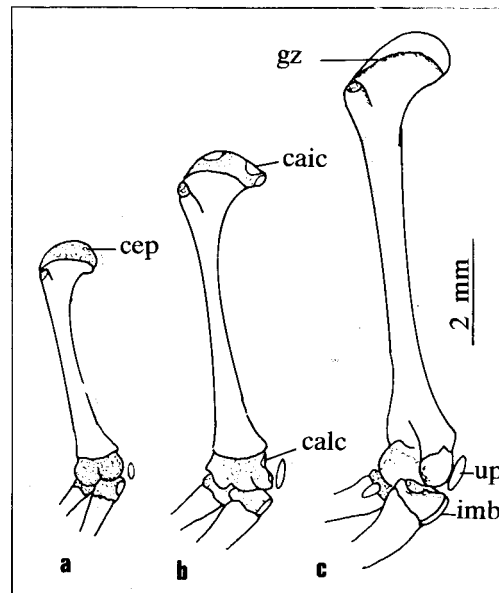
Fig. 1. Pectoral girdle and sternum of *Bunopus.*

Fig. 2. The humerus and the radius/ulna proximal end of *Bunopus.*

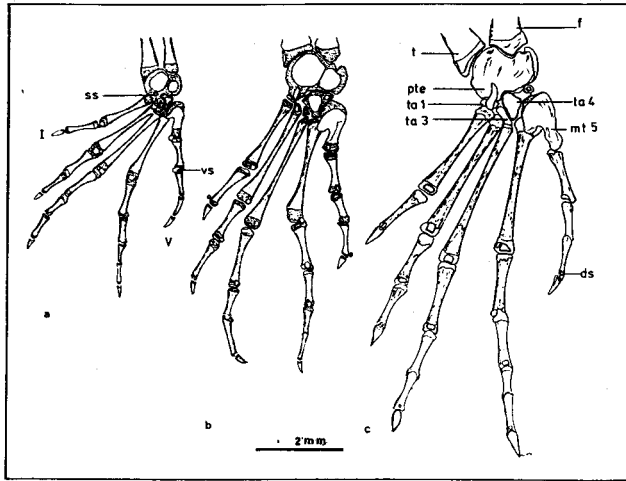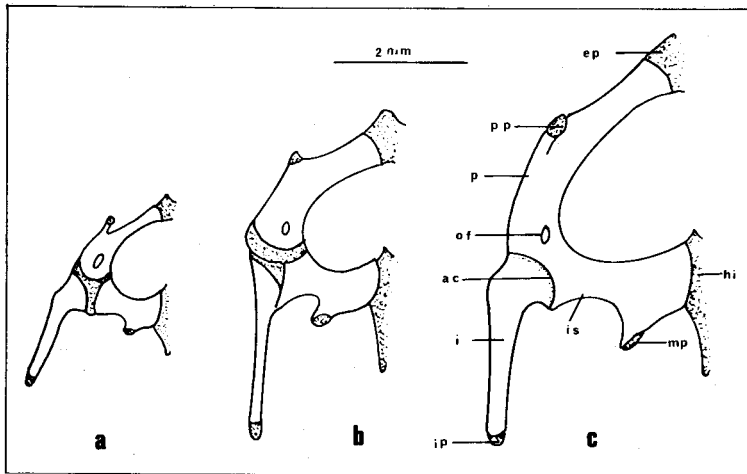Fig. 3. Left manus of Bunopus, ventral view.

Fig. 4. Pelvic girdle of Bunopus, ventral view.

calcified centre proximally on the dorsal side. However, the epiphyses of humerus, radius and ulna are still cartilaginous. In the carpus, the radiale and carpalia-2 have 2 calcified centres while ulnare and carpalia-4 and -5 have one centre. The proximal epiphyses of metacarpal-1 to -5 are cartilaginous while their distal epiphyses show some calcification. The phalangeal epiphyses may be fused or unfused with their shafts. The ulnar patella is calcified and the pisiform and palm sesamoids starting to calcify.

At the age of 12MO, the humeral epiphyses, radius and ulna are fully calcified and a cartilaginous zone occurs between the calcified epiphyses and their shafts. All the carpal elements and pisiform and palm sesamoids are fully calcified. At the same time the mesopodial elements, the long bones epiphyses, ulnar patella and pisiform are enveloped within a thin cartilaginous layer. The ventral and dorsal digital calcified sesamoids are clearly obvious.

