The phylogenetic signal in the skull of New World monkeys

by

Alexander Bjarnason

A dissertation submitted in fulfilment of the degree of Doctorate of Philosophy in Biological Anthropology

University College London

I, Alexander Bjarnason confirm that the work presented in this thesis is my own.

Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Dedicated to my parents

&

The memory of Charlie Lockwood

Acknowledgements

I would like to thank the following for financial support for the research undertaken: Department of Anthropology, UCL, the graduate research fund, UCL graduate school, the central research fund, University of London, and SYNTHESYS. I am indebted to the following institutions for allowing me to study the primate collections in their care: Natural History Museum London**,** Field Museum of Natural History Chicago**,** Museum fur Naturkunde Berlin, Naturhistorische Museum Wien**,** Smithsonian National Museum of Natural History Washington DC**,** Naturhistoriska Riksmuseet Stockholm**,** Anthropological Institue & Museum University of Zurich**.** The staff at the Natural History Museum London in particular (Louise Tomsett, Roberto Portela Miguez and Paula Jenkins) have given me fantastic access to their collections. A big thank you to Bill Stanley who made me feel at home in Chicago on my first research trip, his kindness and warmth meant a great deal to me. Thank you also to the following for providing access to their collections- Bettina Wimmer, Frieder Mayer, Barbara Herzig, Olavi Gronwall, Tea Jashashvili and Marcia Ponce de Leon. I apologise to all those who helped but have been forgotten!

This project was proposed by, and developed with, my late supervisor Charlie Lockwood. His loss was devastating personally and professionally. It would be impossible to describe the positive impact he had on my life, and I will just say that my life has been better for having known him, and I hope I have done him proud. From the sadness of his passing new friendships have been formed and old bonds have been strengthened: thank you to Katharine Balolia, Eric Lewitus, Fire Kovarovic, Nick Walton, Roshna Wunderlich, Rich Lawler, Brenda Bradley, Jeremiah Scott and the Lockwood family. I am deeply grateful to my supervisors Sarah Elton and Christophe Soligo, who stepped in to supervise this project and have provided a great deal of academic and pastoral support throughout the years. They each bring something completely different to my work, and can only apologise to them for my complete inability to get my head around singular & plural forms of taxa and data. Andrea Cardini and Brian Villmoare have also both provided an incredible amount of help and support, and I am grateful to them both. Many other researchers have provided varying input over the years (sometimes just a brief e-mail, but it all helps!) and I thank them all- Jim Rohlf, Chris Klingenberg, Chris Gilbert, Alfie Rosenberger, Walter Hartwig, Susan Ford, Rich Kay, Gabriel Marroig, Jim Cheverud, David Polly, Todd Rae, Andrew Chamberlain, Bill Kimbel, John Lynch, Filippo Aureli, John Aguiar and John Fleagle. Thanks to my friends

who have put up with me for all these years, offering a break from academia and provided a better work-life balance that has helped me stay sane and happy, especially the lads at football and Aude Moffitt, her family and Edward. Thanks to my extended support network, especially Dr Roger Howells, Tina Grigoriou and Sarah Heke. Sorry to anyone who has helped or contributed over the years but I've forgotten to mention.

Thank you to my parents and family. They have given me incredible emotional and financial support over the years, and I could not have done this project or thesis without them.

Finally, thank you to the monkeys & assorted primates sampled in this project, what an interesting and odd bunch you are. I'm sorry that so many of you got shot and killed, which must have been quite annoying.

Abstract

Many phylogenetic relationships based on morphology were rejected following the molecular revolution, yet there is a need for phylogenetic analysis of morphology that reliably infers phylogenetic relationships so that we can understand the evolutionary relationships of extant and fossil taxa. I use geometric morphometric and distance-based phylogenetic methods to study the phylogenetic signal in the skull of a clade of primates, the platyrrhines or New World monkeys, and re-examine congruence between molecular and morphological analyses. I collected digital anatomical landmark data from around 1400 specimens belonging to 16 genera and 50 species of New World monkeys, and nine primate outgroup taxa. I take a modular approach, inferring phylogenies based on the whole skull, face and cranial base, with a range of outgroups and outgroups combinations, and repeat analyses for male, female, pooled sex and separate sex data. Inferred relationships are compared to the most recent platyrrhine molecular phylogeny and past morphology-based analyses. Strepsirrhine outgroups performed slightly better as outgroups, as platyrrhines and Old World monkey or ape outgroups often shared homoplasy that interfered with accurate phylogenetic analysis. Phylogenetic analysis of all platyrrhines recovers a weak phylogenetic signal, but phylogenetic analysis of each of the three major molecular clades, atelids, pitheciids and cebids, finds greater congruence between molecular and morphological analyses. The atelids have a strong phylogenetic signal in the face, the pitheciids in all regions of the skull, and the cebid skull and face support three molecular lineages for callitrichines, cebines and owl monkeys, but infer molecular incongruent relationships within the callitrichines. Phylogenetic analysis of the face holds a stronger phylogenetic signal than expected, whereas the cranial base was more plastic and had a weak phylogenetic signal. In platyrrhines, phylogeny, diet, allometry and encephalization all have an important role in shaping craniodental morphology.

Abstract

Table of contents

List of Figures

List of tables

Chapter 1 Introduction

Phylogenetics is the study and reconstruction of hypothesised evolutionary and genealogical relationships between groups, whether they are populations, species, taxa, or some form of alternative evolutionary unit, based on empirical data from one or multiple sources (Fleagle 1999, Kitching et al. 1998, Schuh & Brower 2009). The origin of the field lies in the 18th century with the acceptance by naturalists and philosophers that the diversity of life on earth was generated via natural processes over large periods of time (Futuyma 2005). Charles Darwin made a lasting contribution to phylogenetics: that evolution proceeds across time through descent with modification from a single common ancestor to create the 'tree of life' (Futuyma 2005). Not only does all life share a single origin, but some groups share a more recent common ancestor and closer relatedness, which creates a hierarchy of tree like relationships between groups (Baum et al. 2005, Gregory 2008).

Phylogenetics is not merely biological stamp collecting, as a reliable phylogeny provides a framework and context within which to study the biology, and evolution, of the organisms of choice and traits they exhibit (Philippe & Telford 2006). Phylogenetic relationships are most often inferred using either morphological or molecular data, and many, but not all, of the phylogenetic relationships proposed based on morphology were usurped following the "molecular revolution", giving rise to the widely accepted belief that there is an inherent clash between molecules and morphology (Patterson 1987). The mammalian order of primates, that includes humans, apes, monkeys, tarsiers, lemurs and lorises, present an intriguing study group for the relationship between morphological and molecular evolution, in no small part because greater understanding of primate evolution informs humans about our own evolutionary history (Purvis 1995, Groves 2001). However, primates are interesting in their own right, encompassing a speciose radiation of long-lived mammals of varying body size that have inhabited a range of habits and climates across the globe (Simons 1972, Fleagle 1999). Primates have large brains relative to body size, complex behaviour, sophisticated social and mating systems, with wide variation in genetics, morphology, behaviour and ecology (Napier & Napier 1967, Napier & Napier 1985, Hartwig 2011).

Whilst cladistic analyses of primate morphology have often proposed monophyletic clades supported by molecular phylogenetics, the specific genera-level phylogenetic relationships regularly contrast, and the primate clade of platyrrhines are one such example (Rosenberger 1984, Ford 1986, Kay 1990). Platyrrhines are a parvorder of South and Central American

monkeys that split from the common ancestor with Old World monkeys, apes and humans between 50-40 million years ago, span two orders of magnitude in body size, and show large variation in diet, locomotion, encephalization, mating systems, social groups and behavioural ecology (Hodgson et al. 2009, Perelman et al. 2011, Wilkinson et al. 2011, Rosenberger 1992, Ford & Davis 1992, Isler et al. 2008). In this thesis, I have carried out new morphology-based phylogenetic analysis of platyrrhine craniodental morphometric data, to evaluate which region of the skull varies in shape with the greatest association to phylogenetic relationships as inferred by current molecular data. Morphological data from the whole skull, face and cranial base were analysed separately to see which matched the branching relationships inferred by molecular data, based on a working assumption that the molecular data accurately reflects the true phylogeny. I have taken a relatively unique approach that combined distance-based phylogenetic methods, an alternative to the more popular cladistic methods, with geometric morphometric data, that quantify morphological variation based on the geometric positions of anatomical landmarks, to infer phylogenetic relationships (Felsenstein 2004, Rohlf & Marcus 1993, Adams et al. 2004). As part of this project, I have collected geometric morphometric landmark data from the skulls of around 1400 adult platyrrhines from 16 genera and 50 species, that formed the basis for the morphological dataset used in phylogenetic analysis.

1.1 Project aims

The aims of this thesis are to:

1. Quantify craniodental morphological variation in the platyrrhine skull using geometric morphometric methods.
2. Evaluate which region of skull shape varies with the greatest association to phylogenetic relationships inferred by molecular genetic data.
3. Assess the impact of outgroup selection on distance-based phylogenetic analysis of geometric morphometric skull data.
4. Examine the effect of sexual dimorphism on relationships inferred by repeating phylogenetic analysis for male-only, female-only, pooled-sex and separate sexes treated as alternative taxonomic units.

1.2 Phylogenetic theory

Phylogenetics operates on the principle that that all biological organisms share a single origin, yet some groups are more closely related to each other and share a more recent common ancestor (Futuyma 2005). Phylogenetics, as one part of the wider field of systematics, has been historically divided into two major schools of phenetics and cladistics (Panchen 1992, Schuh & Brower 2009). A third group, evolutionary systematics, pre-dated these, although it was principally concerned with taxonomy rather than phylogenetic inference (Schuh & Brower 2009). Cladistics and phenetics emerged largely due to the development of computationally intense numerical taxonomy methods for the classification of organisms that allowed for increasingly complex analyses of large datasets (Scott-Ram 1990). The original clash between these two schools centred around whether phylogeny can, or should, be incorporated into taxonomy as the early pheneticists were against the use of "dubiously retrievable phylogenetic information" (Minelli 1993 p8). Today phenetics and cladistics are considered within the context of representing alternative phylogenetic approaches, and although phenetic methods have become increasingly useful for understanding morphological evolution, particularly in the human fossil record (e.g. Manzi et al. 2003, Schillaci 2008, Mounier et al. 2009, Bastir et al. 2010), they are rarely used in vertebrate palaeontology because they group taxa by overall rather than derived similarity.

Cladistics, or phylogenetic systematics, was formally developed by Hennig (1966) and infers phylogenetic relationships using shared derived features between groups (synapomorphies). In cladistic analysis there is a reliance on the use of a closely related outgroup, equally related to all ingroup taxa, to ascertain which state is derived or primitive (Maddison et al. 1984). For cladistic analysis of molecular data, DNA, RNA or protein sequences can be analysed, and for morphology character states or integers are used. In the case of the latter, quantitative/continuous morphological data are converted into character states, although the conversion of continuous data into character states will inevitably lose information that may be phylogenetically informative (Caumal & Polly 2005). When multiple species and traits are used several equally well supported phylogenetic trees may be produced, with several methods used to select which is most likely to be an accurate reflection of the true phylogeny. Parsimony is the most popular method and selects the phylogenetic tree requiring the least amount of evolutionary change (Felsenstein 2004).

In addition to cladistic parsimony methods, maximum likelihood and Bayesian methods incorporate probabilities into phylogenetics, although these methods are nearly exclusively used with molecular data (Nei & Kumar 2000). Maximum likelihood methods are an extension of parsimony methods that use differences in branch lengths and nucleotide substitution rates, evaluating the likelihood observed data occurs from hypothesised evolutionary relatedness and a proposed evolutionary model (Yang 2006). Bayesian methods use a prior assumption of phylogeny (if there is one), model of evolution, branch lengths and given data to produce a posterior probability, a measure of probability for each phylogeny in light of those factors (Ronquist et al. 2009). Maximum Likelihood, borne from classical statistical methods, examines the probability of producing observed data under a particular model whereas the Bayesian approach takes a simpler probabilistic approach investigating the probability a model is correct in light of the observed data (Ronquist et al. 2009).

Sokal & Sneath (1963) and Sneath & Sokal (1973) proposed the major alternative to cladistics, commonly referred to as phenetics, whereby all available characters are included in phylogenetic analysis and equally weighted with groups classified by overall similarity. A major group of phylogenetic approaches known as distance-based (or distance-matrix) methods, separately proposed by Cavalli-Sforza & Edwards (1967) and Fitch & Margoliash (1967), were used by researchers interested in phenetic relationships (Felsenstein 2004). These methods require two steps: calculating distances between groups, with their storage/presentation in a distance matrix, and generation of a phylogeny from those distances. The input data used to generate distances can be qualitative character states or quantitative metric data from molecular, behavioural or morphological sources. For the inference of phylogeny, after measuring evolutionary distances between every species pair (pairwise distances) a tree is found that best reflects these distances, with multiple methods available to either generate one tree or to choose the most appropriate tree from a selection (Felsenstein 2004, Nei & Kumar 2000). The four major distance-based methods are unweighted pair-group method using arithmetic averages UPGMA (Sokal & Michener 1958), minimum evolution (ME, Edwards & Cavalli-Sforza 1963), least squares (LS, Cavalli-Sforza & Edwards 1967) and Neighbor-joining (NJ, Saitou & Nei 1987).

With the exception of UPGMA, distance-matrix methods can be considered phylogenetic when they use an outgroup to provide evolutionary polarity so that taxa are grouped by derived, rather than overall, similarity (Felsenstein 1984, Lockwood et al. 2004). Many systematists make the mistake of dismissing distance-based methods as phenetic due to their

historic origins, even though the use of an outgroup incorporates the primary method of cladistics. Distance-based and cladistic phylogenetic methods differ in how, and when, an outgroup is used. If there is a table with x characters, y ingroup taxa and z outgroup taxa, the cladistic approach will analyse the x characters one-by-one and infer character evolution by comparing the z-outgroup and y-ingroups. At the end of the analysis one of multiple methods is used to infer the most likely evolutionary relationships of the y-ingroup taxa based on the character state evolution of x-characters. The distance-based method will take the same data, but use similarity of all x-characters by each taxon-pair to infer a distance between taxa. The distances will infer a specific topology of the y-taxa on an evolutionary tree based on the relationship with each other and the z-outgroups. The two methods deal with characters differently, but use the outgroup to avoid inferring a tree based on overall similarity.

There are also some important differences between distance-matrix approaches. UPGMA uses a clustering algorithm, with the two closest taxa clustered from the beginning and the next closest added step-by-step until all taxa are included in a single tree, assuming a constant rate of evolution in all lineages (Nei & Kumar 2000). In contrast, the least squares, Neighbor-joining and minimum evolution methods do not assume a constant rate of evolution (Polly 2001, Yang 2006). Least squares attempts to reconcile the difference between given distances, the pairwise distance, and predicted/patristic distances, the sum of branch lengths connecting two taxa, taking the squared differences between the two and fitting them onto a tree (Felsenstein 2004, Yang 2006). There are several versions of least squares: ordinary (applying equal weights to the different observed distances), weighted (weighing each squared difference by dividing it by the observed distance) and generalized (which at great computational cost integrates the variance and covariance of observed distances) (Nei & Kumar 2000, Yang 2006).

The minimum evolution method computes all the possible sums of branch length estimates, using the unweighted least squares method to produce branch lengths, and chooses the tree with the smallest sum of lengths (S value) for all possible topologies (Nei & Kumar 2000, Felsenstein 2004). To put it another way, the phylogeny proposed has the smallest branch lengths and least evolutionary change, which is similar to the maximum parsimony methods (van de Peer 2009). As this method requires investigating all possible trees, the computational demands can be very high, particularly with increasing taxa sampling. The neighbor-joining method approximates the minimum evolution method without generating all possible trees, cutting down computational time (Nei & Kumar 2000, Felsenstein 2004).

Although neighbor-joining is a clustering method it does not assume a molecular clock like UPGMA, and pairs taxa by minimizing the S value (van de Peer 2009). There are a number of tweaked neighbor-joining algorithms including BIONJ, generalized, weighted, relaxed, multi and maximum likelihood variants (van de Peer 2009).

1.3 Geometric morphometrics

The study, and measurement, of shape and shape differences between groups based on statistical analysis are important for our understanding of many different areas of biology (Polly 2008). Historical multivariate morphometrics appled multivariate statistical methods to linear distances, ratios, counts and angles, describing shape variation within and between groups (Adams et al. 2004). The use of these methods lacked consensus on the best approach for size correction and failed to completely capture the spatial arrangement of landmarks that measurements were based on, setting the stage for the latter geometric morphometric revolution and synthesis of a new approach to shape analysis (Rohlf & Marcus 1993, Adams et al. 2004, Slice 2007, Klingenberg 2008). Geometric morphometric methods, that maintain the geometric properties of 2 or 3-dimensional measurements taken between homologous biological landmarks/coordinates, are more adept at capturing and quantifying shape and allow for the use of more powerful and sophisticated statistical methods to test and visualise shape differences (Rohlf & Marcus 1993, Dryden & Mardia 1998).

Superimposition methods are employed to remove non-shape variation from landmark data placing collected morphometric data into a common reference system within a shared scaled size, as direct analyses of coordinates would not account for variation caused by differences in scale, position and orientation (Adams et al. 2004, Slice 2005). Geometric properties of shape are useful because they are maintained regardless of differences in position, orientation or magnification/reduction (Slice 2005). Generalized Procrustes analysis (GPA) is currently the most popular superimposition method used with geometric morphometrics (Adams et al. 2004). GPA uses a least-squares algorithm to translate and rotate the landmark configuration of each specimen, minimising squared summed differences of corresponding homologous landmarks between separate specimens and the consensus mean configuration (Gower 1975, Rohlf & Slice 1990, Goodall 1991, Slice 2005, Slice 2007). GPA also scales all specimens by centroid size, the square root of the sum of squared distances for all landmarks in a configuration based on their average location. Zelditch et al. (2004) considered the process of GPA as three simple steps that do not “alter” shape: translation simply moves shape from one

place to another, rotation turns a specimen on a fixed axis, and scaling enlarges or reduces size, maintaining its shape. The GPA process produces new coordinates that can be used for multivariate statistical tests to compare individuals and groups, with differences in landmark configurations between individuals measured by Procrustes distances- the square root of the sum of squared distances between paired landmarks from separate individuals (Cardini & Elton 2008). Within the context of phylogenetics, geometric morphometric provides quantitative data that can be used to infer phylogenetic relationships (e.g. Polly 2001, Lockwood et al. 2004, Cardini & Elton 2008). The major advantage of a phylogenetic approach with morphology that incorporates geometric morphometric data is the measurement of morphological variation that is difficult to summarise in terms of cladistic character traits or quantify with linear and angle measurements.

1.4 The use of morphology to infer phylogenetic relationships

With the advent of the 'genomic era' and modest pricing of DNA sequencing that rapidly generates large datasets, it is possible to carry out massive phylogenetic analyses of multiple groups and infer relatively well accepted, strongly supported phylogenies based on a wide range of genes and genetic regions (Edwards 2009). Of course, many DNA-based phylogenetic analyses are imperfect, especially due to the variability in rates of molecular evolution, but as more molecular data become available and hypotheses are more readily tested, the phylogenetic relationships between primates have begun to reach near-consensus (Bromham & Penny 2003, Perelman et al. 2011). In comparison to the much-lauded molecular approach, phylogenetic analysis of morphology have lagged far behind. Amongst many problems, morphological phylogenetics are inhibited by the frequency of homoplasy, similarity in groups not a result of shared ancestry, and problems with a character state approach to generating phylogenies (e.g. Lockwood & Fleagle 1999, Lockwood 1999 , Lockwood 2007).

It may be that methodological reasons confound the successful application of morphological phylogenetics and with the resolution of those problems, and development of new methods, there will be greater congruence between morphology and molecules (Wiens 2004). Some biologists find this unnecessary, as DNA sequencing and corresponding phylogenetic inference is cheap, easy and replicable with such overwhelming statistical support that there appears to be little point in using morphology for reconstructing evolutionary relatedness

(Scotland et al. 2003). Whilst true for those interested solely in studying living (extant) species such an approach is difficult for palaeontologists, as due to the rapid deterioration of DNA there are rarely molecular data available from fossil groups (Wiens 2004, Jenner 2004). Therefore, to understand the relationships between living forms and those of the past, it is important to be able to infer with some reliability phylogenetic relationships based on morphology (Wiens 2004).

1.5 Cladistics

All biological organisms display corresponding structures, which Owen (1843: p379) termed homology and defined as "the same organ in different animals under every variety of form and function." (Schuh & Brower 2009, Jardine 1967, Owen 1843). There are two levels of homology for a trait; a primary hypothesis of homology, that each character has a corresponding comparative trait present in multiple groups, albeit potentially in different forms or states (Kitching et al. 1998). A secondary hypothesis of homology is proposed when two groups share a character state via descent from a common ancestor, the basis from which evolutionary relationships are inferred. Homology is potentially troublesome as characters may appear homologous as a result of common ancestry but also due to convergence, a shared response to non-genetic factors, shared function, or similarity in size, shape or position (Lieberman 1999). Any characters strongly influenced by non-genetic factors will be especially inappropriate for phylogenetic inference (Lieberman et al. 1996a).

Characters have typically been the input data for phylogenetic analysis of morphology, and are traits that are scored and analysed to understand trait evolution. Characters must exist in more than one state and can relate to the presence or absence of a trait, binary variables, or multiples states (Kitching et al. 1998). They are preferably distinct traits that are qualitative and discrete derived from morphological, molecular or behavioural data, with low within-taxa variation and little overlap between taxa (Kitching et al. 1998, Schuh & Brower 2009). The recognition of characters and character states for morphology will depend on the judgement of the observer(s), and the overall morphological dataset is an accumulation of comparative data as those researchers interpret it (Schuh & Brower 2009), which may lead to differences in datasets collected and phylogenies inferred by different investigators. Data that are continuous or quantitative are problematic for cladistic analysis as most software relies on input of categorical state data, and the conversion of continuous data into character states will inevitably lose information that may be phylogenetically informative (Kitching et al. 1998).

Stevens (1991) points out that qualitative character states often describe quantitative variation and differences, so metric data is not necessarily ignored in cladistic analysis (Kitching et al. 1998).

There have been numerous morphology-based cladistic analyses of primates and clades within the group. These include cladistic analyses of the primate crown group, lemurs, a clade of lemurs known as the lemuroids (family lemuridae), strepsirrhines, anthropoids, New World monkeys, Old World monkeys, gibbons and siamangs, great apes and humans, and fossil hominins (see Table 1- with several references taken from Groves & Eaglen (1988), Schwartz & Tattersall (1985), Purvis (1995), Strait et al. (1997), Yoder & Irwin (1999). The major accomplishment of early primate cladistic analyses was the acceptance of monophyly in many primate clades later recognised by molecular genetics. However, many of these studies inferred phylogenetic relationships within clades shown to be inaccurate by later molecular genetic work. This suggests that the morphological cladistic approaches strongly reflects phylogenetic relationships at higher hierarchical levels, but are less accurate at lower levels. Clearly cladistic methods and morphology per se are informative for some analyses, but there is also an opportunity to develop and use alternative phylogenetics methods to reliably infer evolutionary relationships in lower hierarchical levels.

Table 1: Examples of morphology-based cladistic analyses of primates

Clade analysed	Reference
Primates	Shoshani et al. (1996)
Lemuroids	Eaglen (1980), Eaglen (1983), Groves & Eaglen (1988), Tattersall & Schwartz (1991), Groves & Trueman (1995)
Lemurs	Tattersall & Schwartz (1974), Tattersall & Schwartz (1975), Stanger-Hall (1997)
Strepsirrhines	Schwartz & Tattersall (1985)
Anthropoids	Ross et al. (1998), Kay et al. (2004)
New World monkeys	Rosenberger (1984), Ford (1986), Kay (1990), Horovitz et al. (1998), Horovitz & MacPhee (1999), Horovitz (1999), Kay et al. (2008)
Old World monkeys	Strasser & Delson (1987), Collard & Wood (2000)
Gibbons & siamengs	Haimoff et al. (1982)
Great apes & humans	Kluge (1983), Schwartz (1984), Creel (1986), Groves (1986), Martin (1986), Andrews & Martin (1987), Groves & Paterson (1991), Begun (1992), Begun (1994), Hartwig-Scherer (1993)
Fossil hominids	Delson et al. (1977), Skelton et al. (1986), Chamberlain & Wood (1987), Stringer (1987), Skelton & McHenry (1992), Lieberman et al. (1996a), Strait et al. (1997), Strait & Grine (2004), Cameron & Groves (2004)

1.6 Character coding

Several researchers have integrated linear or geometric measurements of osteological form (morphometrics) into phylogenetic analysis. There were two major reasons for this: character-based approaches do not fully integrate the quantitative variation observed and there is a question of subjectivity in the scoring of characters. The latter is also a problem with morphometric approaches, as often only a single observer will measure the form of specimens. The two main methods of character coding are gap coding (Mickevich & Johnson 1975, Thorpe 1984, Archie 1985), where character states are coded when metric differences between adjacent group means exceed within group standard deviation by a pre-defined amount, and divergence coding, whereby character states are decided by an overall pattern of statistical differences between groups (Thorpe 1984).

Collard & Wood (2000) ignited interest in the use of character coding with a phylogenetic analysis of hominoid and papionin craniodental morphology, framed as a test of the reliability of craniodental morphology to accurately infer evolutionary relationships. They compared morphology-based phylogenies, consisting of both qualitative characters and quantitative metric measurements converted into character states via character coding, to well resolved molecular phylogenies. The phylogenies inferred by morphological and molecular data were incongruent, leading to the conclusion that "standard craniodental characters cannot be relied on to reconstruct the phylogenetic relationships of the hominoids, papionins, and, by extension, the fossil hominins" (Collard & Wood 2000: p5005). Those authors suggested the incongruence between molecular and morphological analyses lay with the type of characters used rather than cladistics or an inherent problem with craniodental morphology. Strait & Grine (2004) strongly challenged this conclusion in their phylogenetic analysis of living hominoids and fossil hominins, using qualitative character states and quantitative measurements converted into character states with character coding. Phylogenetic analysis of their complete taxon-sample provided congruence between the phylogenies from molecular and morphological data, indicating that the results of Collard & Wood (2000) were not due to a problem with craniodental characters or morphology per se.

The work of Collard & Wood (2000) on papionins was preceded by earlier studies that examined papionin morphology within a cladistic framework (e.g. Strasser & Delson 1987) and recognised three clades: mangabeys, baboons and geladas. Molecular analyses challenged this view (e.g. Disotell et al. 1992, Disotell 1994), as the mangabeys and baboons

were diphyletic, with the mangabeys split into two genera *Lophocebus* and *Cercocebus*, and baboons separated into *Papio* and *Mandrillus*. Gilbert & Rossie (2007) and Gilbert et al. (2009) used similar data to Collard & Wood (2000) but with new character coding methods. The methodological advance was to account for allometry, the relationship between size and shape (see subsection Body Size, Scaling and Allometry), as they examined each linear measurement and, if there was a correlation between the trait and overall size, separated taxa into two groups based on size and character coded the morphological differences against similar-sized taxa. The phylogenetic analyses based on this approach found strong congruence between the phylogenetic trees based on morphology and those inferred by molecular data. By comparing the results of Collard & Wood (2000) to Gilbert & Rossie (2007) and Gilbert et al. (2009) it is clear that in papionins allometry disrupted the phylogenetic signal unless methods were applied to limit its effect. The broad scale applicability of these methods is, however, questionable, as it requires the clade under study to have large variation in size and separation into distinct groups according to size. In the case of hominoids for example, such a method cannot be used (Bjarnason et al. 2011).

1.7 Distance-based phylogenetic analysis & geometric morphometrics

Lockwood et al. (2004) used an alternative methodological approach to the use of morphology in primate phylogenetics. Rather than using a character-based cladistic analysis, they used distances between groups as the basis for inferring evolutionary relationships using distance-based phylogenetic methods. Distance-based methods are advantageous as they use qualitative or quantitative input data, so that metric data can be analysed without character coding, although within-group variation is not considered so there is some data loss. Klingenberg & Gidaszewski (2010) viewed the use of distance-based methods with morphometric data as avoiding the problem of separating quantitative form into characters. The distances separating taxa are calculated between sets of variables in multidimensional space, with no subdivision of variables into character states; therefore, the discussion of characters is simply unnecessary in the context of distance-based methods.

Lockwood et al. (2004) collected 3-dimensional geometric morphometric data from the temporal bone for great apes and humans, with a wide sampling of great ape subspecies. They measured Euclidean distances between mean shapes of taxa which were phylogenetically analysed with distance-based methods. The phylogenies inferred were congruent with the most recent molecular phylogenies of great apes and humans, replicating

the genus and subspecies level relationships. The results were particularly exciting because of the obvious application to the human fossil record, within which the temporal bone is often well preserved. Although often considered a rejection of Collard & Wood (2000), these results could be interpreted as supporting their call for new techniques to be developed. Bjarnason et al. (2011) further investigated the methodological basis for the results presented in Collard & Wood (2000) and Lockwood et al. (2004). This work incorporated phylogenetic analysis of two quantitative datasets- geometric morphometric temporal bone data of hominoids, as used in Lockwood et al. (2004) with additional *Hylobates* data, and craniodental linear morphometric data of hominoids from Chamberlain & Wood (1987), partially used by Collard & Wood (2000). Both datasets underwent phylogenetic analysis with distance-based methods and cladistic analysis (after character coding), and were repeated with *Hylobates* or *Pongo* as outgroup. The results showed that direct phylogenetic analysis of data with distance-based methods produced greater congruence between molecular and morphological phylogenies than character coded cladistic analysis. Outgroup selection was also shown to be a major source of incongruence between morphological and molecular analyses, and likely the major explanation for the results of Collard & Wood (2000).

1.8 Morphological & molecular matrix correlations

The success of Lockwood et al. (2004), in generating a phylogenetic tree with strong support for molecular clades of great apes and humans, was a product of its time as geometric morphometric methods of quantifying morphological variation became increasingly popular, as did phylogenetic analysis of the data collected. For example, an earlier study by Polly (2001) examined the phylogeography of the European shrew *Sorex araneus*. Using morphometric analysis of molar morphology, multivariate measurements described divergence between groups with comparison to molecular genetic data. Procrustes distances were calculated for mean shape of each taxa from 2-dimensional morphometric data, and phylogenetic trees were inferred using UPGMA, neighbor-joining and least squares phylogenetic methods. Correlation of molecular and morphological distance matrices tested for goodness-of-fit, and the relationship between the two was statistically significant. Morphological data accurately resolved phylogenetic relationships where taxa had diverged within a period of 5 million years. Couette et al. (2005) also used Procrustes variables as the basis for morphological distances, comparing morphological and molecular evolution using a subset of callitrichine New World monkeys. Least-squares and neighbor-joining methods

were used following extensive matrix testing, with non-parametric bootstrapping of the morphological data. The morphological and molecular matrices were significantly correlated, but the morphological phylogenetic tree did not support the two major molecular clades.

Several studies of modern humans have also used distances derived from Procrustes residuals and matrix correlation methods for comparison with molecular genetic data. Harvati & Weaver (2006a) examined geometric morphometric data from the face, cranial vault and temporal bone of modern human populations. Morphological distances were measured as Mahalanobis distances, which attempt to control for covariation between coordinates and within-group variation. Correlation of the morphological and molecular matrices found significant relationships for both cranial vault and temporal bone data with the molecular distances. Harvati & Weaver (2006b) employed the same methods with additional population sampling and found greater correlation between the temporal bone and genetic matrices. Smith et al. (2007) examined a similar research question for temporal bone morphology in modern human populations, with one of several analyses comparing morphological and molecular genetic distances by matrix correlation. They used Procrustes distances and not Mahalanobis, rejecting its use due to the assumption that all specimens share similar covariance structures and its vulnerability to the effects of unequal sample sizes. Morphological and molecular matrices were significantly correlated, broadly supporting Harvati & Weaver (2006a,b).

Whilst matrix correlations between morphological and molecular distances are interesting and useful to a degree, the phylogenetic trees inferred by the two types of data are often incongruent even when matrix correlations are high (Klingenberg & Gidaszewski 2010). Considering the primary aim of phylogenetic inference based on morphology is to reach consensus with molecular phylogenetics so that the methods can be reliably applied to phylogenetic analysis of extant and fossil groups, a matrix correlation approach is not used in this project. A possible explanation for the chasm between matrix correlations and phylogenies inferred, where distances are significantly correlated but phylogenetic trees are incongruent, may lie with outgroups. Topology within a phylogeny relies largely on the outgroup and its relationships with the ingroup taxa, so divergence in distance between an outgroup and ingroup taxa could cause a large change in tree topology. This divergence could incorporate just one or a few distances amongst many, and would be enough to produce alternative phylogenetic trees for molecular and morphological data even though the overall pattern of distances are highly correlated.

1.9 Distances from partial warps

The use of matrix correlations by Polly (2001) was itself preceded by Monteiro & Abe (1999) in their work on the scapula of xenarthrans, a superorder clade of American mammals including anteaters, sloths and armadillos. Although Monteiro & Abe (1999) used a matrix correlation approach, other methodological decisions, such as the use of immunological distances and assumed phylogenetic relationships based on the fossil record, make this paper of less interest than subsequent studies. Nicola et al. (2003) measured congruence between morphological and molecular evolution in a 5-taxon clade of spiny rats. Two-dimensional geometric morphometric data was collected from the craniodental region at dorsal, ventral and lateral perspectives, describing somewhat different morphological regions. Following Procrustes analysis, partial warp scores were used as shape variables to compute Euclidean distances. Matrix correlation between Euclidean morphometric and molecular genetic distances measured congruence between the two. Morphological distances of landmarks from the lateral perspective correlated significantly with molecular distances, although dorsal and ventral data did not.

Monteiro & Dos Reis (2005) further investigated *Trinomys* molecular and morphological congruence, using geometric morphometric data from the mandible. Comparison of morphology-based Procrustes distances with molecular-based distances found no congruence between the two. Macholan (2006) examined congruence between mouse molar morphology and molecular genetic distances for 24 groups from nine taxa, using the methodological approach of Nicola et al. (2003) and Monteiro & Dos Reis (2005). There was significant correlation between morphological and molecular distances, yet inference of a morphological tree with neighbor-joining methods did not replicate the most recent molecular phylogeny. The use of partial warps in phylogenetic analysis is rare, has been relatively unsuccessful, and this approach to phylogenetic analysis is not used in this project.

1.10 Distances from principal components

Another approach to phylogenetic analysis of morphometric data is to use principal component scores as the basis for morphological distances between taxa. A non-matrix approach by Viguier (2002) studied six lemur taxa to compare morphological and molecular relationships. 2D geometric morphometric data from the craniodental region was subject to Procrustes superimposition and principal component analysis (PCA), with the principal component scores used to calculate Procrustes distances between taxa. The morphological

distances were analysed with phenetic methods and compared with a lemur molecular phylogeny, with which there was little congruence. Polly (2003) revisited *Sorex araneus* evolution, combining principal component scores with Maximum likelihood methods previously restricted to molecular phylogenetics. Morphological and molecular distances significantly correlated, and the phylogenetic relationships inferred from morphology were broadly consistent with molecular data. Caumal & Polly (2005) examined Eurasian marmot craniodental variation in relation to diet, body size and genetic divergence, using principal component (PC) scores and Maximum likelihood phylogenetic analysis. The morphological dataset was divided into skull, mandible and molar shape, for which skull shape had the greatest, and mandible shape the least, congruence with the molecular tree. Although only 15% of variance in the skull was explained by genetic distance and 25% was explained diet, the phylogenetic analysis of skull shape had strong similarity with the molecular tree.

Cardini & Elton (2008) took a modular approach to investigating the phylogenetic signal in the skull of a clade of Old World monkeys, the guenons, collecting data from the mandible and skull and generating phylogenetic hypotheses from the whole dataset and smaller anatomical regions. They used a combination of phylogenetic approaches, generating distances from both pairwise Procrustes distances and principal components, with distance-based and Maximum likelihood phylogenetic analyses. Morphological matrices were also correlated against a genetic distance matrix, and the morphological region with the highest correlation to genetic distances, the cranial base, was further analysed with clustering, neighbor-joining and maximum likelihood methods. The basicranium shared the highest correlation with molecular distances, and conserved the strongest phylogenetic signal.

Concurrent primate studies continued the use of principal component data for phylogenetic analysis. Smith (2009) examined human phylogenetic signals in regions of the skull, using matrix correlations between molecular and morphological distances. Morphological distances were estimated from extracted principal components, and matrix correlations found significant relationships between molecular distances and those of the whole skull, cranium, mandible and temporal bone. von Cramon-Taubadel (2009) carried out a similar analysis to Smith (2009), with differences in group sampling and genetic data used, using morphological matrices based on principal component data. Although all craniodental regions in the analysis correlated significantly with molecular distances, temporal bone shape had the strongest correlation. Gilbert (2011) approached phylogenetic analysis of papionin morphology with a 3-D geometric morphometric analysis of the basicranium region. Euclidean distances were

extracted from principal component data, and non-corrected data was analysed alongside two allometric correction methods. Allometric correction involved removal of principle components that had a significant correlation with log centroid size and analysis of all PCs that did not significantly correlate, and regression of tangent space coordinates against the natural log of centroid size with PCA on the residuals produced by regression analysis. A bootstrap approach placed confidence intervals on the phylogenies inferred and matrix correlations measured congruence between morphological and molecular data. None of the inferred phylogenies achieved congruence with a papionin molecular phylogeny, possibly due to allometry, and the lack of adequate methods to control for allometry.

1.11 The problem with principal components

The use of principal components to describe shape variation for use in phylogenetic analysis was challenged by Adams et al. (2011) in a reply to a high profile article in *Nature* by Gonzalez-Jose et al. (2008). Gonzalez-Jose et al. (2008) investigated hominin phylogenetics with a proposed 'modular cladistic approach' using 3D geometric morphometric data collected from hominin fossils casts. Anatomical landmarks described four elements of the cranium treated as distinct separate modules: cranial base flexure, facial retraction, neurocranial globularity and masticatory apparatus. These four areas of morphology were individually subjected to Procrustes analysis and principal component analysis, with the principal component scores describing 75% of variation treated as continuous variables in parsimony and Maximum likelihood phylogenetic analyses. The phylogenetic analysis recovered a tree with monophyletic *Homo*, although modern humans were inferred to be more closely related to *Homo erectus* than Neanderthals, and the robust australopiths were paraphyletic.

Adams et al. (2011) critiqued several methodological assumptions of Gonzalez-Jose et al. (2008). They contested the use of a subset of principal components and rank ordering for each species to derive character states as likely to distort the data. Geometric morphometric data places specimens in multivariate shape space after Procrustes analysis, and shape difference exist in tangent space described by Euclidean distances. Principal component analysis rotates the space that holds these shapes and retains the Euclidean distances between shapes, but upon ranking principal components the Euclidean distances are lost and character states can subsequently change according to how the principal components are rotated producing arbitrary character states. A particular problem arises as the rotation, and

subsequent character states and phylogenies inferred, changed with removal or addition of specimens even when mean taxa shapes were identical. In practice, an analysis of 5 taxa with and without a 6th group can give completely different principal component rank orders (and subsequent character states) even though the mean shape of the original 5 taxa are identical in both cases.

Adams et al. (2011) also suggested that Gonzalez-Jose et al. (2008) used an incorrect covariance matrix and weighting to generate principal components for use with Maximum likelihood methods, although this is a practical criticism rather than a methodological one. They also question the choice to use only principal components describing 75% of variation, rather than those describing the full 100%. The 'modular cladistic approach' was challenged in terms of the phylogenetic trees generated, by the reanalysis of their data with alternative phylogenetic methods. A UPGMA tree of the combined landmark dataset based on Procrustes and Euclidean distances produced nearly identical trees to those from the modular cladistic approach and Maximum likelihood methods, suggesting the new method fails to perform better than the alternatives. In a reply to Adams et al. (2011), Gonzalez-Jose et al. (2011) acknowledge the problems raised, agreeing that the effect of rotation on analysis is substantial and requires much further work, although they do question the perceived rejection of modularity (discussed below under 'Modularity & primate phylogenetics'). Instead, they feel modularity and morphological integration should be placed at the core of further phylogenetic work. Whilst they are right to draw attention to the connection between modularity and phylogenetics, their phylogeny of hominins was simply an artefact of the methods employed rather than a new way to view the human fossil record, and ought to be rejected as such.

1.12 Alternative methods for phylogenetic analysis of geometric morphometric data

Several alternative phylogenetic methods have been proposed for use with morphometric data. Cole et al. (2002) proposed a tree building method with a parametric bootstrap for landmark data based on Euclidean Distance Matrix Analysis (EDMA) methods, the major alternative to geometric morphometric methods based on Procrustes analysis. Using landmark data from the midface of atelids as a case study, they compared a molecular phylogeny to a phenetic tree derived from morphological data. Mean shapes of taxa were calculated from interlandmark distances and standardised for size with the geometric mean, and Euclidean distances between groups used to cluster by pairwise dissimilarity. After use of

a clustering method, in this case phenetic UPGMA, tree topologies were compared to give a numerical measure of tree similarity. In the case example used, there was no congruence between molecular and morphological phylogenetic trees. This approach benefits from utilising a bootstrap statistic to statistically support inferred trees, avoiding problems highlighted regarding Procrustes superimposed data being bootstrapped (see Cardini & Elton 2008). However, the EDMA approach has been shown to lack the statistical power of geometric morphometric methods (Rohlf 2000b, Rohlf 2003) and are rarely used in physical anthropology.

Klingenberg & Gidaszewski (2010) developed a method to test whether morphometric data contained a phylogenetic signal by mapping the morphometric data onto a phylogeny. To avoid the problem of dividing morphological data into characters, shape was treated as a single character, with a single character state reflecting the whole shape of the digitised organism/group. A squared-change parsimony method mapped shapes onto terminal nodes of a known molecular phylogeny via estimating shapes of internal nodes and computing their evolution with minimal possible change. A permutation statistic tested for the presence/absence of a phylogenetic signal, based on a null hypothesis of no phylogenetic signal in the morphometric data. Computation of the consistency index (CI) and retention index (RI) provided a measure of the strength of phylogenetic signal in the morphometric data. The CI measured the fit between a character and a phylogeny by comparing the observed number of steps to describe character evolution to the number of steps expected to infer a phylogeny in light of the number of characters and taxa included; a high score means the tree has low homoplasy and required few steps. The RI measured the extent to which a character was synapomorphic, a derived state shared between two or more taxa as a result of descent from a recent common ancestor, and for a dataset with more than one character a measure of overall synapomorphy frequency.

Using these methods, Klingenberg & Gidaszewski (2010) examined the phylogenetic signal of wing landmarks in *Drosophila* species for which there was a well-supported molecular phylogeny. The permutation test supported the presence of a phylogenetic signal, and the high values for consistency and retention indices supported low levels of homoplasy. However, phylogenetic trees generated from the morphological data using Procrustes distances contrasted sharply with the molecular tree. This result is particularly discouraging in light of the attributed presence of a phylogenetic signal and low levels of homoplasy, as an assumption from previous unsuccessful phylogenetic analyses of morphology has been that

homoplasy has disrupted the phylogenetic signal. Alternatively, the model of evolution is assumed to be Brownian motion, which treats evolution as a stochastic process, that changes in separate lineages are independent, and rates of change and variance follow a normal distribution (Felsenstein 2004). This model may be inappropriate with a potential large discrepancy between phylogenetic and phenotypic divergence, with selection in the latter particularly problematic in eroding the connection between morphological and molecular evolution. The method proposed is of deep interest, particularly in its ability to quantify levels of homoplasy but two factors are problematic. First, within the platyrrhines there is not a strongly supported molecular phylogeny at the species level, which would be imperative for the use of the methods described. Second, the methods cannot be applied to the fossil record in the absence of molecular genetic data.

