

Comparative Analysis of Growth and Development in the Hands of Primates

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Abstract

Much of the scientific literature and osteological research on primates focuses primarily on chimpanzee, human, and macaque. There are few comparative analyses across the primate clades of Strepsirrhini and Haplorhini. Early studies on *Macaca mulatta* noted the pattern of ossification to be metacarpals first, followed progressively by more distal elements. However, humans appear to be distinct. Schaefer et al. 2009 show that ossification first appears in the distal row of phalanges, metacarpals, proximal phalanges, and lastly the intermediate phalanges. There are some similarities in the ossification sequence between man and macaque: in both species, MC1 is the last of the metacarpals to ossify. I have conducted a unique, comparative analysis of bone growth and development in the hand across a broader range of non-human primates. I addressed the following questions: Are there differences in ossification and fusion patterns across non-human primates at birth? Do these differences correspond to phylogeny or locomotor behavior? I used virtual reconstructions based on CT imaging and radiographs of the hand taken at birth and juvenile stages from multiple primate species, noting the patterns of ossification and fusion. I hypothesized that ossification of hand elements would be affected by phylogenetic group, but not locomotor behavior.

Introduction

The scientific literature for human ossification and development through fetal and juvenile stages is significant and vast, comparatively there is considerably less written in the scientific literature on such patterns and observations across the Primate order. Biological anthropologists and evolutionary biologists have long been fascinated by evolutionary relationships among the different primate species. Debates continue today as to which species are more closely related on the evolutionary tree as new discoveries and techniques provide unique insight into this topic. Early studies on non-human primates were focused mainly on *Macaca mulatta*, *Macaca nemestrina* (Ruppenthal and Reese 1979; Van Wagenen and Hebert 1964), *Pan troglodytes* (Gavan 1953; Nissen and Riesen 1949), and *Callithrix jacchus* (Phillips 1976). Comparatively, our egocentric mentality has led to countless studies, papers, articles, and books on the growth and development of humans, useful in forensics and pediatrics.

Bones form through the process of ossification which can occur through two methods: endochondral and cartilaginous ossification. The hand materials ossify via endochondral ossification. This process results in growth and healing of bone materials. This process first begins with a cartilage model formed before birth as “embryonic mesenchymal cells condense and differentiate into chondrocytes” (Mackie, et al. 2011). As development progresses, the cartilaginous model is perforated by blood vessels and osteoclasts (which remove bone and cartilage), bone marrow and osteoblasts (which lay down new bone cells, thus resulting in ossification). As this process continues, the materials (phalanges and metacarpals) grow out from the center of the primary ossification center, the shaft. A second ossification center forms proximally for the phalanges and metacarpal (MC) 1 and distally for MCs 2-5. However, it should be noted there was variation in the location of the secondary ossification center for MC 1 for some of the non-human specimens in this study. *See Results.*

The hand is the distal extremity of the upper limb. Within the hand are bones classified as carpals, metacarpals, and phalanges (the fingers, which are again divided into three rows being proximal, intermediate, or distal phalanges). Appearance times of hand materials are meticulously studied and recorded in human juveniles (e.g., Garn et al. 1967; Birkner 1978; reviewed in Schaefer 2009). Garn and colleagues' (1967) study included participants from the Fels Research Institute Program of Human Development and provides information for each ossification center in the hand and assessments for both male and female for the 5th, 50th, and 95th percentiles. For example, they showed that the capitate and hamate are the first two carpals to ossify. This pattern is supported by Birkner's (1978) images which show ossification and development of the hand and wrist elements. Females appear to develop more rapidly than males while there is some overlap between lower female and higher male percentiles (Garn et al. 1967). These two studies were used to compare human growth and development patterns to those observed in the current study in non-human primates.

Ruppenthal and Reese (1979) state that evolutionary scale is inversely proportional to ossification and maturation. The current study attempts to provide more information on this statement, and proves true when humans are included in the discussion. However, humans tend to be an anomaly in terms of evolution, being unique in many attributes. Take humans out of the equation, and this pattern observed by Ruppenthal and Reese (1979) becomes more ambiguous. This work provides insight into important implications from studying non-human hand and wrist ossification patterns as dental estimations are not as reliable "due to large intervals of time between eruptions" (Ruppenthal and Reese, 1979). Ossification and fusion of carpal and metacarpal bones are significantly important and useful in age assessments of nonhuman materials. Both this book and observations from my study show significant acceleration of

development and maturation of bone in nonhuman primates compared to humans. Though not tested in this study, Ruppenthal and Reese (1979) also noted that monkeys with a longer gestation period tended to be heavier and have more advanced skeletal development. My study did not include gestation period nor body weight.