Pelvic girdle (Fig. 4).

At hatching, the pubis, ischium and ilium bones are quite distinct, meeting at the acetabulum with a cartilaginous head. These rudiments are partially fused at the acetabulum at 6MO while at 12MO they are fully fused. On the other hand, the epipubic, hypoischium, metischial process, prepubic process and ilium process are still cartilaginous up to 12MO.

Hind limbs (Figs. 5 and 6 and Tables 1 and 2)

At hatching, long and short bones have cartilaginous epiphyses. At the knee, the patella, cyamella and 2 lunulae are cartilaginous. The cartilaginous distal tibial epiphysis has a metaplastic bone. In the tarsus, the proximal tarsal rudiments has 2 calcified centres. Tarsalia-1 and -3 are fully calcified while tarsalia-4 show slight calcification. The basal sesamoid at the metatarsal distal epiphyses and sole sesamoid are cartilaginous.

At 6MO the femoral proximal epiphysis has 3 calcified areas while the distal epiphysis is still cartilaginous. There is an intertendinous metaplastic bone at the fibular epiphysis. The proximal tarsal element has an obvious suture marking the union of the fibulare (calcaneum) to the intermedium (astragalus). Calcification centres occur in metatarsal-1 to -4 and also for tarsalia-1, -3 and -4 while metatarsal-5 is still fully cartilaginous. The digital epiphyses vary in the presence of calcified centres. The sole sesamoids and dorsal and ventral sesamoids are fully calcified.

At 12MO all the hind limb bones are fully calcified and some calcified digital epiphyses are fused with their shafts. The proximal tarsal element appear as one rudiment with a cartilaginous rim.
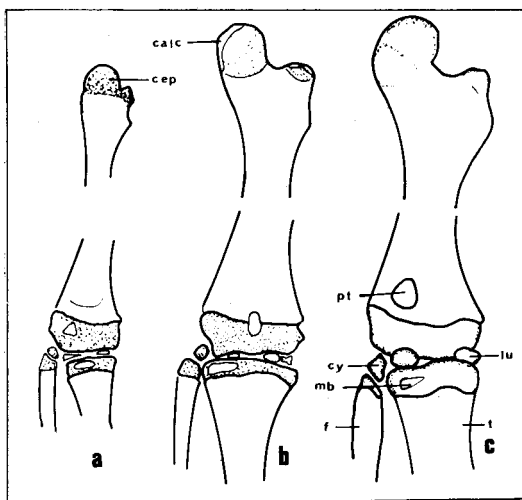
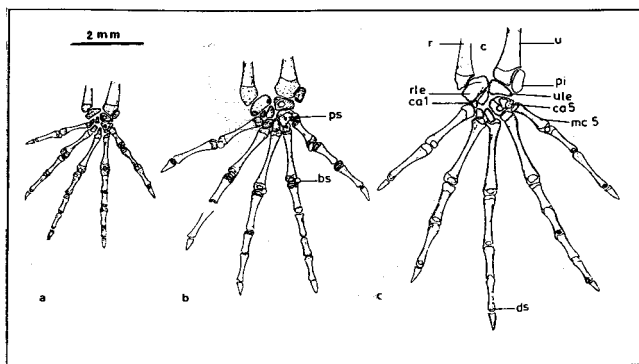Fig. 5. The femur and the tibia/fibula
proximal end of *Bunopus*.

Fig. 6. Left pes of Bunopus, ventral view.

Table 1

The relation between the epiphyses and their shafts; fused (F) or unfused (-), in the fore and hind limbs at 12-Month-Old.