Catalano et al. (2010) proposed a parsimony method based on Farris optimization (Farris 1970), that minimizes tree length using hypothetical taxonomical units, with geometric morphometric data that inferred phylogenetic relationships by estimating shape of hypothetical ancestors. The ancestral position of each landmark was estimated so there was minimal change in all ancestor-descendent relationships. At nodes where an ancestor gives rise to two descendent taxa, a point was calculated in between to give the shortest possible distances. The method used to estimate ancestral phenotype and calculate distances for extant and ancestral shape were extensions of superimposition methods used in geometric morphometrics. For tree building, weighting of non-independent landmarks were proposed by dividing the "score" of each landmark by the "score" of its wider inter-related configuration, contributing to an overall tree "score". These "scores" seem to refer to the coordinate positions of landmarks, although this is not made clear by the authors, and there does not appear to be a criterion for deciding whether landmarks are independent or non-independent.

Theoretically, the proposed method may be problematic in the estimation of ancestral (unknown) phenotypes. In particular, the treatment of landmarks individually when Procrustes analysis is based on treatment as a combined unit of shape will likely be opposed on theoretical grounds by morphometricians. The authors comment on a problem with branch lengths in distance-based methods, originally raised by Farris (1981), that trees may use distances based on mathematically abstract ancestors. Felsenstein (1984) originally defended the use of distance-based methods and challenged Farris (1981) on the interpretation of his results, since which little appears to have changed. The proposal of landmark weighting appears somewhat haphazard, and there is a clear problem with trying to integrate our current

incomplete knowledge of landmark dependence into phylogenetics. If five landmarks are mechanically independent but their change over time in a group of taxa accurately reflects phylogenetic change, they will be considered phylogenetically non-independent by Catalano et al. (2010). They propose weighting such landmarks to minimise their input in phylogenetic analysis, or removal from the analysis all together, which would be counterproductive when the aim of analysis is to reliably infer phylogenetic relationships. Finally, there is a major problem in the practical application of the Catalano et al. (2010) methods, as they are incredibly computationally intensive and no program is currently publically available to run the required analyses.

1.13 Phylogenetic analysis of morphology

It is clear that there is an on-going debate about the most effective method for phylogenetic analysis of morphological data. The emergence of geometric morphometrics, and the ease with which digitised morphometric data is collected from a large numbers of taxa, has provided an impetus for a move away from qualitative cladistics into quantitative phylogenetics. Nevertheless, the increased availability of morphometric data has not led to consensus on the method of choice for phylogenetic analysis. Phylogenetic analysis of geometric morphometric data has either incorporated Procrustes coordinates or further extraction and manipulation of data using partial warp or principal component scores. In the latter two cases, serious methodological issues have been raised (e.g. Adams et al. 2011) and it appears wiser to use Procrustes coordinates as the basis of phylogenetic analysis.

A more pressing topic regards the use distances derived from coordinates as a basis for phylogenetic analysis compared to the alternative approaches of either Klingenberg & Gidaszewski (2010) or Catalano et al. (2010). The Klingenberg & Gidaszewski (2010) approach is attractive due to the integration of classic cladistic methodssuch as retention and consistency indices, but the need for a well-resolved molecular phylogeny at the subspecies and species levels are inappropriate for this project. The method of Catalano et al. (2010) is of interest but it seems likely that methodological issues will be raised in the near future, and the computational processing requirement is simply too great for this project. Instead, a distance-based approach without matrix correlations will be used. Klingenberg & Gidaszewski (2010) have raised a problem with the matrix correlation approach: morphological and molecular distances can strongly correlate, but tree building based on the morphological data can still produce a tree incongruent with the preferred tree derive from

the molecular data. As a result, the approach to phylogenetic analysis of morphological data followed herein is to generate morphological distances from geometric morphometric data and infer trees using distance-based phylogenetic methods as used by Lockwood et al. (2004), Cardini & Elton (2008) and Bjarnason et al. (2011). The morphological and molecular trees are visually compared based on genus-level relationships for which there is a well resolved platyrrhine phylogenetic tree.

1.14 The phylogenetic signal of the primate skull

A phylogenetic signal is present when data, whether morphological, molecular or otherwise, accurately reflects the evolutionary relationships, and history, of a group of organisms under study. When a morphological phylogenetic signal is strong, groups descended from a more recent common ancestor will share phenotypic similarity not present in more distantly related groups, and could include complex structures that may be less vulnerable to homoplasy (Klingenberg & Gidaszewski 2010, Polly 2001). The presence of a phylogenetic signal in the skull is further complicated depending on its treatment as a single indivisible unit, or whether it can be divided into semi-independent, isolated regions known as modules. This is pertinent, because modularity in the craniodental region could lead to different modules becoming prone to homoplasy or maintaining homology, leading to specific regions having a stronger phylogenetic signal.

1.15 Modularity

An organism is a single, biological unit created by a complex interaction between environmental and genetic factors, yet an individual organism also consists of parts which are partially distinct, or heterogenous, in structure and function from each other (Wagner et al. 2007). In studies of morphology, this autonomy of parts has become synonmous with the concepts of integration and modularity, where integration refers to cohesion and covariation of traits as a result of biological processes acting upon the phenotype, and modularity refers to units that have strong integration between traits of the same module but weak interaction between traits from different modules (Klingenberg (2008). Modules are particularly interesting because they are found at different levels of organisation and alternative stages of ontogeny/development, and more broadly may either constrain or facilitate evolution in certain directions (Shirai & Marroig 2010).

From the growing mammalian skull two major, morphologically integrated structures are formed- the face and neurocranium, although they are not completely independent due to the shared link with the cranial base (Cheverud 1982, Cheverud 1995). The neural parts of the skull reach full growth earlier than those of the face, the latter which will continue to grow after the brain has stopped growing (Cheverud 1996b). Brain growth is especially important in skull development; the cranial base forms by endochondral ossification and both support and protects the brain, and the cranial vault forms through intramembranous ossification to protect and cover the brain (Cheverud 1995). Whilst cranial vault morphology is largely dependent on brain growth, the cranial base is influenced by both brain growth and somatic growth factors (Cheverud 1995). In contrast to the regions connected by neural growth, the face is derived from a somatic pattern of growth (Cheverud 1995).

Cheverud (1982) proposed functional craniodental modules in primates based on the neurocranium and orofacial (mouth and face) , with the neurocranium further subdivided into the frontal, parietal and occipital, and the orofacial into masticatory, nasal, orbital and oral parts (Cheverud 1982). When correlations of traits from hypothesised functional and developmental units were compared to average correlations in traits taken from different functional sets, the functional and developmental units had much higher correlations and levels of integration. Cheverud (1995) examined morphological integration in the platyrrhine skull studying *Saguinus fuscicollis*, but functional-developmental modules were separated into the oral, nasal, orbit, zygomatic, cranial vault and cranial base. Morphological integration results from Cheverud (1995) found traits in hypothesised functional-developmental units had higher correlations than those from unrelated units with integration particularly high for oral and cranial vault traits, but low for orbital traits. Cheverud (1996a) examined morphological integration across two *Saguinus* taxa using the same modules as Cheverud (1995), supporting their results of high integration in the cranial vault and oral regions. These six craniodental regions were also used by Ackermann & Cheverud (2000), Marroig & Cheverud (2001), Gonzalez-Jose et al. (2004), Porto et al. (2009), Marroig et al. (2009) and Shirai & Marroig (2010).

Marroig & Cheverud (2001) examined modularity and integration across all 16 genera of the platyrrhines. Correlation of traits within hypothesised functional and developmental modules were 44% higher than correlations between traits that fell outside modules. All genera, except *Callimico*, *Saguinus* and *Aotus*, had high correlations between traits in the face whilst *Callimco*, *Saguinus* and *Aotus* had high correlations between traits from the neural region,

with only the pygmy marmoset and *Callicebus* exhibiting high correlations in both regions. Hallgrimsson et al. (2004) and Goswami (2006a) offered further important contributions to understanding modularity in the primate skull. Hallgrimsson et al. (2004) tested presence of modules by correlation with phenotypic, asymmetry and genetic matrices, using hypothesised modules based on the dermatocranium and chondrocranium, the face, basicranium and neurocranium, and the face, basicranium, neurocranium, palate, temporal, orbit and zygomatic. The results supported the presence of modules originally proposed by Cheverud (1982, 1995).

Goswami (2006a) examined patterns of integration and modularity across the mammals, but is of particular interest due to the sampling of platyrrhine genera (3 atelid, 4 pitheciid and 7 cebid taxa). Goswami (2006a) supported craniodental modules in platyrrhines, via clustering of data and presence of significant correlations, for the anterior oral nasal region, except in *Callimico* and the pygmy marmoset, in half the taxa for the molar region, in the pygmy marmoset for the zygomatic pterygoid region, in all taxa except *Aotus* and *Ateles* for the basicranium, and the cranial vault for the pygmy marmoset and *Alouatta*. The results for the anterior oral nasal region broadly support those of Marroig & Cheverud (2001) and Marroig et al. (2004b), although the presence of statistically significant correlation in the basicranium contrasts. In terms of general patterns, the primate cranial vault had lower integration than in carnivores, that Cheverud (1996a) and Ackermann & Cheverud (2000) have previously linked to brain size increase in the primate radiation.

1.16 Modularity & primate phylogenetics

Several phylogenetic studies have taken an experimental approach to modularity and integrated the two into a single methodological approach, whereas others have concentrated specifically on the basicranium as a source of phylogenetic information. Cardini & Elton (2008) examined the phylogenetic signal in the skull of a clade of Old World monkeys, the guenons, and combined the use of hypothetical functional and developmental modules with phylogenetic analysis of modularised regions from geometric morphometric data in guenons. They split hypothesised modules according to structure (cranium and mandible), ossification (chondrocranium of the cranial base and dermatocranium of cranial vault and face), regions linked to mechanical loading (face, cranial vault, mandible and subdivision within each), and a combined dataset for all landmarks. Correlations measured the relationship between molecular genetic and morphological distances from each module, and the strongest

phylogenetic signal was measured from the chondrocranium (cranial base). The hypothesised modules exhibited quite large variation in the strength of phylogenetic signal, overall skull shape for example had a particularly low phylogenetic signal, and the results justified the experimental approach to modularity and phylogenetics.

In another phylogenetic analysis that integrated modularity and phylogenetic inference, Gonzalez-Jose et al. (2008) investigated fossil human evolutionary relationships based on a partial modular approach. They divided the craniodental region into four distinct separate modules describing basicranial flexion, facial retraction, neurocranial globularity and masticatory apparatus. Adams et al. (2011) tested the justification for separating these regions by creating random modules of 13 landmarks, as a randomly derived module should show lower covariation and infer reduced monophyly of accepted phylogenetic clades than genuine craniodental modules. From the random modules, Procrustes distances were generated and a UPGMA phenogram inferred. The process was repeated 10000 times and found a monophylyletic *Homo* clade 82.91% times. In a second analysis, four random modules were created, principal component analysis of each carried out, and the principal component scores used to generate Euclidean distances from which a UPGMA tree was created. This process was also repeated 10000 times, finding a clade of *Homo* 99.64% of the time. Adams et al. (2011) interpreted the success of random modules in finding a clade of *Homo* as a rejection of the modular approach taken by Gonzalez-Jose et al. (2008). An alternative interpretation is that judging the utility of phylogenetic methods by their ability to reproduce monophyly in one single clade is problematic. A phylogenetic signal ought to be measured across multiple clades, and general skull shape, as measured by a random combination of craniodental landmarks, could itself contain a strong phylogenetic signal, or at least a phylogenetic signal reflective of the single major clade.

1.17 The basicranium as the source of a phylogenetic signal

Olson (1981) proposed that the basicranium was the most strongly conserved, genetically determined area of the skull and was therefore likely to hold key phylogenetic information (Harvati & Weaver 2006a). This position has been strongly supported, as the basicranium has such clear importance for an array of functions (Lieberman et al. 1996a, Lieberman 1997, Strait et al. 1997, Lockwood et al. 2004, Harvati & Weaver 2006a, Harvati & Weaver 2006b). Although there is interaction and integration based on development and function between the different craniodental regions, the cranial base differs significantly from other

regions, due to its pattern of ossification, earlier stage of reaching adult size, and functional importance as the central integrator of the skull (Lieberman et al. 2000a, Hallgrimsson et al. 2007). If the basicranium has a greater effect on facial or cranial vault form than vice versa, it is assumed the cranial base is more stable and is under greater genetic control, making it more phylogenetically informative (Lieberman et al. 2000b).

Empirical testing has supported the theoretical support for the cranial base as a source of phylogenetic information. Lockwood et al. (2004) quantified temporal bone morphology in great apes and humans and measured a strong phylogenetic signal, linked to the numerous functional roles of the cranial base relating to brain size, cognition, posture, mastication and hearing. Due to the numerous functional roles, a single behavioural shift is unlikely to create a sudden shift in morphology or extensive homoplasy (Lockwood et al. 2004). As discussed earlier, studies of modern humans found strong relationships between temporal bone shape and molecular distances (e.g. Harvati & Weaver 2006a, Harvati & Weaver 2006b, Smith et al. 2007). Cardini & Elton (2008) found chondrocranium shape correlated most highly with the molecular genetic distances in guenons, providing evidence from a non-human primate that the cranial base maintains a strong phylogenetic signal. However, Gilbert (2011) conducted phylogenetic analysis of the papionin basicranium based on 3D geometric morphometric data and found no phylogenetic signal. Instead, the region was strongly affected by allometry and associated homoplasy.

1.18 Phylogenetic signal of the face and cranial vault

Compared to the basicranium, the facial skeleton is considered at the mercy of dietary, stress and mechanical factors that mould its morphology, and more developmentally plastic than the cranial base (Smith et al. 2007, Wood & Lieberman 2001, Harvati & Weaver 2006a). Regions of the skull such as the face, with high strain and connection of muscles and tendons, will be vulnerable to homoplasy due to exertion of large functional pressures (Lieberman 1995). Morphological plasticity to non-genetic factors have also been linked to foraging and the need to adapt to the environment (Siebert & Swindler 2002, Martinez-Abadias et al. 2009) which would weaken a phylogenetic signal. Other factors also effect aspects of facial morphology, climate for example is linked to nasal morphology (Harvati & Weaver 2006a,b), whilst dietary shifts and mastication have been shown to affect morphology of the palate and zygomatic arches (Paschetta et al. 2010).

Hallgrimsson et al. (2007) found that the basicranium and neurocranium often act as an integrated complex, whereas facial shape had low correlation with either region. Heavy chewing and related feeding adaptations were linked to major homoplasy in the hominin face, and were hypothesised to have made a disproportionate contribution to inference of hominin phylogenetic relationships (Skelton & McHenry 1992, Skelton et al. 1986). McCollum (1999) suggested that 20 of the derived traits linking robust australopiths in Strait et al. (1997) actually related to three masticatory traits, heavily effecting the overall form of the face and potentially skewing phylogenetic analysis. However, Strait et al. (1997) found little difference between hominin phylogenetic trees inferred with and without masticatory-related traits. Strait (2001) showed that proposed functional correlation between characters needs to be tested, as characters in the cranial base assumed to be correlated were relatively independent, and the same could be true for characters hypothesised as functionally correlated relating to mastication.

Harvati & Weaver (2006a) found the cranial vault had a stronger relationship with genetic distances in modern humans than the temporal region, and proposed that basicranial morphology reflected older population history and slower change and the cranial vault recent population history and faster morphological change. In contrast, the face had a non-significant relationship with genetic distances and was shaped by climate and population history. These results were supported by Harvati & Weaver (2006b), that found temporal bone, neurocranial and overall cranial shape correlated with genetic distances but facial shape did not. Temporal bone morphology shared a greater correlation with genetic distances than the neurocranial morphology, the reverse finding from Harvati & Weaver (2006a). The combined theoretical and practical work described identified the basicranium as most likely to hold a reliable phylogenetic signal. However, the Harvati & Weaver (2006 a,b) results are interesting in the context that different regions may differentially retain phylogenetic information at different levels.

1.19 Body size, scaling and allometry

Allometry is study of size and its consequences on shape or any characteristics linked to physiology, ecology, behaviour and/or adaptations, in particular the relationship between variation in size and variation in non-size traits of interest (Gould 1966, Cheverud 1982, Klingenberg 1998, Dial et al. 2008, Fleagle 1984). Allometry involves a curvilinear relationship between size and shape, in contrast to the linear relationship of isometry, and can

be subdivided into three types; ontogenetic, the relationship between development/ontogeny and size and shape change, intraspecific, size and shape differences between adults of a single taxa, and interspecific, differences between taxa in size and shape (Martin 1990, Fleagle 1984). Within primates, body size varies from around 50g in the mouse lemur to over 100kg in gorillas, and in response to scaling primates have evolved different physical proportions, life history strategies, and physiological adaptations in metabolism, brain size and digestion (Fleagle 1999, Martin 1990, Fleagle 1984). Brain size for example has a negative allometric relationship with body size, so larger primates have smaller brains as a ratio between body and brain size, requiring a wider comparative allometric analysis to study shifts in encephalization (Martin 1990).

There is a broad relationship between size and diet- smaller primates are insectivorous, larger primates folivorous, with either dietary resource providing energy and protein, whereas frugivory provides the desired calorie intake but not protein, requiring additional folivory or insectivory (Fleagle 1984). Insects are an ideal dietary resource as they are high in calories and nutrients, whereas leaves are lower in calories and require extensive digestion and hindgut adaptations (Fleagle 1999). Predation of single insects provides the required calorie intake for small, but not large, primates, whereas folivory allows exploitation of an abundant resource in larger animals that have a reduced basal energetic requirement and large guts, that increase in proportion with body size, whereas folivory would need meet the energy requirements of small primates (Fleagle 1999, Martin 1990).

Size is also important for locomotion, as terrestrial primates are larger than arboreal groups, with size-based diversification between arboreal groups in locomotory systems, with leaping common in smaller primates and suspensory locomotion in larger groups (Fleagle 1984, Fleagle 1999). Life history, such as life span or gestation period, also shares a strong relationship with body size, as does ecology, with smaller primates more susceptible to predation, and larger primates requiring larger home ranges and living in larger social groups (Fleagle 1999, Martin 1990). In the context of morphology and phylogenetics, allometry is of interest because shape similarities may reflect shared functional reactions to size, or shared evolutionary responses, specifically adaptation and selection, or convergence in life history and ecological variables for taxa of the same size (Dial et al. 2008). Allometry would therefore promote similarity between groups that is not a result of common ancestry i.e. homoplasy. However, if body size is influenced by genetics and reflected in phylogeny, then closely related taxa will share similar body-size inherited from a common ancestor, and

allometric variation would help to maintain a phylogenetic signal in morphometric data. Whilst geometric morphometric methods, used in this and many other morphology-based phylogenetic analyses, involve a process of superimposition that removes variation due to scale and the linear relationship between shape and scale, the non-linear curved relationship between shape and allometry will not be removed or controlled for (Hallgrimsson et al. 2008, Brown et al. 2000). Allometry is predicted to be one of many important variables that contribute to primate morphology and accurate phylogenetic analysis.

1.20 Research Aim and Hypotheses

The primary research goal of this project is to investigate the presence, or absence, of a phylogenetic signal in the skull of New World monkeys (platyrrhines). Phylogenetic analyses were repeated for the platyrrhine crown group, and each of three major molecular clades of atelids, pitheciids and cebids. The methodsemployed combines 3-dimensional geometric morphometric methods with distance-based phylogenetic inference. Phylogenetic analysis takes a modular approach to the primate skull, repeating phylogenetic analysis for morphometric data from the whole skull, and the semi-autonomous modules of the face and cranial base that are recognised from extensive testing of modularity in primate and mammalian groups (e.g. Cheverud 1982, Cheverud 1995, Hallgrimsson et al. 2004, Goswami 2006a). The presence of a strong phylogenetic signal in the skull of extant platyrrhines would support application of the same morphometric and phylogenetic methods to the platyrrhine, and wider primate, fossil record to reliably infer the phylogenetic position of fossil taxa alongside living groups.

In this chapter I have outlined phylogenetic theory, geometric morphometric methods, the various methodological approaches to phylogenetic analysis of morphology, modularity and support for alternative phylogenetic signals in different regions of the primate skull. In chapter 2 I provide an introduction to the platyrrhines, incorporating their taxonomy, phylogenetic relationships and evolution. In chapter 3 I describe the materials sampled and methods used for morphometric and phylogenetic analysis, with a more detailed examination of geometric morphometric and distance-based methods. In chapter 4 phylogenetic analysis of the entire platyrrhines clade are presented. In chapters 5, 6 and 7 phylogenetic analysis of the atelid, pitheciid and cebid clades respectively are described. Within each atelid, pitheciid and cebid results chapter, further information is provided on the evolution of the clade, in

addition to detail on the morphology, ecology and behaviour of each genus. Chapter 8 provides a discussion and overview of the results presented in this thesis and their implication for our understanding of platyrrhine and primate evolution, modularity, the presence of alternative phylogenetic signals in the skull, the combination of geometric morphometric methods with distance-based phylogenetic analysis, and future areas of research.

Chapter 2 Platyrrhine phylogenetics and evolution

The New World monkeys, of the parvorder Platyrrhini, are a monophyletic, diverse and speciose group encompassing all known primates native to central and south America, that split from the common ancestor of Old World catarrhines during the Eocene epoch, with the first platyrrhine fossil *Branisella*, from the Bolivian Salla beds, dating back to 26 million years ago (mya) (Fleagle & Kay 1997, Kay et al. 2008). South America was an isolated continent between 80 to 3.5 mya, when South and North America were connected via the Isthmus of Panama, and the ancient Andes uplift created the Amazon basin to the north, the coastal forests to the east and a colder, harsher environment in the south (Flynn & Wyss 1998, Rosenberger et al. 2009). Whilst there has been debate whether platyrrhines arrived in South America via Africa, North America, or even Antarctica, the paleontological evidence strongly supports an African origin (Gingerich 1980, Ciochon & Chiarelli 1980, Wood 1993, Houle 1999, Fleagle & Kay 1997, de Oliveira et al. 2009). How platyrrhines dispersed 2600km across the Atlantic ocean is debated, with the major hypotheses involving floating island(s), island hopping or land bridges, although the latter is generally rejected (de Oliveira et al. 2009).

The current geographic range of platyrrhines spans from Southern Mexico to Northern Argentina with populations in Brazil, Colombia, Venezuela, Peru, Bolivia, Ecuador, Paraguay, Panama, Costa Rica, Nicaragua, Honduras, Guatemala, Guyana, French Guiana, and Suriname (Szalay & Delson 1979, Fleagle & Kay 1997). The platyrrhine fossil record extends distribution into the southern Argentine provinces for the Miocene taxa *Proteropithecia*, *Dolichocebus*, *Tremacebus*, *Carlocebus* and *Homunculus*, and the more recent quaternary Antillean island taxa on Cuba (*Paralouatta*), Jamaica (*Xenothrix*) and the Dominican Republic (*Antillothrix*) that reached the islands via pre-isthmian land routes (Fleagle & Tejedor 2002, MacPhee & Horovitz 2002, Rosenberger et al. 2009). Extant platyrrhines inhabit a variety of wooded habitats but mostly semi-deciduous coastal forest, shrubland, grasslands and tropical savannahs in the Amazonian and Atlantic forests, in all strata, and a range of latitudes (Kinzey 1997, Szalay & Delson 1979, Rosenberger et al. 2009). Platyrrhine taxa of the same genera rarely overlap geographically, are exclusively arboreal and, with the exception of the owl monkey, diurnal (Szalay & Delson 1979, Rosenberger 1977, Sussman 2005). They exhibit extensive diversity in group size, social behaviour, mating systems, locomotion and diet, with dietary preferences including folivory, frugivory, omnivory, insectivory, mycophagy and exudativory (Kinzey 1997). Platyrrhines

range two orders of magnitude in body size from around 100g in the pygmy marmoset to 12kg in muriquis, with large increases in body size in atelids, capuchins and saki-uakaris, and large body size decreases in the callitrichines, with further dwarfing in the pygmy marmoset (Martin 1990, Ford & Davis 1992, Rosenberger 1992, Garber et al. 1996).

2.1 Platyrrhine taxonomy

A relatively conservative taxonomic classification of the platyrrhines from Kinzey (1997), followed here, recognises the following genera (with common names in brackets): *Alouatta* (howler monkeys), *Aotus* (owl/night monkeys), *Ateles* (spider monkeys), *Brachyteles* (muriquis), *Cacajao* (uakaris), *Callicebus* (titi monkeys), *Callimico* (Goeldi's marmosets), *Callithrix* (marmosets), *Cebus* (capuchins), *Chiropotes* (bearded sakis), *Lagothrix* (woolly monkeys), *Leontopithecus* (lion tamarins), *Pithecia* (saki monkeys), *Saguinus* (tamarins) and *Saimiri* (squirrel monkeys). See Figure 1 for a picture of each platyrrhine genus and Table 2 for a list of all extant platyrrhine genera, species, geographical distributions, family taxonomy, diet and mating system. Groves (2001) viewed *Lagothrix flavicauda* as a separate genus *Oreonax*, but a recent investigation by Matthews & Rosenberger (2008) claimed the elevation of this species to a genus was an artefact of the cladistic parsimony method employed by Groves (2001). Nonetheless, due to the scarcity of *Lagothrix flavicauda* specimens the taxa was not sampled in this project. Historically the pygmy marmoset was recognised as belonging to its own genus (Hershkovitz 1977), *Cebuella*, but molecular phylogenetic analyses (e.g. Chaves et al. 1999, Moreira & Seuanez 1999) placed it within the *Callithrix* genus and it is recognised here as *Callithrix pygmaea*. Rylands et al. (2000) and Rylands et al. (2009) maintained this distinction between *Callithrix* and *Cebuella* and dealt with paraphyly, as the Amazonian marmosets (e.g. *Callithrix argentata* and *Callithrix humeralifera*) were more closely related to the pygmy marmoset than to Atlantic marmosets (e.g. *Callithrix jacchus* and *Callithrix penicillata*), by placing Amazonian marmosets into a separate genus *Mico*. van Roosmalen & van Roosmalen (2003) proposed that *Callithrix humilis* should be placed in its own genus *Callibella*, as it was basal to a *Mico-Cebuella* clade. To resolve the issue of *Callithrix* being a single genus, or multiple genera, further work is required on dating the divergence of these lineages incorporating multiple genetic markers: until marmoset taxonomy is resolved a single, diverse *Callithrix* genus is recognised (see section '*Callithrix*- One Genus or Four?' in Chapter 7 for further discussion).

Figure 1: New World monkey genera. Top row from left to right *Alouatta, Ateles, Brachyteles* and *Lagothrix,* second row from left to right *Callicebus, Pithecia, Cacajao* and *Chiropotes, ,* third row from left to right *Leontopithecus, Saguinus, Callithrix* and *Callimico,* fourth row from left to right *Aotus, Cebus* and *Saimiri*

Table 2 List of platyrrhine genera and species, family taxonomic designation, common name, geographic distribution, average size, diet and mating system.

Genus	Species	Family	Common name	Geographic distribution	Average size (kg)	Diet	Mating system
Alouatta	*A. belzebul, A. caraya, A. coibensis, A. fusca, A. palliata, A. pigra, A. seniculus*	Atelidae	Howler monkeys	Argentina, Belize, Bolivia, Brazil, Colombia, Costa Rica, Ecuador, French Guiana, Guatemala, Guyana, Honduras, Mexico, Panama, Peru, Suriname, Uruguay, Venezuela	6.5	Folivore-frugivore	Polygynandry-polygyny
Ateles	*A. belzebuth, A. fusciceps, A. geoffroyi, A. paniscus*	Atelidae	Spider monkeys	Belize, Bolivia, Brazil, Colombia, Costa Rica, Ecuador, El Salvador, French Guiana, Guatemala, Guyana, Honduras, Mexico, Nicaragua, Panama, Peru, Suriname, Venezuela	8.3	Frugivore	Polygynandry-polygyny
Brachyteles	*B. arachnoides*	Atelidae	Muriquis	Brazil	10.8	Folivore-frugivore	Polyandry-polygyny
Lagothrix	*L. cana, L. flavicauda, L. lagothrica, L. lugens, L. poeppigii,*	Atelidae	Woolly monkeys	Bolivia, Brazil, Colombia, Ecuador, Peru, Venezuela	7.0	Frugivore	Polygyny

Aotus	*A. azarae, A. infulatus, A. micronax, A. nancymai, A. nigriceps*	Cebidae	Owl/night monkeys	Argentina, Bolivia, Brazil, Colombia, Ecuador, Panama, Paraguay, Peru, Venezuela	0.9	Frugivore-folivore	Monogamy
Callimico	*C. goeldii*	Cebidae	Goeldi's marmosets	Bolivia, Brazil, Colombia, Peru	0.5	Insectivore-frugivore-fungivore	Monogamy-polyandry-polygyny
Callithrix	*C. aurita, C. argentata, C. emiliae, C. flaviceps, C. geoffroyi, C. humeralifer, C. humilis, C. jacchus, C. kuhli, C. penicillata, C. pygmaea*	Cebidae	Marmosets	Bolivia, Brazil, Colombia, Ecuador, Peru	0.3	Exudativore-insectivore	Monogamy-polyandry-polygynandry-polygyny
Cebus	*C. albifrons, C. apella, C. capucinus, C. libidinosus, C. nigrivittatus*	Cebidae	Capuchins	Argentina, Bolivia, Brazil, Colombia, Costa Rica, Ecuador, French Guiana, Guyana, Honduras, Nicaragua, Panama, Paraguay, Peru, Suriname, Venezuela	2.7	Omnivore	Polygynandry-polygyny
Leontopithecus	*L. chrysomelas, L. chrysophygus, L. rosalia*	Cebidae	Lion tamarins	Brazil	0.6	Frugivore-insectivore	Monogamy-polyandry-polygyny
Saguinus	*S. bicolor, S. fuscicollis, S. geoffroyi, S. imperator, S. inustus, S. leucopus, S. libiatus, S. midas, S. mystax, S. nigricollis,*	Cebidae	Tamarins	Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Panama, Peru, Suriname, Venezuela	0.5	Insectivore-frugivore	Monogamy-polyandry-polygynandry-polygyny

	S. oedipus, S. tripartitus						
Saimiri	*S. boliviensis, S. oerstedii, S. sciureus, S. ustus*	Cebidae	Squirrel monkeys	Bolivia, Brazil, Colombia, Costa Rica, Ecuador, French Guiana, Guyana, Panama, Suriname, Venezuela	0.8	Insectivore-frugivore	Polygynandry-polygyny
Cacajao	*C. calvus, C. melanocephalus*	Pitheciidae	Uakaris	Brazil, Colombia, Peru, Venezuela	3.1	Seed predator	Polygyny
Callicebus	*C. brunneus, C. caligatus, C. cupreus, C.donacophilus, C .modestus, C. moloch, C .oenanthe, C .olallae, C. personatus, C .torquatus*	Pitheciidae	Titi monkeys	Bolivia, Brazil, Colombia, Ecuador, Paraguay, Peru, Venezuela	1.0	Frugivore	Monogamy
Chiropotes	*C. albinasus, C. monachus*	Pitheciidae	Bearded sakis	Brazil, French Guiana, Guyana, Suriname, Venezuela	2.8	Seed predator-frugivore	Polygyny
Pithecia	*P. aequatorialis, P. albicans, P. irrorata, P. monachus, P. pithecia*	Pitheciidae	Saki monkeys	Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Peru, Surinam, Venezuela	2.1	Seed predator	Monogamy

Genus and species listed according to Kinzey (1997), with family taxonomy based on molecular taxonomy from Schneider & Rosenberger (1996), geographical distribution based on IUCN red list, average size based on Kinzey (1997) and Ford & Davis (1992), diet based on Norconck et al. (2009), and mating systems from Kinzey et al. (1997) and Cambpell et al. (2012).

Higher classification of the platyrrhines at the family, subfamily and tribe levels have been the subject of discussion for over a century (see Rosenberger 1981 for a historical account). The modern taxonomy used in this project is the molecular taxonomy of Schneider & Rosenberger (1996) shown in Table 3 that designates family and tribe taxonomy based on molecular phylogenetic relationships. This taxonomy recognises three platyrrhine clades with family status for Atelidae (atelids), Pitheciidae (pitheciids), and Cebidae (cebids). Three subfamilies are recognised within cebids for *Aotus* (Aotinae), *Cebus-Saimiri* (Cebinae) and callitrichines (*Callithrix*, *Callimico*, *Saguinus* and *Leontopithecus*). Atelids include *Alouatta*, *Ateles*, *Lagothrix* and *Brachyteles*, with tribe distinctions between the basal taxon of *Alouatta* and the other atelids. The pitheciid family include *Callicebus*, *Pithecia*, *Chiropotes* and *Cacajao*, and a tribe distinction separates *Callicebus* and remaining pitheciids.

Table 3 Platyrrhine molecular taxonomy from Schneider & Rosenberger (1996)

Family	**Subfamily**	**Tribe**	**Genera**
Atelidae	Atelinae	Atelini	*Ateles, Brachyteles, Lagothrix*
		Alouattini	*Alouatta*
Pitheciidae	Pitheciinae	Pitheciini	*Pithecia, Chiropotes, Cacajao*
		Callicebini	*Callicebus*
Cebidae	Cebinae		*Cebus, Saimiri*
	Callitrichinae		*Callithrix, Cebuella, Leontopithecus, Saguinus, Callimico*
	Aotinae		*Aotus*

2.2 Platyrrhine morphological traits

The platyrrhines share a collection of external characters that separate them from other anthropoids including widely separated nostrils, flattened noses with laterally rather than downward-facing nostrils, reduced opposition (or complete absence) of the thumbs and absence of cheek pouches (Hershkovitz 1977). Unique platyrrhine craniodental characters include a ring shaped external auditory meatus with a thickened lip (Figure 2), a non-extended lateral and reduced medial pterygoid plate (Figure 3), absence of the lateral process

of the malleus with poorly developed muscular process (Figure 4), absent lacerum and spinosa foramina (Figure 5) and a large malar foramen (Figure 6) (Hershkovitz 1977). See Figure 7 for **anterior, lateral, posterior and inferior** views of a sample *Lagothrix* specimen with major bones and anatomical landmarks labelled.

Platyrrhines retain three premolars, a primitive trait lost in catarrhines, and except in howler monkeys have contact between the parietal and zygomatic bones, whereas catarrhines have frontal-sphenoid contact, and skull shape is generally long and narrow (Hershkovitz 1977, Fleagle 1999, see Figure 6). The divergence in howler monkeys towards frontal-sphenoid contact in the cranial vault has received little attention, but is presumably a by-product of restructuring of the skull in response to brain size reduction (Isler et al. 2008) and the enlarged hyoid bone (Kinzey 1997), and highlights the adaptive nature of craniodental form in platyrrhine evolution. In the postcranial skeleton, platyrrhines share an elongated fibular facet on the tibia, a posterior position for the fibular facet, and an epitrochlear notch found on the medial epicondyle for the distal humerus (Ford 1986).

Figure 2 Comparative external auditory meatus morphology of New World monkey *Saguinus* (left) and Old World monkey *Macaca* (right)

From Hershkovitz (1977:p162)

Figure 3 Pterygoid plate morphology of New World monkey *Saguinus* (left) and Old World monkey *Macaca* (right)

Medial (A) and lateral (B) pterygoid plate morphology of *Saguinus* (left) and *Macaca* (right) from Hershkovitz (1977:p162)

Figure 4 Malleus (inner ear bone) morphology of New World monkey *Saguinus* (left) and Old World monkey *Macaca* (right)

From Hershkovitz (1977:p182)

Figure 5 Foramina spinosum and lacerum absence in New World monkey *Saguinus* (left) and presence in humans (right) from Hershkovitz (1977:p162)

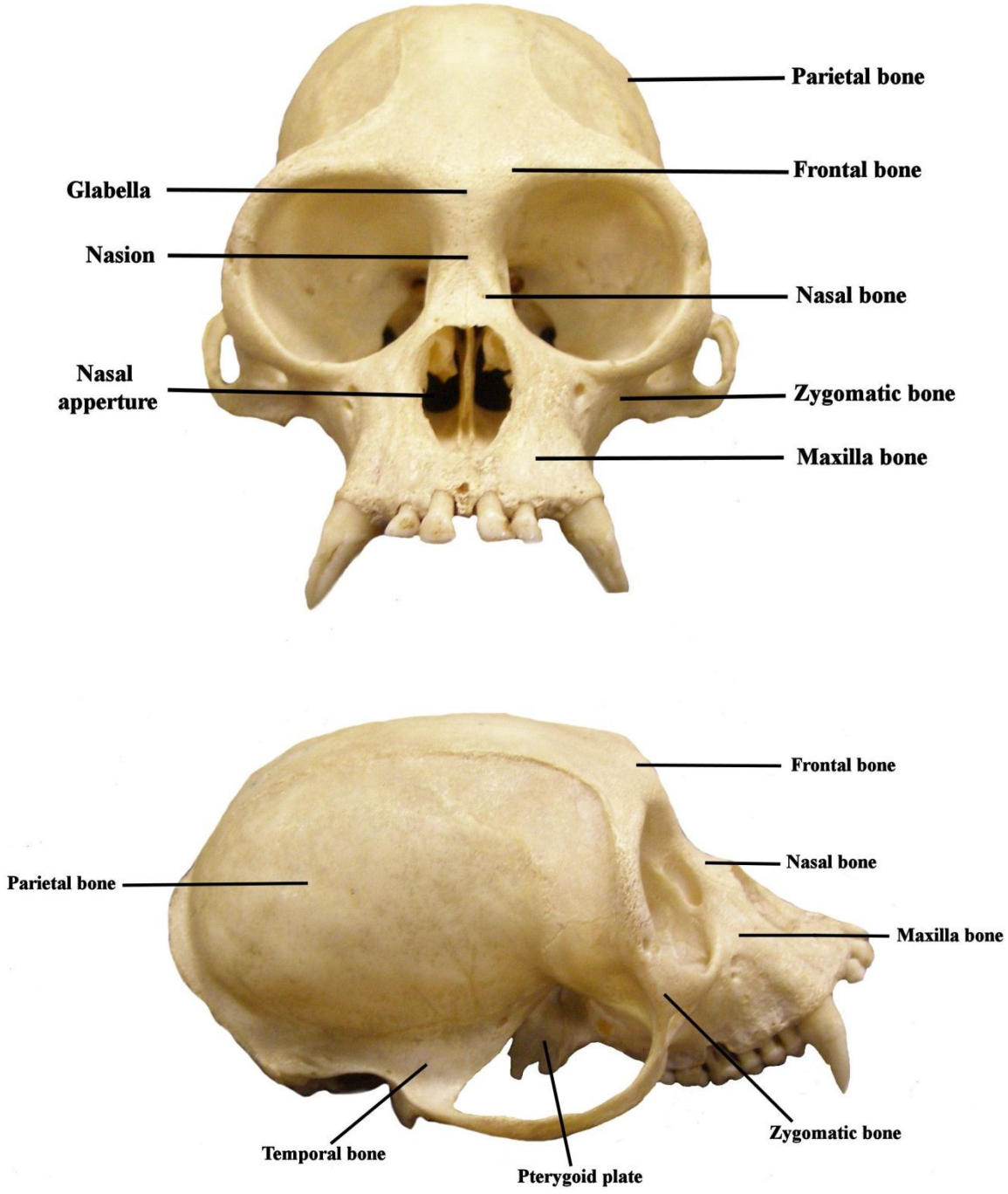
Figure 6 Malar foramen (Zf) size in New World monkey *Saguinus* (left) and Old World monkey *Macaca* (right)

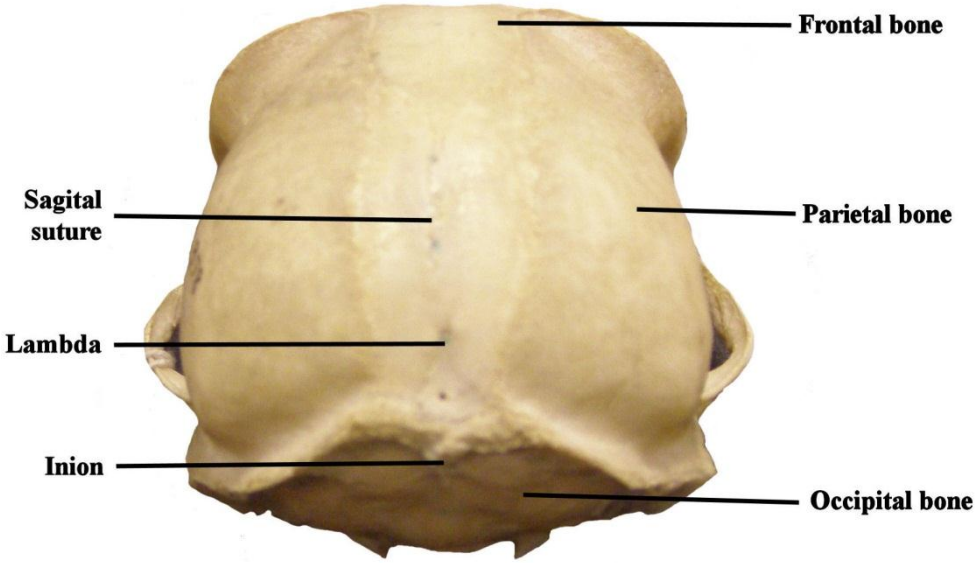
From Hershkovitz (1977:p200)

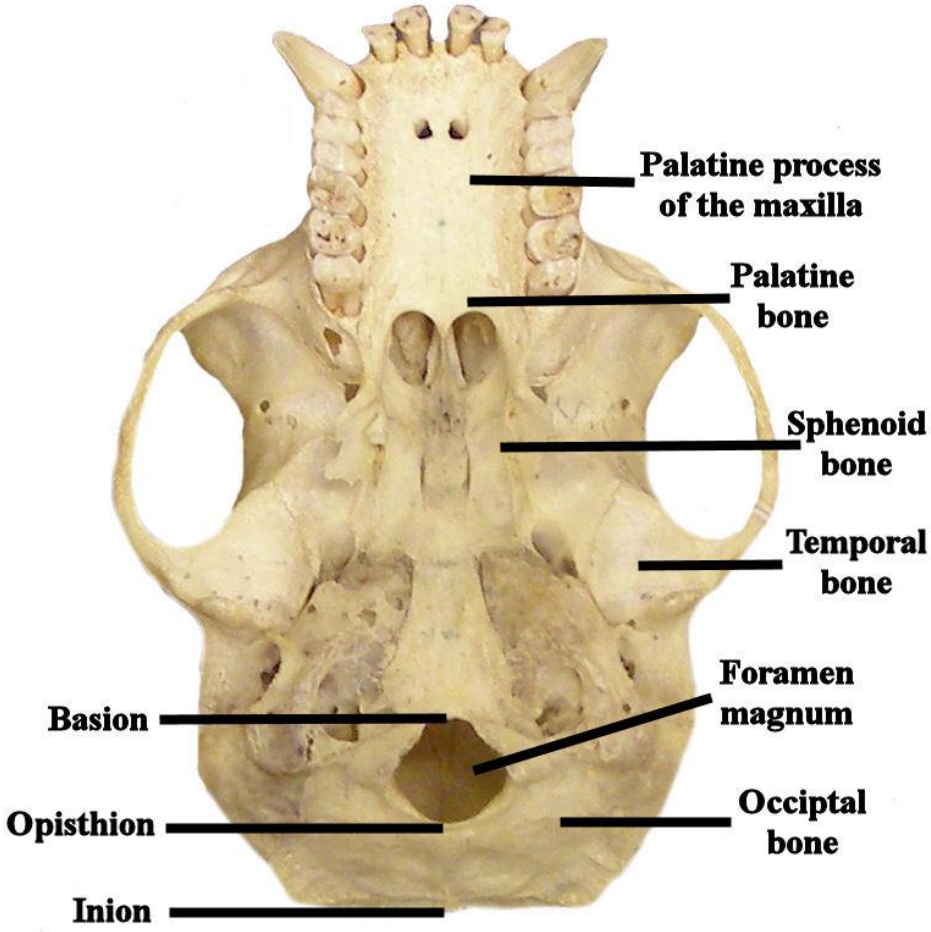
Figure 7 Positions of pterion region bones in platyrrhines (left) and catarrhines (right) from Fleagle (1999:p137)

Frontal (F), Zygomatic (Z), Sphenoid (S) and Parietal (P) bones.