In a study conducted in 1964 by Van Wagenen and Hebert on ossification patterns in *Macaca mulatta*, the researchers documented the ages at which hand and wrist materials ossified through roentgenographic methods. This study was used for comparing ossification patterns in macaques and other specimens in this study. A pattern noted in the study by Van Wagenen and Hebert is that by days 70-72 gestation, ossification centers in the metacarpals begin to appear and by days 120-125, MC3 and MC4 are the most developed in that row of elements (Van Wagenen and Hebert 1964). This pattern observed in their macaque specimens was also seen in some of my specimens. One of the specimens which presented a similar pattern of development as stated in the macaque is *Galago moholi* (P3132) aged 30 days. Observation taken based on the 3D virtual reconstruction of the individual's CT-scans show that all metacarpal epiphyses are present with MC1 being the least developed and MC3 being the most. Another specimen which shows advanced development in MC3 and 4 is *Propithecus verreauxi* (P5640). This specimen is the same age as *G. moholi* previously mentioned secondary epiphyses present only on MCs3 and 4 in this row. Note that both specimens are classified as strepsirrhines.

Chimpanzees are of particular interest to researchers because of their close evolutionary relationship with humans. Nissen and Riesen (1949) used serial x-ray photography to study ossification patterns in nine male and seven female chimpanzees. These data were used to estimate the ages at which ossification began, looking at 70 epiphyses and short bones in the left extremity. The researchers noted a difference in ossification advancement between the male and

female specimens, females being more advanced than males, a pattern also observed in humans. They also noted patterns related to the length of the gestation period, with shorter periods leading to the retardation of appearance of centers (Nissen and Riesen 1949). They found no correlation between timing of appearance of centers and birth weight. However, Ruppenthal and Reese (1979) stated that monkeys that were heavier showed more advanced stages of development. Both agree that gestation impacts progression of ossification and development.

Phillips (1976) conducted a study of skeletal development in fetal and neonatal marmosets. He used methods of radiography and alizarin preparation of 30 *Callithrix jacchus* fetuses. These were at various stages of gestation. In addition, he studied 29 preserved neonates ranging in age from birth to 50 days. The same pattern was observed in the metacarpal row as was previously seen by Van Wagenen and Hebert (1964) in their macaques. What these specimens have in common is that metacarpals are the first (if not among the first) elements in the hand to ossify, beginning with the shaft. From the radiographs in the study, Phillips noted that metacarpals were first visible in fetuses less than 12 days before birth. At 105 to 115 days gestation, the primary ossification centers for MCs 2, 3, and 4 were seen, and MC1 proximal epiphysis was present last, in the oldest specimen of 32 days.

Our knowledge of the evolution of primates is based on numerous traits such as encephalization, orthograde versus pronograde posture, derived dental formulae, increased parental investment, adult morphology, and locomotor behavior etc. Variations in these categories reflected throughout the taxa and numerous classification systems within the primate evolutionary tree. The bones of the hand and feet may provide significant taxonomic markers for later stages of specialized taxa.

Divergence between human ancestors and chimpanzees is estimated to be between 4-6 MYA (million years ago); the divergence between gorillas and the human/ chimpanzee clade is roughly 7-9 MYA, and the hominoids and cercopithecoid divergence is about 30-40 MYA (Yoder & Ziheng 2000). With each divergence, new species emerge with either derived or ancestral traits which may be traced back to a last common ancestor (LCA). Humans share about 95% of their DNA with chimpanzees, our closest relative, a sister taxon (Britten 2002). This high percent of shared DNA shows there are fundamental similarities between the two species on a cellular level, in addition to the numerous behavioral and morphological similarities. This led me to hypothesize that species closely related would be more similar in the ossification and development than species more distantly related, primarily focusing on the hand and wrist. Also, as Beigert (2009) stated, the function of a bone or region of the body impacts the morphology, or shape or appearance, of the material(s). This led me to consider an alternative hypothesis that adult locomotor behavior also influences the development of skeletal elements in the body, as we are adapted to our environment.