Element		Epiphysis	Fore limb	Hund limb
Humerus		p,d	—	—
Radius		p,d	—	—
Ulna		p,d	—	—
Metacarpals		p,d	—	—
Femur		p,d	—	—
Tibia		p,d	—	—
Fibula		p,d	—	—
Metatarsals		p,d	—	—
Digit I	Ph I	p	—	—
		d	F	F
Digit II	Ph I	p	—	—
		d	—	—
	Ph II	p	F	—
		d	F	F
Digit III	Ph I	p	—	—
		d	F	—
	Ph II	p	F	—
		d	F	F
	Ph III	p	F	F
		d	F	F
Digit IV	Ph I	p	—	—
		d	F	—
	Ph II	p	F	—
		d	F	F
	Ph III	p	F	—
		d	F	F
	Ph IV	P	F	F
		d	F	F
Digit V	Ph I	p	—	F
		d	F	F
	Ph II	p	F	F
		d	F	F
	Ph III	p	F	F
		d		F

d, distal epiphysis: p, proximal epiphysis; Ph, phalangus.

Table 2

Different types of sesamoids in the fore and hind limbs
at 12-Month-Old. (+) present and (−) absent.

Sesamoidal type	Fore Limb	Hind Limb
Lunulae	—	+
Cyamellae	—	+
Patellae	+	+
Metaplastc bones	+	+
Dorsal sesamoids:		
tarsus	—	+
digit I to V	+	+
Pisiform	+	—
Palm sesamoid	+	—
Sole sesamoid	—	+
Basal sesamoids:		
metacarpals	+	—
metatarsals	—	+
digit II, Ph I	+	+
digit III, Ph I	+	+
digit IV, Ph II	+	+
digit V, Ph I	+	+

DISCUSSION

The changes occuring in *Bunopus* appendicular skeleton during calcification
appear to resemble those in many geckos (Stephenson and Stephenson, 1956;
Stephenson, 1960) and in many lizards (Heidsieck, 1928; Nauck, 1936; Haines,
1940; 1942, 1969; Mauther and Goel, 1976; Mohammed, 1986).

In the newly hatched *Bunopus*, the pectoral and pelvic elements are highly
comparable to those of other reptiles. The pectoral girdle elements are generally
similar to those described in other geckos (Stephenson and Stephenson, 1956;
Stephenson, 1960; Mohammed, 1986). Unfused scapula and coracoid bones of
Bunopus at hatching and 6MO are recorded in young specimens of *Oedura
lesueurii, Heleronota binoei* and *Phyllurus platurus* (Stephenson, 1960) *Crytodac-
tylus scaber* (Mohammed, 1986). At 12MO, the 2 bones are fused. Similar
observations are recorded in adult geckos *Crytodactylus scaber* (Mohammed, 1986)
and in certain Jamaican genera and the new Zealand genus *Naultinus* (Stephenson

and Stephenson, 1956; Stephenson, 1960). In *Bunopus,* the pectoral girdle has 2 large foramina and a lateral scapular process. In the genera studied by Stephenson (1960), there are 3 large foramina while the lateral scapular process is missing.

The pubis, ischium and ilium in Bunopus are completely fused at 12MO. Separation of these elements was noted in juvenile forms of different New Zealand geckos (Stephenson, 1960). The epipubis process, ilium process, metischial process and hypoischium are cartilaginous. The metischial and pectineal processes are observed in all geckos examined by Stephenson (1960). For the epipubic and hypoischium, Stephenson (1960) recorded that these 2 rudiments of the juvenile forms may be only slightly calcified.

In the present gecko, the epiphyseal calcification pattern of the appendicular skeleton is significant. The epiphyses of the phalanges calcify before those of the longer bones, a sequence which is observed also in the agamid lizard *Calotes versicolor* (Mathur and Goel, 1976) and in the gecko *Crytodactylus* (Mohammed, 1986). This sequence is in contrast with Haine's (1969) suggestion that the larger epiphyses calcify before the smaller ones. Also, in *Bunopus,* the calcified epiphyses may be separated from the diaphyses by cartilaginous zones or fused with them. Such epiphyseal arrangement occurs in most lizards at the approach of maturity (Heidsieck, 1928); agamids and gekkonids (Vialleton, 1919); *Lacerta* (Nauch, 1936); *Calotes* (Mathur and Goel, 1976). This is in general agreement with the typical lacertilian condition, and supports the observations that the phalanges grow only on their proximal ends (Haines, 1969; Mathur and Goel, 1976) and the contention that the metacarpals grow only at their heads (Haines, 1969). Bellairs (1969) noted that some lizards may grow very slowly after maturity if the epiphyses do not fuse completely with the diaphyses. Such phenomena was recorded in Sphenodon lizards and in *Gephrosaurus* (Evans, 1981).