Figure 8 *Lagothrix* sample specimen in anterior, lateral, posterior and inferior views with major bones and anatomical landmarks




Frontal bone
Sagital suture
Parietal bone
Lambda
Inion
Occipital bone


Palatine process of the maxilla
Palatine bone
Sphenoid bone
Temporal bone
Foramen magnum
Basion
Occiptal bone
Opisthion
Inion

2.3 Platyrrhine evolution

Primates are split between the strepsirrhines, including lemurs, lorises, bushbabies and galagos, and haplorhines including tarsiers and anthropoids, with anthropoids subdivided into the platyrrhine monkeys of central and south America and catarrhine monkeys, apes and humans of the Old World (Kay et al. 1997, Fleagle 1999). The estimated divergence time between platyrrhines and catarrhines falls within the Eocene Epoch: Steiper & Young (2006) dated the split to 42.9 mya with a confidence interval of 52.4-37.3 mya, Hodgson et al. (2009) to 43.9 million years with 95% Bayesian credibility intervals of 52.3 and 36.1 mya, Chatterjee et al. (2009) 44.8 mya (45-44.3 mya at 95% Bayesian posterior probability) or 42.8 mya (45-40.1 mya at 95% Bayesian posterior probability) depending on a strict or relaxed molecular clock, and Perelman et al. (2011) to 43.47 mya and confidence intervals between 48.4-38.6 mya. However, alternative models of fossil calibration date the divergence deeper in evolutionary time, and Wilkinson et al. (2011) produced five averages ranging between 49.6-44.1 mya with highest and lowest confidence intervals of 58.8-36.7 mya. The first known New World primate, *Branisella boliviana*, and confirmation of platyrrhine divergence dates to 26 mya from the Salla beds of Bolivia, even though there is a well-recorded mammalian fossil record dating back much further (MacFadden 1990, Fleagle & Kay 1997, Kay et al. 1998, Kay et al. 2008). Molecular estimates for the last common ancestor of the platyrrhines vary: Steiper & Young (2006) estimate 20.8 mya with 95% credibility intervals of 26.0-16.5 mya, Hodgson et al. (2009) 19.5 mya with 23.4-16.8 Bayesian 95% credibility intervals, Chatterjee et al. (2009) 24.2 (25.4-23 mya 95% Bayesian posterior probability) or 26.6 mya (30-23.5 95% Bayesian posterior probability), Perelman et al. (2011) at 24.82 mya with confidence intervals 29.25-20.55 mya, and Wilkinson et al. (2011) provided five estimates between 26.3 and 23.4 mya, with the lowest and highest confidence intervals of 32.2 and 18.7 mya.

There are two divergent views on the evolution and emergence of modern platyrrhines: the deep-time, long lineage or morphological stasis hypothesis, and layered, successive radiations hypothesis (Kay et al. 2008, Hodgson et al. 2009). The deep-time hypothesis placed all platyrrhine fossil groups into extant family groupings with a more ancient timing of coalescence for extant platyrrhines, and extant genera belonging to long lived lineages with slower rates of morphological change over time (Rosenberger 1979, Rosenberger 1980, Rosenberger 1992, Rosenberger 2002, Delson & Rosenberger 1984, Rosenberger et al. 2009).

Morphological stasis was supported by similarity shared between extant and extinct groups such as *Saimiri* and *Neosaimiri* or *Alouatta* and *Stirtonia* (Rosenberger 2010).

The layered hypothesis predicted a more recent coalescence date of around 20 million years and viewed the earliest platyrrhine fossils as stem platyrrhines sharing adaptive strategies with modern platyrrhines whilst exploiting very different niches (Kay et al. 2008, Hodgson et al. 2009). Accordingly, adaptation and morphological specialisations create homoplasy at different periods of geological time, making taxa morphologically similar when they are phylogenetically distant. In a phylogenetic analysis of extant and extinct platyrrhine taxa, Kay et al. (2008) inferred a phylogenetic tree that supported the layered hypothesis, with *Dolichocebus* and other ancient platyrrhines falling outside the crown group of extant platyrrhines. Whilst Rosenberger (2002) rejected the layered hypothesis due to the need for a higher level of homoplasy, Kay et al. (2008) rejected the deep-time hypothesis as requiring greater homoplasy, yet it seems implicit that both hypotheses require extensive homoplasy.

Molecular phylogenetic analysis by Hodgson et al. (2009) attempted to test the predictions from the two hypotheses using mitochondrial sequences from a representative sample of all major anthropoid clades. The deep-time hypothesis predicted evolutionary stasis in platyrrhines compared to catarrhines, that platyrrhine common ancestry would pre-date most of platyrrhine fossil record, and a more ancient origin for each of the major platyrrhine clades. In contrast, the layered hypothesis predicted equal rates of change between platyrrhines and catarrhines, platyrrhine common ancestry more recent than *Branisella*, and more recent evolution of the major platyrrhine clades. The branch lengths extracted from genetic data showed the branch leading to platyrrhines is 64% longer than that leading to catarrhines, so platyrrhines either evolved more recently or had a faster rate of molecular evolution than catarrhines. However, within catarrhines the evolutionary rate was slower in hominoids lowering the catarrhine average, so that Old and New World monkeys shared similar rates of evolution. The branch lengths for each platyrrhine family were also very short, strongly indicating they have recently diversified very rapidly, supporting the layered hypothesis. Estimated divergence dates proposed the most recent common ancestor of all platyrrhines was dated to 19.5 mya with a 95% CI of 16.8-23.4mya. This date does not reject the deep-time hypothesis, as the confidence interval either side could support either hypothesis.

The dates placed on the emergence of the platyrrhine clades by Hodgson et al. (2009) appear to reject the deep-time hypothesis, as the fossil taxa predated the emergence of the living groups they were associated with. The cebid clade was dated to 16 mya (CI 14.1-19.3), with *Cebus* and *Saimiri* splitting at around 14.3 mya (CI 12.6-17.5 mya) and *Aotus* and *Saguinus* splitting at practically the identical time. *Dolichocebus* and *Tremacebus*, linked in the deep-time hypothesis to extant cebids, existed before the splits took place and must be stem platyrrhines- they cannot be more closely related to any living cebids. Such evidence, however, relies upon the accuracy of dating divergence/splitting events and the consistency of the molecular clock. Wilkinson et al. (2011) have found much deeper divergence times for major primate clades including the platyrrhines, so the divergence times should be considered estimates rather than definitive.

Rosenberger (2010) challenged elements of Hodgson et al. (2009) and Kay et al. (2008), revisiting the major themes of the original deep-time hypothesis: that *Aotus, Saimiri, Cebus* and *Alouatta* had evolved during or potentially before 11-20 mya, that the distinction between *Stirtonia* & *Alouatta* and *Neosaimiri* & *Saimiri* were controversial, that within those two pairs of taxa there was evidence for morphological stasis, and that platyrrhines diverged earlier than catarrhines. Kay & Fleagle (2010) re-examined features of *Dolichocebus* and *Saimiri* that Rosenberger (1979) had previously claimed were shared derived features. Such an academic pursuit highlights a problem with morphological, character-state cladistic analysis: two groups of highly trained morphologists, with extensive experience of describing fossil and living platyrrhines, can reach very different conclusions upon analysing specific traits and specimens. It seems there is enough ambiguity associated with changes in the rates of change and dating divergence times, with very different dates from Wilkinson et al. (2011) and Hodgson et al. (2009), that neither the deep-time or successive radiations hypotheses can be outright rejected, although the evidence slightly favours successive radiations.

2.4 Molecular phylogenies of the New World monkeys

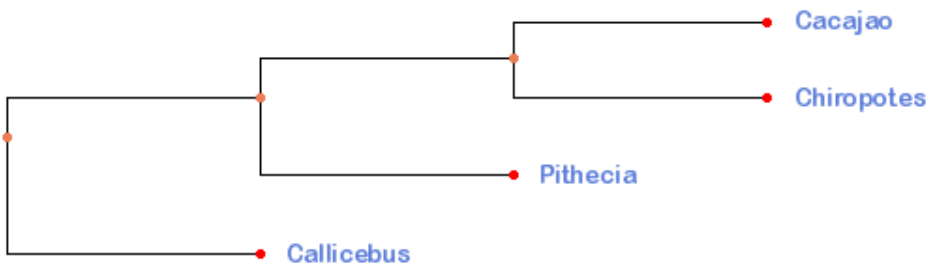
Molecular phylogenetics offers hope for resolving the true phylogeny of all living organisms, incorporating rapid, cheap sequencing of DNA and generation of huge amounts of biological data with increasingly complex mathematical models of genome evolution and statistical methods to test the accuracy and stability of phylogenetic inference (Whelan et al. 2001). In platyrrhines, original molecular analyses in the form of immunological distances by Cronin & Sarich (1975), Cronin & Sarich (1978), Sarich & Cronin (1976), Sarich & Cronin (1980),

Baba et al. (1979) and Baba et al. (1980) were quickly surpassed by phylogenetic analyses of sequenced DNA, with the current consensus of platyrrhine phylogenetic relationships shown in Figure 9. Schneider et al. (1993) carried out the first 15 genera study of platyrrhines, using the nuclear gene epsilon-globin. Their phylogeny (Figure 10) recovered three clades synonymous in nearly every DNA-based platyrrhine phylogeny: cebids, pitheciids and atelids. Which of the groups were most closely related was one of the enduring controversies in platyrrhine evolution prior to its recent resolution.

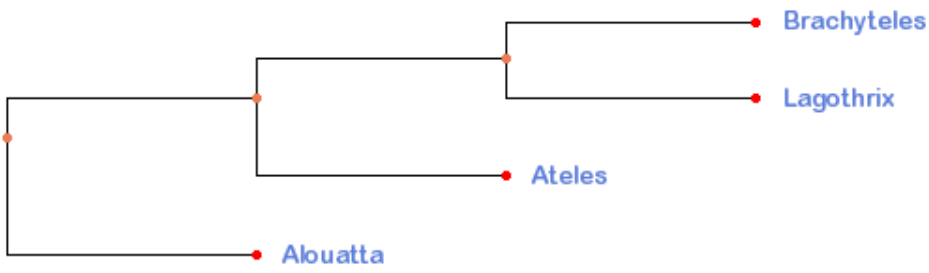
Schneider et al. (1993) placed pitheciids and atelids as the most closely related families. Within the pitheciid clade, *Cacajao* and *Chiropotes* was sister to *Pithecia*, with *Callicebus* the most basal taxon. For atelids, *Brachyteles* and *Lagothrix* formed a clade sister to *Ateles*, with *Alouatta* as the most basal taxon . Relationships within the callitrichines were disputed within earlier studies prior to reaching current consensus with *Callithrix-Callimico* sister to *Leontopithecus* and *Saguinus* the basal most lineage with support from Harada et al. 1995, Horovitz & Meyer 1995, Schneider et al. 1996, Barroso et al. 1997, Porter et al. 1997, Canavez et al. 1999b, Schneider et al. 2001, Singer et al. 2005, Schrago 2007).

Figure 9 Consensus phylogenetic relationships of platyrrhines based on molecular data (see text for references)

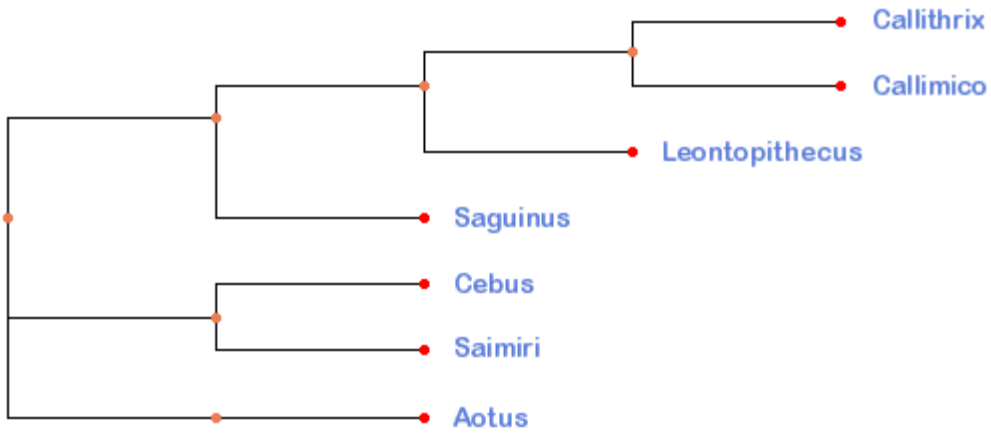
a) Phylogenetic relationships of the pitheciids



b) Phylogenetic relationships of the atelids



c) Phylogenetic relationships of the cebids



In nearly all subsequent molecular analyses, those within-clade relationships for pitheciids and atelids were repeated whilst the phylogenetic relationships within cebids have been variable. For Schneider et al. (1993) the cebids were split into five lineages for *Saguinus-Leontopithecus*, *Callimico–Callithrix*, *Saimiri*, *Cebus* and *Aotus* (Figure 10). Harada et al. (1995) added epsilon-globin sequences from more species and used a second gene, the nuclear-based IRBP. Phylogenetic analysis (Figure 11) of just epsilon-globin and joint analysis of both gene sequences supported atelids and pitheciids as sister clades, but reduced the cebid relationships to a dichotomy with *Aotus*-callitrichines sister to *Cebus-Saimiri*.

Schneider et al. (1996) carried out combined analysis of IRBP and epsilon -globin with additional species sampled for IRBP. In isolation, the phylogeny of IRBP intron 1 had atelids and cebids as sisters. Within cebids, there were two clades, one for the callitrichines and another for *Aotus* and *Cebus-Saimiri*. Combined analysis of both datasets (Figure 12) had atelids and pitheciids as sister clades, and cebids with a trichotomy between *Aotus*, *Cebus-Saimiri* and the callitrichines.

Figure 10 Molecular phylogeny of platyrrhines according to Schneider et al. (1993) and Barroso et al. (1997)

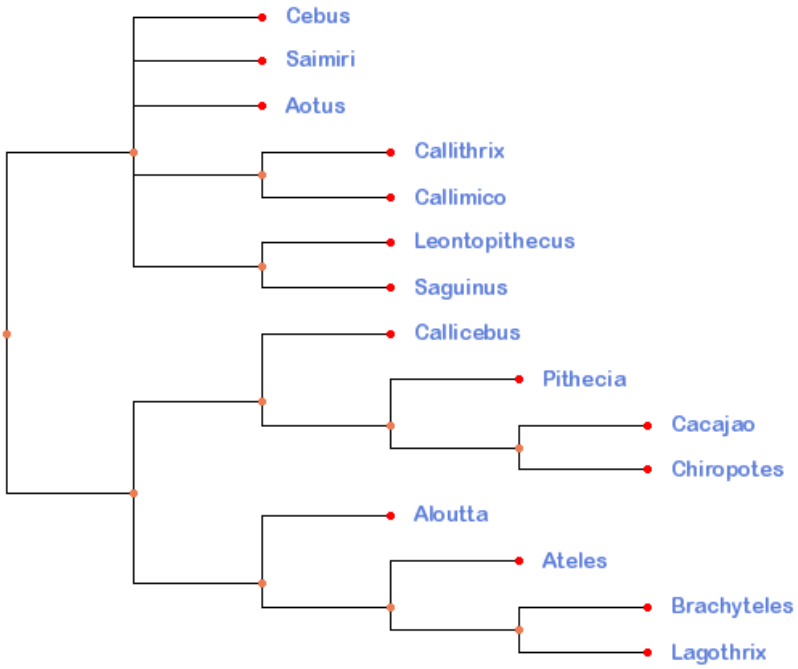


Figure 11 Molecular phylogeny of platyrrhines according to Harada et al. (1995)

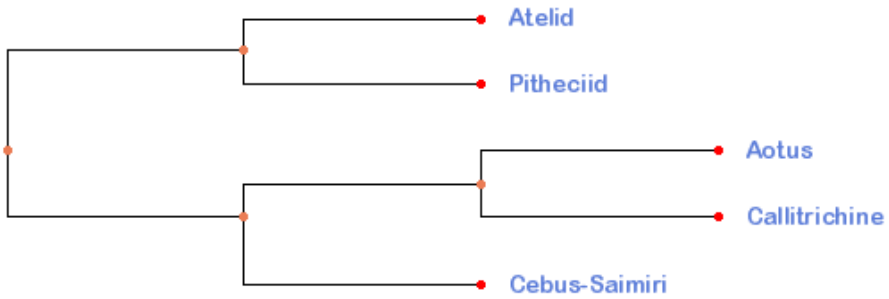
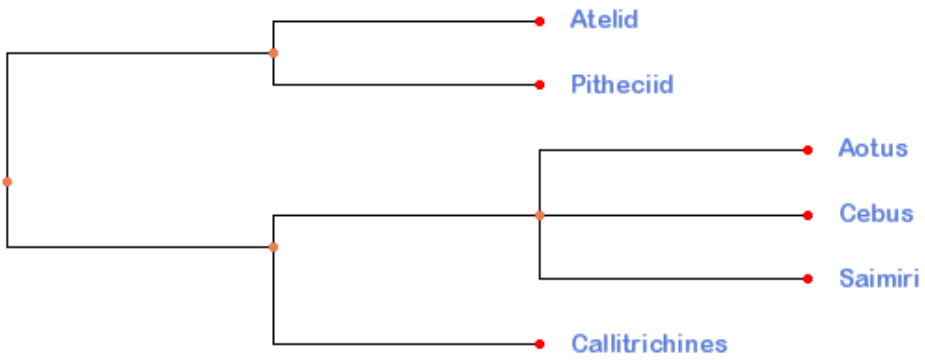


Figure 12 Molecular phylogeny of platyrrhines according to Schneider et al. (1996) and Barroso et al. (1997)



Several studies concentrated on the phylogeny inferred by analysis of just a single gene. Porter et al. (1997) and Barroso et al. (1997) examined epsilon-globin and IRBP genes respectively with added *Callithrix* species sampled. Barroso et al. (1997) proposed the same genera level relationships as the Schneider et al. (1996) analysis of the IRBP gene sequences (Figure 12), with cebids and pitheciids sister clades. Porter et al. (1997) supported the combined IRBP and epsilon-globin analysis of Schneider et al. (1996) with pitheciids and atelids sister clades, and *Saimiri-Cebus* were basal-most with *Aotus* sister to callitrichines in the cebid clade (Figure 13). With IRBP and epsilon-globin studies failing to reach consensus, von Dornum & Ruvolo (1999) examined the glucose-6-phosphate dehydrogenase (G6PD) gene. The consensus tree inferred atelids and cebids as sister clades, and a trichotomy formed between *Aotus*, *Saimiri-Cebus* and callitrichines within cebids (Figure 14).

Figure 13 Molecular phylogeny of platyrrhines according to Porter et al. (1997)

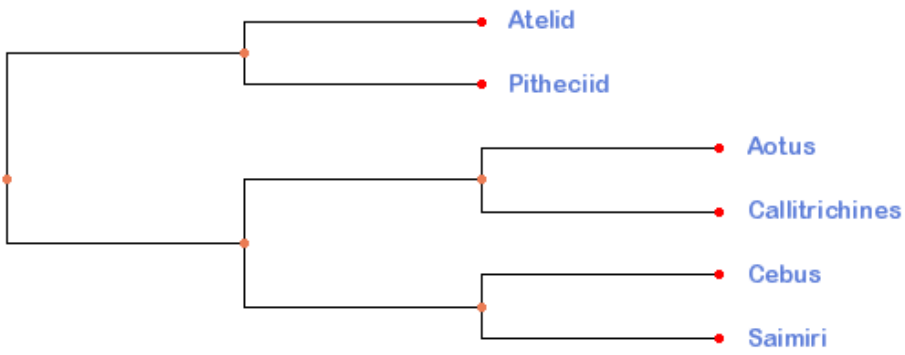
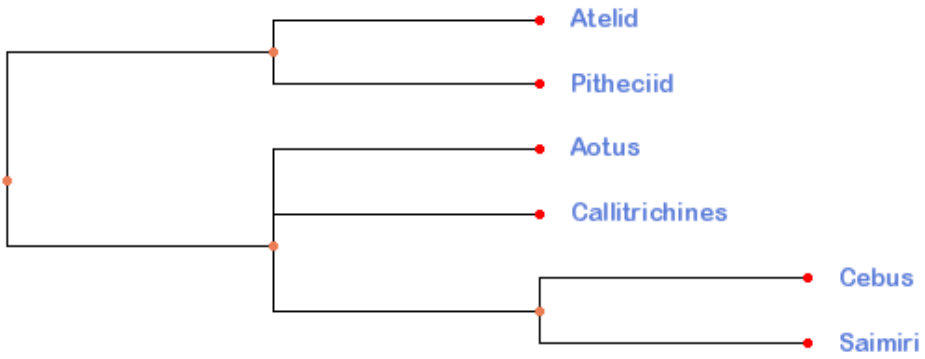


Figure 14 Molecular phylogeny of platyrrhines according to von Dornum & Ruvolo (1999)

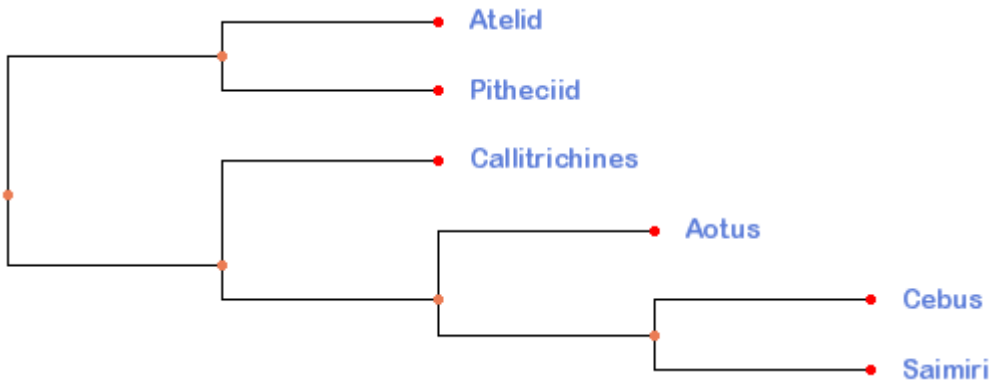


Further work on G6PD by Steiper & Ruvolo (2003) produced multiple different phylogenies depending on the phylogenetic method used. Parsimony and distance-based analyses had

cebids and atelids as sister clades, Bayesian analysis cebids and pitheciids as sisters, and maximum likelihood reproduced a trichotomy of the three. Relationships within cebids varied, parsimony inferred *Aotus* as sister to *Cebus-Saimiri* and a separate clade for callitrichines, the distance-based tree switched the position of *Aotus* to sister of callitrichines, Bayesian analysis had *Aotus* basal and a dichotomy between callitrichines and *Cebus-Saimiri*, while maximum likelihood returned a trichotomy.

Another candidate gene, beta 2-microglobulin, was investigated by Canavez et al. (1999b). Their phylogeny retained the monophyly of the three families with atelid and pitheciids sister clades. Within cebids, *Aotus* was sister to *Cebus-Saimiri* separate from the callitrichines (Figure 15). Prychitko et al. (2005) generated a phylogeny from beta-globin sequences of 10 platyrrhine genera using both maximum parsimony and maximum likelihood approaches. This phylogeny had cebid paraphyly with *Callimico* and *Callithrix* forming a clade with the atelids. This result, in addition to those of Steiper & Ruvolo (2003), highlighted the problem of conducting platyrrhine phylogenetics without sampling taxa from every platyrrhine genus.

Figure 15Molecular phylogeny of platyrrhines according to Canavez et al. (1999b) and Horovitz et al. (1998)

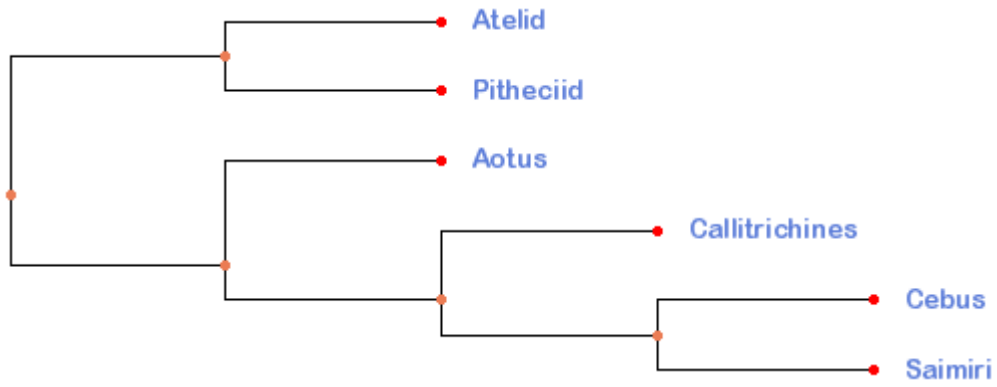


The rapidly increasing DNA sequence data publicly available led to platyrrhine phylogenetic analyses incorporating sequences from four or more genes. Schneider (2000) used both the G6PD and beta 2-microglobulin sequences with IRBP and epsilon-globin for a combined 6700 base sequence analysis. The phylogenetic relationships produced, using Neighbor-joining and maximum parsimony, had an atelid-pitheciid clade sister to cebids with a trichotomy of *Aotus*, *Cebus-Saimiri* and callitrichines (as in Figure 14), whilst the maximum parsimony method placed *Aotus* sister to *Cebus-Saimiri*, in turn sister to the callitrichines (as in Figure 15). In an apparently near identical analysis, Schneider et al. (2001) reproduced

these results, adding maximum likelihood phylogenetic analysis that produced the same phylogeny as the maximum parsimony phylogeny.

Opazo et al. (2006) used representatives of all platyrrhine genera to phylogenetically analyse sequences from epsilon-globin, IRBP, beta 2-microglobulin, G6PD, beta-globin and von Willenbrand factor (vWF). All phylogenetic methods produced the same monophyletic within-family relationships, the cebids with *Aotus* sister to *Cebus-Saimiri* separate from the callitrichines. For maximum parsimony, cebids and atelids were sister clades, whereas maximum likelihood and Bayesian phylogenies had atelids and pitheciids as sister clades. Another analysis of multiple datasets (12S rRNA, epsilon-globin, intron 1 of IRBP and chemokine co-receptor 5) by Schrago (2007) produced a phylogeny with pitheciids and atelids as sister clades, and cebids with *Aotus* basal to a dichotomy of *Cebus-Saimiri* and callitrichines (Figure 16).

Figure 16 Molecular phylogeny of platyrrhines according to Schrago (2007)



In addition to nucleus-based DNA phylogenies there have also been mitochondrial studies. Horovitz & Meyer (1995) used a fragment of the mitochondrial 16S ribosomal gene to propose relationships for 12 platyrrhine genera. Weighting techniques were investigated, producing multiple trees, many of which had low resolution, and lacked consensus on platyrrhine phylogenetic relationships. Horovitz et al. (1998) added 12S mitochondrial gene sequences except from *Cacajao*. The phylogeny had a paraphyletic cebid clade sister to atelids. Cebid paraphyly was resolved with the addition of further gene sequences and morphological data (including fossil taxa). Three monophyletic clades were produced with atelids and pitheciids sister clades, and cebids with basal *Aotus* sister to *Saimiri-Cebus* separate from callitrichines (Figure 15).

Another alternative molecular phylogenetic approach, highlighted in Xing et al. (2007), involved the use of DNA mobile elements. Singer et al. (2005) found that six of 74 *Alu* insertions were phylogenetically informative, with three shared elements supporting platyrrhine monophyly, one shared element between *Aotus-Cebus-Saimiri* and another for the callitrichines. Ray et al. (2005) took a wider sample of 174 *Alu* elements, with 124 present in at least two species, with parsimony analysis proposing a single most parsimonious tree. This phylogeny supported a sister relationship of the atelids and cebids although they only sampled nine platyrrhine genera.

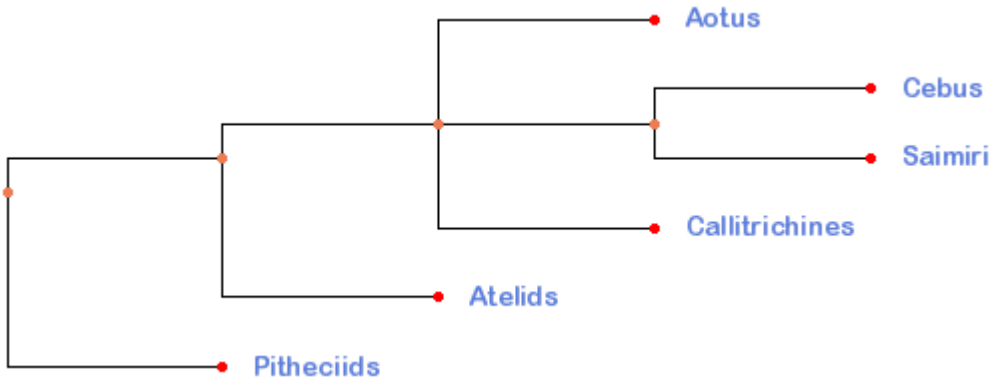
More recently, several important papers by Wildman et al. (2009), Hodgson et al. (2009) and Perelman et al. (2011) have resolved the branching pattern of the platyrrhine clades using very large datasets. Wildman et al. (2009) added two more nuclear genes (PRKCE and DICER1) to the seven used in Opazo et al. (2006) to make a 10144 base pair dataset and sequenced a second dataset of non-coding markers of 7665 base pairs that lacked repetitive or duplicated regions. In phylogenetic analyses the trees produced for parsimony, maximum likelihood and Bayesian methods produced the same topology, albeit often with different statistical support for clades. The inferred phylogeny (Figure15) had atelids and cebids as sister clades, and within cebids, the callitrichines and *Cebus-Saimiri* were sisters to the exclusion of *Aotus*. For the dataset of non-coding sequences, again, atelid and cebids formed a clade, but within cebids, *Aotus* was sister to *Cebus-Saimiri*. A third dataset, of merged coding and non-coding markers, produced a cebid-atelid clade, with *Aotus* sister to *Cebus-Saimiri*. However, the latter clade was only supported by 55% bootstrap support with parsimony, 53% bootstrap support with maximum likelihood, and 0.70 Bayesian posterior probabilities.

Hodgson et al. (2009) examined fully sequenced mitochondrial genomes to resolve the branching pattern of the three platyrrhine families, the cebid trichotomy, and estimate the time of the most recent common ancestor for platyrrhines. As there was an emphasis on timing divergence and common ancestry they only sampled single representative taxa for callitrichines (*Saguinus*), atelids (*Ateles*) and pitheciids (*Callicebus*), in addition to *Cebus, Saimiri* and *Aotus,* and representative outgroup taxa from several major primate clades. Both Bayesian and maximum likelihood phylogenetic methods inferred atelids and cebids as a clade sister to pitheciids (Figure 17). Within cebids, *Aotus* was sister to callitrichines, with *Cebus-Saimiri* basal, although these relationships are not well resolved. From the inferred phylogeny, the branch length leading to the platyrrhine common ancestor from the anthropoid

common ancestor was much longer than that to the catarrhines common ancestor. As platyrrhines and cercopithecoids have very similar rates of substitution this implied a more recent origin for the New World monkeys. Within platyrrhines, the internodes between groups were also very small implying rapid diversification.

Perelman et al. (2011) supported Wildman et al. (2009) and Hodgson et al. (2009), inferring a cebid-atelid clade with pitheciids basal-most. The phylogenetic analysis sampled 54 nuclear genes and around 35000 base pairs of sequence for each taxa, sampling 186 species and 61 genera of primates. Within cebids, phylogenetic analysis supported *Aotus* as sister to callitrichines, supporting Hodgson et al. (2009), although the very high levels of indel mutations and high nucleotide substitution rates indicates a unique evolutionary history in owl monkeys that may distort the phylogenetic position of *Aotus*. The cebid phylogenetic relationships are best treated as a trichotomy between callitrichines, cebines and owl monkeys, and may actually reflect the true relationships of the three groups. Earlier molecular phylogenetic work was constrained largely by a lack of data, and the minor differences between sequences of taxa were probably responsible for inference of alternative phylogenies according to the phylogenetic method used. It is apparent from the work of Wildman et al. (2009), Hodgson et al. (2009) and Perelman et al. (2011) that the major platyrrhine relationships are now resolved.

Figure 17 Molecular phylogeny of platyrrhines according to Wildman et al. (2009) , Hodgson et al. (2009) and Perelman et al. (2011)



2.5 The adaptive evolution of platyrrhines

Platyrrhine body size spans two orders of magnitude, a level of variation unique amongst extant primates, and shifts in body size are linked to adaptations within alternative feeding niches (Rosenberger 1992). The platyrrhines have several distinct adaptive radiations: seed

eating pitheciids, postcranial-modified atelids, small and clawed, clinging callitrichines, big brained, predator cebines (Rosenberger 2002), and the nocturnal, monogamous owl monkeys. In primates, body size has a negative relationship between dietary quality and consumption/predation of other animals, so larger bodied primates consume a poorer quality diet consisting of fewer predated animals, and size also affects locomotion, habitat, predation strategy and use of space (Ford & Davis 1992). Platyrrhines are primarily frugivores to varying degrees, with intake ranging from 16% in *Callithrix* and *Pithecia* to 86% in *Ateles*, alongside consumption of insects, vertebrates, leaves, flowers, nectar, fungi and exudates (Norconk et al. 2009). Body size averages from Ford & Davis (1992) for each genus are provided in Table 4, and average dietary proportions of each genus from Norconk et al. (2009) are listed in Table 5.

The physical properties of the major food source in a primates diet may shape craniodental morphology, as several clades are clearly adapted to their respective diets, for example seed predation in saki-uakaris (Kay 1975). Yet, platyrrhine diets can vary significantly and may be shaped by adaptations for dietary flexibility particularly in response to seasonal variation, although few taxa are dietary generalists, and morphology is probably shaped by an interplay between phylogeny, function and adaptation (Rosenberger 1992). Rosenberger (1980) proposed the adaptive radiation of platyrrhines have occurred within two distinct adaptive zones. These zones, hypothesised to be occupied at the beginning of the platyrrhine radiation, created a dichotomous split between callitrichines-*Cebus*-*Saimiri* (frugivorous-insectivorous zone) and atelid-pitheciid-*Aotus* (frugivorous-folivorous zone), with an initial selective pressure operating on acquisition of dietary protein and mastication-linked morphology. Within the zones, there was proposed further niche specialisation, accounting for variation in diet, foraging, and locomotion within clades.

Callitrichines have experienced secondary size reduction, molar reduction and twinning that are indicative of dwarfing, but have also evolved claw-like nails and vertical hanging allowing for exploitation of canopy and subcanopy and expansion into new feeding niches that have driven size change (Ford 1980, Rosenberger 1992). *Callithrix* taxa weigh around 250-300g, although the pygmy marmosets are closer to 100g, *Callimico* 500g, *Saguinus* 400-600g and *Leontopithecus* 600g (Kinzey 1997, Ford & Davis 2009, Ford & Davis 1992). The callitrichines may have endured a body size decrease up to an order of magnitude in the pygmy marmoset, a two-thirds reduction in non-pygmy marmosets, and a 50% decrease in *Leontopithecus*, *Saguinus* and *Callimico* (Ford & Davis 1992). Although there has been a

shift in callitrichine body size, the smallest cebine *Saimiri* and largest callitrichine *Leontopithecus* individuals are close in body size, and it is possible that *Leontopithecus* have experienced a body size gain (and are "mega-marmosets") following the initial size reduction (Rosenberger 1992, Garber 1992). There are several unique dietary specialisations in callitrichines, as marmosets are specialised for exudate feeding and *Callimico* consume significant amounts of mushrooms (Rosenberger 1992). *Callithrix* consume 45% exudates and 39% insects, although the pygmy marmoset have increased gummivory (60%), *Callimico* consume 41% insects, 29% fungi, and 29% fruit, *Leontopithecus* are frugivorous (53%) with increased insectivory (25%), and *Saguinus* have lower frugivory (35%) and higher insectivory (45%) (Norconk et al. 2009). Although Rosenberger (1992) considered *Aotus* as belonging in the same dietary-adaptive zone as pitheciids, sharing the opportunist adaptive strategy of *Callicebus*, they have converged into insectivory-folivory independent of *Callicebus* with a diet that has a high proportion of leaves (41%) and fruit (45%).

Within the cebines, capuchins are around 4kg, four times larger than its sister taxa *Saimiri* that are closer to 1kg, with a suite of adaptations including a prehensile tail, partially opposable thumbs for extractive foraging of insects, and large thick-enamelled molars to crunch branch-ends that may have insects on them (Rosenberger 1992, Kinzey 1997, Ford & Davis 1992). Capuchins use their larger size and strength, alongside stabilisation via their prehensile tail, to predate on larger organisms and extract insects in large amounts (Rosenberger 1992). The closely related cebines *Saimiri* and *Cebus* are noticeably distinct- *Saimiri* are small, non-herbivorous frugivores isolated to the Amazon, whereas *Cebus* are much larger omnivores with a wider geographical distribution (Rosenberger et al. 2009). *Saimiri* have high insectivory (60%), with moderate frugivory (25%) and some folivory (10%), and *Cebus* have reduced insectivory (33%), higher frugivory (47%), similar folivory (8%) and some seed consumption (8%) (Norconk et al. 2009). Large-bodied capuchins target small insects whilst smaller-bodied callitrichines prefer larger insects, although *Cebus* exploit social animals present in colonies whilst callitrichines preferred non-flying, slow prey (Rosenberger 1992). Capuchins also have a higher quality diet with larger amounts of insects and vertebrates than expected for its relatively large size (Ford & Davis 1992).

In pitheciids, the basal lineage *Callicebus* is around 1kg, likely the ancestral pitheciid body size, with the other pitheciids (the saki-uakaris) having experienced a large size increase, with *Cacajao* the largest (Ford & Davis 1992). Pitheciids are split between mixed insectivory-folivory-frugivory opportunists, *Callicebus*, and seed harvesting *Pithecia*, *Cacajao* and

Chiropotes (Rosenberger 1992). *Callicebus* have low insectivory, moderate seed (27%) and high fruit (59%) diets, *Pithecia* and *Cacajao* have high seed (61% and 67%) with low fruit (16% and 18%) diets, and *Chiropotes* have both high fruit (42%) and seed (51%) consumption (Norconk et al. 2009). The seed-eating specialization in *Cacajao* and *Chiropotes* isolates them to habitats of the Rio Amazonas, whereas *Callicebus* have a wider geographical spread due to consumption of leaves and unripe fruit (Rosenberger et al. 2009).

The largest platyrrhines, the atelids, experienced a large ancestral shift to bigger body size, with *Alouatta*, *Lagothrix* and *Ateles* around 7-8kg and *Brachyteles* having experienced secondary size increase with an average body size of around 12kg (Ford & Davis 1992). Within atelids the large body size and benefit of a prehensile tail promote the evolution of energetic, acrobatic locomotion in *Brachyteles* and *Ateles*, compared to energy conserving, slower behaviour in *Alouatta* (Rosenberger 1992). Atelids evolved within a frugivore-folivore adaptive zone; *Alouatta* and *Brachyteles* have high folivory (54% and 51%) and reduced frugivory (34% and 27%), *Ateles* have very high frugivory (86%) and low folivory (11%), and *Lagothrix* have high frugivory (64%) (Rosenberger 1992, Norconk et al. 2009). Atelids rely heavily on large trees for foraging and consuming leaves , and *Alouatta* and *Brachyteles* have spread into semi-deciduous forests in Mata Atlantica due to their leaf consumption and associated adaptions, with *Alouatta* groups especially adept in woodlands and savannah with low concentrations of trees (Rosenberger et al. 2009).

The evolution of size and diet, and associated life history and behavioural diversification , will also affect other areas of platyrrhine biology such as locomotion, within which the interaction between morphology phylogeny, function and adaptation promotes high levels of homoplasy, best exemplified by convergence in the platyrrhine postcrania (Lockwood 1999, Rosenberger 1992). It is clear from character evolution there has been extensive parallel evolution between pitheciids and atelids, both clades having evolved increased body size, with especially high levels of homoplasy within the *Ateles* and *Cacajao* taxa (Lockwood 1999). The high levels of platyrrhine postcranial homoplasy may be promoted by canopy structure, which differentially creates a selective advantage for either climbing or suspensory behaviour, and could explain the homoplasy and variation of atelids and pitheciids (Lockwood 1999). Homoplasy in the postcrania, as a response to behavioural specialisations and size evolution, are also likely reflected in high levels of homoplasy in platyrrhine craniodental morphology.

Platyrrhine sexual dimorphism, as measured by the ratio between average body size of males to females, are reproduced from Ford & Davis (1992) in Table 4, and highlight the variation within the platyrrhine clade. The dimorphism ratios were not statistically tested for significant differences between male and female body weights, so designation of monomorphism and dimorphism are based on an arbitrary distinction. There was positive sexual dimorphism, with males larger than females, in nearly all genera except *Ateles* and *Callicebus*, which were both monomorphic. *Cebus*, *Saimiri*, *Pithecia*, *Alouatta* and *Brachyteles* all had relatively high dimorphism, with *Lagothrix* the greatest. No genera had negative sexual dimorphism, although the species *Ateles paniscus* did. Measuring sexual dimorphism in genera that incorporated multiple species or subspecies from different populations was clearly problematic, as several genera show variable levels of dimorphism between species such as in *Callithrix*, *Saguinus* and *Ateles*. A problem with these measurements of dimorphism was that sample sizes were generally low, and for groups with low dimorphism there was a tendency for quite large changes in measured dimorphism as more data were added, indicating low sample sizes may not accurately represent wider populations.

Plavcan & van Schaik (1998), using much of the same data as Ford & Davis (1992), published the platyrrhine levels of body size dimorphism at the species level with multiple values for taxa signifying dimorphism in different populations of the same species (reproduced in Table 6). This highlighted the variation in dimorphism estimates, due to either a real variation within taxa or resulting from error or variation introduced by data collection. *Alouatta seniculus* in particular have a large range of sexual dimorphism estimates, ranging from only slight dimorphism at 1.08 to very large dimorphism at 1.73. Another atelid, *Ateles geoffroyi*, ranged from dimorphism of 1.125 to reverse dimorphism of 0.87, whilst *Lagothrix lagothrica* ranges from 0.73 to 1.53, and within pitheciids *Callicebus torquatus* displayed negative dimorphism. *Cebus apella* have a range of medium (1.32) to high (1.82) dimorphism, which may reflect their large geographical variation and response of populations to ecological variables, and *Saimiri* show large variation between species and populations of *Saimiri sciureus*, which ranges from 1.22 to 1.76, reflecting their complex and variable social groups (Kinzey 1997). The range of variation in sexual dimorphism for the two closely related genera of *Cebus* and *Saimiri*, considering the wide geographical variation and variation in social group and mating systems, would place these groups as good candidates for further study of the genetic and environmental basis for sexual dimorphism.