In addition to supplementing evolutionary analytical methods, the results from subsequent studies can be used in estimating age of primates, as the ossification and fusion of carpals and metacarpals are more accurate than dental age estimations (Ruppenthal and Reese 1979). Dental age estimations for humans have been studied extensively and can be accurate and useful in forensic contexts. Age estimations of non-human primates through the ossification patterns in the hands and wrists may be useful in primatology and biological anthropological studies of development, shedding more light on the broader story of evolution.

I have conducted a unique, comparative analysis of bone growth and development in the hand across a broad range on non-human primates. Studies show that bone development is

influenced by evolutionary history but animals are also skeletally adapted to their environment. I hypothesized that ossification of hand elements would be affected by phylogenetic group, not locomotor behavior.

Materials and Methods

Primate specimens were individually selected from the Newborn Primate Database of Dr. DeLeon and her colleagues, which includes micro-CT scans of primates- at different developmental stages. Specimens were grouped into Haplorhine and Strepsirrhine clades. There were more specimens in the dataset in the Strepsirrhini suborder (n=16) than in Haplorhini (n=8).

I used virtual reconstructions based on those micro-CT images of the hand taken at birth and juvenile stages from multiple primate species, noting the pattern of ossification. Each specimen's micro-CT images were uploaded to the Amira program, where they were virtually reconstructed by extracting isosurfaces based on density threshold. Notes and observations on patterns were placed in an Excel chart for easy access and organizational purposes. Specimens were chosen based on the inclusion of an entire right hand, left hand or both hands in the micro-CT scans of the specimen. The left hand was preferred for consistency, but if it was unavailable, (i.e., not included in the scan or partially included in the scan) then the right hand was used. The majority of specimens were perinatal (late fetal through 6-days-old). Percent total ossification (%TO) represents the number of ossified elements present divided by the total materials that would be expected to ossify later in life. Carpals, epiphyses, and shafts were considered separate elements.

Results

Observations on Ossification in Haplorhines

General observations reported for the Haplorhine suborder include wide variation in perinatal primates, as well as continued greater development when compared to Strepsirrhines of similar age. Note these descriptions focus on secondary epiphyses and the presence of carpals because metacarpal and phalanx shafts are all present in these micro-CT images. Their presence is not listed here for each individual, but were included in calculations and comparisons in this study. *Aotus nancymae*, stillborn, had no secondary ossification centers present in both the left and right hands. There were two carpals that had begun to ossify in the left and right hands (possibly three in the right). *Callicebus cupres*, stillborn, similar to *A. nancymae*, had no secondary ossification centers, or epiphyses, present in either hand. *Saguinus midas*, stillborn, presented different patterns in its development than those observed in the other two stillborn Haplorhine individuals. *S. midas*'s left hand included the presence of three carpals and the epiphyses of the metacarpals. The right hand included three carpals, clear ossification of metacarpal distal epiphysis for ray two, three and four, with a possible epiphysis for the first metacarpal just beginning the process.

Other Haplorhines included in the study were two *Tarsius syrichta*, two *Cebuella pygmaea*, and one *Saguinus oedipus*. The fetal Philippine Tarsier showed no carpals or epiphyses present, similar to the pattern observed in *A. nancymae* and *C. cupreus*. The second tarsier (age P0) is more developed. Materials present in this specimen's micro-CT images include the presence of five carpals and epiphyses for the first, second, and third metacarpals in the right hand. In the left hand (which was used for %TO comparisons) includes five carpals and epiphyses for metacarpals one, three, and four.

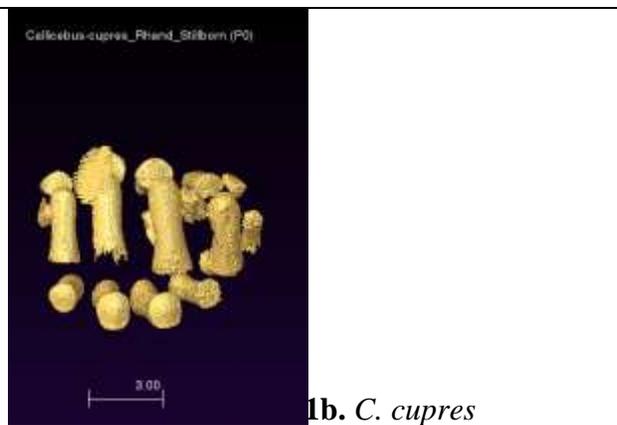
The newborn *Cebuella pygmaea*, or pygmy marmoset, had both hands included in the scans. The right hand materials included five (possibly six) carpals ossified, all metacarpal epiphyses, proximal row epiphyses, and intermediate row epiphyses ossified. The left hand used for comparisons included five (possibly seven) carpals ossified. All metacarpal materials, epiphyses for proximal phalanges one-four, all intermediate row epiphyses, and distal epiphyses for phalanges one, three, and four were present. The second *C. pygmaea* was described as “subadult” but the exact age is unknown. Here, all materials are present, as well as sesamoid bones present anteriorly on all metacarpals.