The carpus elements of *Bunopus* are the radiale, ulnare, centrale and the carpalia 1 to 5. Similar observations are obtained in the agamids *Calotes* (Mathur and Goel, 1976); *Ascalabotes fascicularis* (Sweertzoff, 1908); *Varanus* (Porter, 1972); in several geckos (Stephenson and Stephenson, 1960; Stephenson, 1960; Mohammed, 1986) and in many lizards (Romer, 1956; Mohammed, 1986). As pointed out by Romer (1956), most authors have considered embryonic fusion to be the main mode of reduction in the number of skeletal elements in the autopodium. Holmgren (1933) contented that the entire primitive reptile series of eleven elements appeara in turtle embryo. In *Agama colonorum,* Holmgren (1933) reported as many as 12 elements in the embryo as compared with 7 in the adult, the centrale-3 and -4 and the intermedium fuse with the ulnare, and the carpalia-1 fuses with the metacarpal-1. On the other hand, cartilaginous elements do not usually appear as independent foci but very often appear as branches or buds from the

existing chondrgenic or prechondrogenic blastemas (Burke and Alberch, 1985). Therefore, the gecko embryonic carpus probably has a certain number of elements, some of which fuse during development with the result of a reduced number of such elements in the adult.

In *Bunopus,* the tarsal elements are the proximal tarsal element, tarsalia-1, -3 and -4. At hatching, the proximal tarsal element has 2 calcified areas while at 6MO may jointed at obvious suture but at 12MO they appear as one segment. In *Calotes* (Mathur and Goel, 1976) and *Ascalabotes* (Sewertzoff, 1908), embryonic evidence indicates that the proximal tarsal element incorporates the astragalus, the fibulare and a distal centrale. In *Agama* (Holmgren, 1933), the proximal tarsal element is made of the astragalus (centrale-1 + intermedium), the fibulare (centrale-4), distal centrale (centrale-2) and probably the tarsalia-1 and -2. As illustrated by primitive reptilian Captorhinus tarsus, the astragalus is made up of the tibiale, the proximal centrale, and the intermedium (Peabody, 1951) or it is represented by only the tibiale or only the intermedium (Romer, 1956). Schaeffer (1941) has postulated that the tibiale has been lost from the tarsus of later reptiles. Burke and Alberch (1985) noted that the tarsus of 2 chelonian, *Chelydra* and *Chrysemys,* the intermedium is clearly separated from the fibulare. The fusion may also occur in the embryo, resulting in hatching with a single proximal element. Zug (1971) concluded that the single proximal element in the tarsus of trionchids was composed only of astragalus. He rejected the contribution of a calcanium (fibulare) because he found only one centre of ossification in this element in juvenile specimens. Also, Stephenson (1960) recorded a single ossification centre in the juvenile geckos *Nephrurus* asper, *Nephrurus laevis* and *Oedura lesueurii.* In *Bunopus,* comparison of the proximal tarsal element with those described in the literature for other reptiles indicates that there are 2 or may be more rudiments merging to form the proximal element in the gecko.

The distal tarsal row in *Bunopus* has 3 elements, the tarsalia-1, -3 and -4 which increase in size in that order. The same situation exists in all the geckos described by Stephenson (1960) and Mohammed (1986) and in the agamids *Calotes* (Mathur and Goel, 1976), *Asalabotes* (Sewertzoff, 1908) and *Uromastix microlepis* (Mohammed, 1980). Tarsalia-5 is absent in *Bunopus.* In all reptiles, the tarsalia-5 is always missing. Possibly, this is the means of adjusting a broad foot to a narrow tarsus, bringing the divergent fifth toe into line with the other digits (Romer, 1956) or to help broaden the base of the foot (Bellairs, 1969).