Table 4 Body weight of male, female and pooled sex platyrrhines and dimorphism ratio from Ford & Davis (1992)

Family	Genus	Male mean body weight	Female mean body weight	Pooled mean body weight	Dimorphism ratio
Atelidae	*Alouatta*	7.564	5.438	6.501	1.391
	Ateles	8.273	8.28	8.276	0.999
	Brachyteles	12.125	9.45	10.788	1.283
	Lagothrix	8.335	5.75	7.043	1.45
Cebidae	*Aotus*	0.932	0.91	0.921	1.025
	Callimico	-	-	-	-
	Callithrix	0.286	0.261	0.274	1.095
	Cebus	3.093	2.315	2.704	1.336
	Leontopithecus	0.58	0.556	0.568	1.103
	Saguinus	0.482	0.468	0.475	1.03
	Saimiri	0.911	0.703	0.807	1.296
Pitheciidae	*Cacajao*	3.45	2.81	3.13	1.228
	Callicebus	1.048	1.049	1.049	0.999
	Chiropotes	3.06	2.555	2.808	1.198
	Pithecia	2.384	1.763	2.074	1.352

Table 5 The average dietary consumption (%) of platyrrhine genera over the course of a year, presented in Norconk et al. (2009) based on multiple sources

Family	Taxa	Fruit	Leaf	Insect	Seed	Exudate	Fungi	Flowers
Atelidae	*Alouatta*	34	54					9
	Ateles	86	11					3
	Lagothrix	64	6	9	1			4
	Brachyteles	27	51		5			11
Cebidae	*Callithrix*	16		39		45		
	Callimico	29		41		1	29	
	Leontopithecus	53		25		9		7
	Saguinus	35	3	45		10		
	Saimiri	25	10	60				5
	Cebus	47	8	33	8			
	Aotus	45	41					14
Pitheciidae	*Callicebus*	59	6	4	27			4
	Pithecia	16	5	3	61			2
	Cacajao	18			67			6
	Chiropotes	42		4	51			1

Table 6 Species body weight dimorphism from Plavcan & van Schaik (1998) with multiple values given for different populations of same species

Family	Taxa	Dimorphism ratio	Family	Taxa	Dimorphism ratio
Atelidae	*Alouatta belzebul*	1.39	Cebidae	*Aotus lemurinus*	0.97
		1.35		*Aotus trivirgatus*	0.99
	Alouatta caraya	1.53		*Callithrix jacchus*	0.87
		1.32		*Callithrix pygmaea*	0.92
	Alouatta fusca	1.37			1.09
	Alouatta palliata	1.28			0.92
		1.32			1.07
		1.16			1.14
		1.30		*Cebus apella*	1.38
		1.21			1.32
		1.25			1.72
	Alouatta pigra	1.84			1.45
		1.69			1.82
	Alouatta seniculus	1.26			1.34
		1.73		*Cebus olivaceous*	1.40
		1.43			1.41
		1.31		*Leontopithecus rosalia*	1.06
		1.22	Pitheciidae	*Cacajao calvus*	1.20
		1.27		*Callicebus brunneus*	0.99
		1.08		*Callicebus moloch*	1.09
	Ateles geoffroyi	1.25			1.16
		0.93		*Callicebus personatus*	0.92
		0.98		*Callicebus torquatus*	0.84
		0.87		*Chiropotes satanas*	1.08
	Ateles paniscus	1.10			1.12
		1.16			1.40
		1.02		*Pithecia pithecia*	1.19
	Brachyteles arachnoides	1.15			1.20
		1.20			1.27
	Lagothrix lagothrica	1.20			1.24
		1.41			
		0.73			
		1.33			
		1.53			

2.6 The role of phylogeny, diet and size in platyrrhine morphological evolution

Marroig & Cheverud (2001) investigated morphological integration and the evolution of trait inter-relationships in the platyrrhine skull, based on a series of linear craniodental measurements, and the contribution of phylogeny, using molecular genetic distances, ecology as measured by dietary proportions, and development to patterns of variation at different taxonomic levels. The results showed that platyrrhines shared a correlation and covariance structure, with higher levels of similarity at the taxonomic family level, although the subfamilies of Aotinae and Callitrichinae have reduced similarity. Generally, platyrrhine taxa had high facial integration and low neural integration, or the reverse as in *Saguinus*, *Callimico* and *Aotus*, with the exception of *Callithrix pygmaea* and *Callicebus*, which had high integration in both regions. Regarding the evolution of integration in platyrrhines, there was a general shared pattern across platyrrhines, with specialisation and alternative patterns in several taxa.

Morphological distances correlated significantly with both phylogeny and diet, and the results of the dietary analysis found a negative correlation between dietary similarity and phylogenetic distance; closely related genera share similar diets (Marroig & Cheverud 2001). Within the four broad dietary clades for atelids, pitheciids, cebines and callitrichines, correlation matrices show that size accounts for 30% of variation and was correlated between dietary types, indicating broad similarity in craniodental allometry in each dietary group. Following removal of "general" size and division of traits into craniodental regions, facial trait correlations were responsible for most of the differences between the four dietary groups. For callitrichines, correlations with other dietary groups/clades and between facial traits indicated that the secondary body size reduction had decreased facial integration.

Marroig & Cheverud (2005) explored diet, size, evolutionary time, and the amount and tempo of evolutionary change in the context of platyrrhine phylogeny, testing the theory of lines of least evolutionary resistance (LLER), that underlying genetic and developmental variation can promote or restrict morphological evolution and control the pathway and tempo of evolutionary change (Schluter 1996). Skull size differences correlated with molecular genetic branch lengths and the amount and pace of morphological change. Branch lengths positively correlated with dietary and morphological amounts of change; the longer the time since a common ancestor between two groups then the greater the differences they would have in diet and morphology. The amount of morphological change positively correlated with the

pace of morphological change and amount of dietary change, but not with the LLER. Marroig & Cheverud (2005) interpreted the results as evidence that all platyrrhine lineages maintained a shared allometric pattern of variation along the LLER. Size was clearly important for the divergence of platyrrhines: diversification followed size along LLER, whilst skull size strongly correlated with the amount of morphological evolution. The direct implication was that LLER facilitated large scale, high tempo evolutionary change in size and morphology in the platyrrhine radiation, with movement away from the LLER producing an opposite pattern.

The relationship between adaptive evolution in platyrrhines and diet were supported by the correlation results that showed a link between dietary change (tempo and amount), size differences and morphological change (tempo and amount), and between group t-tests showing differences between major clades thought to have invaded new dietary zones against those that had not (Marroig & Cheverud 2005). The conclusion of Marroig & Cheverud (2005) was that platyrrhines evolved craniodental diversity along a LLER, resulting from either selection or constraint along the line. Several groups follow an alternative evolutionary path (e.g. *Lagothrix* and *Leontopithecus*), so clearly the lines do not completely constrain taxa, but these groups did not stand out as having undergone extensive diversification from common ancestors indicating constraints acting upon them had not changed.

Perez et al. (2011) also examined the link between cranial shape with size, ecology and phylogenetic relationships, measuring craniodental morphology with landmark and semi-landmark geometric morphometric methods that allowed more extensive quantification of platyrrhine variation, especially in cranial vault morphology. Morphometric data was compared to body mass, diet, life history and molecular phylogenetic data, and regression of cranial size and shape against body size and ecological variables tested the relationship between those two sets of variables. The relationship between phylogenetic relationships and cranial morphology were strong, although they find low association between body mass and cranial shape, and little association between cranial shape and diet or life history.

Marroig & Cheverud (2001), Marroig & Cheverud (2005) and Perez et al. (2011) all found a strong connection between molecular genetic and morphological distances, but the absence of correlation between morphology, size and diet in Perez et al. (2011) contrasts sharply with Marroig & Cheverud (2001) and Marroig & Cheverud (2005), that found craniodental morphology was interconnected with diet, size and phylogeny. The difference in results could relate to the morphological data used, as Perez et al. (2011) sample the skull in greater detail

using geometric morphometric methods and Marroig & Cheverud (2001) and Marroig & Cheverud (2005) used linear measurements, but there are also differences in methodsused to connect morphology with phylogeny, diet and size. If craniodental morphology was as strongly correlated with genetic distances as proposed by Perez et al. (2011), it seems unusual that morphology-based phylogenetic analyses (e.g Rosenberger 1984, Ford 1986, Kay 1990) are incongruent with current molecular phylogenies of platyrrhines and exhibit such high levels of homoplasy (e.g. Lockwood 1999). The strong relationship between morphology, diet and size in Marroig & Cheverud (2001) also has greater power in explaining the adaptive radiation of the five platyrrhine clades that incorporated size, diet and phylogeny. Considering size reduction in the callitrichines, and size increase in cebines, atelids and pitheciids, the lack of correlation between size and morphology from Perez et al. (2011) is especially peculiar, and seems to indicate a methodological problem in their study.

This chapter has provided a summary of platyrrhine taxonomy, morphology, phylogeny and evolution. In the next chapter, the materials and methods section of the thesis are presented. This includes description of the anatomical landmark collected, taxa sampled, and detailed presentation of geometric morphometric and distance-based phylogenetic methods. Measurement error for single landmarks and overall shape are also provided. After the materials and methods chapter, four chapters are presented for phylogenetic analysis of platyrrhines, atelids, pitheciids and cebids, followed by a final discussion chapter.

Chapter 3 Materials and Methods

3.1 Summary

The aim of this thesis is to investigate the phylogenetic signal of the platyrrhine craniodental region, and this chapter describes the taxa sampled, morphological data collected and methods used to address this research question. Digital landmark morphological data were collected from 1500 individual specimens belonging to fifty platyrrhine species and nine outgroups. A selection of seventy-two anatomical landmarks were used to quantify morphological variation in the craniodental region of each specimen, and all landmarks are listed and illustrated in a series of photographs (Figures 18-22). The 3D anatomical landmarks were originally collected in Microsoft Excel and saved in a format allowing for geometric morphometric analysis, using two computer packages- Morphologika (O'Higgins & Jones 2006) and MorphoJ (Klingenberg 2011). Geometric morphometric analysis was applied, using a mathematical procedure called Generalized Procrustes Analysis (GPA) that produces Procrustes residuals, removing several sources of non-biological variation and allows for comparison of landmark positions between individuals or groups (Adams et al. 2004, Gower 1975, Rohlf & Slice 1990, Goodall 1991). The geometric morphometric methods are described in detail, both due to their fundamental importance for this project and growing application in biological anthropology more generally (Adams et al. 2004, Lawing & Polly 2010).

The mean shape of species, as described by geometric morphometric data, were used to quantify a morphological Euclidean distance separating any two species, and these distances were generated between all species involved in phylogenetic analysis (Zelditch et al. 2004). Morphological distances were stored in a distance matrix and used for distance-based phylogenetic analysis in the computer program Phylip (Felsenstein 2005). Distance-based phylogenetic methods are quite poorly understood and are often erroneously described as phenetic, and like geometric morphometric methods are described in detail due to their prominence in this work (Lockwood et al. 2004). As many phylogenetic methods rely on the use of an outgroup to infer a phylogenetic tree, the general philosophy of using an outgroup is outlined and the biology of each outgroup is briefly summarised (Lockwood et al. 2004, Felsenstein 2004). As part of a thorough testing of phylogenetic trees inferred, combinations of outgroups were outlined for use in phylogenetic analysis. The core question in this thesis is whether different regions of the skull hold an alternative phylogenetic signal when compared

to each other and overall skull shape. This subdivision into regions is based on theories of cranial modularity, with the hypothesised regions outlined in reference to past work on primates and mammals (e.g. Cheverud 1982, Hallgrimsson et al. 2004, Goswami 2006a). Another question of interest is whether sexual dimorphism has noticeable effects on phylogenetic analysis, and the separation of data according to sex is also outlined.

Several approaches are described that test whether individual landmarks were susceptible to error, and a measure of overall error is made (Polly 2001, Lockwood et al. 2002, Cardini & Elton 2008). Several landmarks were removed from the dataset on the basis of these error estimates, but mean error accounted for less than 10% of observed variation within a single *Lagothrix* taxa and is not expected to have a large impact on phylogenetic analysis (Polly 2001). Due to potential differences between two types of morphological distance, Euclidean and Procrustes, a correlation between the two was computed that shows they are very highly correlated (Rohlf 1999a).

3.2 Materials

The platyrrhine taxa sampled with sample sizes are listed in Table 7, and a more exhaustive list of taxa sampled, including subspecies and geographical location for wild captured specimens, the museum collection visited, and the sample size for male, female and pooled-sex specimens are all provided in the Appendix. Overall 16 genera (18 for taxonomy that splits *Callithrix* into *Mico*, *Cebuella* and *Callithrix*) and 50 species of platyrrhines were sampled alongside nine outgroups. The taxonomy and species identification used follows that of Kinzey (1997), except for *Lagothrix* where sampling of subspecies allowed for their treatment as separate taxonomic units supported by molecular phylogenetic evidence (Ruiz-Garcia & Pinedo-Castro (2010), and the elevation of *Cebus libidinosus* to a species distinct from *Cebus apella*. Sexing of specimens was based on museum collection records. Adult specimens were used based on last molar eruption and, where possible, full fusion of the spheno-occipital portion of basicranium (the spheno-occipital synchondrosis). Nearly all specimens were wild and hunted, except in the case of *Leontopithecus rosalia*, *Callimico goeldii* and *Callithrix pygmaea*, where captive specimens were sampled because of the scarcity of wild specimens. This inevitably introduced a potential source of error, as morphology in these taxa may reflect any number of variables linked to captivity. However, the alternative, to not sample two genera and some of the most important platyrrhine taxa, would have been far more problematic in attaining the fundamental goals of this thesis.

For phylogenetic analysis, separate individual taxa were represented at the species but not subspecies level due to sample size considerations (i.e. subspecies of the same species were combined in a single taxon). The expected effect of sampling from a wide range of subspecies, and/or geographical regions, would be to increase variation at the species level. For phylogenetic analysis at the genus-level, it seems unlikely that increasing variation at the species-level will have a large effect on analysis, and it is preferable to the detrimental effect of smaller sample sizes. Sample sizes of male, female and pooled sex taxa are provided in the Appendix. The ideal sample size aim was 10 male and 10 female specimens for each taxon, with increased sample sizes where/when time allowed. For some taxa, only lower sample sizes were available, and in the case of *Brachyteles arachnoides* (seven males and five females) including the group in the analysis was considered more important than potential error introduced by low sample size.

Table 7 List of taxa sampled and sample sizes

Family	Genus	Species	Male	Female	Pooled
Platyrrhines					
Atelidae	*Alouatta*	*belzebul*	10	10	20
		caraya	9	11	20
		coibensis	8	9	17
		fusca	9	9	18
		palliata	18	13	31
		pigra	8	10	18
		seniculus	22	10	32
	Ateles	*belzebuth*	11	10	21
		fusciceps	10	10	20
		geoffroyi	10	10	20
		paniscus	7	12	19
	Brachyteles	*arachnoides*	7	5	12
	Lagothrix	*cana*	10	11	21
		lagothrica	10	10	20
		lugens	8	10	18
		poeppigii	10	10	20
	Atelidae		167	160	327
Cebidae	*Cebus*	*albifrons*	10	10	20
		apella	92	60	152
		capucinus	10	10	20
		libidinosus	11	10	21
		nigrivittatus	10	10	20
	Saimiri	*bolviensis*	10	10	20
		oerstedii	11	9	20
		sciureus	33	15	48
		ustus	10	6	16
	Aotus	*azarai*	6	10	16
		lemurinus	10	10	20
		trivirgatus	13	11	24
		vociferans	10	10	20
	Leontopithecus	*rosalia*	11	13	24
	Callithrix	*argentata*	11	10	21
		humeralifer	11	9	20
		jacchus	8	7	15
		penicillata	18	14	32
		pygmaea	10	9	19
	Callimico	*goeldii*	11	11	22
	Saguinus	*fuscicollis*	27	11	38
		geoffroyi	10	9	19

		leucopus	9	9	18
		midas	12	10	22
		mystax	10	11	21
	Cebidae		384	304	688
Pitheciidae	*Callicebus*	*cupreus*	10	9	19
		hoffmannsi	9	10	19
		moloch	13	15	28
		torquatus	12	9	21
	Cacajao	*calvus*	13	10	23
		melanocephalus	13	17	30
	Chiropotes	*satanas*	14	9	23
	Pithecia	*monachus*	14	13	27
		pithecia	12	10	22
	Pitheciidae		110	102	212
Outgroups					
Cercopithecidae	*Cercopithecus*	*aethiops*	10	10	20
	Colobus	*guerza*	11	10	21
	Macaca	*mulatta*	9	10	19
	Trachypithecus	*obscura*	10	10	20
	Cercopithecidae		40	40	80
Galagidae	*Galago*	*senegalensis*	10	11	21
	Otolemur	*garnetti*	10	9	19
	Galagidae		20	20	40
Hylobatidae	*Hylobates*	*lar*	10	10	20
Lemuridae	*Eulemur*	*fulvus*	10	10	20
Lorisidae	*Perodicticius*	*potto*	10	10	20
All platyrrhines			661	566	1227
All outgroups			90	90	180
All specimens			751	656	1407

3.3 Methods

3.4 Summary

Data collection for this project involved collecting 3D digital craniodental anatomical data from primate specimens. In subsequent sections, geometric morphometric and distance-based phylogenetic methods are described in detail to explain how morphological data were standardised and used to infer phylogenetic relationships. Several phylogenetic analyses of primate craniodental morphology have previously combined geometric morphometrics and distance-based methods (e.g. Lockwood et al. 2004, Cardini & Elton 2008, Bjarnason et al. 2011) and a similar approach was followed. Three major methodological decisions are also explained- outgroup selection, cranial modularity and separate sex analyses.

Digital data from the skulls of a range of New World monkey and primate outgroup taxa were originally collected using a Microscribe G2X in a Microsoft Excel file, with 72 landmarks collected from each specimen, and 0 0 0 coded for any missing landmarks. The data was transferred into Morphologika (O'Higgins & Jones 2006) file format, as the Morphologika program provides a user-friendly interface for geometric morphometric analysis of coordinate data. Geometric morphometric analysis involves a mathematical superimposition process (Generalised Procrustes Analysis- GPA) that removes scale, orientation and position, and generates new (Procrustes) shape residuals (Adams et al. 2004, Gower 1975, Rohlf & Slice 1990, Goodall 1991). In the course of this project, an alternative computer program, MorphoJ (Klingenberg 2011), replaced Morphologika for geometric morphometric analysis due to its faster processing speeds.

Following geometric morphometric analysis of coordinate data in MorphoJ, average taxa shapes were saved and loaded into Microsoft Excel. An Excel macro, provided by Charles Lockwood, was used to calculate the Euclidean distance separating mean shapes of all taxa analysed. Euclidean distances were stored in a distance matrix that was transferred into the phylogenetics program Phylip (Felsenstein 2005). All phylogenetic analyses were based on the neighbor-joining method, and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis was also used to quantify phenetic relationships and draw a contrast with the results from phylogenetic analyses. Both UPGMA phenetic clustering and neighbor-joining phylogenetic analyses were carried out in Phylip, generating tree files that were either saved in Treeview (Page 1996) or copied onto Phyfi (Fredslund 2006), an online tool for drawing phylogenies.

The phylogenetic analyses and results were split into four chapters according to the platyrrhine clade studied: platyrrhines, atelids, pitheciids and cebids. For each clade, phylogenetic analyses were repeated with each single outgroup (of which there were nine) or in a variety of combinations. Phenetic analyses were also carried out without the inclusion of any outgroup taxa. To test the phylogenetic signal of different craniodental regions distance data were analysed according to craniodental morphology of the whole skull, or subdivision into facial and cranial base modules. Finally, these analyses were also repeated for pooled sex, male-only, female-only and separation of male and female specimens into separate taxa. In every instance the geometric morphometric analysis was repeated for each variation of outgroup, module and sex.

3.5 Landmark selection

The landmarks selected to describe morphological variation of the craniodental region were largely the same as those of Cardini & Elton (2008), with the addition of several landmarks from the face, cranial vault, temporal bone, foramen magnum and other parts of the basicranium. In full, 72 landmarks were selected and are listed in Table 8, although some taxa were missing landmarks. Rather than estimating missing landmarks, specimens were either removed from analysis or a smaller landmark list was used. The landmarks broadly described morphology of the face, dental positions, basicranium and cranial vault. More specifically, the landmarks cover the nasal aperture and palate, along with zygomatic, frontal, palate, sphenoid, temporal, occipital and parietal bones. Several landmarks are midpoints between two other landmarks, and were determined by measuring the distance between landmarks (using a tape measure) and marking the midpoint in pencil. In Figures 18-22 the landmarks are shown on a variety of spider monkey specimens that correspond to the original landmark list that follows. Landmarks that have a * were later removed from the dataset for phylogenetic analysis due to concerns about repeatability and associated error (see Error subsection at the end of the chapter).

Table 8 Original anatomical landmark list

1. Nasospinale, inferior-most mid-line point of nasal aperture
2. Point of greatest width for the nasal aperture
3. Meeting point of nasal and pre-maxilla on the border of the nasal aperture
4. Rhinion, anterior-most midline point of suture connecting nasal bones

5. Nasion, suture meeting point between frontal and nasal bones
6. Glabella, midline point of the greatest projection on the frontal bone along the supraorbital ridges
7. Greatest projection of the supraorbital ridge
8. External frontomalare orbitale, where frontozygomatic suture meets the inner orbit
9. External rontomalare temporale, where frontozygomatic suture meets the lateral part of zygomatic bone
10. External zygomaticomaxillary superior, the antero-superior point where orbital rim meets zygomaticomaxillary suture
11. External zygomaticomaxillary inferior, the lateral point of zygomatic on the zygomaticomaxillary suture
12. Inferior-most point of zygomatic foramen
13. Inferior-most point of infraorbital foramen
14. Inferior-most point of lacrimal duct fossa
15. Inferior-most point of optic foramen
16. Ventral-most point of suture between maxilla and sphenoid
17. Maximum point of curvature on interior side of zygomatic portion of zygomatic arch
18. External superior point of zygomaticotemporal suture on lateral part of zygomatic arch
19. External inferior point of zygomaticotemporal suture on lateral part of zygomatic arch
20. Junction between the external sutures of the of sphenoid and zygomatic bones
21. Junction between the external sutures of the sphenoid, zygomatic and parietal bones
22. Junction between the external sutures of the sphenoid, parietal and temporal zygomatic bones
23. Junction between the external sutures of the zygomatic, parietal and frontal bones
24. External midpoint between glabella and bregma
25. Bregma, the external junction between the coronal and sagittal sutures

26. Midpoint between the bregma and lambda

27. Lambda, the external junction between the lamboid and sagittal sutures

28. Asterion, the external junction between the external sutures of the mastoid part of the temporal, parietal and occipital bones

29. Midpoint between 23 and 26/lambda*

30. Anterior-most part of the external auditory meatus

31. Posterior-most part of the external auditory meatus

32. Inferior-most part of the external auditory meatus

33.Lateral incisor I1 septum

34. Lateral incisor I2 septum*

35. Lateral canine C1septum

36. Lateral premolar P2 septum

37. Lateral premolar P3 septum*

38. Lateral premolar P4 septum*

39. Lateral molar M1 septum

40. Lateral molar M2 septum*

41. Lateral molar M3 septum*

42. Midpoint of septum at end of dentition

43. Posterior-most point of incisive foramen

44. External midline meeting point of maxilla and palatine

45. Posterior-most point of palatine foramen

46. Point of maximum curvature on posterior edge of palatine

47. Midpoint of posterior part of nasal spine

48. Midpoint of external suture connecting basiosphenoid and basioccipital

49. Junction between the external sutures of the petrous, basiosphenoid and basioccipital bones

50. Lateral-most point of foramen lavelli

51. Junction between the external sutures of the zygomatic process of temporal, petrous and sphenoid bones

52. Greatest central projection of the external petrous part of the temporal bone

53. Medial-most part of the stylomastoid foramen

54. Distal-most point of the jugular foramen

55. Medial-most point of jugular foramen

56. Anterior-most point of carotid foramen
57. Midpoint between the external basion and basisphenoid-basioccipital bones
58. Basion, anterior-most part of the foramen magnum
59. Anterior-most point on the occipital condyle
60. Posterior-most point on the occipital condyle
61. Medial-most point of the hypoglossal canal
62. Opisthion, posterior most part of the foramen magnum
63. External midway between opisthion and inion
64. Inion, the most posterior part of the cranium
65. Greatest point of curvature on interior of the external posterior zygomatic process of temporal bone
66. External meeting point between sphenoid and zygomatic process of temporal
67. External tip of the post glenoid process
68. Deepest external point within the mandibular fossa
69. Medial-most part of articular eminence
70. Midpoint of articular eminence
71. Lateral-most part of articular eminence
72. External meeting point between occipital crest and occipital-frontal suture*

Figure 18: Facial landmarks from the frontal, nasal, maxilla, zygomatic and sphenoid bones on a *Lagothrix* specimen

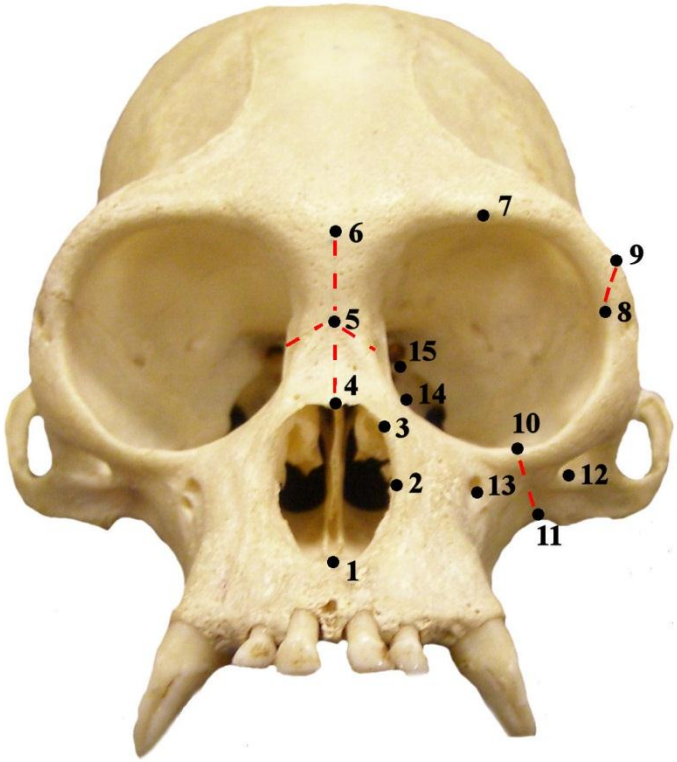


Figure 19: Cranial vault and zygmomatic landmarks on a *Lagothrix* specimen

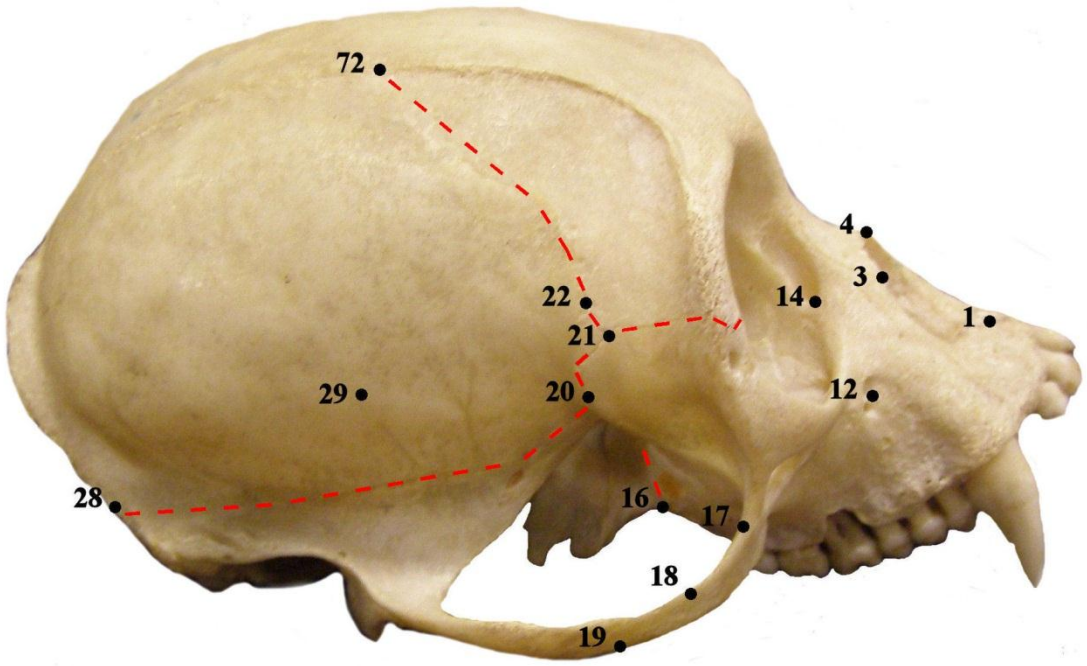


Figure 20: Cranial vault landmarks from the frontal and parietal bones on a *Lagothrix* specimen

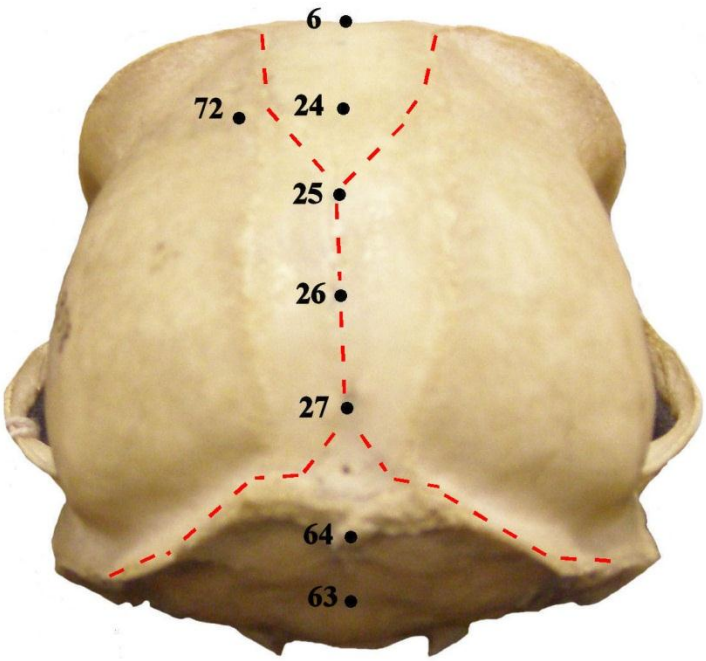


Figure 21: Dental, oral and basicranium landmarks on an *Ateles* specimen on a *Lagothrix* specimen

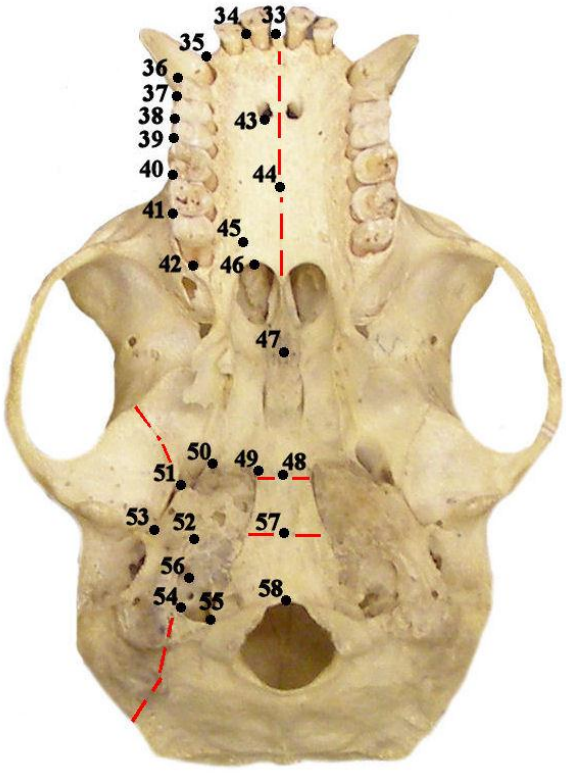
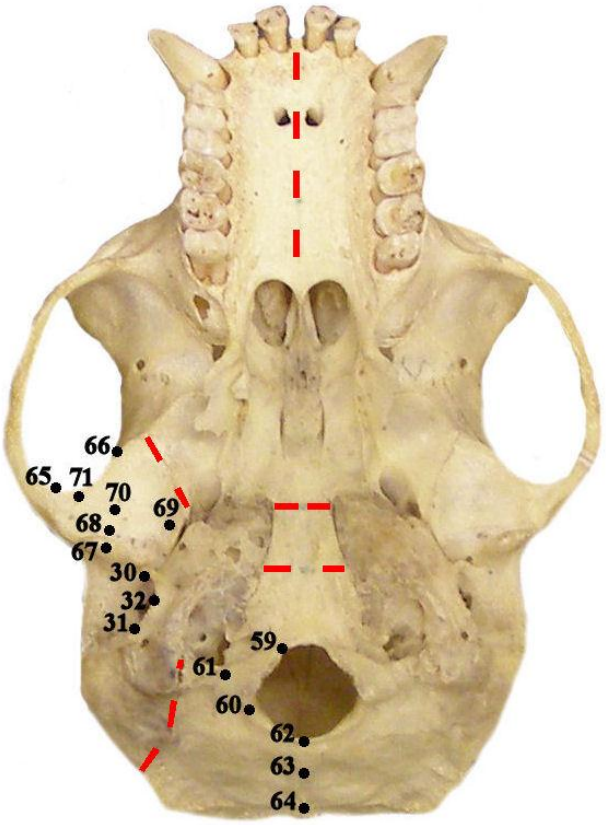


Figure 22: Basicranium landmarks on temporal and occipital bones



3.6 Geometric morphometrics

Broadly defined, morphometrics is the quantitative description, statistical analysis and interpretation of variation found in biological organisms, and the study of covariation between shape and variables of interest (Rohlf 1990, Rohlf & Marcus 1993, Adams et al. 2004). Morphometrics have been used to investigate biological questions relating to micro- and macro-evolution, genetics, allometry, evo-devo, ontogeny, heterochrony, phylogenetics and systematics, phylogeography, integration, modularity, asymmetry, sexual dimorphism and forensics (Slice 2007, Klingenberg 2010, Lawing & Polly 2010). Morphometrics can be broadly separated into historical multivariate and modern geometric morphometrics. Multivariate morphometric approaches use multivariate statistics on variables, usually metric measurements or angles, but experience problems with size correction, the homology of distance measures not defined by homologous biological landmarks, and the efficiency of methods to capture spatial positions of landmarks from which measurements are taken (Adams et al. 2004, Slice 2007).

The alternative of geometric morphometrics places emphasis on measuring and preserving the geometry of structures being studied, combined with the use of multivariate statistics and visualisation of biological form not possible with older methods (Rohlf & Marcus 1993, Adams et al. 2004, Mitteroecker & Gunz 2009). The geometric morphometric revolution (Rohlf & Marcus 1993) has led to the development of methods to analyse either the outline or surfaces of shapes or biological landmarks described by two- or three-dimensional coordinates (Adams et al. 2004, Klingenberg 2010). Landmarks can be considered as simply points on an object/form/specimen that can be accurately located and have a clear, shared correspondence between specimens being studied (Klingenberg 2010). Landmarks come in three broad forms: type I of strongest support from local or histological structures such as the meeting of several bones, type II with geometric support that exhibit functional homology such as the tip of a structure, or type III located on an outline or surface such as a point of maximum curvature (Bookstein 1991, O'Higgins 2000). The type III landmarks are expected to have greater variation as a result of error (O'Higgins 2000).

With landmark-based approaches, direct analysis would include variation relating to orientation, scale and position of landmark configurations. Therefore, a mathematical approach is required to remove this variation so that remaining, meaningful shape variation can be used in statistical analysis (Adams et al. 2004). Superimposition methods remove

orientation, scale/size and position according to optimization criteria, with Generalized Procrustes Analysis (GPA) the most common method based on a least squares approach (Adams et al. 2004, Gower 1975, Rohlf & Slice 1990, Goodall 1991). Procrustes superimposition scales coordinate data to a standard size using a measure of size (most often the centroid), moves them to a standard position so the centre of gravity are at a coordinate system's origin, and rotate from the centre of gravity so that there is the lowest possible sum of squared distances between different landmark configurations (Klingenberg 2010). Procrustes analysis provides a measure of size in the form of centroid size, defined as the square root of the sum of squared distances of landmarks from the centroid (Zelditch et al. 2004).

Following Procrustes superimposition, variation in landmark coordinates describes shape variation to be used in subsequent multivariate statistical analysis- the use of multivariate statistics is especially important because of the correlation between coordinates/landmarks and the myriad of ways that shape can vary (Klingenberg 2010). It is important to note that with geometric morphometrics emphasis is on comparing configurations of landmarks between individuals and groups rather than single landmarks in isolation (Zelditch et al. 2004). The most popular multivariate analyses of geometric morphometric data include principal component analysis to look at patterns of variation, canonical variate analysis for group separation, multivariate regression for studies of allometry and change in shape related to time, and partial least squares for studying covariation in shape which is especially useful for integration and modularity (Klingenberg 2010).

The shape coordinates generated by Procrustes analysis do not exist in a flat plane but in curved space similar to a spherical surface, in what is known as Kendall's shape space, with data points projected into a space that exists tangentially to Kendall's shape space (Adams et al. 2004, Mitteroecker & Gunz 2009). Within the tangent space, Procrustes distances separate pairs of landmark configurations; landmark configurations represent the biological forms being studied, and shape variables can be scored on tangent axes for use in multivariate statistical analyses (Adams et al. 2004). Put another way, after Procrustes superimposition all the coordinates of all the specimens will exist within shape space where any single point will represent a different shape and superimposed shapes are an approximation within this nonlinear, multidimensional space (Klingenberg 2010). Prior to multivariate analysis, landmark configurations need to be projected onto a Euclidean shape space as they reside in Kendall's shape space that is spherical, and multivariate statistics are carried out in Euclidean

space (Cardini et al. 2007, Monteiro et al. 2000). The geometric morphometric program MorphoJ, that was used for data analysis in this thesis/project, projects Procrustes output data into tangent space automatically following Procrustes superimposition (Klingenberg 2011).

There are alternatives to a geometric morphometric approach based on landmark based superimposition that aim to quantify morphology with greater accuracy than historical multivariate morphometrics, specifically interlandmark approaches such as Euclidean distance matrix analysis (EDMA, Lele & Richtsmeier 1991) or the use of interior angles by Rao & Suryawanshi (1996) and Rao & Suryawanshi (1998) (Adams et al. 2004). EDMA uses Euclidean distances between landmarks to describe, and study change, in form, collating the average interlandmark distances for a group into a single form matrix (Richtsmeier et al. 1992, Adams et al. 2004). The form matrix of separate groups can then be compared in a form difference matrix, quantifying the similarity/difference between form of different groups based on the ratios between corresponding interlandmark distances (Adams et al. 2004). EDMA methods are split into earlier EDMA-I (Lele & Richtsmeier 1991) and later EDMA-II (Lele & Cole 1996) methods, as the earlier methods assumed equal variance-covariance in groups being compared whereas the later methods did not (Lele & Cole 1996). The internal angle approach of Rao & Suryawanshi (1996) and Rao & Suryawanshi (1998) creates triangles from landmarks and extracts information on the angles of coordinates to describe shape (Adams et al. 2004). These two approaches are unaffected by the location and orientation of landmarks so do not require superimposition, unlike geometric morphometric methods (Adams et al. 2004). The latter requires a means of scaling, with Rao & Suryawanshi (1996) using log distances for internal angle approaches and Cole et al. (2002) suggesting scaling for EDMA by either a single distance the experimenter decides on, the maximum distance or geometric mean from all distances. The EDMA approaches are interesting as they concentrate on the variance associated with particular landmark points and have been used extensively in medical morphometrics (Lawing & Polly 2010). For a more detailed exploration of EDMA see Lele & Richtsmeier (1991), Lele (1993), Richtsmeier & Lele (1993), Lele & Cole (1996) and Richtsmeier et al. (2002). However, EDMA or internal angle approaches are not used in this project due to the ubiquity of geometric morphometric methods in physical anthropology, and the statistical justification of Rohlf (2000a), Rohlf (2000b) and Rohlf (2003).

Rohlf (2000a) examined the shape space data occupied for different morphometric ordination methods that allow comparison of shapes. The EDMA and angle methods suffer from a

problem linked to non-linear space where average shape and true shape contrast sharply. It is possible that landmark data will fall outside of shape space to create impossible shapes with serious distortion of results, a problem avoided with Kendall's shape space and Procrustes methods by projection of shape onto Kendall's tangent shape space. Rohlf (2000b) tested the statistical power for the various morphometric methods when comparing shape differences of two groups. The superimposition based methods performed much better with higher statistical power than the interlandmark methods. The EDMA-I method performed poorly with increasing landmarks and unequal interlandmark distances, which would be particularly problematic in this study as the landmarks on the platyrrhine skull are often distributed unequally. The EDMA-II method had especially high type 1 errors and performed poorly in comparison of groups.

Within the superimposition methods the Goodall F test outperformed Kendall's tangent space, although both performed strongly (Rohlf 2000b). The Goodall F test requires that very strict assumptions be met, that variance across all landmarks is small and variation within and between landmarks is independent, which is unlikely in a biological dataset such as that collected for this project, making Kendall tangent space a preferable alternative. Rohlf (2003) looked at the accuracy of mean shape estimation and the pattern of associated bias for the different morphometric methods. Procrustes superimposition performed strongest, with the estimate of mean shape unbiased and more accurate than the alternative morphometric methods. Rohlf (2000a,b, 2003) has clearly shown that geometric morphometrics using Procrustes superimposition have the greatest power to test for differences in mean shape between populations, with the greatest accuracy in predicating mean shape, and the lowest error estimate with no bias: they are the most powerful and accurate methods with which to quantify and statistically analyse shape. For this reason, such methods are used in this project.

3.7 Distance-based phylogenetic analysis

As described in the first chapter, Cavalli-Sforza & Edwards (1967) and Fitch & Margoliash (1967) originally proposed the major alternative to cladistic phylogenetic methods in the form of distanced-based methods (also called distance matrix methods). Distance-based methods can be broadly split into two parts: first distances are calculated between every possible pairing of taxa being studied, and then a phylogenetic tree that best suits the distances is either created or searched for (Felsenstein 2004, Yang 2006). Distance between taxa can be genetic and a measure of accumulated DNA differences or morphometric and a measure of

distance between a collection of data points (Nei 1972, Zelditch et al. 2004). Felsenstein (2004) described distance-based methods as a means to inferring a full tree of N taxa from a collection of pairwise distances (for all possible two- taxa combinations), with the taxa pair separated by variable branch lengths in an unrooted tree. These branch lengths measure the amount of change that has occurred along that evolutionary branch and will not necessarily reflect time. If two sister branches diverge from a shared common ancestor at the same time, one branch can be longer if more evolutionary change has occurred along it, although the evolutionary differences between the two taxa could be reduced, rather than increased, by the change.

The major distance-based methods are least squares, minimum evolution, neighbor-joining and UPGMA. These methods are often incorrectly referred to as non-phylogenetic and phenetic, but with the exception of UPGMA, they use an outgroup to infer polarity and apply a root to the phylogenetic tree (Lockwood et al. 2004). Distances between taxa are required for phylogenetic analysis, and these can be derived from molecular or morphological data using an array of techniques. With molecular sequences, a model of evolution will be applied to sequences to estimate similarity/dissimilarity, whilst morphological distances are based on the metric difference between mean shapes of separate taxa using either linear or geometric morphometric data.