Placed in the same age category as the subadult *C. pygmaea*, *Saguinus oedipus*, a cotton-top tamarin also presented a high development of ossification. At age P27, all carpals are present. All metacarpal material, proximal row and intermediate row materials, and epiphyses for distal phalanges two, three and four are present. The epiphysis for the distal phalanx in ray two appears to beginning its ossification process, while those for ray one and five have not yet begun at this age.

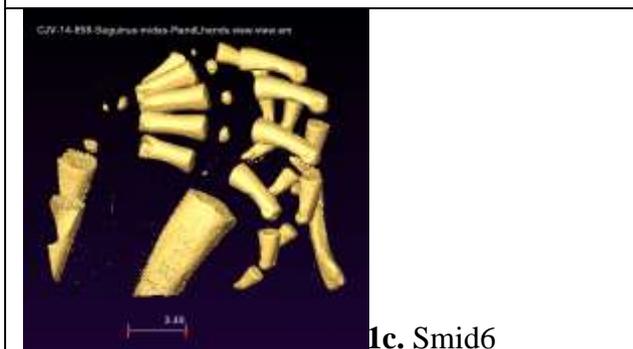
The images in this section (1a-h) show the ossified elements of the Haplorhine specimens used in this study.



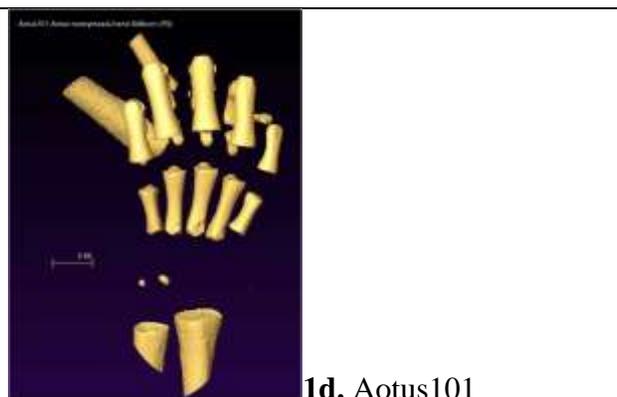
1a. P96



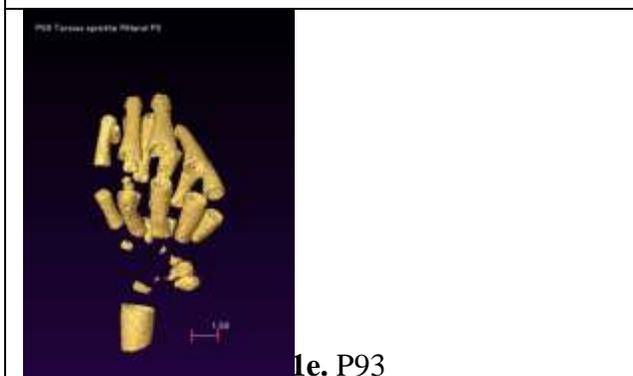
1b. *C. cupres*



1c. Smid6



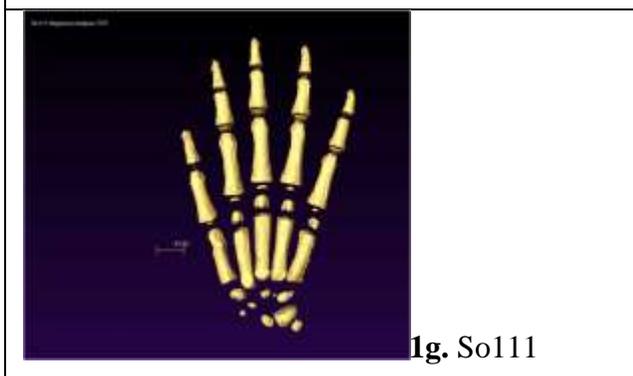
1d. Aotus101



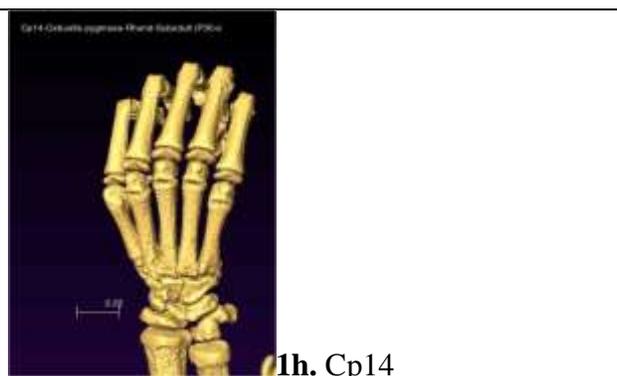
1e. P93



1f. Cp17



1g. So111



1h. Cp14

Observations on Ossification in Strepsirrhines

There were more specimens in the dataset in the Strepsirrhine suborder, 16 individuals, than in Haplorhine.

Perinatal Strepsirrhines: Two individuals, *Hapalemur griseus* and *Propithecus coquereli*, were classified as “neonate”. There were no secondary ossification centers present in either of the specimens’ hands, apart from a possible carpal in the right hand of *P. coquereli*.

At the fetal stage, *Loris tardigradus* also presented no epiphyses or carpals. Also observed in this individual were the undeveloped, “ball-like” appearances of distal phalanges two and five. Late fetal strepsirrhines include *Loris tardigradus*, *Otolemur crassicaudatus*, and *Lemur catta*. Observations on both left and right hands of these three specimens note the absence of ossified carpals and secondary centers.

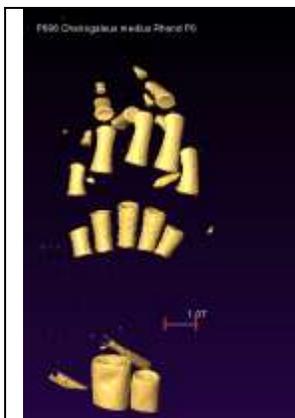
Cheirogaleus medius and *Galago moholi* micro-CT images were taken at age P0. The right hand was used for analysis of *C. medius* and did not show any carpals or epiphyses. The left hand for *G. moholi* presented the same pattern. The second *C. medius*, aged P1, and *Eulemur mongoz*, aged P1, presented the same stage of development as observed at age P0.