The sesamoids are well recognized in *Bunopus.* The pisiform is recorded in all early tetrapods with well ossified hands (Romer, 1956; Haines, 1969). The pisiform is recorded in geckos of New Zealand (Stephenson and Stephenson, 1956) and in other lizards (Romer, 1956; Haines, 1969; Mohammed, 1986). The lunulae appear

in young *Bunopus* as cartilage and in older animals as calcified centres as in *Lacerta* and gecko *Hemidactylus angularis* (Haines, 1969). In *Sphenodon*, there is a single lunula and which appears in the varanus-like fossils (Nopsca, 1903). Banchi (1900) considered the cyamella is a remnant of a fin ray comparable to the tibia or fibula. Heidsieck (1928) described mineralized tissue at the attachments of tendons, capsules, and ligaments in lizards, with the ground substance being bony while the cells are cartilaginous. Schaffer (1888) believed that such tissue could be considered as true bone. Haines (1942) termed them intradentinous centres, developing by direct ossification of the tendons where they are inserted into the cartilaginous epiphyses; he believed them to be distinct from sesamoids in their origin.

The basal sesamoid pads of *Bunopus* were recorded in Crytodactylus (Mohammed, 1986) and in *Varanus* (Haines, 1969 and the ventral palm and sole sesamoids were recorded in *Calotes* (Muther and Goel, 1976), *Uromastix microlepis* and *Crytodactylus* (Mohammed, 1986). Romer (1956) has indicated the presence of such sesamoids in reptiles but Haines (1969) did not make any reference to them. The dorsal sesamoids of *Bunopus* were not recorded before in any lizards.

The typical reptilian phalangeal formula of 2 3 4 5 3 for manus and 2 3 4 5 4 for the pes is the primitive formula for reptiles (Romer, 1956). The phalangeal formula in *Bunopus* is 2 3 4 5 3 for manus and 2 3 4 5 4 for pes. This formula is recorded in gecko genera of New Zealand, Jamica, Australian and Solomon Islands (Stephenson and Stephenson, 1956; Stephenson, 1960).

Based on comparison between *Bunopus* and other lizard species, appendicular skeleton calcification has involved changes in the timing and sequence of appearance of different bones. Information such as this will likely prove to be invaluable for better understanding of the mechanism of skeleton development and evolution.

ABBREVIATIONS

Cartilage is shown by stippling. a, at hatching; b, 6-month-old; c, at 12-month-old.

ac, acetabulum; bs, basal sesamoid; c, centrale; ca 1, ca 5, carpalia 1 and carpalia 5; calc, calcified centre; cep, cartilaginous epiphysis; cf, coracoid fenestra; ce, clavicle; co, coracoid; cy, cyamella; ds, dorsal sesamoid; epc, epipubic; epc, epicoracoid; f, fibula; gc, glenoid cavity; hi, hypoischium; ic, interclavicle; il, ilium; imp, intratendinous metaplastic bone; ip, ilium process; isc, ischium; lu, lunulae; lsp, lateral scapular process; mb, metaplastic bone; mc 5, metacarpal 5; mp, metischial process; mt 5, metatarsal 5; of, obturator foramen; pis, pisiform; pp, prepubic (pectineal) process; ps, palm sesamoid; pt, patella; pte, proximal tarsal element; pu, pubis; r, radius; ri, rib; rle, radiale; sc, scapula; scf, scapulo-coracoid fenestra; sf, supracoracoid foramen; ss, sole sesamoid; ssc, suprascapula; st,

sternum; str, sternal rib; t, tibia; ta 1, ta 2 and ta 4, tarsalia 1, 3 and 4; up, ulnar patella; vs, ventral sesamoid; xst, xiphisternum.

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الجهاز الطرفي في فقس وصغار البرص
بنوبس تبروكيولاتس بلانفورد ١٨٧٤
(البرصيــــات ، الزواحــف)

محمـد بهجت حسين محمد

دُرس تركيب ونظم التعظم في الجهاز الطرفي في فقس وصغار البرص **بنوبس تبركيولاتس** عند الاعمار الآتية : حديثة الفقس ، عمر ٦ أشهر ، وأيضاً ١٢ شهراً وأوضحت النتائج تباين قطع الطرف الأمامي وكذلك قطع الطرف الخلفي في زمن تعظيمها وأيضاً في أشكال التعظيم عند الاعمار الثلاث . وللبرصيات أنواعاً متباينة في العظام السمسمية .

وأشارت النتائج إلى تباين البرصيات في نظم التعظيم داخل النوع الواحد أو بين الانواع المختلفة منها .