The use of neighbor-joining in molecular phylogenetics has experienced a renaissance due to the rapidly increasing size of genetic data being analysed and taxa included in analysis, whereby the speedy phylogenetic inference of neighbor-joining are preferable to alternative computationally intense methods (Tamura et al. 2004). Neighbor-joining is considered a simplified, faster version of the minimum evolution method that estimates a phylogenetic tree according to the smallest sum of branches i.e. the true tree is likely that which requires the least amount of evolutionary change (Nei & Kumar 2000). In simulation studies neighbor-joining and minimum evolution methods have very similar performances, with neighbor-joining's faster computational speed favoured at little extra cost (Nei & Kumar 2000). The neighbor-joining method is statistically consistent, meaning that it infers the correct evolutionary tree when distances are accurate reflections of phylogeny (Mihaescu et al. 2009).

Simulation studies provide experimental support for phylogenetic methods in different evolutionary scenarios, as an initial phylogeny is known and phylogenetic methods can be

analysed on their ability to replicate the tree. In a comparison of maximum likelihood, parsimony and distance-based methods based on consistency, efficiency and robustness, Huelsenbeck (1995a) found maximum likelihood methods performed best with little separating parsimony and distance-based methods (although UPGMA performed poorly). Takahashi & Nei (2000) found, more generally, that neighbor-joining performed well compared to maximum parsimony and maximum likelihood methods. Where transitional mutation rate is high neighbor-joining performed better than maximum parsimony (Jin & Nei 1990), and where low mutation rates and low number of sequences were used neighbor-joining outperformed maximum parsimony (Sourdis & Nei 1988). Maximum parsimony is more susceptible than distance-based methods to inaccuracy arising when rates vary between taxa being studied (Kuhner & Felsenstein 1994) and are also more vulnerable to the problem of long branch attraction (Felsenstein 1997).

Neighbor-joining and the minimum evolution methods have similarly strong performance in simulation experiments and outperform maximum likelihood if rates of evolution are constant (Saitou & Imanishi 1989). However, a direct comparison of maximum likelihood to neighbor-joining methods found support for the superiority of maximum likelihood methods (Huelsenbeck 1995b). An analysis of phylogenetic methods with morphological data, comparing neighbor-joining to UPGMA and maximum parsimony alternatives found that no single method was more accurate than the other (Kim et al. 1993). More recently, in molecular phylogenetics, there has been a move towards Bayesian and maximum likelihood methods when taxa number are low, with the use of neighbor-joining when higher taxa number make computationally intensive methods unenviable. A recent study by Roch (2010) provided hope for major developments of the distance-based method that incorporates correlation of distances based on shared phylogenetic history, essentially allowing for more informative data to be retrieved from phylogenetic distances (Allman & Rhodes 2010).

Considering there are multiple distance-based methods available, the use of only neighbor-joining requires justification. Although neighbor-joining is faster than least squares, the difference in time is slight and makes little difference. However, in a pilot study it became clear that the least-squares and minimum-evolution methods often inferred paraphyletic relationships for platyrrhine taxa with a large number of constituent species, the reasons for which are unclear. Problems with inference of monophyly were also found in Lockwood et al. (2004) and Bjarnason et al. (2011) with least squares analysis of hominoid geometric

morphometric data. As a result of these observations, only neighbor-joining was used here for phylogenetic analysis.

In the descriptions of distance-based phylogenetic methods above, one issue not covered is the initial step in the phylogenetic analysis- generation of distances. Molecular and morphological distance-based analyses deal with very different data and have different methods of generating distances. Two types of distance measure, Euclidean and Procrustes, are most often used for geometric morphometric data and in this project Euclidean distances were used (Zelditch et al. 2004). Euclidean distances, measured as the square root of the sum of squared distances between two configurations of landmarks, exist within linear Euclidean tangent space where multivariate analysis of geometric morphometric data takes place (Zelditch et al. 2004). Several past phylogenetic analyses of geometric morphometric data have used Procrustes distance, measured by the square root of the sum of squared differences between two configurations of landmarks within the curved space of Kendall's shape space (Polly 2001, Cardini & Elton 2008). Euclidean distances were used because it is more consistent to use Euclidean distances present in Euclidean space considering multivariate statistics (and any subsequent analyses) are based in this space. Any concern about the use of Euclidean rather than Procrustes distances should be minimal, as when variation in samples is small Euclidean distances and multivariate statistical techniques in tangent space can be used as approximations of Procrustes distances in Kendall's shape space (Marcus et al. 2000, Zelditch et al. 2004). For the platyrrhine dataset used in this study the correlation between the two types of distance was very high with a tiny error, so the use of Euclidean over Procrustes distances is highly unlikely to affect phylogenetic inference.

Another issue is reproducibility and statistical node support of inferred clades and phylogenetic relationships. Lockwood et al. (2004), Couette et al. (2005) and Bjarnason et al. (2011) used bootstrapping of morphometric data to provide statistical support for clades, although Caumal & Polly (2005) and Cardini & Elton (2008) have objected to resampling morphometric data without repeating Procrustes superimposition. In this thesis, with such a wide breadth of analyses based on craniodental region, outgroup selection and sex of specimens, results are reported according to genus-level phylogenies. Providing additional bootstrap support for clades, or use of an alternative statistical measure, would provide an overwhelming amount of data for the results presented, which would hinder the presentation and analysis of results. Instead, the preference is to test consistency of phylogenetic

relationships inferred according to variation in outgroup selection, sexual dimorphism and modularity.

3.8 The neighbor-joining method

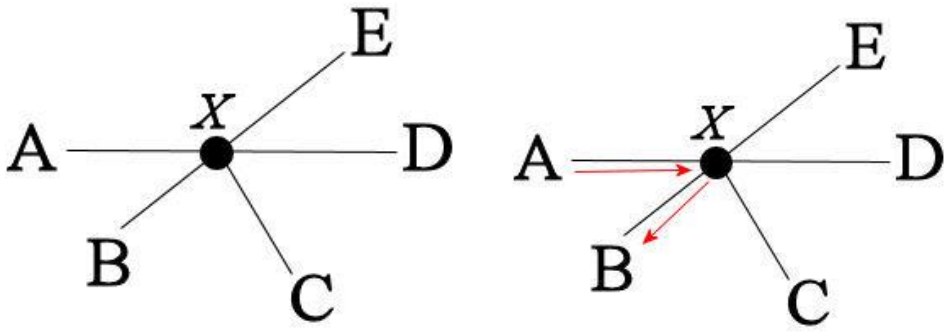
Neighbor-joining, developed by Saitou & Nei (1987), like Unweighted Pair Group Method using Arithmetic averages (UPGMA), uses an agglomerative clustering algorithm. This means that it constructs a phylogenetic tree with a stepwise additive method that converts a star tree into a phylogeny using a divisive cluster algorithm (i.e. taxa are separated from each other into clades) that minimizes overall branch length (Kuhner & Felsenstein 1994, Yang 2006, Desper & Gascuel 2005). The 'additive' part of the method includes an assumption that the distance between two taxa is equal to the distance between each respective taxa and a shared node. Whereas some phylogenetic methods use tree searching, neighbor-joining uses distance data to build a single tree.

Unlike UPGMA, neighbor-joining does not assume a molecular/morphological clock and steady rate of evolution (ultrametricity), but instead a minimum evolution criterion (see Desper & Gascuel 2005). At each step of the computation of a phylogeny the shortest tree of minimal length, as measured by the sum of all branch lengths, is selected (Yang 2006, Desper & Gascuel 2005). Beginning with a star tree where all taxa are equally related, the algorithm calculates all possible tree topologies created by a single taxa pairing; the topology which has the smallest tree length (i.e. the pairing of taxa which requires the least amount of evolution to have occurred) with two taxa separated by the smallest distance is chosen (Desper & Gascuel 2005). In other words, the algorithm has chosen the two taxa most closely related according to the smallest distance between them which will reduce tree length and overall branch lengths by the most (Yang 2006). This continues round by round with branch lengths on the tree and overall tree length updated for every round of clustering (Yang 2006). When the algorithm calculates new branch and tree lengths, the input distance data will change each time because the paired taxa will be treated as one group and all distances recalculated (Desper & Gascuel 2005).

3.9 How neighbor-joining works

The phylogenetic analysis begins with a star radiation (as in the left diagram) operating on the assumption that phylogenetic distance between taxa can be measured in additive terms by adding the combined lengths of each taxon to a shared node (as in the right diagram). The

distance between taxa A and B is calculated by addition of the distance from taxon A to node x and taxon B to node x, node x being the node both taxa share.



The first step of the algorithm is to calculate all distances between taxa pairs by adding the distance from each taxon to node X. After calculating all possible distances, the two taxa separated by the smallest distance are designated as neighbors, with a new node inserted to connect the two. The branch length and extent of evolutionary change needs to be controlled for, so the sum of distances for each taxon are divided by taxa sample size minus two. These controlled-for measures are then included in quantifying the distance between each taxon and the shared internal node. The equation for these two steps are as follows- where A and B refer to taxa A and B, x refers to the internal node x connecting taxa A and B, r refers to the correction factor that incorporates amount of evolution, and Dist is distance between the internal or external nodes stated:

$$rA = (\text{sum of all distances for taxon A})/(N_{taxa} - 2)$$

$$rB = (\text{sum of all distances for taxon B})/(N_{taxa} - 2)$$

$$Dist.Ax = (Dist.AB + rA - rB)/2$$

$$Dist.Bx = Dist.AB - Dist.Ax$$

The neighbor-joining algorithm then removes distances between A and all taxa, and B and all taxa, and computes a new set of distances between the neighbor pair AB and all remaining groups. The new distance, for say taxa C, will be the original distance AC plus the original distance BC minus the original distance AB, divided by two. The generation of new distances is carried out for all remaining taxa and a new, updated distance matrix is created with a

single taxon AB replacing the two taxa of A and B. The algorithm then starts the neighbor-joining process again, until the tree is fully resolved with no more distances to analyse. The outgroup, as selected by the experimenter, is then used to root the tree with a phylogeny of hypothetical evolutionary relationships inferred.

A partial example is displayed below. Let the initial distance matrix be:

	A	B	C	D	E	Sum
A	0	2.3	12.9	2.9	5.9	24
B	2.3	0	10.6	0.6	3.6	17.1
C	12.9	10.6	0	10	7	40.5
D	2.9	0.6	10	0	3	16.5
E	5.9	3.6	7	3	0	19.5

The smallest distance separating any taxa is that between B and D, so the first step is to calculate rB, rD, Dist.Bx and Dist.Dx:

$$rB= (17.1)/(3)= 5.7$$

$$rD= (16.5)/(3)= 5.5$$

$$Dist.Bx= (0.6 + 5.7 – 5.5)/2= 0.4$$

$$Dist.Dx= 0.6-0.4= 0.2$$

Then the distances between BD and remaining taxa are recalculated, with original distances for the neighbor pair to each remaining taxon added together, with the BD distance subtracted, and a division by 2:

$$BD \text{ to } A= 2.3 + 2.9 – 0.6/ 2= 2.3$$

$$BD \text{ to } C= 10.6 + 10 -0.6/ 2= 10$$

$$BD \text{ to } E= 3.6 + 3 – 0.6/ 2= 3$$

An updated matrix is produced:

	A	C	E	BD	Sum
A	0	12.9	5.9	2.3	21.1
C	12.9	0	7	10	29.9
E	5.9	7	0	3	15.9
BD	2.3	10	3	0	15.3

The shortest distance connects A to BD, and the algorithm would continue as above and proceed until the tree is fully resolved, and then rooted with the outgroup.

3.10 Outgroups

In phylogenetic analysis a selection of ingroup taxa of interest are studied to understand the patterns of monophyly and evolutionary relationships between taxa in the group of interest (Maddison et al. 1984). Typically, characters, whether molecular or morphological, are analysed to understand polarity- which character states are ancestral or "primitive" and gave rise to character states that evolved more recently and are considered "derived". The inference of polarity and evolutionary relationships of groups being studied requires the use of an outgroup that falls outside the group of study (Colless 1995). Understanding polarity helps to infer relationships between groups that requires the least amount of evolutionary novelty and convergent evolution, with an emphasis on derived traits shared between groups inferring ancestry that is more recent (Maddison et al. 1984).

The hypothesis of polarity can use an ontogenetic method, patterns of evolution within ingroups, or use of an outgroup that falls outside the ingroup taxa of interest (Maddison et al. 1984). There is disagreement as to whether one or multiple outgroups should be used, and whether the outgroup ought to be the closest sister group to ingroup taxa or not (Nixon & Carpenter 1993). If an outgroup is too distant to the ingroup, long branch attraction can occur whereby the outgroup and an ingroup are both very distant and divergent from all other ingroup taxa, with the two divergent lineages drawn together even though they may not share similarity (Sanderson & Shaffer 2002). Also, if the sister taxon to the ingroup being studied is highly divergent, a more distant but less divergent outgroup will be more appropriate (Sanderson & Shaffer 2002).

The language of cladistics can be transferred to that of distances to a degree: the use of an outgroup provides polarity for inferring phylogenetic relationships, and for some methods trees are inferred according to the least amount of change across the tree (parsimony). Where an outgroup, or collection of outgroups, display a character state, it is viewed as the ancestral primitive form for the ingroup taxa, which is no different in a distance-based approach. In the discussion of results the morphological connection between members of a clade is often referred to as derived, and that of basal lineages as primitive, which has the same meaning here as it does in a cladistic analysis.

More generally, there can be a theoretical, rather than practical, problem when living/extant outgroups are viewed as representing primitive ancestral forms. This is common in palaeoanthropology where chimpanzees are used as a proxy for the ancestral phenotype for the shared ancestor of *Pan-Homo*, ignoring the often highly derived nature of morphology displayed by the outgroup. More widely this can lead to gibbons and siamangs being viewed as primitive compared to highly evolved apes and humans, ignoring the complex derived morphology of the gibbons or siamangs, with the same problem when comparing hominoids to monkeys, or anthropoids to strepsirrhines. For the phylogenetic analysis described in this project, multiple outgroups (either by themselves or in combinations) are used to examine how consistent inferred phylogenies are. By using representatives of multiple major primate clades no assumptions are made about which outgroup is least derived or similar to an ancestral phenotype.

The outgroup taxa selected for phylogenetic analysis in this project include one ape, four Old World monkeys and four strepsirrhines, including *Hylobates lar*, *Macaca mulatta*, *Chlorocebus aethiops*, *Colobus guerza*, *Trachypithecus obscurus*, *Otolemur garnetti*, *Galago senegalensis*, *Eulemur fulvus* and *Perodicticus potto*. The ape taxa is the lar gibbon, *Hylobates lar*, found in south-eastern Asia, that are relatively monomorphic, have a size range of 4.5-7.5 kg, and a mainly frugivorous diet (50-71%) with some folivory and insectivory (Bartlett 2007). Gibbons are arboreal, exhibiting a mix of brachiation, leaping and bipedal locomotion, and are behaviourally territorial, pair bonded, and use regular vocal displays (Bartlett 2007). From the Old World monkeys two taxa are taken from each of the two major radiations; *Macaca mulatta* (macaque) and *Chlorocebus aethiops* (guenon) from the Cercopithecinae, and *Colobus guerza* (guereza)and *Trachypithecus obscura* (leaf monkey) from the Colobinae. The vervet monkey, *Chlorocebus aethiops*, has a wide sub-Saharan distribution, with gum, leaves, fruit, seeds and flowers all contributing to a varied diet (Enstam & Isbell 2007). Rhesus macaques, *Macaca mulatta*, have an average male weight of 11kg and female weight of 8.1kg, with a flexible diet including variable levels of fruit, leaves and seeds depending on seasonal availability (Thierry 2007). *Trachypithecus obscurus* are Asian leaf eating monkeys, with a highly folivorous diet and an average weight for females of 6.1kg and males of 7.5kg (Kirkpatrick 2007, Fleagle 1999). *Colobus guerza* are an African colobine, around 8kg in body weight, with folivory ranging from 50-80% and varying amounts of fruit in the diet (Fashing 2007, Milton & May 1976).

From the strepsirrhines, two Galaginae genera were sampled- *Otolemur garnetti* and *Galago senegalensis*, a lemur *Eulemur fulvus* and a loris *Perodicticus potto*. *Eulemur fulvus* is a lemur within the infraorder Lemuriformes, which is diurnal, with an average weight of 2.2kg, a largely frugivorous diet, and locomotion by quadrupedal walking and leaping (Gould & Sauther 2007). *Perodicticius*, *Galago* and *Otolemur* are all African, arboreal nocturnal primates in the infraorder Lorisiformes. *Galago* and *Otolemur* fall within the family Galagidae, whilst *Perodicticius* is in the Lorisidae family. *Perodicticus potto* has an average size of 1.5 kg, a mixed diet of around 50% fruit and 40% animal prey, and slow climbing locomotion (Nekaris & Bearder 2007). *Otolemur garnetti* are on average 0.8kg, with a diet 50% animal prey and 50% fruit, and locomotion mixed between quadrupedal running, leaping and bipedal hopping (Nekaris & Bearder 2007). *Galago senegalensis* have an average male body weight of 180g in males and 160g in females, with a mixed exudativory-insectivory diet (Nekaris & Bearder 2007, Harcourt 1986).

Phylogenetic analysis was repeated for the entire platyrrhine group (chapter 4) and atelid (chapter 5), pitheciid (chapter 6) and cebid (chapter 7) clades using each of the nine individual outgroups. Phylogenetic analysis was also repeated using multiple outgroups in a series of combinations, as the general consensus is that phylogenetic analysis is helped by the use of multiple outgroups. Note that an outgroup combination does not mean that different taxa are combined into one single, non-existent morphotype, but refers to combinations of different taxa included in the same analysis as outgroups. Unless otherwise stated in the respective results sections, rooting the phylogenetic tree with any of the alternative outgroups does not alter tree topology. Outgroup pairings used in phylogenetic analysis included *Chlorocebus-Macaca* (Cercopithecinae), *Trachypithecus-Colobus* (Colobinae), *Galago-Otolemur* (Galaginae) and *Eulemur-Perodicticius* (non-Galaginae strepsirrhines). Larger combinations of monophyletic groups were also used for Old World anthropoids (including *Hylobates*, *Chlorocebus*, *Macaca*, *Colobus* and *Trachypithecus*), Old World monkeys (*Chlorocebus*, *Macaca*, *Colobus* and *Trachypithecus*), strepsirrhines (*Perodicticius*, *Eulemur*, *Galago* and *Otolemur*) and all nine outgroups together. In the case of this latter outgroup combination, the rooting of the outgroup often affected ingroup topology, leading to numerous alternative phylogenies being inferred in the results sections of several chapters.

3.11 Craniodental regions and modularity

Studies of modularity in the primate skull have provided relatively strong support for the presence of distinct, semi-autonomous craniodental modules. Cheverud (1982) examined the neurocranium and orofacial regions, with subdivision into frontal, parietal, occipital, nasal, orbital and oral regions. Cheverud (1995, 1996a), Ackermann & Cheverud (2000), Marroig & Cheverud (2001), Marroig et al. (2004b, 2009), Gonzalez-Jose et al. (2004), Porto et al. (2009) and Shirai & Marroig (2010) settled on the use of oral, nasal, orbit, zygomatic, cranial vault and cranial base regions. Correlation results (e.g. Marroig & Cheverud 2001) show that traits within these functional-developmental modules have greater integration than traits from separate modules, justifying the treatment of modules as somewhat autonomous units.

The regions originally proposed by Cheverud (1995) are divided according to those derived developmentally from cranial neural crests and the viscerocranium that form the face, or those of paraxial mesoderm that forms the cranial base and vault. The comparative modularity work of Hallgrimsson et al. (2004), which formed the basis for the phylogenetic work of Cardini & Elton (2008), provided further evidence that modules of the dermatocranium and chondrocranium, face, basicranium and neurocranium were also present in addition to those of the palate, temporal, orbit and zygomatic. Using a distinctly different method, Goswami (2006a) found strong support for the presence of six modules in the mammalian skull: basicranium, cranial vault, zygomatic-pterygoid, molar, orbital and anterior oral-nasal regions.

All of these studies have provided extensive evidence for the presence of modularity within the primate, and more generally, mammalian, skull. However, Cardini & Elton (2008) raised a particularly troubling issue relating to error. They found that modules described by fewer than 20 landmarks had much higher standard error associated with matrix correlations than those regions described by 30 or more landmarks. In a preliminary pilot study of atelids, where phylogenetic trees were generated from geometric morphometric data for distinct modules, the trees derived from modules described by under 10 landmarks failed to maintain monophyly in many genera. Interpreted in light of the error observation of Cardini & Elton (2008), these pilot results appear to support the problem of error and low landmark number when integrating phylogenetic studies with modularity. Unfortunately, as a result, the modular approach taken with this project is highly restricted and uses only two major regions.

As is explained below, although the cranial vault is clearly well supported as a module, the relative lack of landmarks makes adequately quantifying the region especially difficult.

3.12 Modules used in phylogenetic analysis

The approach taken towards modularity in this project is the use of two major modules, in addition to landmarks describing overall morphology of the entire craniodental region. One can simplify the embryological origins of the skull into three distinct units- the face, which develops from the splanchnocranium with additional development of dematocranial parts, the basicranium, which develops from the chondrocranium, and the neurocranium, consisting of the dermatocranial bones (Hallgrimsson et al. 2007, Lieberman et al. 2000a).

The first hypothesised module used for phylogenetic analysis is that of the face; ontogenetically the face is distinct and Marroig & Cheverud (2001) have shown that the patterns of integration in the platyrrhine face are especially strong. The facial module includes the first 15 landmarks from the list of anatomical points, and are graphically illustrated in Figure 18. This region does not include landmarks related to the teeth, largely because of uncertainty as to whether the strong modular support for the face would be detrimentally affected by joining it with the teeth, which themselves likely form a separate module and have a unique ontogenetic trajectory. The facial module also contains a potentially controversial landmark from the optic foramen (landmark 15), which is included due to the importance of the eye to orbital and facial morphology.

The second module is described here as the basicranium(landmarks 48 to 71) and are illustrated graphically in Figures 19 and 20. The basicranium has a partial developmental independence (Hallgrimsson et al. 2007), and has a significant pattern of integration in platyrrhines (Goswami 2006a), justifying treatment of the region as a semi-autonomous, independent module. The landmarks used to quantify the basicranium nearly exclusively belong to the temporal and occipital bones, with only the basal portion of the temporal bone sampled.

The ideal third module, present as a distinct unit in embryological development (Hallgrimsson et al. 2007) and used in much of the modular work previously described, is the cranial vault. However, there are two major problems with including this module in phylogenetic analysis. Cranial vault morphology is particularly difficult to quantify, with only around seven landmarks present, and preliminary analysis on atelids found particularly

low levels of monophyly based on phylogenetic analysis of this region. As a result, a third module for the cranial vault was not used even though it is recognised as a distinct module. In addition to the modules of the face and basicranium, phylogenetic analyses was also carried out on the combined set of skull landmarks that requires no hypotheses of modularity, including cranial and dental traits for 63 landmarks. In phylogenetic analysis of the platyrrhines (chapter 4), atelids (chapter 5), pitheciids (chapter 6) and cebids (chapter 7) each chapter presents results for the whole skull, face and cranial base. Each are repeated for all outgroups and outgroup combinations, and for four data partitions based on sex.

3.13 Male, female, pooled and separate sex analyses

Until recently, phylogenetic analysis based on morphology had tended to ignore sexual dimorphism and its potentially confounding effect on analyses. Lockwood et al. (2004) investigated hominoid relationships, comparing a molecular phylogeny to that derived from temporal bone geometric morphometric data. Due to the sexual dimorphism exhibited in gorillas and orangutans, they chose to analyse male and female data separately. Of the four phylogenetic trees generated, three were congruent with the molecular tree, but the female tree generated by the least-squares phylogenetic method was slightly incongruent due to the placement of the *Pan paniscus* taxon. Further testing of hominoid morphometric data by Bjarnason et al. (2011), both temporal bone geometric morphometric and craniodental linear measurements, found that sexual dimorphism, and the splitting of morphological data according to sex, had little to no effect on the accuracy of phylogenetic inference. Phylogenetic analysis of guenons by Cardini & Elton (2008) also separated male and female specimens, where observed matrix correlations between genetic and morphological distances for male and female data showed quite large differences. This supported analysing male and female data separately for phylogenetic analysis, although they did not provide results for matrix correlations between molecular and morphological distances when sex was pooled.

Work on papionins by Gilbert & Rossie (2007), Gilbert et al. (2009) and Gilbert (2011) provided examples of how sexual dimorphism can have a large effect on phylogenetic analysis. Papionins exhibit large sexual dimorphism, with Gilbert & Rossie (2007) highlighting the problem of male and female morphologies that are extensively divergent being pooled into a single, non-existent morphology. Gilbert & Rossie (2007) used an allometric coding method to assign character codes for use in phylogenetic analysis, and

found that phylogenetic analysis of male and pooled-sex specimens were congruent with the molecular phylogeny but not the female data.

In a more extensive study, integrating extra craniometric data and qualitative characters in addition to a new character coding method, Gilbert et al. (2009) found that although there was greater agreement between male and female analyses the latter had much lower bootstrap support for molecular congruent clades. In the case of papionins, sexually dimorphic males retained a stronger phylogenetic signal. The authors advocated phylogenetic analysis based on morphology that keeps male and female data separate, avoiding the problem of creating a phantom morphotype with pooled-sex data, potentially carrying out analyses where male and females are included in the same analyses but coded as separate taxa. Separating species that contain interbreeding individuals, with the obvious genetic homogeneity of being in a single (potential) breeding group, seems difficult to justify but sexual dimorphism must have some genetic basis which could support the idea of dividing the two groups. The separation of specimens by sex could potentially have one of two effects- to provide a greater amount of information that is phylogenetically informative or lead to repetition and weighting with a presumably negative effect on the accuracy of phylogenetic inference (Gilbert et al. 2009). Gilbert (2011) extended the phylogenetic analysis of papionins with distance-based analysis of the basicranium, with the result that male and female data inferred slightly different phylogenetic trees.

Considering the papionin and guenon cases, it seems clear that it is important to run four types of phylogenetic analysis for this project: male-only, female-only, pooled-sex, and a combined analysis with treatment of male and female specimens as separate taxa. Although levels of sexual dimorphism are lower in platyrrhines, with the potential problem of creating a non-existent morphotype by pooling of specimens less likely, it is an important area that needs to be fully tested. Note that for the phylogenetic analysis of the entire platyrrhine group (chapter 4), only pooled sex data were used, because phylogenetic analysis produced such a large number of trees that it would otherwise have been impossible to present and discuss all the results within the limits of this dissertation.

3.14 Error

3.15 Outliers

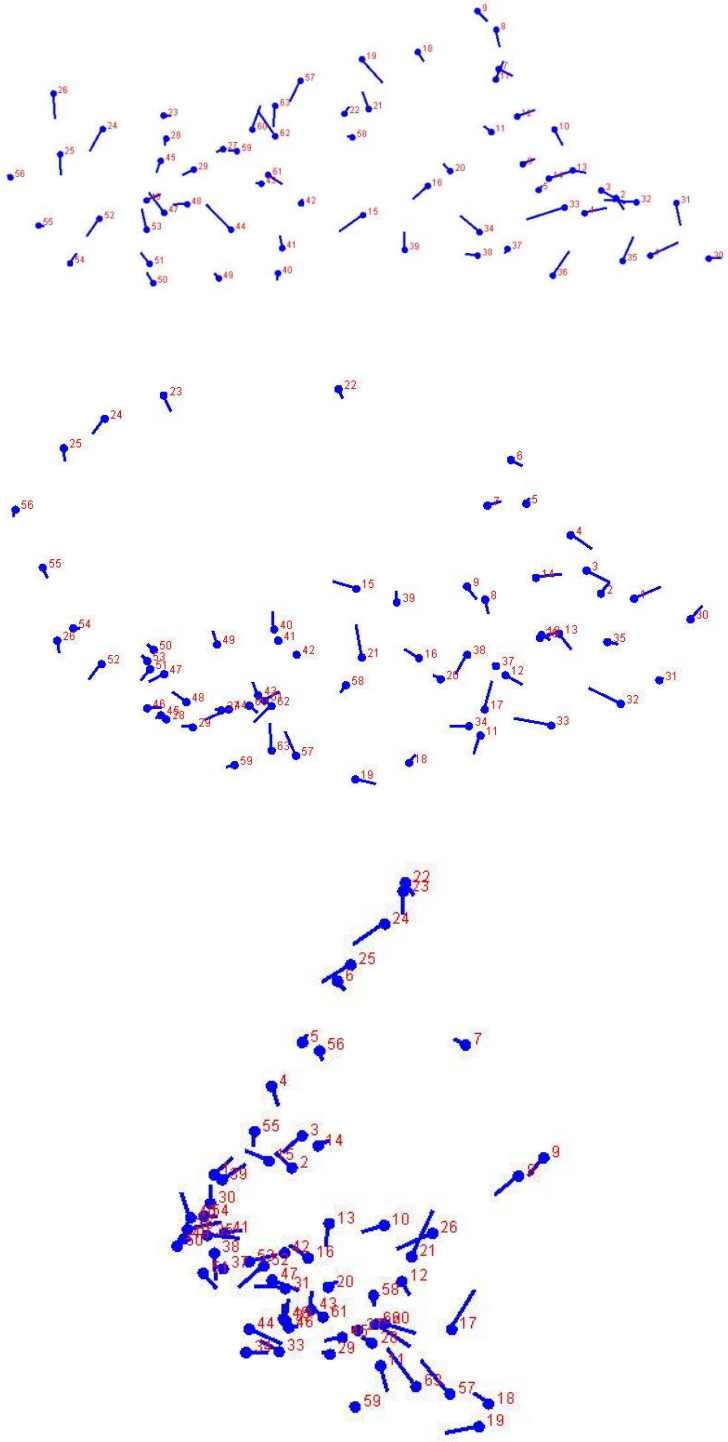
As phylogenetic analysis of geometric morphometric data requires the use of taxa mean shapes, it was important to ensure that digitised specimens with landmarks incorrectly placed were either corrected or removed. This was analysed with Morphologika (O'Higgins & Jones 2006) where the position of coordinates transferred onto the axis of a principal component analysis (PCA) can be examined. It is clear on the visualised PCA when an individual has one or several landmarks that cause it to fall far outside the group mean. Where possible, such specimens were redigitised and maintained in the dataset, although occasionally this was not possible due to the time overlap between digitising individuals and examining the data. PCA's were generated with all taxa and specimens present, with all specimens of a single genus, and with all specimens of a single species. This may be considered an arbitrary attempt at resolving the problem of outlier-based error, but it did ensure obvious mistakes were noticed and resolved.

3.16 Tests of landmark error

There is currently no single, accepted method to measure landmark repeatability or to identify which anatomical points can (and cannot) be located within an accepted degree of error. Several exploratory approaches were used on a dataset with a single *Lagothrix poeppigii* specimen digitised 10 times on the left side of the skull. By using a single specimen, the variation measured should correspond to error associated with landmark repeatability. For the first approach, Procrustes analysis and principal component analysis were used to view landmark variation. Principal component analysis is a useful statistical tool that takes the entirety of a dataset and extracts smaller packs of information to describe overall patterns of variation. With geometric morphometric data this allowed a range of morphological data to be condensed and visualised to show which landmarks were especially variable. The principal component analysis was carried out in MorphoJ (Klingenberg 2011), which has an option to visualise the first principal components as lines moving from the position of each landmark. Procrustes analysis of the original full 72 landmark dataset for 10 repeated measurements of the same *Lagothrix poeppigii* specimen produced the visualisation in Figure 23. From this PCA it was clear that landmark 29, a midway point between two other landmarks, was considerably more variable than any other landmark. Although there were clearly some landmarks that vary little, especially those associated with teeth septa,

landmarks with higher variation displayed a consistent level of variation and their repeatability was not of concern.

Figure 23 Visualised first principal component for 72 landmarks based on repeated sampling of a single specimen, with axis 1 against axis 2 (top), axis 1 against axis 3 (middle) and axis 2 against axis 3 (bottom)



An alternative approach to assessing landmark error was to examine the average standard deviation for the x-y-z coordinates of each landmark. This was based on the premise that an unreliable landmark would show greater variation in its coordinates when compared to other landmarks, or potentially greater variation in repeats of the same specimen when compared to a taxon-level of variation. When this rough measure of variation/error was generated from repeats of the same specimen, the average landmark standard deviation was ordered to see whether any landmarks stood out as having larger variation than the rest of the dataset. The results from 10 repeats of a *Lagothrix poeppigii* specimen are shown in Table 9. Landmark 29 was the only anatomical point that appeared to have a greater variability than the rest of the landmarks.

When the average standard deviation of landmarks were compared for repeats of the same specimen (within-specimen variation) against the variation within-taxa, a comparison was made to three groups: a species sample of *Lagothrix poeppigii*, a wider *Lagothrix* group with all specimens from four species, and a combined dataset with the single specimen repeats added to the whole *Lagothrix* group so that all data was combined into a single analysis. Any reliable landmark ought to vary less when measured on the same specimen than between separate specimens; results are shown in Table 10. In no case did any landmark measured in the same specimen have a greater average standard deviation than that observed in the *Lagothrix poeppigii*, entire *Lagothrix* or combined dataset groups. There does appear to be increased variation for several landmarks in *Lagothrix poeppigii* compared to the entire *Lagothrix* or combined dataset groups. The reduced sample size of looking at one species means that a single outlier individual could be skewing the position of landmarks and increasing the overall standard deviation in the group. Although these proposed methods may be considered subjective, they are preferable to the common alternative whereby landmark repeatability is not considered or reported at all.

Table 9: Average x-y-z standard deviation for each landmark in a single *Lagothrix* specimen

Landmark number	Average standard deviation	Landmark number	Average standard deviation	Landmark number	Average standard deviation
29	0.00362	59	0.00125	24	0.00095
62	0.00234	15	0.00123	3	0.00095
30	0.00232	23	0.00119	9	0.00094
31	0.00231	16	0.00118	57	0.00094
32	0.00223	58	0.00118	12	0.00093

28	0.00212	54	0.00117	51	0.00092
63	0.00179	67	0.00117	44	0.00092
60	0.00177	56	0.00114	2	0.00089
64	0.00167	25	0.00113	10	0.00088
65	0.00166	17	0.00112	33	0.00085
55	0.0016	22	0.00112	6	0.00085
69	0.00154	7	0.00111	36	0.00084
19	0.00146	39	0.0011	34	0.00083
52	0.00144	45	0.00106	38	0.00082
72	0.00143	40	0.00106	35	0.00081
50	0.0014	66	0.00106	37	0.0008
43	0.00139	42	0.00102	21	0.0008
71	0.00136	13	0.001	11	0.00078
70	0.00136	46	0.00099	4	0.00076
53	0.00135	1	0.00098	20	0.00073
26	0.00134	61	0.00098	48	0.0007
68	0.00132	41	0.00097	5	0.00069
8	0.00129	14	0.00096	49	0.00067
27	0.00126	18	0.00096	47	0.00065

Table 10: Comparison of average landmark standard deviations in four datasets

Landmark no.	**Same specimen**	***Lagothrix*** *poeppigii*	**All *Lagothrix* specimens**	**Repeats & all specimens**	**Difference between same specimen and *L. poeppigii***
1	0.00098	0.00297	0.00338	0.00357	0.00199
2	0.00089	0.00301	0.0033	0.00342	0.00212
3	0.00095	0.00259	0.00307	0.00333	0.00164
4	0.00076	0.00233	0.00328	0.00397	0.00157
5	0.00069	0.00304	0.00302	0.00309	0.00235
6	0.00085	0.00405	0.00394	0.0039	0.0032
7	0.00111	0.00513	0.00573	0.00562	0.00402
8	0.00129	0.00378	0.00435	0.00416	0.00249
9	0.00094	0.00602	0.00552	0.00547	0.00508
10	0.00088	0.0029	0.00395	0.00374	0.00202
11	0.00078	0.00375	0.00385	0.00377	0.00297
12	0.00093	0.0038	0.00372	0.00363	0.00287
13	0.001	0.00394	0.00398	0.00396	0.00294
14	0.00096	0.0034	0.0031	0.00312	0.00244
15	0.00123	0.00319	0.00328	0.00333	0.00196
16	0.00118	0.00309	0.00333	0.00359	0.00191
17	0.00112	0.00446	0.00377	0.00409	0.00334
18	0.00096	0.00446	0.00446	0.00537	0.0035

19	0.00146	0.00412	0.00473	0.00543	0.00266
20	0.00073	0.00504	0.00457	0.00467	0.00431
21	0.0008	0.00385	0.00501	0.00517	0.00305
22	0.00112	0.00504	0.00515	0.00509	0.00392
23	0.00119	0.00529	0.00537	0.00551	0.0041
24	0.00095	0.0054	0.00521	0.00517	0.00445
25	0.00113	0.00586	0.00643	0.00651	0.00473
26	0.00134	0.00468	0.00489	0.00547	0.00334
27	0.00126	0.00619	0.00598	0.00963	0.00493
28	0.00212	0.00377	0.00413	0.00408	0.00165
29	0.00362	0.00763	0.00734	0.00794	0.00401
30	0.00232	0.00248	0.00288	0.00293	0.00016
31	0.00231	0.00239	0.00296	0.00309	8E-05
32	0.00223	0.00244	0.00282	0.00295	0.00021
33	0.00085	0.00316	0.00345	0.00357	0.00231
34	0.00083	0.00289	0.00317	0.00352	0.00206
35	0.00081	0.00327	0.00336	0.00345	0.00246
36	0.00084	0.00279	0.00276	0.00278	0.00195
37	0.0008	0.00275	0.00264	0.00261	0.00195
38	0.00082	0.00304	0.00265	0.00259	0.00222
39	0.0011	0.00299	0.00275	0.00268	0.00189
40	0.00106	0.00333	0.0029	0.00293	0.00227
41	0.00097	0.00385	0.00352	0.00356	0.00288
42	0.00102	0.00403	0.00368	0.00399	0.00301
43	0.00139	0.00246	0.00267	0.00264	0.00107
44	0.00092	0.00263	0.00303	0.00293	0.00171
45	0.00106	0.00315	0.00453	0.00444	0.00209
46	0.00099	0.00342	0.00341	0.00337	0.00243
47	0.00065	0.00647	0.00625	0.00618	0.00582
48	0.0007	0.00271	0.00289	0.00283	0.00201
49	0.00067	0.00284	0.00299	0.00288	0.00217
50	0.0014	0.00313	0.00309	0.0032	0.00173
51	0.00092	0.00368	0.00323	0.00326	0.00276
52	0.00144	0.00536	0.00683	0.0067	0.00392
53	0.00135	0.002	0.00294	0.00296	0.00065
54	0.00117	0.00487	0.00454	0.00452	0.0037
55	0.0016	0.0042	0.00365	0.00365	0.0026
56	0.00114	0.00326	0.00375	0.00384	0.00212
57	0.00094	0.00278	0.00277	0.00274	0.00184
58	0.00118	0.00317	0.00319	0.00309	0.00199
59	0.00125	0.00316	0.00336	0.00321	0.00191

60	0.00177	0.00317	0.00304	0.00316	0.0014
61	0.00098	0.00342	0.00339	0.00332	0.00244
62	0.00234	0.00326	0.00342	0.00344	0.00092
63	0.00179	0.00344	0.00373	0.00395	0.00165
64	0.00167	0.0049	0.00546	0.00566	0.00323
65	0.00166	0.00273	0.00332	0.0037	0.00107
66	0.00106	0.00387	0.00383	0.00364	0.00281
67	0.00117	0.00296	0.00318	0.00381	0.00179
68	0.00132	0.00371	0.00339	0.00336	0.00239
69	0.00154	0.00338	0.00335	0.00344	0.00184
70	0.00136	0.00306	0.00306	0.00315	0.0017
71	0.00136	0.00353	0.00356	0.00352	0.00217
72	0.00143	0.0106	0.00986	0.01092	0.00917

3.17 Removal of landmarks

The initial landmark list included 72 anatomical points. Dental traits were originally measured for each of the septum separating teeth and a landmark at the end of the dental arcade, but due to the absence of teeth in some groups the ten landmarks were replaced by five. These five landmarks related to the septum of the I1 incisor, the canine, the P2 premolar, the M1 molar and a midpoint at the end of the dental arcade. This allows comparison of primates with a range of dental formulas, as the callitrichids have 2-1-3-2, other platyrrhines 2-1-3-3, and the Old World anthropoid outgroups 2-1-2-3. Two anatomical landmarks (21 and 23) around the pterion were removed as homologous points were absent in outgroups; throughout the primates only the connection between the sphenoid, parietal and zygomatic portion of the temporal bone were homologous. *Alouatta* also has restructuring of this region unique for a platyrrhine and as found in Old World monkeys, so the pterion landmarks were not completely homologous within the platyrrhine clade. A midway landmark (number 29) placed between one of the pterion landmarks and the lambda was also removed, both because of the problem measuring it in *Alouatta* and the previous evidence of high levels of error in its measurement. A final landmark (number 72) for cranial cresting was removed due to the observation of very large within-taxa variation indicating questionable anatomical stability. With the removal of these nine landmarks (numbers 21, 23, 29, 34, 37, 38, 40, 41 and 72), 63 landmarks remained and were used to describe craniodental variation in the platyrrhine skull for this project. When *Perodicticus potto* was used as an outgroup landmark 20 was also removed, and landmark 12 was removed when either galago taxon was used.

3.18 Sample error

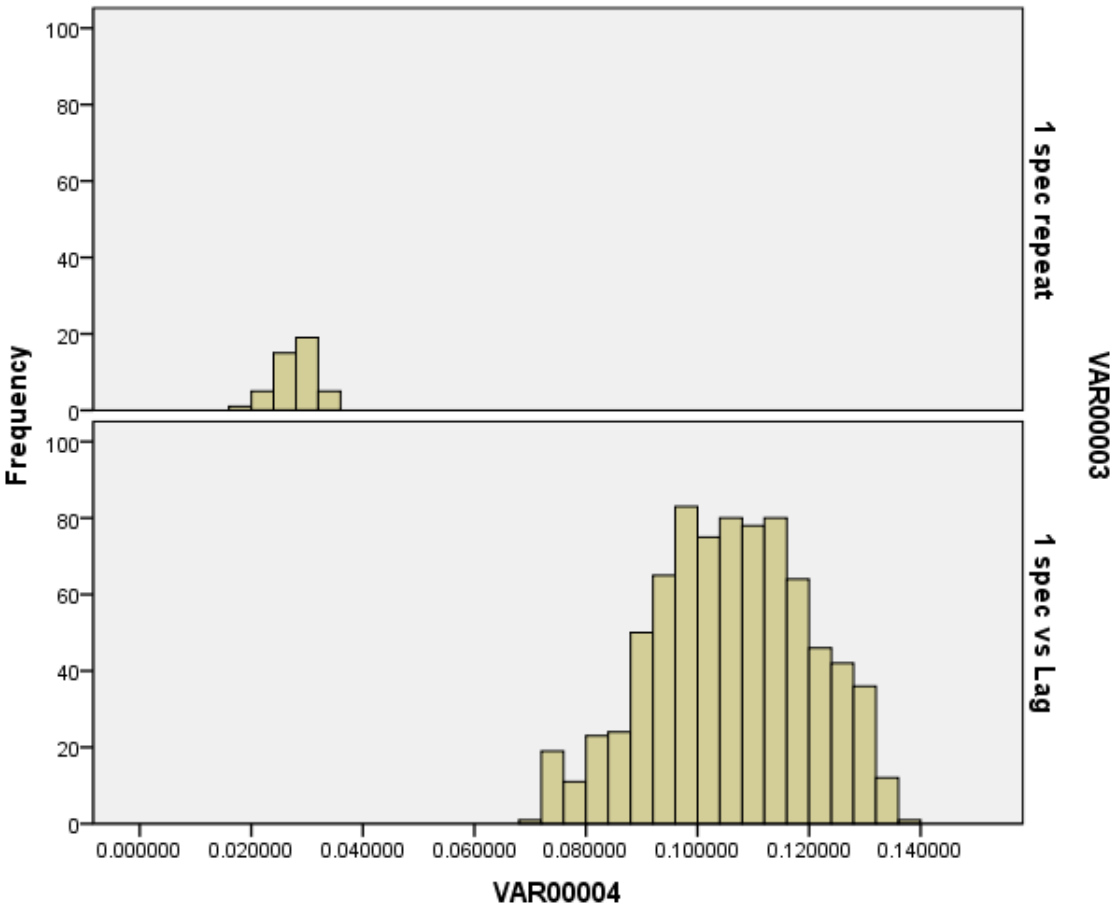
It is necessary to assess the extent to which shape data collected will be affected by individual sampling error. One approach to this problem is that of Lockwood et al. (2002), whom compared within-specimen to between-specimen variation to act as a measure of intra-observer error. This approach used shape, as described by Procrustes residuals, to calculate Euclidean distances between repeats of the same specimen (within-specimen variation) and Euclidean distances between different specimens from the same genera. Based on the 63 landmark dataset, a single *Lagothrix* specimen was compared to repeats of that individual and 89 *Lagothrix* specimens. The Euclidean distances were plotted in a bar chart in SPSS 17.0 as shown in Figure 24, showing considerably lower Euclidean distances between repeats of the same specimen compared to the distances derived from comparing the single individual to other *Lagothrix* specimens. These results show that it is highly unlikely that intra-observer bias would skew data collection and mean shape estimates used in phylogenetic analysis.