Juvenile Strepsirrhines: The left hand of *Galago senegalensis* at age P7 showed two carpals in the micro-CT images but no other secondary centers appeared to be ossifying. At age P23, *G. senegalensis*, secondary ossification centers were beginning to appear as the individual developed. The left hand included all carpals, epiphyses for metacarpals two and four, intermediate phalanges two, three, and four, as well as the epiphysis for distal phalanx one. There were no epiphyses present in the proximal row of phalanges, nor for the other four distal phalanges. *Eulemur mongoz*’s scans included both right and left hands; the left was used for

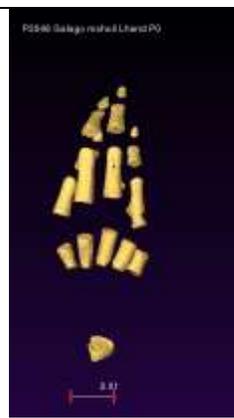
analysis and comparison. The right and left hand materials were at the same stage of development, and included five carpals, epiphyses for metacarpals two, three and four, all epiphyses in the proximal and intermediate rows. The distal row in the right hand did not present and epiphyses at this stage.

Galago moholi and *Propithecus coquereli* included images taken at age P30. All epiphyses for the metacarpals, and proximal and intermediate phalanges were present for *G. moholi*. All carpals were also present, as well as epiphyses for distal phalanges one, three and four. It appears that some of these epiphyses have also begun the fusion process though they are still clear distinguishable from their respective shafts. For *P. coquereli*, the right hand was used. This specimen was significantly less advanced in ossification; no carpals were present and no epiphyses in the proximal and distal rows. Those materials that did have ossifying epiphyses include metacarpals three and four and intermediate phalanges three and four.

The left and right hands for *Galago moholi* at age P97 were included in the scans. The right hand materials included all carpals and all secondary epiphyses. There were also two sesamoid bones present on each metacarpal shaft. The materials in the distal row were fused. Similarly, the left hand materials included all carpals, metacarpal sesamoid bones, and all epiphyses. The intermediate and distal materials appeared further developed because these materials were fused while materials in the proximal row have not yet completed fusion. The images in this section (2a-p) show the ossified elements of the Strepsirrhine specimens in this study.