Polly (2001), applying the method of Bailey & Byrnes (1990) also compared variation within and between specimens, whereby variation due to measurement error was quantified as a percentage. The equation is:

Percentage measurement error= 100 x (within-sample error/(within-sample error x between-sample error))

Measuring within-sample error as the average standard deviation of coordinates from the same *Lagothrix* specimen sampled ten times, and between sample error as the average standard deviation from all *Lagothrix* specimens, the percentage measurement error was 7.2%. This means that in the population measured 7.2% of variation was measurement error, and the remaining 92.8% of variation related to differences between individuals sampled. This amount of sampling error is below that measured by Polly (2001), similar to that of Cardini & Elton (2008), and is relatively low and not expected to have a serious effect on subsequent analyses.

Figure 24 Average Euclidean distances between specimen repeats and within taxon variation



Euclidean distances between repeated measurement of the same specimen (top) and between *Lagothrix* individuals

3.19 Distances and spaces

Euclidean and Procrustes distances are both measured as the square root of the sum of squared distances between two configurations of landmarks, but Euclidean distances are measured within linear Euclidean tangent space and Procrustes distances within the curved Kendall's shape space (Zelditch et al. 2004, Polly 2001, Cardini & Elton 2008). In TPSsmall it is possible to generate a bivariate plot of Euclidean distances in tangent space against Procrustes distances in shape space (Rohlf 1999a). The relationship between the two distances tests whether variation in data is small enough for tangent space to be used as an estimate, or approximation, of data in shape space (Rohlf 1999a). Previously Marcus et al. (2000) showed that even with mammal skulls where maximum distance between landmarks

ranged from 31mm in the smallest skull to 498mm in the largest skull, variation was still small enough for Euclidean distances to be used as an approximation of Procrustes distances. In MorphoJ (Klingenberg 2011), Procrustes analysis of the separate-sex dataset of 63 landmarks (with *Perodicticus potto* and galagonids removed due to missing landmarks 12 and 20) generated mean shapes for each taxon, which were transferred into Ntys file format and loaded into TpsSmall. Here Euclidean and Procrustes distances were generated and correlated (see Figure 25), with results showing that variation is sufficiently low to allow for the use of data in tangent space as an approximation for shape space with the distances highly correlated:

Statistic Procrustes d Tangent d

Min 0.053342 0.053316

Max 0.245361 0.242907

Mean 0.103609 0.103337

Regression through the origin for distance in tangent space, Y, regressed onto Procrustes distance (in radians), X

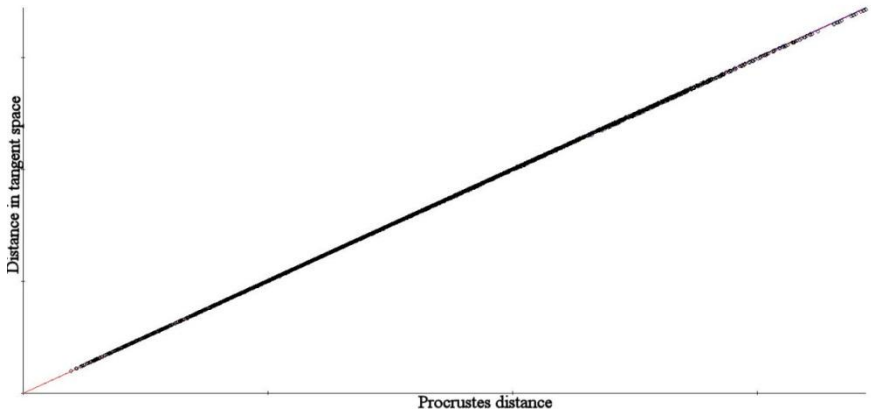
Y-intercept: 0.000000

Slope: 0.996570

Correlation (uncentered): 0.999997

root MS error: 0.000064

Figure 25 Correlation between Procrustes and Euclidean distances



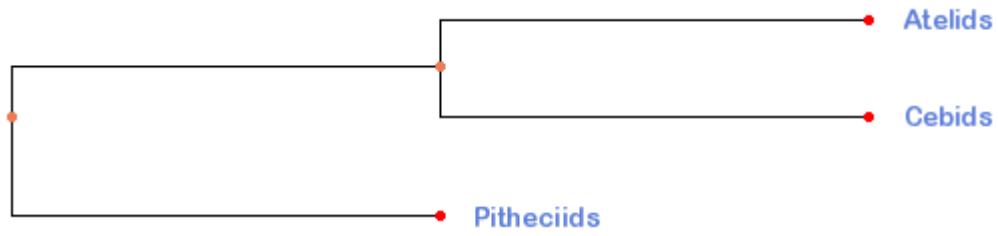
Chapter 4 Platyrrhine phylogenetic analysis

4.1 Introduction

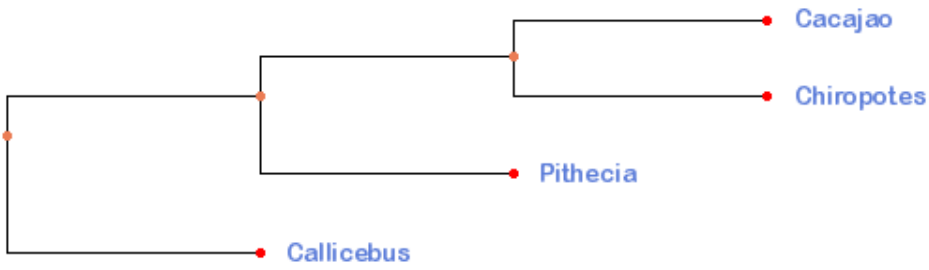
The evolutionary relationships of platyrrhines have been inferred using both morphological and molecular data (e.g. Rosenberger 1984, Ford 1986, Kay 1990, Schneider et al. 1993, Schneider 2000, Kay et al. 2008, Perelman et al. 2011), with the two sources of data combined in several analyses (e.g. Horovitz 1999, Kay et al. 2008). Platyrrhine molecular phylogenetic relationships were reviewed in chapter 2 and are reproduced in Figure 26, supporting three clades for pitheciids (*Callicebus*, *Pithecia*, *Cacajao* and *Chiropotes*), atelids (*Alouatta*, *Ateles*, *Brachyteles* and *Lagothrix*), and cebids, that are subdivided into callitrichines (*Saguinus*, *Leontopithecus*, *Callimico* and *Callithrix*), cebines (*Cebus* and *Saimiri*) and owl monkeys (*Aotus*), with the cebids and atelids sister clades. The aim of this chapter is to provide a synthesis of past morphology-based phylogenetic analyses and present new phylogenetic analysis of the platyrrhine clade. The methods used, previously described in chapter 3, used a combination of distance-based phylogenetic analysis with geometric morphometric data for the entire platyrrhine clade, the first application of these methods to the phylogenetic inference of platyrrhines. Phylogenetic analyses were repeated for morphology of the whole skull and modules of the face and cranial base to ascertain whether alternative phylogenetic signals were maintained in different craniodental regions. The results of phylogenetic analyses are interpreted in comparison to both the accepted molecular phylogenetic relationships and past morphology-based phylogenetic analyses, with consideration of the biological factors that contribute to platyrrhine craniodental morphology and may affect accurate phylogenetic analysis.

Figure 26 Consensus molecular phylogenetic relationships of platyrrhines

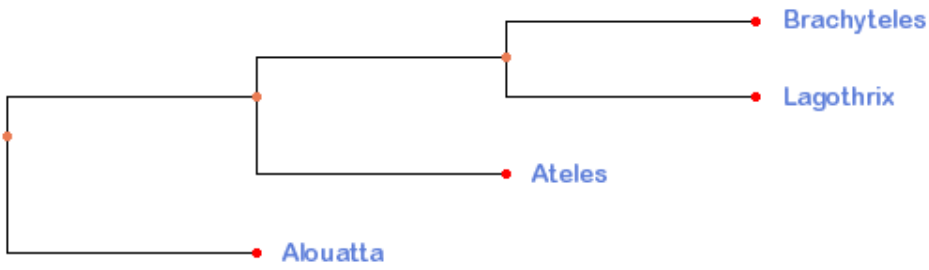
a) Phylogenetic relationships between the three family clades



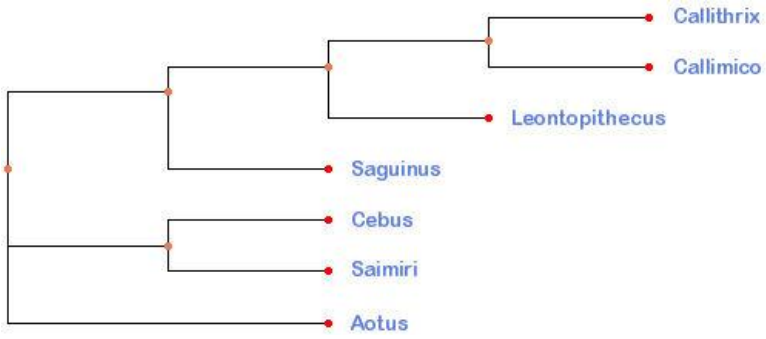
b) Phylogenetic relationships within the pitheciids



c) Phylogenetic relationships within the atelids



d) Phylogenetic relationships within the cebids



The classical view of platyrrhine evolutionary relationships based on morphology, championed by Hershkovitz (1977), recognized two grades separated by size- one for callitrichines and another for all remaining taxa, the Cebidae (Ford & Davis 1992, Rosenberger 1980). The clawed callitrichines were separate from nailed cebids, with the latter split into an intermediate callitrichine-cebid grade for *Aotus*, *Saimiri* and *Callicebus*, and true cebids including saki-uakaris, *Cebus* and atelids (Rosenberger 1980). The central tenet of the size-based separation was that callitrichines are primitive and retained the ancestral platyrrhine phenotype, whereas substantial evidence supports callitrichine size reduction as derived linked to several unique traits- loss of the third, evolution of claws, twinning, and exudativory (feeding on gum) in several taxa (Ford 1980, Rosenberger 1980, Martin 1992). Both molecular and morphological analyses acknowledged that several taxa

placed in Cebidae were more closely related to callitrichines, rejecting a phylogenetic split between platyrrhines by body size, with the new Cebidae family incorporating a molecular phylogenetic clade of callitrichines, owl monkeys and cebines (Schneider & Rosenberger 1996).

Three major morphology-based phylogenetic analyses by Rosenberger (1984), Ford (1986) and Kay (1990) preceded the molecular phylogenetic revolution and are the major comparative studies to the phylogenetic analyses presented in this thesis. Rosenberger (1984) used a "synthetic" approach to character analysis and phylogenetic inference that maintained cladistic principles but did not use an actual algorithm, whereas Ford (1986) and Kay (1990) used cladistic computer programs to infer phylogenetic relationships. The synthetic approach used ingroup-outgroup comparisons to examine the morphocline of traits, involving a-priori judgements on homology with morphological comparison to the wider primate group (Rosenberger & Strier 1989). The dataset of Rosenberger (1984) consisted of mostly cranial and dental characters, many of which were used by Ford (1986), who added extra characters mostly relating to postcranial morphology. Kay (1990) also used some traits from Rosenberger (1984), but added a large range of dental characters (Ford & Davis 1992, Schneider & Rosenberger 1996). There is clearly overlap between these analyses in the data used but the datasets were not identical, often using different outgroups, and in the case of Rosenberger (1984) used an unconventional phylogenetic approach, all of which contributed to alternative phylogenetic relationships being inferred. The phylogenetic placement of four taxa in particular, *Cebus*, *Saimiri*, *Aotus* and *Callicebus*, contrasted between the different analyses.

Prior to Rosenberger (1984), earlier phylogenetic hypotheses were developed in Rosenberger (1977, 1981). Rosenberger (1977) proposed a "provisional" platyrrhine phylogenetic tree with callitrichines as the basal platyrrhine clade separate from *Cebus*-*Saimiri* and *Aotus*, with *Callicebus* outside the pitheciids and more closely related to atelids (see Figure 27). Small body size and long claws on all digits bar the hallux supported callitrichine monophyly, and the abundance of nails throughout primates indicated claw-like nails in callitrichines was derived and part of an adaptive response to a small body size niche in the callitrichine common ancestor (Rosenberger 1977). Within the callitrichines, the pygmy marmoset was linked to *Callithrix* by shared adaptations in the anterior teeth and mandible for tree gouging and accessing exudates. Whilst there was strong evidence for callitrichine monophyly, no single shared derived trait supported the alternative clade that included owl monkeys,

cebines, pitheciids and atelids, except increased body size. Rosenberger (1981) slightly altered the phylogeny of Rosenberger (1977), recognising the cebines (*Cebus-Saimiri*) were closely related to the callitrichines and rejecting the separation of platyrrhines into two body-size clades (see Figure 28). With the exception of the phylogenetic position of owl monkeys, connected to atelids and pitheciids via dental and mandibular traits, Rosenberger (1981) recognised many of the now accepted molecular phylogenetic clades for atelids, pitheciids, callitrichines and cebines.

Rosenberger (1984) proposed a complete platyrrhine phylogeny that included phylogenetic positions for all genera. This phylogeny placed callitrichines and cebines as sister clades, and atelids (*Alouatta*, *Brachyteles*, *Ateles* and *Lagothrix*) sister to a clade of *Aotus*, *Callicebus* and saki-uakaris (see Figure 29). Within the callitrichines, *Callithrix* was most closely related to *Leontopithecus*, sister to *Saguinus*, and *Callimico* basal-most, and atelids inferred *Ateles-Brachyteles* sister to *Lagothrix* and *Alouatta* basal-most. An *Aotus-Callicebus* sister relationship was basal-most to the saki uakaris, within which *Cacajao-Chiropotes* was sister to *Pithecia*. This tree shared several major similarities with the current consensus molecular phylogeny, such as callitrichine, cebine and atelid monophyly, the phylogenetic relationships between saki-uakaris, and placement of *Alouatta* as the basal-most atelid. The major disagreement with the recent molecular phylogenies was the placement of *Aotus* outside the cebids in a sister relationship with *Callicebus*, and relationships within callitrichines and between *Lagothrix-Brachyteles-Ateles*.

A major criticism of Rosenberger (1979, 1981, 1984) was the use of non-cladistic methods as clades were decided upon based on adaptive zones. Although cladistics principles were incorporated by placing emphasis on character polarity, the method strongly weighed certain characters due to an emphasis on key traits linked to adaptive trends (Lockwood 1999). By not incorporating a systematic treatment of characters within a parsimony-cladistic computational framework, the experimental work was difficult to verify or repeat, which creates significant scientific objections. Such a fundamental problem with the method, however, is more remarkable considering the inference of several molecular phylogenetic relationships and clades by Rosenberger (1979, 1981, 1984).

Figure 27 Platyrrhine phylogeny according to Rosenberger (1977)

Figure 28 Platyrrhine phylogeny according to Rosenberger (1981)

Figure 29 Platyrrhine phylogeny according to Rosenberger (1984)

Ford (1986) produced a cladistic phylogenetic analysis of platyrrhines using a combined dataset consisting of cranial, postcranial, dental, brain, karyotypic and hair follicle data. The craniodental data were largely the same as Rosenberger (1977, 1981, 1984), agreeing with the original morphoclines and character polarities, although postcranial data in particular provided new data for phylogenetic analysis. Ford (1986) supported three molecular clades-atelids, saki-uakaris (minus *Callicebus*) and callitrichines, with atelids and saki-uakaris sister and callitrichines basal-most, all sister to a clade of *Aotus*-*Callicebus* and *Saimiri*, and *Cebus* basal to all other platyrrhines (see Figure 30). Atelid monophyly was supported by multiple traits, with *Alouatta* basal most and the relationship of the other three taxa unresolved. A sister relationship between *Brachyteles* and *Lagothrix* (as inferred by molecular DNA analysis) was not proposed or considered, with discussion focussing on the sister taxa to

Ateles. This personifies the upheaval that molecular phylogenetics created, inferring close phylogenetic relationships between groups that lacked clear derived anatomical traits. The saki-uakaris were proposed as a three genus clade with *Pithecia* sister to *Chiropotes-Cacajao*, without *Callicebus*. Ford (1986) noted that many of the traits that link the three pitheciid taxa were also present in atelids and interpreted as convergent traits related to allometry. Within the monophyletic callitrichines, *Callithrix-Leontopithecus* was sister to *Saguinus* with *Callimico* basal-most.

For the relationships between major clades, atelids and pitheciids were sister clades, supported by 12 postcranial traits and a single unique derived trait (a rounded deltopectoral crest on the humerus), which was more strongly supported than a sister relationship of callitrichines with atelids (2 shared derived traits) or pitheciids and callitrichines (6 shared derived traits). Several traits supported a clade of atelids, pitheciids and callitrichines to the exception of the four problematic taxa *Aotus*, *Callicebus*, *Cebus* and *Saimiri*, including similarity in sulcal pattern, dental eruption of the M3 molar and 17 postcranial traits. A close relationship between *Callicebus* and the three pitheciids would have required reversals in 20 traits, and a close relationship between *Callicebus* and *Aotus* was proposed instead on the strength of 13 shared traits. *Saimiri* and *Cebus* each acquired several autapomorphies, 6 in *Saimiri* and 1 in *Cebus*, and 11 *Cebus* traits were shared with atelids, linked to allometry, with just as many shared with pitheciids relating to the dentition.

From this dataset, a close relationship of *Cebus* to the callitrichines would require 13 character reversals, although they do share 8 derived traits with callitrichines. *Saimiri* had 6 atelid, 8 pitheciid and 6 callitrichine synapomorphies, and of the 6 shared with callitrichines, 3 were also present in *Cebus* relating to canine and incisor morphology. The relationship of *Saimiri* with *Cebus* or *Aotus-Callicebus* was left partially unresolved, the main phylogeny placed *Saimiri* with *Aotus-Callicebus*, but a close link between *Cebus* and *Saimiri* was also indicated by a dotted line. The phylogeny proposed by Ford (1986) agreed with the phylogenetic hypotheses of Rosenberger (1984) in several key areas; callitrichines formed a monophyletic group with *Callimico*, atelids grouped with *Alouatta*, a saki-uakari clade of *Pithecia*, *Chiropotes* and *Cacajao*, and support for a quasi atelid-pitheciid clade (Ford & Davis 1992). Although Ford (1986) and Rosenberger (1984) both viewed *Callicebus* and *Aotus* as closely related, Ford (1986) suggested these two taxa were early offshoot lineages along with *Cebus* and *Saimiri*, whereas Rosenberger (1984) linked *Aotus* and *Callicebus* to saki-uakaris (Ford & Davis 1992).

Figure 30 Platyrrhine phylogenetic relationships as proposed by Ford (1986) (from Ford & Davis 1992)

Kay (1990) examined the phylogenetic relationships of platyrrhines whilst investigating the monophyly of pitheciids, although little information was given for character descriptions, character states and the number of traits supporting clades. Kay (1990) inferred a trichotomy between owl monkeys, atelids and a callitrichine-*Saimiri* clade, sister to saki-uakaris, in turn sister to *Cebus*, and *Callicebus* as the basal-most platyrrhine taxa (see Figure 31). The atelids consisted of a dichotomy between *Alouatta-Brachyteles* and *Lagothrix-Ateles*, and the callitrichines had *Callithrix-Saguinus* sister to *Leontopithecus*, with *Callimico* basal and lone *Saimiri* sister to this clade.

As with Ford (1986), Kay (1990) found homoplasy and evolutionary reversal were common across the platyrrhines (Ford & Davis 1992). The *Aotus-Callicebus* clade proposed in Rosenberger (1979, 1984) was supported by no postcranial characters, and Kay (1990) suggested the proposed derived cranial features (presence of paraoccipital process, robusticity in the pyramidal process of the palatine, nasal bone shape) were not due to the presence of a greater range of variation in characters than previously acknowledged. Two cranial and four

dental derived traits that were contested actually connected *Callicebus* with *Pithecia-Chiropotes-Cacajao* in a pitheciid clade, whilst three other derived traits linked those four taxa with *Aotus*. By challenging the polarity of traits, whether they were derived or primitive retentions, highlighting potential homoplasy, and drawing attention to increased variation in character states or potentially incorrect character designations, Kay (1990) removed both phylogenetically informative data linking *Callicebus* and the saki-uakari clade and phylogenetically misleading data that supported a false clade between pitheciids and owl monkeys.

There was congruence with both Rosenberger (1984) and Ford (1986) in callitrichine monophyly, ateline monophyly, and *Pithecia-Cacajao-Chiropotes* monophyly. As with Ford (1986), *Cebus* and *Callicebus* were viewed as basal lineages, but with atelids, *Aotus* and callitrichines as a trichotomy to the exception of pitheciids. Four postcranial and three dental traits, the latter all related to the cheek teeth, separated all other platyrrhines from *Callicebus* and *Cebus*. The phylogenetic tree proposed by Kay (1990) supported a close relationship between atelids and cebids, although the latter was paraphyletic due to the exclusion of *Cebus*, and this atelid-cebid clade broadly reflects the branching relationships found in the recent molecular phylogenies (Hodgson et al. 2009, Wildman et al. 2009, Perelman et al. 2011).

Figure 31 Platyrrhine phylogenetic relationships from Kay (1990) from Ford & Davis (1992)

To summarise, at the family/subfamily level, Rosenberger (1984) proposed four groups/clades congruent with molecular phylogenetics- atelids, saki-uakaris, callitrichines and cebines. Ford (1986) recognised atelids, saki-uakaris and callitrichines, but favoured a basal *Cebus* lineage and a separate clade for *Aotus*, *Saimiri* and *Callicebus*. Kay (1990) also supported atelid, saki-uakari and callitrichines clades but placed *Callicebus* and *Cebus* as basal offshoot lineages and *Aotus* in a trichotomy with atelids and callitrichines. These three studies clearly had broad consensus on the existence of three major clades of atelids, saki-uakaris and callitrichines, with major disagreement regarding the phylogenetic positions of *Cebus, Saimiri, Aotus* and *Callicebus*. Rosenberger (1984) and Ford (1986) viewed saki-uakaris and atelids as the most closely related clades whilst Kay (1990) favoured a trichotomy of atelids, callitrichines and *Aotus*. For atelids, all three studies recognised *Alouatta, Ateles, Lagothrix* and *Brachyteles* as forming a single monophyletic clade.

Rosenberger (1984) had *Ateles-Brachyteles* as derived taxa sister to *Lagothrix* whereas Kay (1990) favoured a dichotomy between *Ateles-Lagothrix* and *Alouatta-Brachyteles*, and Ford (1986) proposed an unresolved trichotomy between *Ateles*, *Lagothrix* and *Brachyteles*. Whilst all three recognised a saki-uakari clade, with *Cacajao-Chiropotes* sister to *Pithecia* as supported by most platyrrhine molecular phylogenies, only Rosenberger (1984) recognised *Callicebus* was closely related. For callitrichines, Rosenberger (1984) and Ford (1986) supported a *Callithrix-Leontopithecus* clade sister to *Saguinus*, whilst Kay (1990) had a *Callithrix-Saguinus* clade sister to *Leontopithecus*, and all three studies placed *Callimico* as the basal-most callitrichine. Only Rosenberger (1984) supported a sister relationship between *Cebus-Saimiri*, although Ford (1986) alluded to a possible close relationship.

Although morphology-based phylogenetic analysis of platyrrhines has become synonymous with the three studies discussed, subsequent studies have continued to add to our understanding of platyrrhine evolution and the relationships inferred by study of morphology. MacPhee et al. (1995) used a restricted 32 craniodental character dataset that sampled only 13 extant genera, inferring atelid, cebid and saki-uakari monophyletic clades, with the atelids and *Callicebus* sister taxa. Horovitz & Meyer (1997) conducted a phylogenetic analysis of all extant platyrrhine genera with several fossil taxa, incorporating nuclear and mitochondrial genes for extant taxa and 66 morphological characters for all taxa (with some missing characters for fossil taxa). Analysis of just the morphological dataset reproduced the three molecular clades, with atelids and pitheciids as sister clades. Atelids had a dichotomy between *Alouatta-Brachyteles* and *Ateles-Lagothrix*, and pitheciids had *Chiropotes-Cacajao* sister to *Pithecia* with *Callicebus* basal. Within cebids the callitrichines formed a clade sister to *Saimiri*, itself sister to *Cebus* and *Aotus* basal-most. The total evidence tree, combing trees inferred from morphology, mitochondrial DNA and nuclear DNA, was true to a modern molecular tree for within-clade phylogenetic relationships but with atelids and pitheciids as sister clades.

The phylogeny of Horovitz & Meyer (1997) was supported by similar analysis in Horovitz et al. (1998) with the addition of several morphological characters and extra DNA sequences. This analysis was unique in positioning *Callimico* as sister to *Callithrix* and *Lagothrix* sister to *Brachyteles*, relationships strongly supported by molecular phylogenetics. Taken in its entirety, the joint morphological-molecular tree supported clades that are congruent with the platyrrhine molecular tree, but the authors link this to high consistency of the nuclear genetic data rather than a particular benefit of bringing alternative data sources together. Cladistic

analysis of 80 morphological characters sampling platyrrhine extant and fossil groups by Horovitz & MacPhee (1999) supported platyrrhine monophyly, recognising atelid, pitheciid and cebid clades. The characters were mostly craniodental, with several postcranial and soft tissue traits, and several linear measurements character coded with gap coding. Phylogenetic relationships within the pitheciids were congruent with the most recent molecular phylogenies but were unresolved for atelids. Within the cebids, *Aotus* was basal-most, preceded by *Cebus* and then *Saimiri*, and within callitrichines, *Callithrix* and *Leontopithecus* formed a clade with *Saguinus* sister and *Callimico* basal.

The phylogenetic analyses of Horovitz (1999) used all extant platyrrhine genera with 18 fossil taxa, much more extensive sampling of fossil taxa than any previous analyses, in addition to four extant and one fossil outgroup taxa. Phylogenies were generated from morphological data only or a combined dataset including both morphological and DNA data. For phylogenetic analysis of morphology, the addition of fossil taxa was problematic, as their inclusion lowers tree resolution by enlarging the number of most parsimonious trees due to the fragmentary nature of the taxa described with fewer characters. One solution was to use only a few fossil taxa and re-run phylogenetic analyses with different fossil combinations, with the inference of alternative arrangements between the major clades. When the molecular and morphological data were analysed together the addition of fossil taxa was less problematic, although phylogenetic relationships of extant genera were the same whether fossil taxa were included or not. This latter point is of interest as there has been evidence in phylogenetic analysis of hominoids (e.g. Begun 1994, Strait & Grine 2004) that the inclusion of fossil taxa was especially important for accurately inferring relationships of living groups. The phylogenetic tree inferred by the combined morphological-molecular dataset had a pitheciid-atelid clade, the relationships in each clade the same as for nearly all platyrrhine molecular phylogenies. For the cebids, callitrichines were sister to *Cebus*-*Saimiri*, with *Aotus* basal-most. Within the callitrichines, *Callithrix*-*Callimico* were sister to *Saguinus* with *Leontopithecus* basal-most.

The most recent phylogenetic analysis based on morphology, Kay et al. (2008), carried out a phylogenetic analysis combining both molecular and morphological data, investigating the placement of a 20 million year old specimen of *Dolichocebus gaimanensis* within the platyrrhine evolutionary tree. Phylogenetic analysis included all extant genera and 8 platyrrhine fossil taxa including *Dolichocebus*. The morphological dataset included 85 cranial, 114 lower dental and 69 upper dental traits of which 199 were parsimony-

informative. A molecular backbone was implemented, so the phylogenetic relationships of extant genera were rigid and decided by molecular DNA with morphological data added to this strict tree, although the fossil taxa were free to move anywhere on the constrained tree. The phylogeny generated had a clade of *Dolichocebus*, *Carlocebus*, *Tremacebus* and *Soriacebus* sister to living platyrrhines (with *Proteropithecia* within the pitheciids), and *Branisella* basal to all other platyrrhines. When the molecular backbone was removed, and the morphological dataset was phylogenetically analysed on its own, there was support for a platyrrhine crown group (with *Proteropithecia*) separate to stem platyrrhines, alongside clade support for atelids, callitrichines, and *Pithecia-Cacajao-Chiropotes*. Atelids and saki-uakaris were the most closely related clades, with *Callicebus-Aotus-Cebus* basal. To this group *Saimiri* was basal, with callitrichines as the earliest platyrrhine offshoot. Within atelids, *Alouatta-Brachyteles* were sister to *Lagothrix*, for callitrichines *Callithrix-Leontopithecus* and *Saguinus-Callimico* formed a dichotomy, and in the saki-uakaris *Cacajao-Chiropotes* were sister to *Pithecia*.

From the phylogenetic analyses described, it is clear that there is relatively large variation in the phylogenies inferred. Although several of the morphology-based phylogenies have supported the major molecular clades, relationships within these clades are rarely congruent with molecular phylogenies. A growing number of phylogenetic analyses have combined molecular and morphological data to accurately infer phylogenetic relationships for extant taxa, but the position of the fossil groups relies solely on morphological data and phylogenetic methods shown to be, at least partially, unreliable in platyrrhines if you compare the relationships inferred by morphology in Rosenberger (1984), Ford (1986) and Kay (1990) to the molecular phylogenetic relationships supported in Hodgson et al. (2009), Wildman et al. (2009) and Perelman et al. (2011) . There is, therefore, justification in conducting further morphology-based phylogenetic analyses to try and find greater congruence between molecular and morphological phylogenies. Even if such endeavours prove unsuccessful, they may provide important insight, into both the underlying biological and specific methodological reasons, as to why morphological and molecular phylogenetic analyses regularly clash. This may lead to more accurate and reliable analyses in the future, moving closer to the goal of understanding the true phylogenetic relationships of extant and fossil taxa.

4.2 Materials and Methods

Full explanation of the methods used in this project were provided in chapter 3, including the full set of anatomical landmarks used to quantify craniodental variation, justification for subdivision of the skull into semi-autonomous modules of the face and cranial base, and a full description of both geometric morphometric and distance-based phylogenetic methods. The taxa sampled, and sample sizes, for phylogenetic analysis of all platyrrhine taxa are listed in Table 11. Geometric morphometric analysis was carried out in the MorphoJ program and phylogenetic analysis in the Phylip software package. The entire platyrrhine dataset included 50 species, resulting in a very large range of trees inferred depending on the outgroup used, the region of the skull examined, and sex of specimens. In practical terms the variety of phylogenies produced would be very difficult to present, describe and draw coherent conclusions from. Therefore, phylogenetic analyses of the platyrrhines are presented from pooled sex specimens for the entire skull, face and cranial base, and single outgroups of a representative Old World monkey taxa (*Chlorocebus*), ape taxa (*Hylobates*) and strepsirrhine taxa (*Otolemur*), and outgroup combinations including all Old World monkeys, all strepsirrhines and all nine outgroups. UPGMA clustering analysis of platyrrhine data are provided to highlight the phenetic relationships between platyrrhine taxa. Using pooled sex specimens ought to be less of a problem in platyrrhines than it has been in papionins (see Gilbert & Rossie 2007, Gilbert et al. 2009), as craniodental size differences between males and females are lower in platyrrhines (Perez et al. 2011). The results in later chapters for atelids, pitheciids and cebids, where sex-specific results are reported, also support this position. Past phylogenetic analyses of platyrrhines based on morphology have also used pooled sex samples, so results presented are comparable to past work.

Table 11: Taxa and sample sizes used in phylogenetic analysis of entire platyrrhine clade

Genus	Species	Male	Female	Pooled
Alouatta	*belzebul*	10	10	20
	caraya	9	11	20
	fusca	9	9	18
	palliata	18	13	31
	seniculus	22	10	32
	pigra	8	10	18
	coibensis	8	9	17
Ateles	*paniscus*	7	12	19
	belzebuth	11	10	21

	fusciceps	10	10	20
	geoffroyi	10	10	20
Lagothrix	*lagothrica*	10	10	20
	lugens	8	10	18
	poeppigii	10	10	20
	cana	10	11	21
Brachyteles	*arachnoides*	7	5	12
Callicebus	*moloch*	13	15	28
	torquatus	12	9	21
	cupreus	10	9	19
	hoffmannsi	9	10	19
Cacajao	*melanocephalus*	13	17	30
	calvus	13	10	23
Chiropotes	*satanas*	14	9	23
Pithecia	*pithecia*	12	10	22
	monachus	14	13	27
Cebus	*capucinus*	10	10	20
	albifrons	10	10	20
	apella	92	60	152
	nigrivittatus	10	10	20
	libidinosus	11	10	21
Saimiri	*sciureus*	33	15	48
	oerstedii	11	9	20
	bolviensis	10	10	20
	ustus	10	6	16
Aotus	*trivirgatus*	13	11	24
	azarai	6	10	16
	lemurinus	10	10	20
	vociferans	10	10	20
Leontopithecus	*rosalia*	11	13	24
Callithrix	*jacchus*	8	7	15
	argentata	11	10	21
	humeralifer	11	9	20
	penicillata	18	14	32
	pygmaea	10	9	19
Callimico	*goeldii*	11	11	22
Saguinus	*midas*	12	10	22
	fuscicollis	27	11	38
	mystax	10	11	21
	leucopus	9	9	18
	geoffroyi	10	9	19

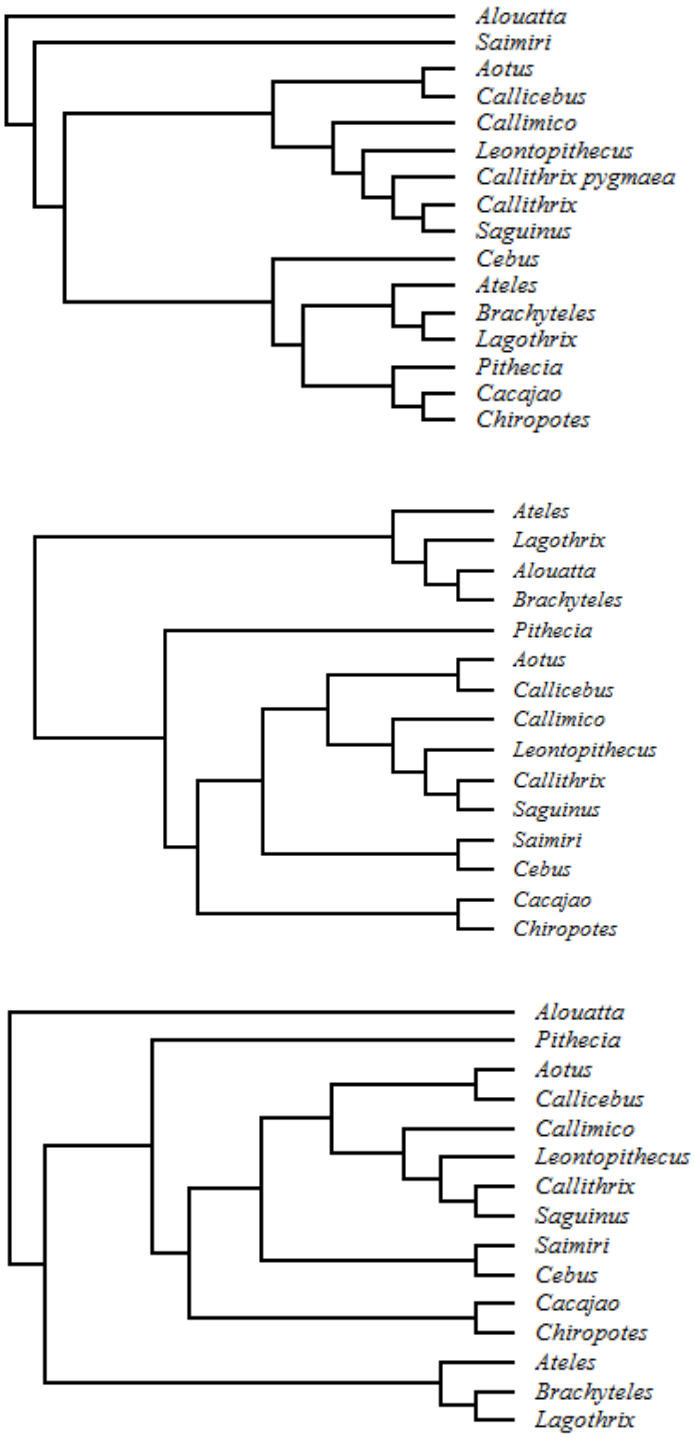
Outgroups				
Hylobates	*lar*	10	10	20
Macaca	*mulatta*	9	10	19
Perodicticus	*potto*	10	10	20
Colobus	*guereza*	11	10	21
Chlorocebus	*aethiops*	10	10	20
Trachypithecus	*obscura*	10	10	20
Otolemur	*garnetti*	10	9	19
Galago	*senegalensis*	10	11	21
Eulemur	*fulvus*	10	10	20

4.3 Results

4.3.1 Whole skull morphology

One UPGMA phenetic tree and two phylogenetic trees are presented in Figure 32, based on distances derived from shape data describing the entire skull. The phenetic tree inferred two major clades, callitrichines sister to *Aotus-Callicebus*, and *Ateles-Lagothrix-Brachyteles* (minus *Alouatta*) sister to *Pithecia-Chiropotes-Cacajao* (minus *Callicebus*) with *Cebus* as a basal lineage. At the root of the tree, *Saimiri* was sister to both these major clades, preceded by *Alouatta* as the basal-most lineage. With the exception of the two basal lineages, *Saimiri* and *Alouatta*, the phenetic tree was mostly split by size with *Cebus* sister to the large atelids and pitheciids, whilst *Aotus-Callicebus* were sister to the smaller callitrichines. The callitrichine clade was notable for the placement of *Callimico* as the basal-most lineage and *C. pygmaea* falling outside the *Callithrix* clade. Phylogenetic analysis using *Chlorocebus*, *Hylobates* or the multiple Old World monkey outgroup produced a callitrichine clade sister to *Aotus-Callicebus*, with *Saimiri-Cebus* sister to this clade preceded by *Cacajao-Chiropotes*, then *Pithecia*. At the base of the phylogenetic tree the atelids formed a monophyletic clade with *Alouatta-Brachyteles* sister to *Lagothrix*. There was support for molecular clades of *Saimiri-Cebus*, *Cacajao-Chiropotes*, atelids and a cebid group although this included *Callicebus*. The phylogenetic tree inferred using *Otolemur*, multiple strepsirrhine outgroups or the entire set of outgroups produced an identical phylogenetic tree, except *Alouatta* fell outside the atelid clade and was the basal-most lineage for platyrrhines.

Figure 32 UPGMA phenetic tree (top) inferred with platyrrhine whole skull morphology, phylogenetic relationships inferred using *Chlorocebus*, *Hylobates* and multiple Old World monkey outgroups (middle), and *Otolemur*, multiple strepsirrhines and all possible outgroups (bottom)



4.3.2 Facial morphology

Phylogenetic analysis of the platyrrhines using facial morphology alone produced a wide range of inferred trees. Figure 33 displays the UPGMA phenetic tree and phylogenetic trees inferred with Chlorocebus and Hyloboates outgroups, and Figure 34 displays phylogenetic trees inferred with Otolemur, Old World monkey, Strepsirrhine and all outgroups. UPGMA cluster analysis formed two broad clades, and placed *Alouatta* at the base of the tree. One clade included the callitrichines with *Aotus* and *Callicebus*, the other had *Cebus*, *Saimiri* and the remaining atelids and pitheciids. Within the callitrichine clade the pygmy marmoset fell outside a group of *Callithrix-Saguinus* and *Callimico*, and *Callicebus* was sister to the callitrichines. Within the other clade, *Cacajao-Chiropotes* and the three taxa atelid clade were the only molecular congruent relationships inferred (in addition to the monophyletic callitrichine clade).

Phylogenetic analysis of facial landmarks using *Chlorocebus* as outgroup produced a phylogeny with *Brachyteles-Lagothrix* as the basal-most clade, preceded by *Alouatta-Ateles*. Two major clades were inferred; the first with *Saimiri-Chiropotes* sister to *Cebus* and *Cacajao* basal-most. The second had a callitrichine clade with *Aotus* and *Callicebus*, sister to *Callithrix.jacchus-Callithrix penicillata*. Use of a *Hylobates* outgroup placed *C.jacchus* and *C.penicillata* within *Callithrix*, with near identical phylogenetic relationships as for the phylogeny with *Chlorocebus* as outgroup. Use of all four Old World monkeys as outgroups moved the *C.jacchus-C.penicillata* clade outside *Callithrix*, sister to *Callithrix-Saguinus-Callimico*. The use of *Otolemur* as outgroup inferred a phylogeny with monophyletic atelids sister to the *Saimiri-Chiropotes*, *Cebus* and *Cacajao* clade and callitrichines with *Callicebus* basal-most as a sister clade. *Pithecia* was basal to both these clades, with *Aotus* at the root of the tree. Use of multiple strepsirrhine outgroups moves *Callicebus* sister to the atelid and *Saimiri-Chiropotes*, *Cebus* and *Cacajao* clade. *Pithecia* was the next sister lineage to this wider group, with *Aotus* the basal-most lineage. The phylogeny inferred using all possible outgroups had a series of atelid genera branching off at the base of the tree. Again, a clade is present with *Chiropotes-Saimiri* sister to *Cebus* and *Cacajao* basal-most. This clade was sister to a group including a monophyletic callitrichine clade, sister to *Callicebus* and *Aotus-Pithecia* basal-most. The relationships within the callitrichines had *C.jacchus-C.penicillata* sister to *Saguinus leucopus*.

Figure 33 UPGMA phenetic tree (top) inferred by facial morphology, and phylogenetic relationships inferred using *Chlorocebus* (middle) and *Hylobates* (bottom) outgroups

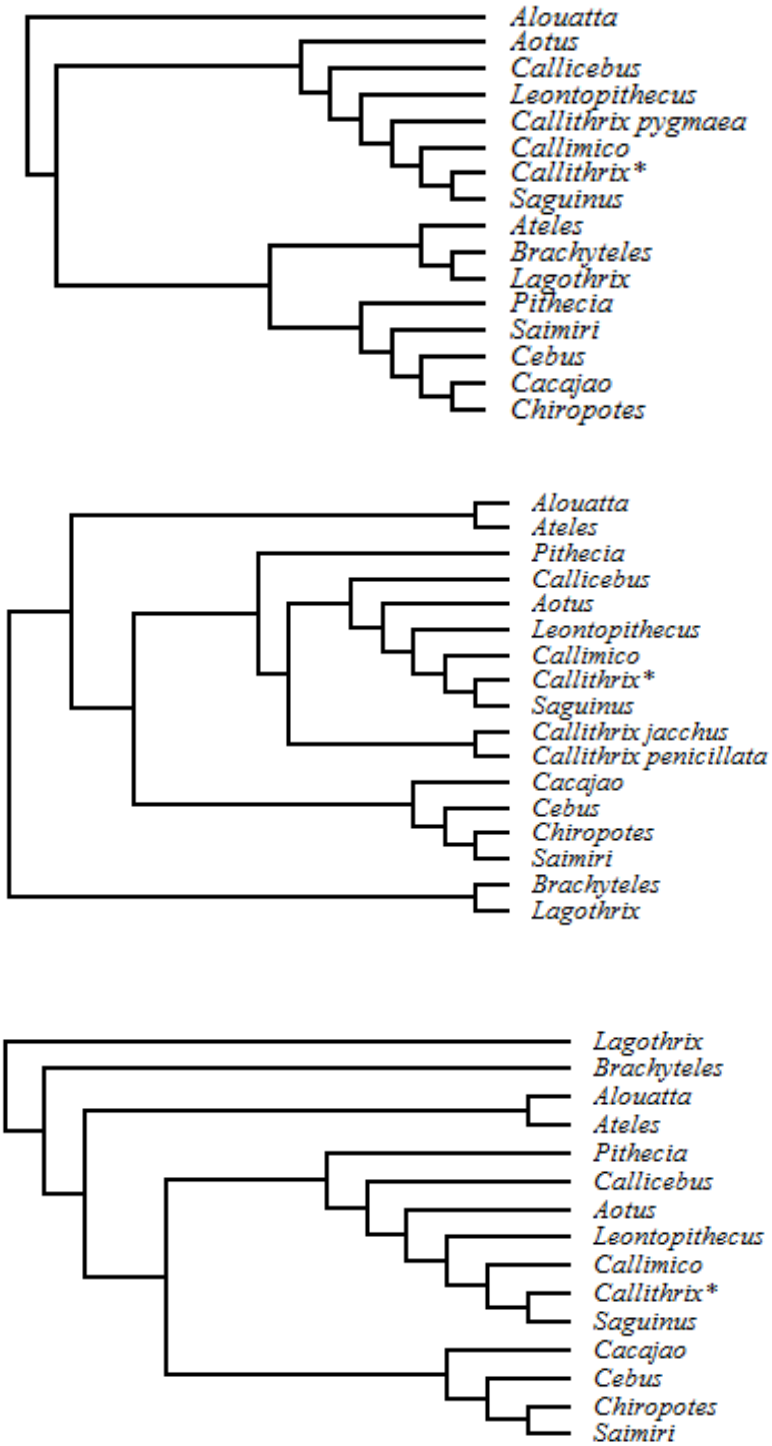
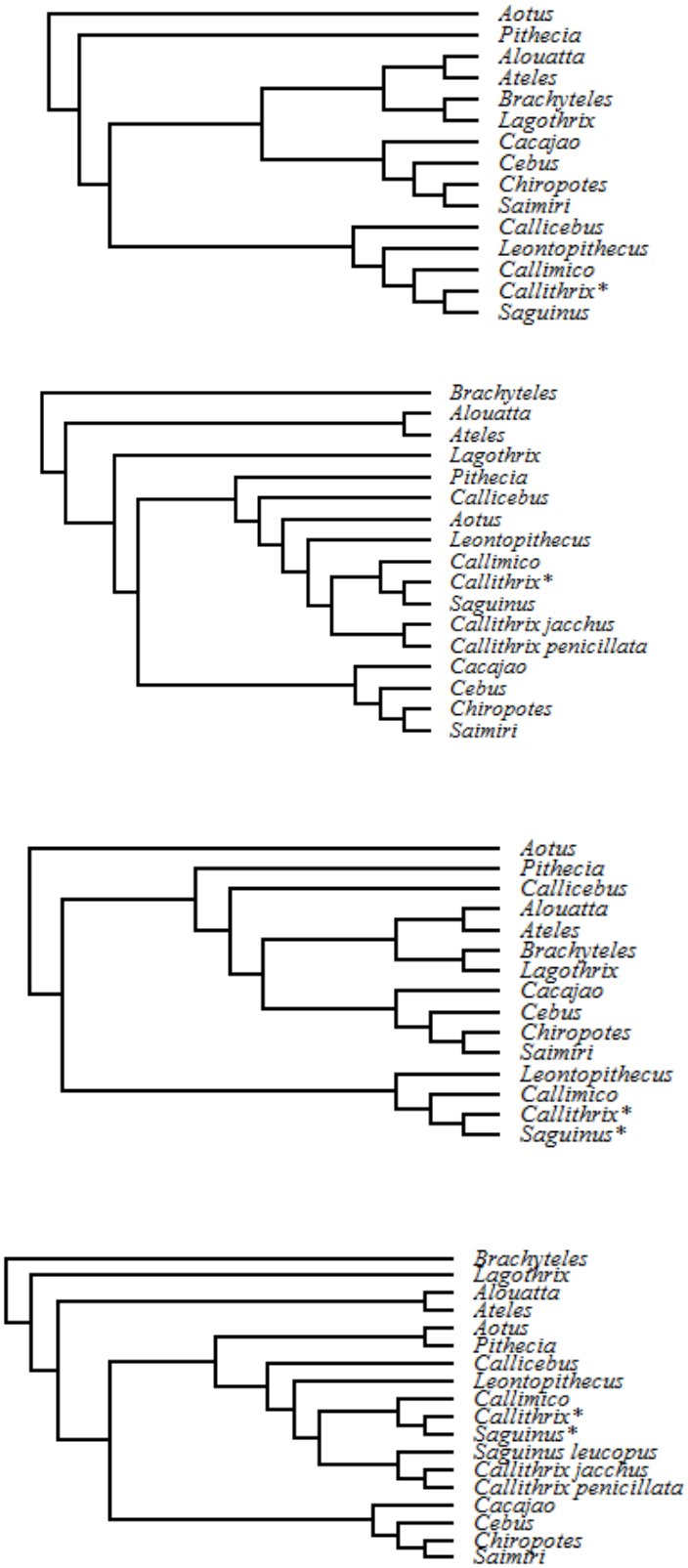


Figure 34 Phylogenetic relationships inferred from facial morphology with *Otolemur* (top), Old World monkey (second top), strepsirrhine (second bottom) and all outgroups (bottom)



4.3.3. Cranial base morphology

As with phylogenetic analysis of the face, numerous trees were inferred from distances based on the cranial base. The phenetic tree and phylogenetic trees inferred with Chlorocebus and Hylobates outgroups are shown in Figure 35, with phylogenetic trees inferred with Old World monkey, Strepsirrhine and all outgroups displayed in Figure 36 . The phenetic tree placed *Saimiri* as the basal-most lineage, preceded by *Alouatta*, then the pygmy marmoset. Two major clades are formed, one with the three atelid taxa in a clade sister to a three-taxon group of *Chiropotes-Cacajao* sister to *Cebus*. The second clade had *Aotus-Callicebus* basal-most, and two further groups- a partial callitrichine clade with *Callimico* and both *Saguinus* and *Callithrix* paraphyletic, sister to *Leontopithecus, S.midas* and *Pithecia*.

Phylogenetic analysis using *Chlorocebus* as outgroup had *Saimiri* as the basal-most taxon, preceded by *Cacajao-Chiropotes*, then *Cebus*. The atelids formed a monophyletic group (with *Alouatta-Brachyteles* sister to *Lagothrix*) sister to a clade with *Aotus-Callicebus* and *Leontopithecus-Pithecia* forming one group, and *Callimico-S.midas* and *Callithrix-Saguinus* forming another. The phylogeny produced when *Hylobates* was outgroup had *Cacajao-Chiropotes* basal-most, preceded by a monophyletic atelid clade (*Brachyteles-Alouatta* sister to *Lagothrix*). *Saimiri-Cebus* was sister to two clades, one with *Aotus-Callicebus* and *Leontopithecus-Pithecia*. The other clade had *Callithrix* and paraphyletic *Saguinus* sister to *Callimico-S.midas*. When multiple Old World monkey taxa were used as outgroup, *Cebus* monophyly disintegrates. *Cacajao-Chiropotes* was basal most, preceded by *C.apella-C.libidinosus*. These offshoot lineages were preceded by *Saimiri* and remaining *Cebus* taxa. The rest of the tree was identical to that of the *Hylobates* phylogeny, with support for two clades of *Aotus-Callicebus* and *Leontopithecus-Pithecia* on one branch, and *Callimico-S.midas* and *Callithrix-Saguinus* on the other. This group was also inferred in phylogenetic analysis with *Otolemur* as outgroup. That clade was sister to *Saimiri-Cebus*, the latter of which was paraphyletic, with *Cacajao-Chiropotes* the next sister taxa. There was no atelid monophyly, with successively more basal branches of *Ateles*, then *Brachyteles-Lagothrix*, with *Alouatta* as the basal-most taxon of the platyrrhine tree.

Using a strepsirrhine combination outgroup inferred a monophyletic atelid sister to *Cacajao-Chiropotes*, then *Saimiri-Cebus*. *Leontopithecus-Pithecia* was sister to this clade, with further branching of a clade for *Callimico-S.midas* and *Callithrix-Saguinus*. *Aotus-Callicebus* were basal to these groups, with the pygmy marmoset the basal-most taxon of all platyrrhines. This

tree contrasted with that inferred using all possible outgroups which had *Saimiri* basal most, preceded by paraphyletic *Cebus*, then *Cacajao-Chiropotes*. *C.apella* and *C.libidinosus* were sister to a clade comprising monophyletic atelids and a mix of taxa. The latter had *Leontopithecus-Pithecia* basal-most, with the pygmy marmoset and *Aotus-Callicebus* forming one clade, and *Callimico-S.midas* and *Callithrix-Saguinus* another.

Figure 35 UPGMA phenetic tree (top) inferred by cranial base morphology, and phylogenetic relationships inferred using *Chlorocebus* (middle) and *Hylobates* (bottom) outgroups

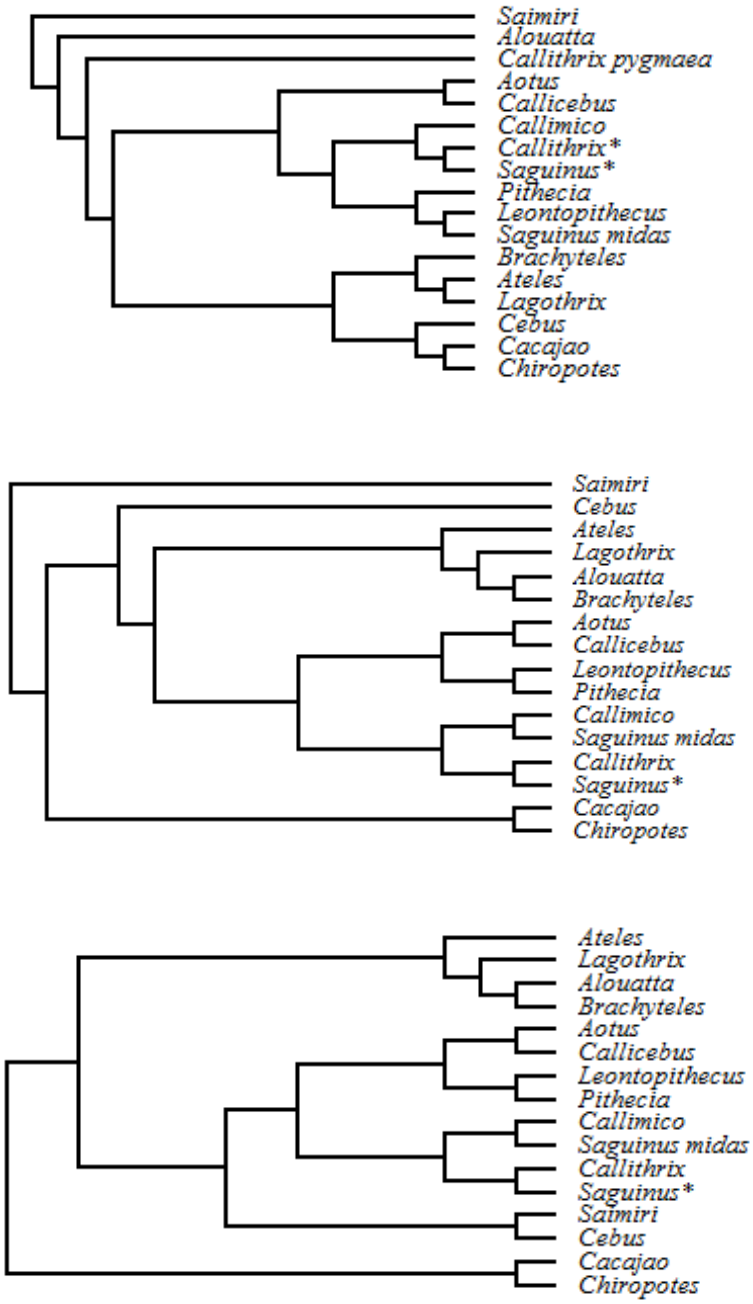
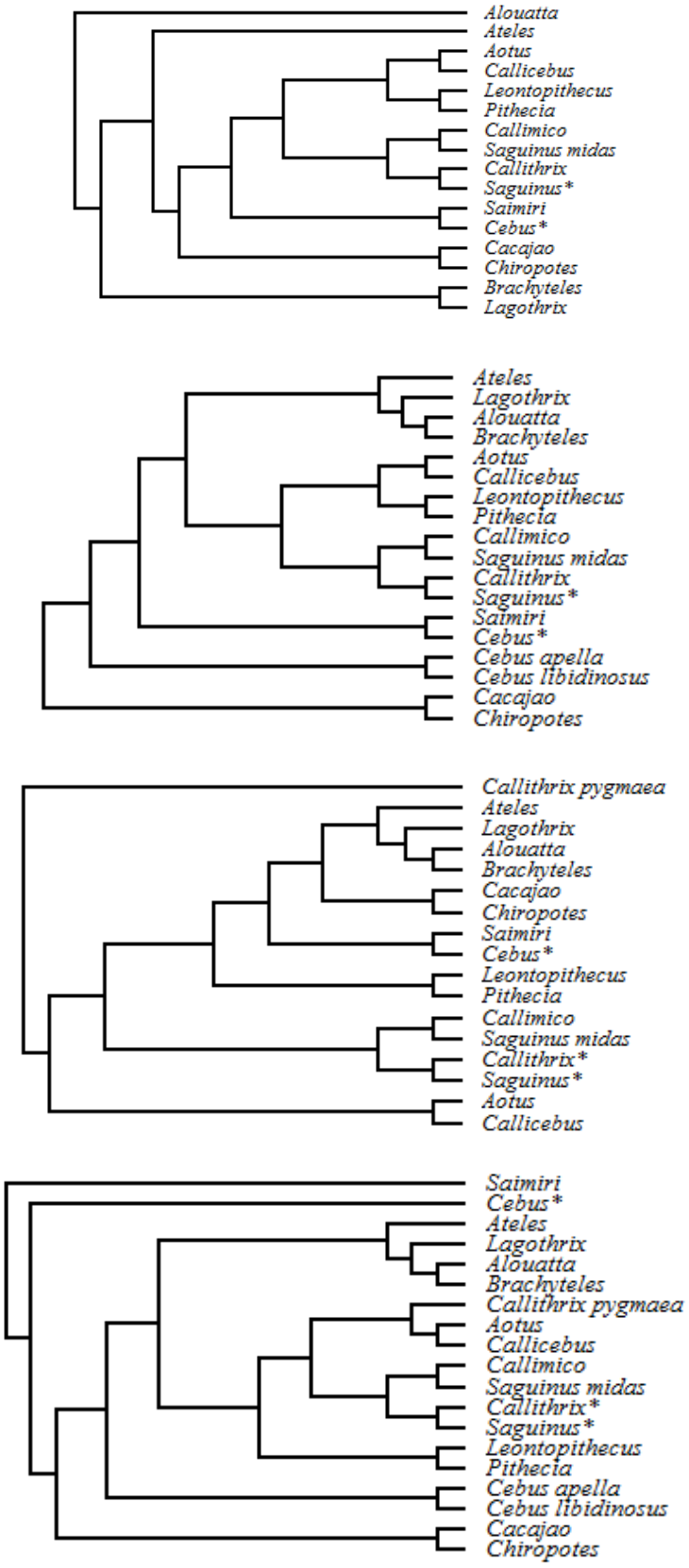


Figure 36 Phylogenetic relationships inferred from cranial base morphology with *Otolemur* (top), Old World monkey (second top), strepsirrhine (second bottom) and all outgroups (bottom



4.3.4 Summary of results

To clarify the congruence between molecular and morphological phylogenetic analyses, a summary (Table 12) is provided highlighting which morphological analyses supported each molecular clade. Figure 37 displays the most recent molecular phylogeny (e.g. Hodgson et al. 2009, Wildman et al. 2009, Perelman et al. 2011) of platyrrhines, with each clade assigned a node number. In Table 12 the molecular clades and respective node numbers are listed on the left hand side, and the morphological analyses that inferred those clades are listed on the right hand side, including outgroup (or UPGMA for phenetic analyses) and craniodental region used. In addition, the most common molecular incongruent clades are provided in Table 13.

Figure 37 Current molecular phylogeny of platyrrhines based on Hodgson et al. (2009), Wildman et al.(2009) and Perelman et al. (2011) with numbered nodes

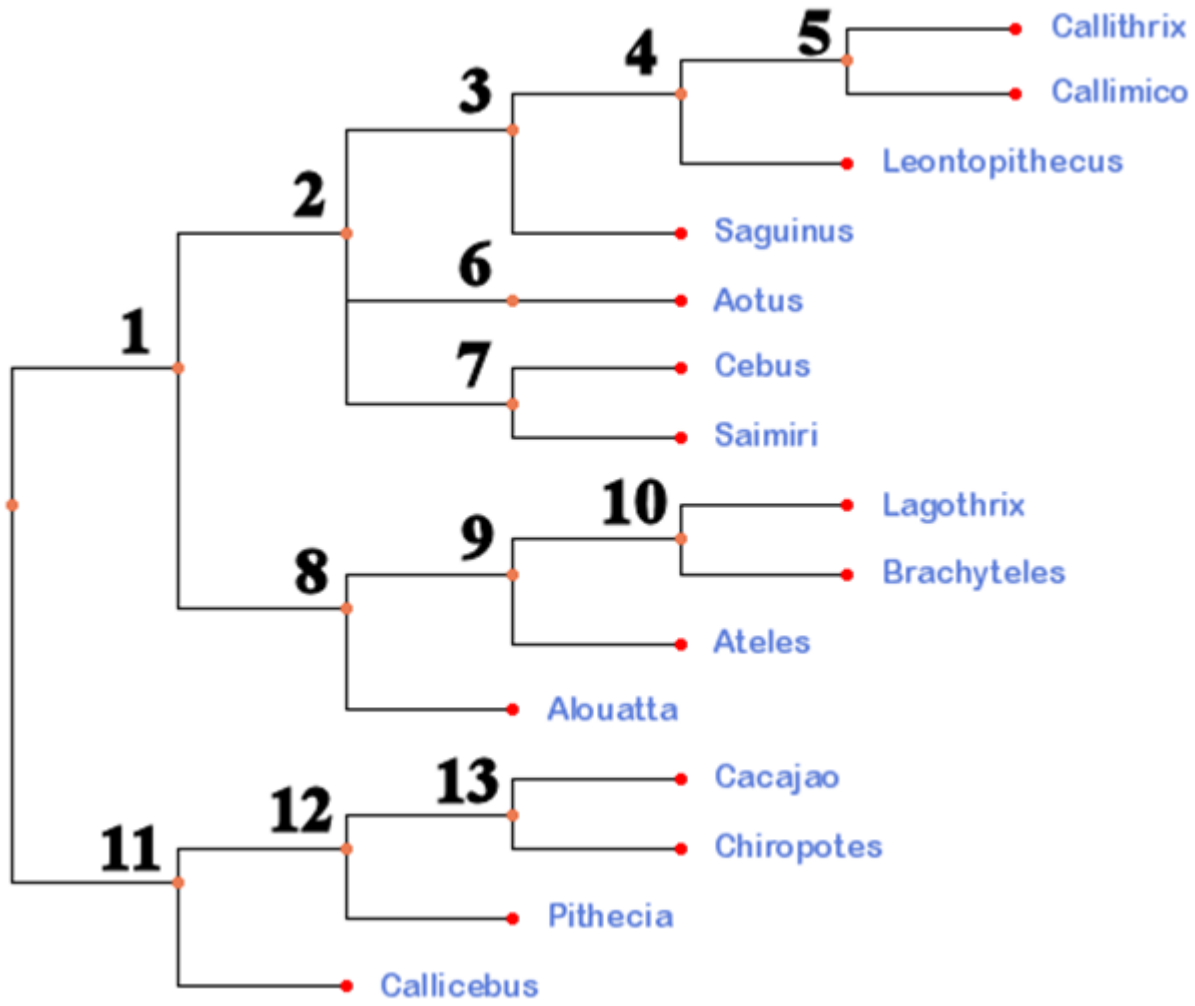


Table 12 Summary of congruence between molecular and morphological analyses, node numbers refer to Figure 37

Molecular clades	**Morphological analysis & region**
Atelid-Cebid (node 1)	None
Cebids (node 2)	None
Callitrichines (node 3)	UPGMA whole skull *Chlorocebus* whole skull *Hylobates* whole skull Old World monkey whole skull *Otolemur* whole skull Strepsirrhines whole skull All outgroups whole skull *Hylobates* face *Otolemur* face Old World monkey face Strepsirrhine face All outgroups face
Callithrix-Callimico-Leontopithecus (node 4)	None
Callithrix-Callimico (node 5)	None
Owl monkeys (node 6)	All
Cebines (node 7)	*Chlorocebus* whole skull *Hylobates* whole skull *Otolemur* whole skull Old World monkey whole skull Strepsirrhines whole skull All outgroups whole skull *Hylobates* cranial base *Otolemur* cranial base
Atelids (node 8)	*Chlorocebus* whole skull *Hylobates* whole skull Old World monkey whole skull *Chlorocebus* cranial base *Hylobates* cranial base Old World monkeys cranial base Strepsirrhine cranial base All outgroups cranial base
Brachyteles-Lagothrix-Ateles (node 9)	UPGMA whole skull UPGMA face UPGMA cranial base *Otolemur* whole skull Strepsirrhines whole skull All outgroups whole skull *Otolemur* cranial base
Brachyteles-Lagothrix (node 10)	UPGMA face UPGMA whole skull UPGMA face *Otolemur* whole skull Strepsirrhines whole skull All outgroups whole skull

	Chlorocebus face *Otolemur* face Strepsirrhine face *Otolemur* cranial base
Pitheciids (node 11)	None
Cacajao-Chiropotes-Pithecia (node 12)	UPGMA whole skull
Cacajao-Chiropotes (node 13)	UPGMA whole skull UPGMA face *Chlorocebus* whole skull *Hylobates* whole skull *Otolemur* whole skull Old World monkey whole skull Strepsirrhines whole skull All outgroups whole skull *Chlorocebus* cranial base *Hylobates* cranial base *Otolemur* cranial base Old World monkeys cranial base Strepsirrhine cranial base All outgroups cranial base

Table 13 Summary of molecular incongruent clades inferred

Molecular clades	**Morphological analysis & region**
Callithrix-Saguinus	UPGMA whole skull *Chlorocebus* whole skull *Hylobates* whole skull Old World monkey whole skull *Otolemur* whole skull Strepsirrhines whole skull All outgroups whole skull *Hylobates* face *Otolemur* face Strepsirrhine face
Callimico-Saguinus midas	*Chlorocebus* cranial base *Hylobates* cranial base *Otolemur* cranial base Strepsirrhine cranial base
Callithrix-Saguinus-Leontopithecus	UPGMA whole skull *Chlorocebus* whole skull *Hylobates* whole skull Old World monkey whole skull
Callithrix-Saguinus-Callimico	UPGMA face *Hylobates* face *Otolemur* face Old World monkey face Strepsirrhine face All outgroups face *Chlorocebus* cranial base *Hylobates* cranial base *Otolemur* cranial base Old World monkey cranial base All outgroups cranial base
Alouatta-Ateles	*Chlorocebus* face *Hylobates* face *Otolemur* face Old World monkey face Strepsirrhine face All outgroups face
Alouatta-Brachyteles	*Chlorocebus* whole skull *Hylobates* whole skull Old World monkey whole skull *Chlorocebus* cranial base *Hylobates* cranial base Old World monkey cranial base Strepsirrhine cranial base
Alouatta-Brachyteles-Lagothrix	*Chlorocebus* whole skull *Hylobates* whole skull Old World monkey whole skull *Chlorocebus* cranial base *Hylobates* cranial base

	Old world monkey cranial base Strepsirrhine cranial base All outgroups cranial base
Aotus-Callicebus	UPGMA whole skull *Chlorocebus* whole skull *Hylobates* whole skull Old World monkey whole skull *Otolemur* whole skull Old World monkey face All outgroups whole skull All outgroups face *Chlorocebus* cranial base *Hylobates* cranial base *Otolemur* cranial base Old World monkey cranial base All outgroups cranial base
Leontopithecus-Pithecia	*Chlorocebus* cranial base *Hylobates* cranial base Old World monkey cranial base Strepsirrhine cranial base All outgroups cranial base
Cacajao-Chiropotes-Cebus-Saimiri	UPGMA face *Chlorocebus* face *Hylobates* face *Otolemur* face Old World monkey face Strepsirrhine face All outgroups face

4.4 Discussion

The phenetic and phylogenetic results presented highlight the problematic nature of using distance-based phylogenetic and clustering methods in a clade like the platyrrhines with such wide genera sampling, and where ecological, behavioural, morphological and size variation is so large. It seems inevitable that distantly related groups will overlap morphologically due to plasticity and shared responses to non-genetic factors creating homoplasy, and inference of clades that reflect morphological, but not phylogenetic, similarity. Whilst there is not a strong phylogenetic signal overall, multiple phenetic and phylogenetic trees did support molecular clades, so there is a form of underlying phylogenetic signal present. Phylogenetic analysis provided quite strong support for callitrichine, cebine and atelid groups, and sister relationships between *Cacajao* and *Chiropotes*, and *Lagothrix* and *Brachyteles*. Yet, molecular incongruent clades were also prominent, especially *Callithrix-Saguinus*, *Callithrix-Saguinus-Callimico*, *Aotus-Callicebus*, and a sister relationship of *Alouatta* with either *Brachyteles* or *Ateles*.

Phylogenetic relationships inferred from morphology of the whole skull mostly support atelid and callitrichine monophyly and two molecular clades of *Saimiri-Cebus* and *Cacajao-Chiropotes*, whereas the cranial base supports atelid monophyly, *Saimiri-Cebus*, *Cacajao-Chiropotes* and occasionally *Lagothrix-Brachyteles* clades. The results from the face highlight the potential problem of describing a region with a low number of landmarks, as genera are often paraphyletic, and few molecular congruent clades are inferred. However, whilst error may be responsible, the facial landmarks could register genuine morphological variation that are reflected in the phylogenetic trees. Generally, the trees from the face, cranial base and whole skull generated alternative phylogenetic relationships, justifying the treatment of separate craniodental regions as partially autonomous modules.

When considering the phylogenetic trees alongside the phenetic relationships, size variation and allometry significantly affect morphological similarity shared between platyrrhines, although they are one of many factors that contribute to convergent evolution. Size variation and allometry are considered problematic for accurate phylogenetic analysis (e.g. Gilbert 2011), but they can also be viewed as another source of information that can both hinder and help reliable phylogenetic analysis, as strong support for callitrichine and atelid clades are supported by respective decreased and increased body sizes. The problem with allometry is that there is no agreement on how to measure and correct for it, and the studies that have

controlled for allometry with geometric morphometric data are based on principal component scores (e.g. Cardini et al. 2010, Elton et al. 2010, Gilbert 2011) that are problematic when applied to phylogenetic analysis (Adams et al. 2011). The effect of morphological variation on phylogenetic analyses has received even less attention, as have ecological and behavioural variation. These are important areas for future study; in particular, platyrrhine field studies will provide a clearer picture of platyrrhine behavioural ecology.

4.4.1 Phenetic craniodental evolution

Phenetic analysis of the whole skull (Figure 32) supported a morphological disparity between *Alouatta* and *Saimiri* and the other platyrrhines. The howler monkeys are certainly distinct from all other platyrrhines, they are the only taxa with frontal-sphenoid contact in the pterion, and have an enlarged hyoid bone, extremely robust faces, are slow-moving and energy conserving with reduced brain size relative to body size (Hartwig et al. 1996, Kinzey 1997, Isler et al. 2008). Squirrel monkey specialisations are likely linked to ontogeny and brain evolution, as they are born with nearly fully developed brains, relating to high predation and resource competition, and have large brains relative to body size, which could also be linked to having the largest social groups and behavioural flexibility of any platyrrhine (Hartwig 1995, Hartwig 1996, Kinzey 1997, Isler et al. 2008). The remaining platyrrhines were separated into two size clades, a smaller sized clade for callitrichines, *Aotus* and *Callicebus*, and a larger-bodied clade for *Cebus* with the remaining pitheciids and atelids. However, these two clades are also split between a clade with relative decrease in brain size for *Aotus*, *Callicebus* and callitrichines, and a group with relatively larger brains (Isler et al. 2008). Considering the basal position of *Alouatta* and *Saimiri* may also relate to brain size changes, the pattern of craniodental similarity could reflect encephalization rather than body size changes.

The pygmy marmoset fell outside the *Callithrix* group in all three phenetic analyses. Presumably, this morphological diversification relates to dwarfing and small body size, as the pygmy marmosets have experienced a significant size reduction, but they also have increased gummivory which could contribute to diversification (Ford & Davis 1992, Rosenberger 1992). The presence of callitrichine, atelid and saki-uakari groups in phenetic analyses match the morphology-based results of Rosenberger (1984), Ford (1986), Kay (1990), MacPhee et al. (1995), Horovitz (1999) and Kay et al. (2008). Either those phylogenetic analyses recorded largely phenetic relationships, or the phenetic and phylogenetic relationships are

both influenced by a shared underlying biological factors. The phenetic relationships reflected a mix of phylogeny, allometry and brain size changes, all of which are responsible for similarity in craniodental morphology between platyrrhine taxa.

4.4.2 Phylogenetic analysis of platyrrhine morphology

Phylogenetic analysis of overall skull morphology (Figure 32) supported molecular clades of *Cacajao-Chiropotes, Cebus-Saimiri* and callitrichine clades, with atelid monophyly or a *Lagothrix-Brachyteles* clade sister to *Ateles* depending on outgroup selection. If not for the presence of *Callicebus* as sister to *Aotus,* the phylogenetic analyses would support a monophyletic cebid clade as well. Clearly, there is some form of phylogenetic signal present in these data, but many molecular incongruent clades are also supported. The basal position of *Alouatta* with a strepsirrhine outgroup appears to be due to a large scale adaptive shift in the howler monkeys whereby their shape has become similar to strepsirrhines. This is especially interesting, as it highlights the position of *Alouatta* as one of the most specialised primates, with extreme specialisation in craniodental morphology.

For phylogenetic analysis of the face (Figures 33 and 34), use of non-strepsirrhine outgroups supported two molecular incongruent clades- a callitrichine clade with *Aotus* and *Callicebus* and another clade for *Saimiri-Chiropotes*, *Cebus* and *Cacajao*. This latter clade,also present with the strepsirrhine outgroups, could reflect the four groups sharing increased brain size relative to body size, as encephalization is linked to orbital orientation which could connect the four groups in facial morphology (Isler et al. 2008, Ross & Ravosa 1993). In several of the analyses of facial morphology there is divergence between *Callithrix jacchus* and *Callithrix penicillata* from the other *Callithrix* taxa. This separation relates partially to a phylogenetic distinction between the eastern Brazilian *jacchus*-taxa and the Amazonian pygmy and *argentata*-taxa (Rylands et al. 2009, Ford & Davis 2009), although the reasons for facial divergence are unknown, and do not relate to diet, as the jacchus groups share increased gummivory with pygmy marmosets (Ford & Davis 2009).

The position of *Callicebus* close to *Aotus* and callitrichines may be explained by homoplasy with *Aotus,* and shape similarity linked to smaller size and allometry, and shared adaptations for frugivory at the smaller range of body sizes- the size difference with *Leontopithecus* is not great and the amount of fruit consumed in the diet is similar. The position of *Pithecia* is more difficult to explain, as *Pithecia* is predominantly a seed eater and is much larger, but it may

share morphological similarity with *Callicebus* as a result of their more recent shared ancestry, pulling *Pithecia* into the clade with *Callicebus* and the callitrichines.

The results of phylogenetic analysis of the cranial base (Figures 35 and 36) often supported divergent morphology of *Saguinus midas* linking it to *Callimico* and a close morphological relationship between *Leontopithecus* and *Pithecia* that are relatively unexpected and difficult to explain. The divergent cranial base morphology of *Saguinus midas* clearly needs further investigation, and it is of note that Ford & Davis (2009) found overlap between *Callimico* and *Saguinus midas* in the first factor from discriminant function analysis of principle components extracted from postcranial traits. Possibly *Saguinus midas* and *Callimico* overlap in positional and locomotory behaviour with associated adaptations reflected in cranial base morphology. This is, of course, highly speculative, but much work remains to be done on callitrichine positional behaviour (Ford & Davis 2009) and the morphological similarities registered with the analyses described in this chapter may have clear behavioural correlates upon further study.

The connection between *Leontopithecus* and *Pithecia* was both phenetic and phylogenetic, and is a quantitative similarity that henceforth requires attention, but the biological reasons for this similarity remain enigmatic. Possibly the increased size and dietary flexibility of *Leontopithecus* has led to cranial base developments linking the group with *Pithecia*. The use of a non-strepsirrhine outgroup pulled *Cacajao-Chiropotes* to the base of the phylogeny, indicating a shared morphological similarity with Old World monkeys and gibbons. This could provide an interesting comparison for future work on cranial base evolution in anthropoids.

Phylogenetic analysis of the cranial base that used a combined strepsirrhine outgroup had the pygmy marmoset as the basal most lineage, which appears to be driven by similarity with *Galago senegalensis*. Both these small primates engage in extensive leaping behaviour, with possible cranial base adaptation and associated shape changed linked to locomotion measured in the phylogenetic analysis. When Old World monkey outgroups were used *Cebus* apella and *Cebus libidinosus* were separate from the other *Cebus* species. - *Cebus apella* are specialized for destructive foraging and have significantly different locomotor style with associated morphological differentiation when compared to *Cebus olivaceous*, both of which could contribute to cranial diversification (Rosenberger et al. 2009, Wright 2007). The sister relationship of *Cebus apella* with *Cebus libidinosus* is not unusual considering the latter was

historically seen as a subspecies of the former. When all outgroups were used for cranial base morphology *Saimiri* and *Cebus* were not inferred as sister taxa, the reason for which is unclear.

4.4.3 Synthesising past and present analyses

How have the geometric morphometric analyses discussed added to our understanding of the work on platyrrhine phylogenetics? The phenetic trees support a callitrichine clade as sister to a pitheciid-atelid clade much like the phylogeny proposed by Ford (1986). Ford (1986) and Rosenberger (1984) also inferred a sister relationship between *Aotus* and *Callicebus* that was strongly supported by whole skull and cranial base morphology, but not the face, likely due to the adaptation of *Aotus* to nocturnality with larger orbits. Although the molecular genetic data strongly indicate that these two genera are not sister taxa, a position that no amount of morphological analysis will supplant, geometric morphometric data also infers the shared derived morphology that Rosenberger (1984) and Ford (1986) acknowledged and Rosenberger et al. (2009) continues to support. It is clear that the morphological similarity shared by the two taxa is a quantitative reality, and is not simply linked to the cladistic methods or problems involved in those analyses. *Aotus* and *Callicebus* share a diet with a high proportion of fruit, and similarities in diet and mastication, and convergence upon similar body size, likely interact to infer a close relationship between the two groups. It is apparent that these two groups display one of the prime examples of morphological homoplasy in the primate group, serving as key taxa for further study of homoplasy in craniodental evolution.

Regarding other taxa, Ford (1986) was the only analysis that placed *Saimiri* near the base of the platyrrhine phylogeny, a position supported by phenetic and two phylogenetic analyses of the cranial base presented here, that probably relates to the unique ontogeny and large brain size in *Saimiri*. Rosenberger (1984), Ford (1986) and Kay (1990) all placed *Callimico* as the basal-most callitrichine, a position supported by the whole skull data, but not by other craniodental regions in the present analyses. For the atelids, when a monophyletic group was present, most often either the molecular clade of *Lagothrix-Brachyteles* was inferred or *Alouatta-Brachyteles*, the clade supported by Kay (1990) which corresponds to a shared, highly folivorous diet. Cluster analysis of the cranial base connected *Lagothrix* and *Ateles*, which share many behavioural and dietary adaptations, whilst several phylogenetic analyses of the face supported a sister relationship between *Alouatta* and *Ateles*. The molecular clade

of *Cacajao-Chiropotes* sister to *Pithecia*, that Rosenberger (1984), Ford (1986) and Kay (1990) was also supported by phenetic analysis of the whole skull, but not by any of the phylogenetic analyses. The *Cacajao-Chiropotes* clade was, however, inferred by phylogenetic analysis of the whole skull and cranial base, but not the face. The rare inference of saki-uakari and pitheciid clades are due to homoplasy with non-pitheciid groups, for example *Pithecia* appears to share a close relationship to *Leontopithecus* in cranial base morphology, which previous analyses have not reported, and the strong connection between *Aotus* and *Callicebus* in craniodental morphology is obvious.

The recent analysis of platyrrhine cranial morphology by Perez et al. (2011) also need to be considered in light of the results presented. Perez et al. (2011) stated that patterns of cranial shape in platyrrhines were not explained by size and allometry, but by molecular phylogenetic relationships. The results presented here contrast quite sharply, and agree with Marroig & Cheverud (2001) and Marroig & Cheverud (2005) that platyrrhine morphology is influenced by an interaction of phylogeny, size and diet. Results of platyrrhine phylogenetic analysis do not solely reflect size but it does have a clear role in shaping craniodental morphology and effecting accurate phylogenetic analysis, a prime example being the sister relationship between *Aotus* and *Callicebus*. Perez et al. (2011) suggested the differences in result with Marroig & Cheverud (2001, 2005) may have related to the use of geometric morphometrics in the former and more historical multivariate morphometrics in the latter, but this would not explain the differences between the phylogenetic analyses presented in this chapter and Perez et al. (2011).

If cranial shape is as closely linked to phylogeny as proposed in Perez et al. (2011), then past phylogenetic analyses and those detailed here would find greater congruence between molecular and morphological analyses. The disjuncture could be explained by the apparent contradiction that lies at the heart of the approach used by Perez et al. (2011), as highlighted by Klingenberg & Gidaszewski (2010). The tree length approach can measure a strong phylogenetic signal in morphological data, but phylogenetic analysis based on that morphological data may infer phylogenies inconsistent with molecular phylogenies. This raises difficult questions about methods, both those proposed by Klingenberg & Gidaszewski (2010) and the phylogenetic methods used to infer phylogenetic relationships from morphological and morphometric data. This explains, to a degree, how Perez et al. (2011) found a strong correlation between molecular and morphological distances, but the phylogenetic analysis in this chapter do not measure a strong phylogenetic signal overall.

Chapter 5 Atelid phylogenetic analysis

5.1 Introduction

Molecular and morphological analyses support the presence of a large bodied, monophyletic clade of platyrrhines, the atelids (Rosenberger 1984, Ford 1986, Kay 1990, Schneider et al. 1993, Barroso et al. 1997, Schrago 2007, Wildman et al. 2009, Perelman et al. 2011). Atelids are a four-genera monophyletic group including the howler monkeys (*Alouatta*), spider monkeys (*Ateles*), woolly monkeys (*Lagothrix*) and muriquis (*Brachyteles*), that inhabit the upper forest canopy across south and central America, and have a prehensile tail able to completely support body weight during feeding that is used to varying degrees in locomotion (Hartwig 2005, Di Fiore et al. 2011). *Alouatta* have extensive sympatry with other atelids; they overlap with *Ateles* in the eastern Amazon and central America, whereas *Ateles* and *Lagothrix* are isolated to the western Amazon and *Brachyteles* the Atlantic coastal forest (Strier 1992). Atelids display large variation in body size, with average muriquis body size (10.8kg) nearly 70% larger than average howler monkeys (6.5kg), and wide variation in sexual dimorphism within and between genera (Hartwig et al. 1996, Ford & Davis 1992). The group also display diversity in dietary preference, social organisation, mating systems and life histories, have increased relative brain size in *Ateles*, *Lagothrix* and *Brachyteles* but a large decrease in *Alouatta*, and locomotor adaptations for slow, energy conserving quadrupedalism in *Alouatta*, and extreme acrobatic suspensory locomotion in *Ateles* (Hartwig et al. 1996, Isler et al. 2008).

Although atelids are divided by dietary preference into frugivorous *Lagothrix* and *Ateles*, and folivorous *Brachyteles* and *Alouatta*, *Ateles* will increase leaf eating in times of scarcity and *Brachyteles* will increase fruit consumption in times of abundance (Rosenberger & Strier 1989, Norconk et al. 2009). Size differences between *Alouatta* and *Brachyteles* are large, but *Brachyteles* do not simply consume more leaves, they also have greater dietary flexibility and a frugivorous foraging strategy similar to *Lagothrix* and *Ateles* (Rosenberger & Strier 1989). Whilst *Alouatta* and *Brachyteles* share high relief beneficial for shearing, the shearing mechanisms are unique for each taxon, *Alouatta* using buccal and *Brachyteles* lingual shearing, which may indicate convergent evolution of folivory (Rosenberger & Strier 1989). The digestive abilities of *Brachyteles* and *Alouatta* could also vary (Rosenberger & Strier 1989), which seems likely considering their dietary proportions are similar, yet the two groups are very different in behaviour, body size and morphology.

Rather than subdividing atelids between frugivores and folivores, it appears that howler monkeys and the remaining atelids have taken two distinct evolutionary paths. *Alouatta* are specialised for minimal energy use, including a large reduction in brain size and slow locomotion, and have exaggerated, extreme craniodental specialisations, whilst the remaining atelids have experienced increased body and brain size, use more energy and have used more complex climbing and suspensory behaviours (Rosenberger & Strier 1989, Hartwig et al. 1996, Isler et al. 2008). The distinct evolutionary trajectory of *Alouatta*, folivory mixed with strategies for energy conservation, could be viewed as either a forced fall back to escape competition with other atelids, or a highly derived suite of adaptations that have helped their wide geographic spread and colonisation of new habitats (Strier 1992).

A comparative sample of atelid craniodental photographs are provided, with a specimen of each atelid genus shown in photographs from frontal (Figure 38), lateral (Figure 39), and basal (Figure 40) views. *Alouatta* are distinct from the rest of the atelids in braincase size and shape, foramen magnum position and basicranium flexion, linked to adaptive shifts in folivory and communication (Rosenberger & Strier 1989). They have a large face that is tilted upwards, a catarrhine-like configuration of the pterion with frontal-sphenoid rather than zygomatic-parietal contact, and are distinct from other platyrrhines with extreme anatomy including extremely robust maxilla and zygomatic bones (Kinzey 1997, Fleagle 1999, Hartwig et al. 1996). *Alouatta* are also quite prognathic, have an elongated muzzle, a wide palate, a very posteriorly positioned foramen magnum, and an occipital that connects to the parietal at a steep angle to give the cranial vault a non-globular shape (Rosenberger & Strier 1989).