2a. P696



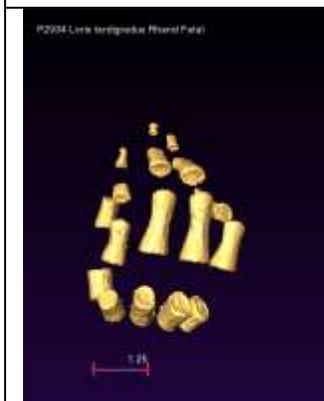
2b. P2046



2c. P1308



2d. DLC6905



2e. P2934



2f. P2935



2g. P2810



2h. P6888



2i. P1600



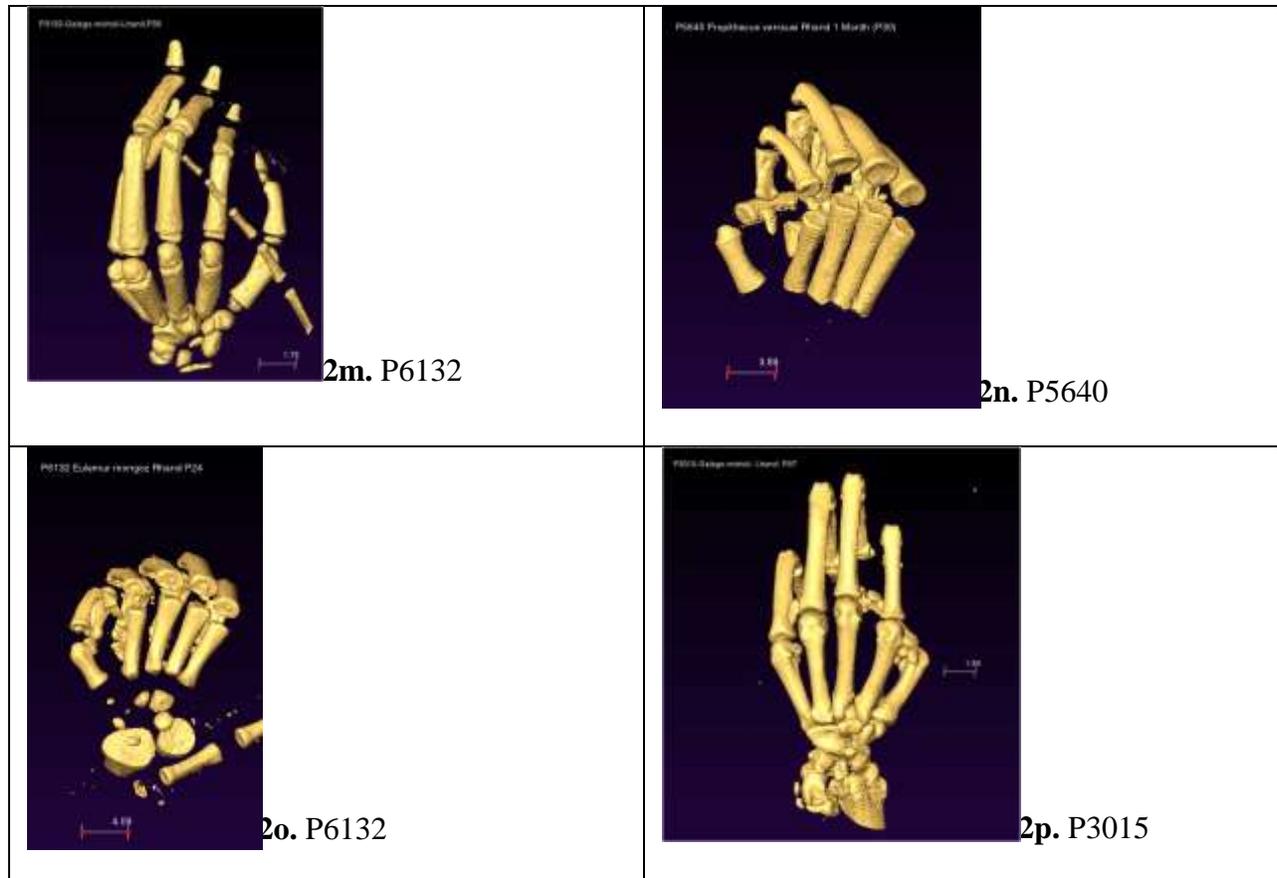
2j. P6426



2k. Is1020



2l. Ry2018



Comparative Analysis

Most of the primates in this study showed an MC1 epiphysis at the base- rather than at the head, similar to the pattern seen in humans. Those which exhibit a distal MC1 different to humans include *Cebuella pygmaea* (Cp17) and *Saguinus midas* (Smid6). This pattern is interesting because it is different from the pattern observed in humans but is rare in non-human primates. This is of further interest because Cp17 and Cp14 are both of the same species but one shows a distal epiphysis while the other shows a proximal one. It is unclear if this is individual variation.

A similarity between both phylogenetic groups, Haplorhini and Strepsirrhini, is the ossification pattern observed at birth. Primary ossification centers (shafts) of the metacarpals and

phalanges are present, with secondary centers (epiphyses) and carpals appearing in the older age groups. This study had comparatively more strepsirrhine specimens than haplorhines (16 specimens in Strepsirrhini and eight specimens in Haplorhini). Suborder Haplorhini showed more variability in ossification at birth, whereas the roles were switched in the older age groups with strepsirrhines showing more variability around P30 (day 30 after birth). Among perinatal specimens, strepsirrhines had a mean %TO of **40.62%**, and haplorhines had a mean %TO of **54.90%** (**Table 1**). This apparent difference in mean %TO shows that, on average, haplorhines show earlier ossification at or around the time of birth than do strepsirrhines.

Among older juveniles (P11-P30), the strepsirrhine *G. moholi*'s %TO (95.7%) is closer to that of haplorhine *S. oedipus* (97.9%) than it is to the more closely related *G. senegalensis* (80.9%), contrary to the hypothesis that ossification patterns are related to phylogeny (**Table 1**). This could be due to individual variation. Also among older juveniles (P23-P30), the haplorhines still appear to be more advanced (97.9-100%) than strepsirrhines, although the sample size is very small. The strepsirrhines showed a high level of variation at this later stage, ranging from 53.2% in *Propithecus* to 96.7% in *Galago* (**Table 1**). This may also be attributed to individual variation. An effect of locomotor behavior is not clear.

Table 1. A chart showing each taxon grouped by age and suborder classification with %TO and locomotor behavior.

Taxon	Perinatal			Juvenile			Locomotor Behavior
	Specimen	Age	%TO	Specimen	Age	%TO	
<i>Tarsius pyrichta</i>	P96	fetal	38.30%				vertical clinging and leaping
<i>Callicebus cupreus</i>	Callicebus1	stillborn	40.40%				arboreal quadruped, leaping
<i>Saguinus midas</i>	Smid6	stillborn	59.60%				quadrupedal running and walking, capable of leaping
<i>Saguinus oedipus</i>				So111	P27	97.90%	arboreal quadruped, vertical clinging and leaping
<i>Aotus nancymae</i>	Aotus101	stillborn	44.70%				arboreal quadruped, leaping
<i>Tarsius pyrichta</i>	P93	P0	57.40%				vertical clinging and leaping
<i>Cebuella pygmaea</i>	Cp17	newborn	89.40%	Cp14	P30	100.00%	arboreal quadruped, vertical clinging and leaping
Average Haplorhine			54.90%				
<i>Cheirogaleus medius</i>	P696	P0	40.40%				arboreal quadruped, no leaping
<i>Galago moholi</i>	P2046	P0	40.40%	P3132	P30	96.70%	arboreal quadruped, semi-terrestrial
<i>Galago moholi</i>				P3015	P97	100.00%	arboreal quadruped, semi-terrestrial
<i>Galago senegalensis</i>				Is1020	P7	44.70%	arboreal quadruped, semi-terrestrial
<i>Galago senegalensis</i>				Ry2018	P23	80.90%	arboreal quadruped, semi-terrestrial
<i>Haplorhina griseus</i>	P1308	neonate	40.40%				vertical climbing and leaping
<i>Propithecus coquereli</i>	DLC6905	neonate	42.60%	P5640	P30	53.20%	vertical clinging and leaping
<i>Loris tardigradus</i>	P2934	fetal	40.40%				slow, arboreal quadruped, above and below branches
<i>Loris tardigradus</i>	P2935	late fetal	40.40%				slow, arboreal quadruped, above and below branches
<i>Otolemur crassicaudatus</i>	P2810	late fetal	40.40%				semi-terrestrial quadruped
<i>Lemur catta</i>	P6888	late fetal	40.40%				arboreal quadruped, semi-terrestrial
<i>Cheirogaleus medius</i>	P1600	P1	40.40%				arboreal quadruped, no leaping
<i>Eulemur mongoz</i>	P6426	P1	40.40%	P6132	P24	76.69%	arboreal quadruped, leaping
Average Strepsirrhine			40.62%				

Discussion

Haplorhines appear to show a more advanced stage of ossification than strepsirrhines at birth. Haplorhines showed more variability at birth, whereas strepsirrhines showed more variation at one-month of age. My limited sample size of older juveniles did not allow me to discern significant patterns of ossification related to phylogeny or locomotor behavior. An interesting observation was the similarity between humans and most of the other primates in this study sharing a ~40.40%TO at birth (Schaefer et al., 2009). Human development is particularly interesting, in that, due to the derived characteristic of having a prolonged gestation period and long childhood, human development takes a considerably longer time to progress when compared with all other primates. As expected, when the stage of development between humans and other primates are compared at the same, or similar, ages, humans have a slower rate of

development. In comparison, *Galago* (P97 with 100% TO) appeared to have all elements ossified (**Figure 2**). Figure 2 also presents data contradicting the stated hypothesis that ossification patterns are related to phylogeny. Here, *Galago moholi*, a strepsirrhine species (95.7% TO) is closer in development to *Saguinus oedipus*, a haplorhine species (97.9% TO) than to the more closely related *Galago senegalensis* (80.9% TO). It is unclear if this pattern relates to function of the hand.

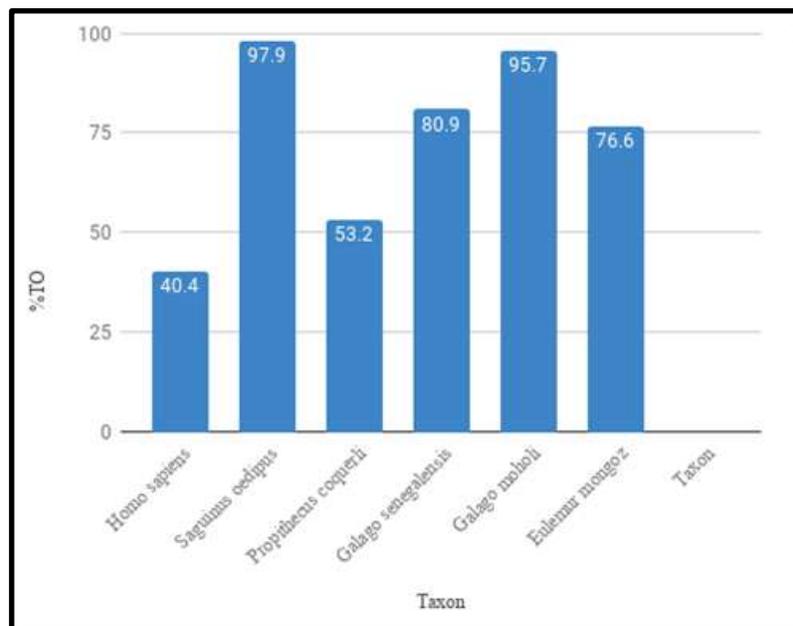


Figure 2. A bar graph showing the %TO observed in the older juvenile age group.

Conclusions

This study was restricted by the limited data set and could not fully answer the questions put forth in the beginning. While there were some patterns and observations which can be attributed to phylogeny, supported the initial hypothesis, there were also those that did not agree with the hypothesis. There is yet to be a clear explanation for these and other outliers that were present. To begin with, more specimens would need to be added to the existing Newborn Primate

Database from which these data were collected. Including apes would help in determining the potential influence of phylogenetic relationships between humans and the other primates. Being the only habitual bipedal primate did not allow me to determine if the locomotor behavior was related to ossification patterns in humans.

With more specimens added to the database, researchers could better control for interspecific variation in ossification. There should also be background knowledge of each birth of the specimens, such as the health of the mother, the duration of the pregnancy, the sex of the baby, and how the individual died. These variables could have also factored into the advancement or delay of ossification in these primates. It is unclear why some of the specimens were stillborn, leading to the question whether a complication with the pregnancy led to a delay in development, thus producing a lower %TO.

This is just one part in the greater picture of evolution. Primate hand ossification is helpful for age estimations and would be useful in biological anthropology, ecology, and primatology. This and other research from this database could help us better understand the specifics of ossification and development in the hand and wrist as related to evolution.

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