The non-howler atelids share a rounded occipital and neurocranium, partially developed orbital torus and short basicranium (Rosenberger & Strier 1989). *Ateles* have a small head and face with gracile craniodental morphology including a narrow face with large orbits, a narrow but distinct snout and a globular braincase (Rosenberger & Strier 1989). In contrast, *Lagothrix* and *Brachyteles* both have large, broad faces, and less rounded braincases than seen in *Ateles* (Rosenberger et al. 2008, Rosenberger & Strier 1989). *Brachyteles* are more robust in the face than *Ateles* but less so than *Alouatta*, have a wide palate, as seen in *Alouatta* but without the curvature, a foramen magnum placed slightly more anteriorly than in *Alouatta* and like *Lagothrix* and *Ateles*, and a braincase which is relatively globular and more similar to *Lagothrix* and *Ateles*. *Lagothrix* have large heads with a slightly prognathic muzzle, share a relatively narrow palate with *Ateles*, although *Lagothrix* have a wider, more

robust face. In dental morphology, *Ateles* molars are relatively small with well-developed incisors, whereas *Alouatta* and *Brachyteles* have large molars and small incisors, and *Lagothrix* have both large incisors and molars (Rosenberger et al. 2008).

Figure 38 Frontal view of male *Alouatta* (top left), *Ateles* (top right), *Lagothrix* (bottom left) and *Brachyteles* (bottom right)

Figure 39 Lateral view of male *Alouatta* (top), *Ateles* (second top), *Lagothrix* (second bottom) and *Brachyteles* (bottom)

Figure 40 Basal view of female *Alouatta* (top left), *Ateles* (top right), *Lagothrix* (bottom left) and *Brachyteles* (bottom right)

5.1.1 Howler monkeys

Alouatta have the widest distribution of any platyrrhine, from southern Mexico to south-eastern Brazil and Argentina with populations in Colombia, Ecuador, Peru, Venezuela, Bolivia, Costa Rica, Guatemala, Honduras, Panama and Nicaragua (Kinzey 1997). They inhabit a diverse range of forest habitats including swamp, seasonally flooded, gallery, semideciduous and dry deciduous forest, with occasional long distance terrestrial travel between patches of forest (Kinzey 1997). They have an enlarged hyoid bone that functions as a resonator to increase the volume of long calls likely used to communicate with other group members and solitary individuals, strengthen pair bonds, advertise group composition, and space out competing groups (Kinzey 1997). The howler monkeys are the smallest atelids with an average body weight of 6.5kg, and are the most sexually dimorphic platyrrhines with an average dimorphism ratio of 1.39 but a range of 1.08-1.84 and considerable variation in dimorphism between populations of the same species (Ford & Davis 1992, Plavcan & van Schaik 1998).

Howler monkeys generally live in cohesive groups with several adult females and one adult male, although there is wide variation in group size and sex proportions dependent on population and species studied (Kinzey 1997). They have a polygynous mating system where dominant males monopolise mating opportunities with aggression and infanticide common as a result, and both sexes often disperse from the natal group prior to maturation although there is a slight female bias in dispersal patterns (Kinzey 1997, Di Fiore et al. 2011). They have a diet with high levels of folivory (54%) and significant proportions of frugivory (34%) with some flower consumption (9%), although field studies indicate dietary preference is often linked to seasonality (Norconk et al. 2009, Kinzey 1997). Howlers have evolved a strategy of energy conservation, with slow moving energy-efficient quadrupedalism, much smaller home ranges than other atelids and a large relative brain size reduction (Kinzey 1997, Rosenberger & Strier 1989, Isler et al. 2008).

5.1.2 Spider monkeys

Spider monkeys also have a wide distribution and are the northern most platyrrhine, spanning southern and eastern Mexico into Brazil and Bolivia, and populations in Colombia, Belize, Costa Rica, El Salvador, Panama, Nicaragua, Honduras, Peru, Ecuador, Guyana, Surinam and French Guiana (Kinzey 1997). They inhabit evergreen tropical forests with a preference for humid, lowland, primary forest, although they are also present in secondary highland, dry,

swamp and deciduous forests (Kinzey 1997). They are around 8kg in size, are effectively monomorphic, and are the most frugivorous platyrrhine group with nearly 90% fruit consumption, specialising in ripe fruit and feeding in large trees (Ford & Davis 1992, Norconk et al. 2009, Kinzey 1997, Di Fiore et al. 2011). They have very long limbs, a long tail, and have a vestigial or completely absent thumb, all of which are linked to their acrobatic and highly energetic form of locomotion and suspension (Kinzey 1997, Di Fiore et al. 2011). Although they have been observed utilising quadrupedalism, bipedalism, climbing and leaping, they are known for suspensory locomotion with brachiation, often suspending from only the tail (Kinzey 1997, Fleagle 1999). They exhibit fission-fusion organization, living in large multimale-multifemale groups which break down into smaller foraging units, with philopatric males staying in their breeding group whereas females disperse upon maturation (McFarland Symington 1990, Kinzey 1997).

5.1.3 Muriquis

Brachyteles are found in the eastern Brazilian Atlantic coastal forests with a preference for primary or secondary-tall forests, where habitat destruction has caused populations to drop very low (Kinzey 1997). The muriquis are the largest platyrrhines, with an average size above 10kg and low levels of sexual dimorphism, consuming a mainly folivorous diet with a preference for immature leaves and dental adaptations for lingual shearing, although fruit consumption is high when available (Kinzey 1997, Ford & Davis 1992, Norconk et al. 2009). Their social organisation is complex as they have been observed to have both fission-fusion and cohesive group structures, although the cohesive groups were later observed to break into smaller groups when group size became large, indicating underlying fission-fusion structure, in addition to a polygamous mating system, and are noteworthy for their low levels of aggression (Kinzey 1997, Strier 1987, Di Fiore et al. 2011). They used a mix of quadrupedal walking and running, in addition to climbing, leaping and suspension, with suspensory locomotion in particular allowing for rapid movement between patches of high value foods (Kinzey 1997, Strier 1987). Like spider monkeys, and linked to their shared use of fast semi-suspensory brachiation, muriquis will either have an absent or vestigial thumb, with shoulder adaptations and elongated tail and limbs (Kinzey 1997, Di Fiore et al. 2011).

5.1.4 Woolly monkeys

Lagothrix are relatively large platyrrhines, with an average weight around 7kg and relatively high levels of sexual dimorphism depending on the population studied (Ford & Davis 1992,

Plavcan & van Schaik 1998). They are found in the upper Amazon basin of western Brazil and Venezuela, and eastern Peru, Columbia and Ecuador, at altitudes between sea level and 2.5km in primary forests (Kinzey 1997). The woolly monkey diet is largely frugivorous (64%), mostly ripe fruits, with additional feeding on leaves (6%) and insects (9%) (Norconk et al. 2009, Kinzey 1997). They are almost exclusively arboreal but for short periods travelling between forests, and spend most of their time in the upper canopy (Kinzey 1997, Ramirez 1988). Locomotion is largely by quadrupedal walking and running, with some use of the tail but without the dexterity of *Ateles* or *Brachyteles*, and the tail is instead often used to anchor the body in postures (Kinzey 1997, Ramirez 1988). Socially, *Lagothrix* are polygamous, with large variation in group size and flexibility in social organisation and grouping patterns, male dominance hierarchies, and mostly female dispersal, although it appears male dispersal is more common than originally thought (Kinzey 1997, Ramirez 1988, Di Fiore 2009, Di Fiore et al. 2011). They are also the most active atelid, in terms of time spent per day in activity compared to rest, spending 60% of their activity time on subsistence, due to their need for large amounts of fruit, large group sizes, and slower locomotion (compared to *Ateles*) making it more difficult to meet their dietary needs (Di Fiore et al. 2011).

5.1.5 Atelid phylogenetic relationships

Molecular phylogenetic analyses of platyrrhines have repeatedly supported the atelids as a monophyletic clade with *Lagothrix*-*Brachyteles* sister to *Ateles* and *Alouatta* the basal-most lineage (Schneider et al. 1993, Harada et al. 1995, Horovitz & Meyer 1995, Schneider et al. 1996, Barroso et al. 1997, Porter et al. 1997, Horovitz et al. 1998, von Dornum & Ruvolo 1999, Canavez et al. 1999a, Porter et al. 1999, Schneider 2000, Schneider et al. 2001, Opazo et al. 2006, Schrago 2007, Hodgson et al. 2009, Wildman et al. 2009, Perelman et al. 2011). The phylogenetic relationships as inferred by morphological data have historically contrasted sharply with the molecular view. In his excellent review of atelid phylogenetic relationships and evolution, Hartwig (2005) viewed the study of atelid brachiation by Erikson (1963), which described shared similarity in *Brachyteles* and *Ateles*, as the forbearer of a new era of morphological study starting with the dental analysis of Orlosky (1973), that found *Ateles* and *Lagothrix* shared dental similarity distinct from *Brachyteles*.

Hartwig (2005) noted that the theses of Rosenberger (1979) and Ford (1982), upon which their later published work was largely based, had few specimens of *Brachyteles* to study, with

Ford (1982) basing postcranial analysis on a single immature *Brachyteles* specimen; this likely had significant (detrimental) effects on phylogenetic analyses. *Alouatta* was proposed as the basal most atelid taxon by Rosenberger (1977), Rosenberger (1981) and Rosenberger (1984) and supported by Ford (1986). Ford (1986) recognised a close relationship between *Alouatta* and *Ateles*, *Brachyteles* and *Lagothrix* that was inferred by multiple shared derived features. These included a posteriorly reduced metacone, lingual cleft on upper molars, deep narrow bicipital grooves on the upper arm, trochlear process on the posterior part of the heel bone, and increases in elements of femoral and humeral indices. In all, the four taxa shared 5 dental and 29 postcranial traits. Although many traits confirmed the monophyly of the atelids, *Alouatta* had many unique autapomorphic traits, 14 dental and 13 postcranial, in addition to karyotype and hair follicle data.

Although *Alouatta* was clearly the basal taxon for the atelid group, the relationships between *Brachyteles*, *Ateles* and *Lagothrix* were less well resolved. Rosenberger (1977, 1981,1984) and Ford (1986) viewed *Lagothrix* as sister to *Ateles-Brachyteles*. Ford (1986) found *Brachyteles* had 8 dental and 12 postcranial unique derived traits, although 5 dental and 2 postcranial traits were shared with *Alouatta*- the dietary similarity and associated adaptations linking the two groups. *Ateles* shared many traits with either *Brachyteles* or *Lagothrix*; for *Brachyteles* there were four dental derived traits and shared karyotype number, but though they shared locomotory behaviour there were no shared postcranial traits. In contrast, *Ateles* and *Lagothrix* shared 9 derived features including two that were related to the femoral index, a low rounded mound on the posterior part of the femur neck, and a slight bow of the femoral shaft. Most of the postcranial traits connecting *Ateles* and *Brachyteles* related to the ankle, whilst those connecting *Lagothrix* and *Ateles* were on the hip and knee.

There was no evidence from Ford (1986) to support closer proximity of *Lagothrix* and *Brachyteles*.Hartwig (2005) found *Ateles* and *Brachyteles* to be significantly different from *Lagothrix* in his own thesis (Hartwig 1993), whilst the thesis of Cole (1995) found synapomorphic brain size increase in *Ateles* and *Brachyteles*. Rosenberger & Strier (1989) viewed several shared postcranial traits between *Ateles* and *Brachyteles*, including suspensory adaptations for long metacarpals, loss of thumb function and midcarpal grasping, as proof of recent common ancestry. The reliance on platyrrhine postcranial data as phylogenetically informative was rejected by Lockwood (1999), who showed that high levels of homoplasy minimise the phylogenetic signal, with the locomotory functional system overtly shaping postcranial similarity.

Kay (1990) challenged the earlier phylogenetic proposals and viewed atelids as a dichotomy between *Alouatta-Brachyteles* and *Ateles-Lagothrix*, and the phylogenetic analysis of Horovitz & Meyer (1997) inferred the same relationships. Kay et al. (2008) placed *Alouatta-Brachyteles* sister to *Lagothrix* when only morphological data was analysed, although in common with Horovitz et al. (1998), when molecular and morphological data were combined they both supported a sister relationship between *Brachyteles* and *Lagothrix* due to the strength of the molecular data. Cole et al. (2002) carried out a largely methodological study, examining cluster analysis of morphometric interlandmark distances from the atelid face, and found strong support for a phenetic relationship between *Lagothrix-Ateles* and *Brachyteles*. Following Rosenberger & Strier (1989), they hypothesised the *Lagothrix-Ateles* clade related to shared primitive adaptations linked to a frugivorous diet, but also noted the inferred relationship may be a result of the basal-most lineage *Alouatta* being so different to the other taxa involved in analysis.

5.2 Methods & materials

Phylogenetic analysis of atelids included 16 ingroup taxa and 9 outgroup taxa, all of which are listed in Table 14 alongside sample sizes for male, female and pooled sex. Geometric morphometric analysis was carried out in the MorphoJ program and phylogenetic analysis in the Phylip software package. Each ingroup-outgroup combination was repeated for data with only males, only females, pooled sex and treatment of male and females of the same species as separate taxa. Phylogenetic analyses were carried out for atelids only, with both NJ using *Alouatta* as outgroup and UPGMA phenetic trees using no outgroup, and with a single outgroup for each of the 9 taxa selected. Different combinations of outgroups were also used; all outgroups (9 taxa), all strepsirrhines (4 taxa), all Old World anthropoids (5 taxa), all Old World monkeys (4 taxa), and two-taxon combinations for Cercopithecinae, Colobinae, Galagonidae and *Eulemur-Perodicticus*. Using geometric morphometric data and distance-based phylogenetic methods, consensus trees were inferred using three datasets: the whole skull as described by 63 landmarks, 15 landmarks describing the face and 24 landmarks describing the cranial base. Note that for some craniodental regions there were more/fewer phylogenetic trees inferred, as in some cases where multiple outgroups were used the tree topology changed depending on which outgroup was used to root the tree, while in others it did not.

Tables 14 Atelid and outgroup sample sizes for male, female and pooled sex analyses

Ingroups			
Taxa	**Male**	**Female**	**Pooled**
Alouatta belzebul	10	10	20
Alouatta caraya	9	11	20
Alouatta coibensis	8	9	17
Alouatta fusca	9	9	18
Alouatta palliata	18	13	31
Alouatta pigra	8	10	18
Alouatta seniculus	22	10	32
Ateles belzebuth	11	10	21
Ateles fusciceps	10	10	20
Ateles geoffroyi	10	10	20
Ateles paniscus	7	12	19
Brachyteles arachnoides	7	5	12
Lagothrix cana	10	11	21
Lagothrix lagothricha	10	10	20
Lagothrix lugens	8	10	18
Lagothrix poeppigii	10	10	20

Outgroups			
Taxa	**Male**	**Female**	**Pooled**
Chlorocebus aethiops	10	10	20
Colobus guerza	11	10	21
Eulemur fulvus	10	10	20
Galago senegalensis	10	11	21
Hylobates lar	10	10	20
Macaca mulatta	9	10	19
Otolemur garnetti	10	9	19
Perodicticus potto	10	10	20
Trachypithecus obscura	10	10	20

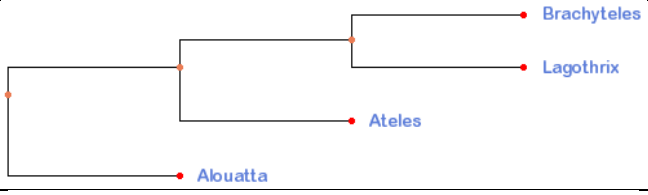
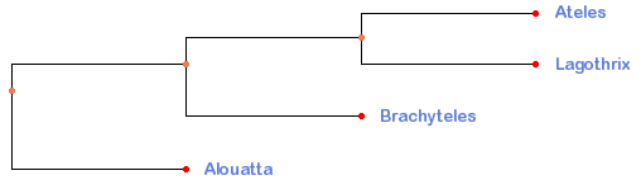
5.3 Results

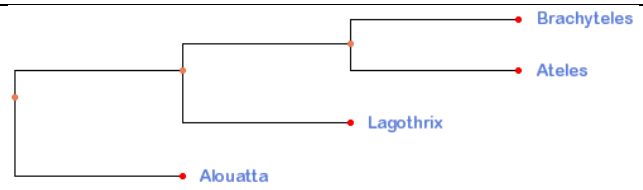
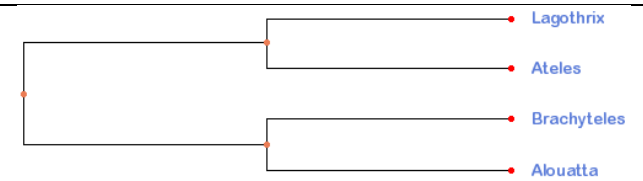
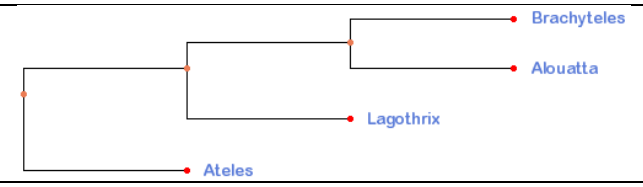
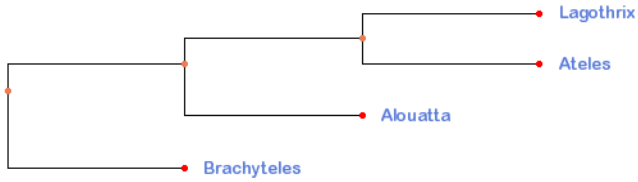
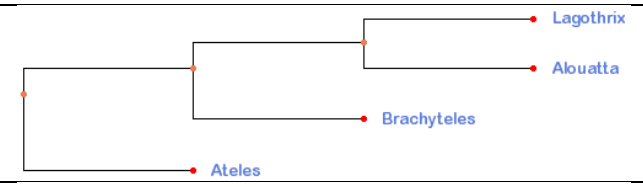
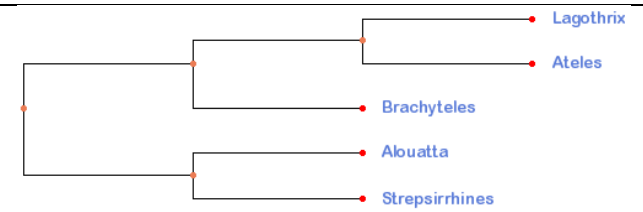
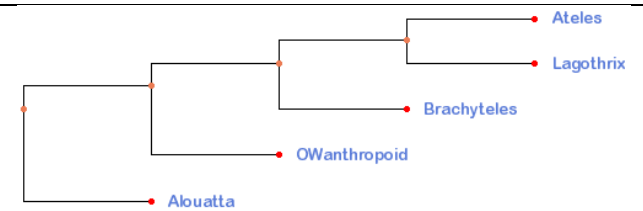
5.3.1 Whole skull

The phylogenies inferred by phenetic and phylogenetic analysis of the whole skull are shown in Table 15, with genus-level phylogenies of atelids inferred on the left hand side and the analyses and outgroups that produced the trees listed on the right hand side.

The cluster analyses (UPGMA) of whole skull shape were the only analyses of the entire skull that reproduced the strongly supported molecular phylogenetic relationships of atelids. The vast majority of analyses (54 trees out of 77) inferred a tree with *Ateles* and *Lagothrix* sister to *Brachyteles* with *Alouatta* the basal-most lineage. For male specimens, the use of strepsirrhine outgroups inferred an *Ateles-Brachyteles* clade sister to *Lagothrix.* Analyses of female specimens with *Hylobates* as outgroup inferred a dichotomy of *Ateles-Lagothrix* and *Alouatta-Brachyteles*. Several of the datasets analysed with *Macaca* as outgroup produced a tree with *Alouatta-Brachyteles* sister to *Lagothrix*. Female and separate sex analysis with *Trachypithecus* as outgroup inferred *Ateles-Lagothrix* sister to *Alouatta*, and male *Hylobates* as outgroup inferred *Lagothrix-Alouatta* sister to *Brachyteles*. When all outgroups were included rooting with an Old World anthropoid places *Alouatta* in a clade with strepsirrhines, and rooting with a strepsirrhines places Old World anthropoids with *Ateles*, *Lagothrix* and *Brachyteles* to the exclusion of *Alouatta*.

Table 15 Atelid phylogenetic relationships inferred from whole skull morphology

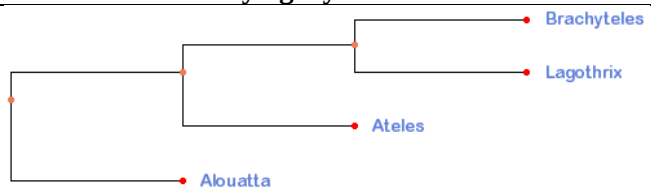
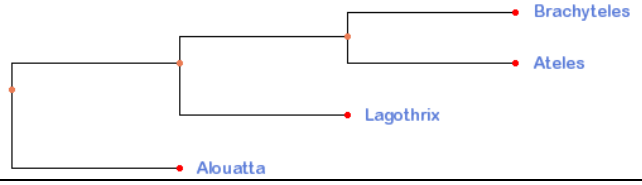
Phylogeny inferred	Outgroup(s) used
	UPGMA (all)
	*Alouatta* (female, pooled, separate) *Chlorocebus* (female, pooled, separate) *Colobus* (all) *Trachypithecus* (male, pooled) *Macaca* (male, pooled, separate) *Otolemur* (female, pooled, separate) *Galago* (all) *Eulemur* (female, pooled, separate) *Perodicticus* (female, pooled, separate) Cercopithecoidea (all) Colobinae (all) Galagonidae (female, pooled, separate) *Eulemur-Perodicticus* (female, pooled,

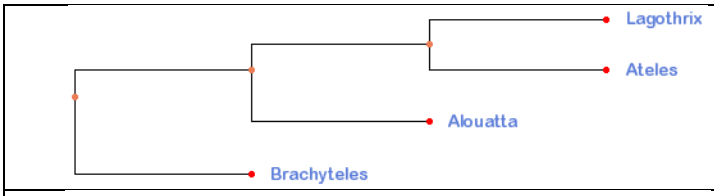
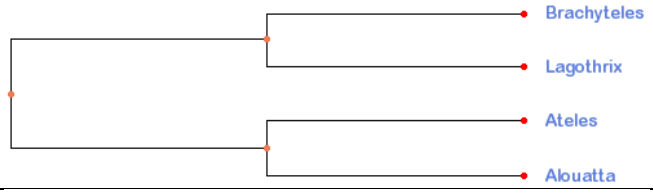
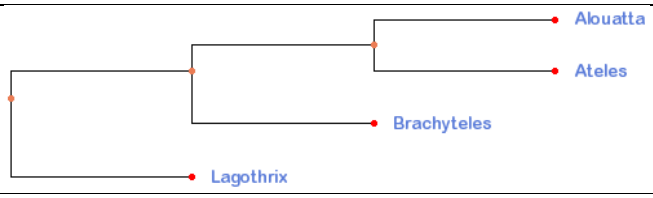
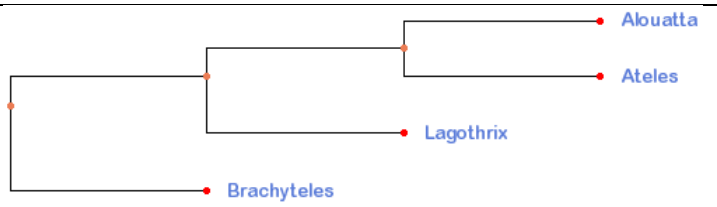
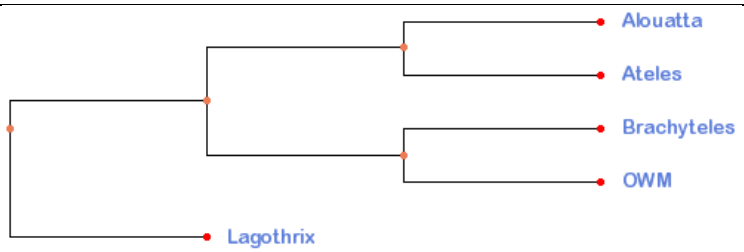
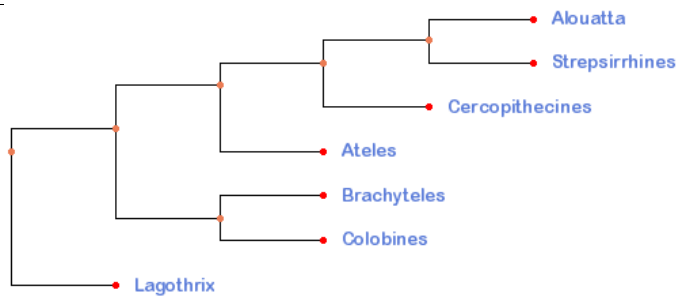
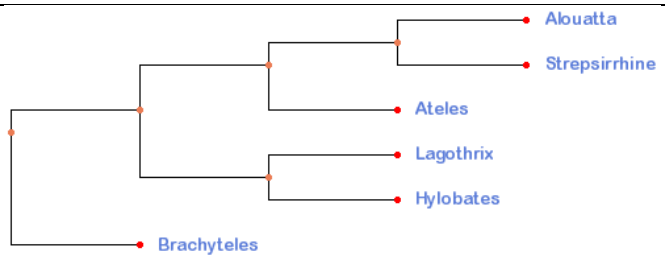
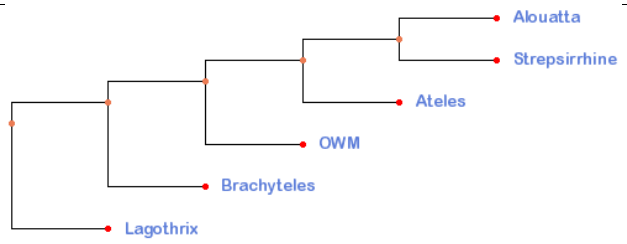
	separate) Old World anthropoid (all) Old World monkeys (all) Strepsirrhine (all)
	*Alouatta* (male) *Otolemur* (male) Galagonidae (male) *Eulemur* (male) *Perodicticus* (male) *Eulemur-Perodicticus* (male)
	*Hylobates* (female)
	*Macaca* outgroup (female, pooled, separate)
	*Trachypithecus* (female, separate)
	*Hylobates* (male)
	All outgroups Old World anthropoid root (female, male, pooled)
	All outgroups strepsirrhine root (all)

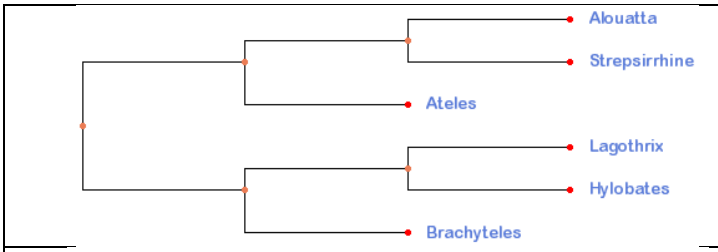
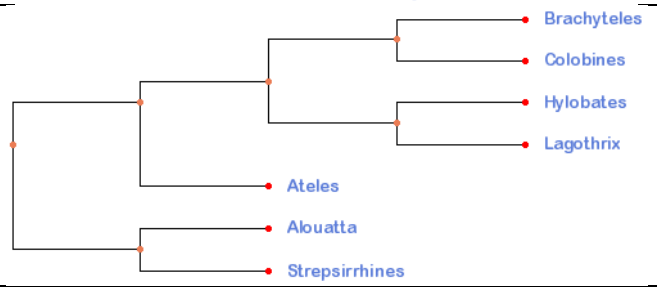
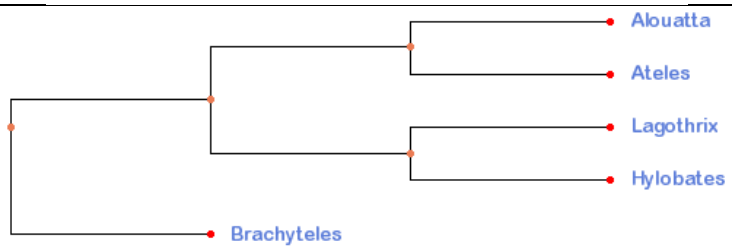
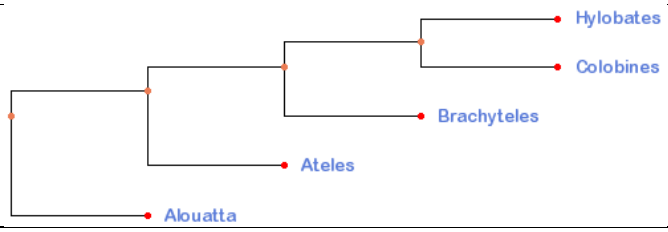
5.3.2 Facial morphology

The results of phenetic and phylogenetic analysis of the face are shown in Table 16. The tree most commonly inferred (41 of 83 analyses) was congruent with the strongly supported atelid molecular phylogeny; *Lagothrix-Brachyteles* sister to *Ateles* with *Alouatta* as the basal-most lineage. The phenetic clustering produced the molecular phylogeny for female, pooled and separate sex datasets, but male data inferred a *Brachyteles-Ateles* clade sister to *Lagothrix*. Use of the basal atelid *Alouatta*, *Macaca*, *Eulemur*, *Galago*, *Otolemur*, *Perodicticus*, or combination of *Eulemur-Perodicticus*, Cercopithecinae, Old World monkey (male only) or strepsirrhines inferred the same phylogenetic relationships as moleculardata. Several female and/or pooled sex analyses with *Chlorocebus*, *Colobus*, Old World monkey and Cercopithecinae outgroups inferred the molecular *Brachyteles-Lagothrix* clade but as a dichotomy with *Ateles-Alouatta*. The use of *Hylobates* as outgroup inferred *Alouatta-Ateles* sister to *Brachyteles*. A tree with *Alouatta-Ateles* sister to *Lagothrix* was inferred with *Trachypithecus* as outgroup, and a mix of separate sex, male and pooled analyses with *Chlorocebus*, *Colobus*, Colobinae and Old World monkey combination outgroups. The use of large combinations of outgroups was particularly problematic, with all analyses with 5 or more outgroups inferring paraphyletic atelid clades. This most commonly inferred a relationship between *Alouatta* and strepsirrhines or *Lagothrix* and *Hylobates*.

Table 16 Atelid phylogenetic relationships inferred from facial morphology

Phylogeny inferred	Outgroup(s) used
	UPGMA (female, pooled, separate) *Alouatta* (female, separate) *Macaca* (all) *Eulemur* (all) *Perodicticus* (all) *Galago* (all) *Otolemur* (all) Cercopithecoidea (male, pooled, separate) Galagonidae (all) *Eulemur-Perodicticus* (all) OWM (male) Strepsirrhine (all)
	UPGMA (male)

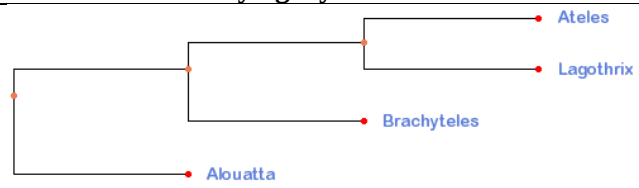
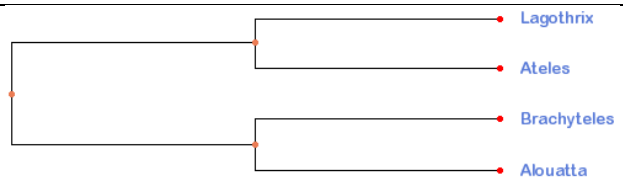
	Colobinae (female)
	*Chlorocebus* (female, pooled) *Colobus* (female, pooled) Cercopithecinae (female) Old World monkey (female)
	*Hylobates* (all)
	*Chlorocebus* (male, separate) *Trachypithecus* (all) *Colobus* (male, separate) Colobinae (pooled, separate) Old World monkey (pooled, separate)
	Old World anthropoid *Hylobates* root (all)
	All outgroups *Hylobates* root (pooled)
	All outgroups *Perodicticus* root (pooled)
	All outgroups *Hylobate* root (female, male)

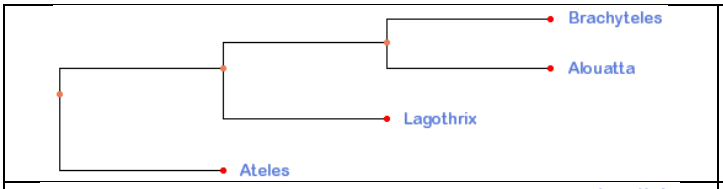
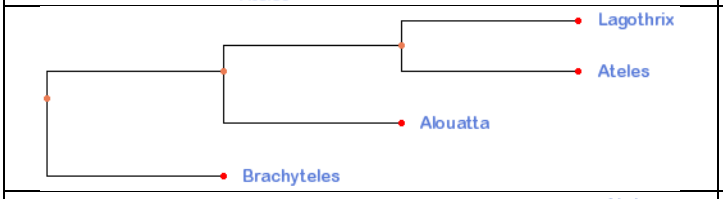
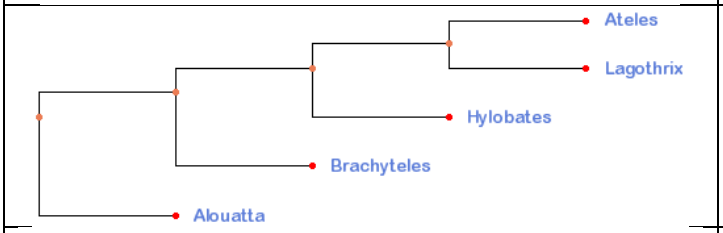
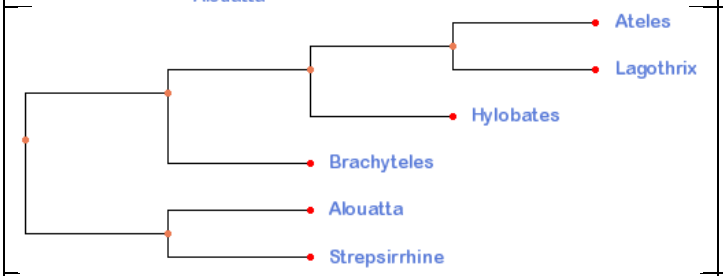
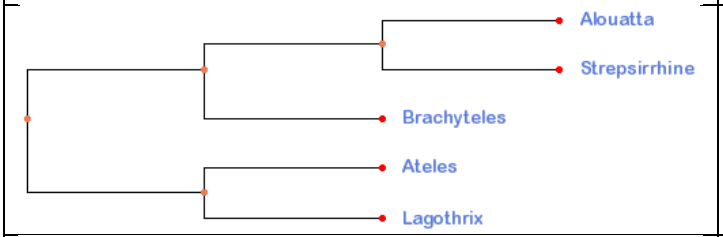
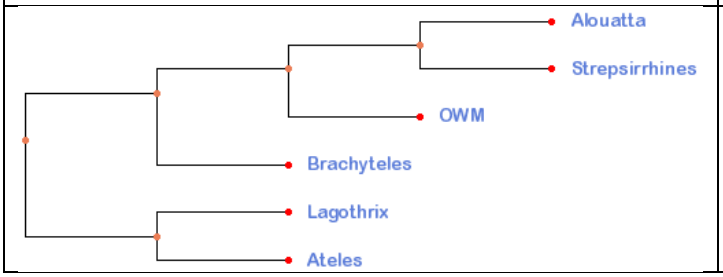
	All outgroups *Macaca* root (female)
	Old World anthropoid *Macaca* root (all) All outgroups *Macaca* root (pooled)
	All outgroups (separate)
	All outgroups *Macaca* root (male)

5.3.3 Cranial base

Results of the phylogenetic analysis of the cranial base are shown in Table 17. The relationships inferred by molecular datawere not inferred by any analysis of the cranial base. The vast majority of inferred trees either had a dichotomy between *Alouatta-Brachyteles* and *Lagothrix-Ateles* (26 trees), or have *Brachyteles* sister to *Ateles-Lagothrix* (40 trees). The phenetic tree and phylogenetic trees with *Alouatta*, *Colobus*, *Trachypithecus* (male only), *Otolemur*, *Eulemur*, *Perodicticus*, *Otolemur* (male only), *Eulemur-Perodicticus*, strepsirrhines and colobinae (except separate sex) supported *Brachyteles* as sister to *Lagothrix-Ateles*. The use of *Chlorocebus*, *Macaca*, *Trachypithecus* (except male), *Otolemur* (except male), cercopithecinae, colobinae (separate sex only) and Old World anthropoids (except female) inferred an *Ateles-Lagothrix* and *Brachyteles-Alouatta* dichotomy. The use of *Hylobates* as outgroup inferred a tree with *Alouatta-Brachyteles* sister to *Lagothrix*. The use of all outgroups produced trees where atelids were paraphyletic, with *Alouatta* in a clade with strepsirrhines for a *Macaca* (except male) rooted tree, or the Old World anthropoids within a clade with *Ateles*, *Lagothrix* and *Brachyteles* when *Eulemur* or *Macaca* (male only) were used to root the tree.

Table 17 Atelid phylogenetic relationships inferred from cranial base morphology

Phylogeny inferred	Outgroup(s) used
	UPGMA (all) *Alouatta* (all) *Colobus* (all) *Trachypithecus* (pooled) *Otolemur* (all) *Galago* (male) *Eulemur* (all) *Perodicticus* (all) Colobinae (female, male, pooled) Galagonidae (all) *Eulemur-Perodicticus* (all) Strepsirrhines (all)
	*Chlorocebus* (all) *Macaca* (all) *Trachypithecus* (female, male, separate) *Galago* (female, pooled, separate) Cercopithecinae (all) Colobinae (separate) Old World monkey (all) Old World anthropoid (male, pooled, separate)

	*Hylobates* (all)
	Old World anthropoid (female)
	All outgroups *Eulemur* root (all)
	All outgroups *Macaca* root (male)
	All outgroups *Macaca* root (female, pooled, separate)
	All outgroups *Hylobates* root (all)

5.3.4 Summary of results

Phylogenetic results from atelids for all craniodental regions, outgroups and outgroup combinations are summarised in Table 18.

Table 18 Summary of inferred atelid phylogenetic relationships

		Outgroup or Outgroup combination	UPGMA	*Chlorocebus*	*Colobus*	*Eulemur*	*Galago*	*Hylobates*	*Macaca*	*Otolemur*	*Perodicticus*	*Trachypithecus*	Cercopithecoidea	Colobinae	Galagonidae	*Eulemur-Perodicticus*	Old World anthropoid	Old World monkeys	Strepsirrhine	All outgroups
Whole skull	**Molecular clades**	*Lagothrix-Brachyteles-Ateles*	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
		Lagothrix-Brachyteles	✓																	
	Molecular incongruent clades	*Ateles-Lagothrix & Alouatta-Brachyteles*						✓												
		Ateles-Lagothrix		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
		Ateles-Brachyteles						✓												
		Atelid paraphyly																		✓
Face	**Molecular clades**	*Lagothrix-Brachyteles-Ateles*	✓			✓	✓		✓	✓	✓		✓		✓	✓			✓	
		Lagothrix-Brachyteles	✓	✓	✓	✓	✓		✓	✓	✓		✓		✓	✓			✓	
	Molecular incongruent clades	*Ateles-Lagothrix-Alouatta*										✓					✓	✓		
		Ateles-Brachyteles-Alouatta						✓												
		Lagothrix-Brachyteles-Alouatta						✓				✓						✓		
		Alouatta-Ateles				✓	✓	✓				✓					✓	✓		
		Atelid paraphyly																✓		✓
Cranial base	**Molecular clades**	*Lagothrix-Brachyteles-Ateles*	✓		✓	✓	✓			✓	✓	✓		✓	✓	✓			✓	
		Lagothrix-Brachyteles																		
	Molecular incongruent clades	*Ateles-Lagothrix & Alouatta-Brachyteles*		✓					✓								✓	✓		
		Ateles-Brachyteles-Alouatta						✓												
		Ateles-Lagothrix	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
		Ateles-Brachyteles		✓				✓	✓								✓	✓		
		Atelid paraphyly																		✓

5.4 Discussion

The phenetic and phylogenetic results presented in Tables 15-18 highlight the benefit of morphological analyses on monophyletic clades with a restricted number of genera that have evolved over a smaller scale of time compared to the analyses of the entire platyrrhine clade described in the previous chapter. Morphological analysis of the atelid skull found a strong relationship between diet, mastication and stress with overall craniodental and cranial base shape, supporting a close relationship between the frugivorous *Ateles* and *Lagothrix*, and to a lesser extent between folivorous *Alouatta* and *Brachyteles*. In an unexpected finding, atelid facial morphology reflected phylogeny and supported a *Lagothrix* and *Brachyteles* clade sister to *Ateles*. The phylogenetic signal in the face was supported by divergence of the howler monkey at the base of the clade, which may be due to their reduced size, as they are the smallest atelid, or extreme adaptations for folivory and energy conservation, which includes a large decrease in brain size (Ford & Davis 1992, Rosenberger & Strier 1989, Isler et al. 2008). The brain size reduction is important for understanding the phylogenetic signal of the face, because size change can affect orbital orientation (Isler et al. 2008, Ross & Ravosa 1993), and the remaining atelids have relative brain size increases (Isler et al. 2008), which will further contribute to the divergence of *Alouatta* away from the other atelids. Facial morphology also differentiates the remaining atelids between gracile morphology of *Ateles* and increased size and robusticity in *Lagothrix* and *Brachyteles* (Ford & Davis 1992, Rosenberger & Strier 1989, Rosenberger et al. 2008, Hartwig 2005).

5.4.1 Atelid phenetic evolution

The phenetic shape of the atelid skull maintains a phylogenetic signal, as the phenetic trees from overall skull (Table 15) and facial morphology (Table 16) were congruent with the molecular phylogenetic relationships. It is well documented that *Alouatta* are divergent from the remaining atelid taxa, explaining its basal phenetic position, whilst *Lagothrix* and *Brachyteles* share similarity in the face and cranial vault compared to the more gracile *Ateles* (Rosenberger et al. 2008). The congruence between phenetic morphological and molecular phylogenetic is relatively rare, and ought to be of interest for the wider understanding of morphological evolution in primates, especially because phenetic patterns are often considered distinctly separate, often in outright disagreement, with phylogenetic relationships. In molecular phylogenetics, the UPGMA phenetic method will infer the correct phylogenetic tree when the assumptions of a molecular clock are met (Nei & Kumar 2000),

and to extrapolate this to morphology, the congruence between phenetic and phylogenetic data indicates that for atelids, morphological shape has evolved in a steady clocklike manner.

However, not all the atelid phenetic relationships were congruent with molecular phylogenetic relationships. Phenetic analysis of facial morphology in males (Table 16) inferred a *Brachyteles-Ateles* clade, indicating that in males sexual dimorphism masks the underlying phylogenetic signal, as the two taxa are linked by reduced sexual dimorphism compared to *Alouatta* and *Lagothrix* (Ford & Davis 1992, Rosenberger et al. 2008). The phenetic relationship from the cranial base (Table 17) linked *Ateles* and *Lagothrix*, which are both highly frugivorous, and mastication and stress associated with diet appears to shape cranial base morphology. It is clear for example that *Ateles* and *Lagothrix* are more gracile, and *Brachyteles* and *Alouatta* more robust, in the mandibular fossa of the temporal bone, which contributed to the phenetic patterns inferred.

5.4.2 The atelid phylogenetic signal

Multiple phylogenetic analyses of the atelid face (Table 16), using a variety of anthropoid and strepsirrhine outgroups and outgroup combinations, inferred the accepted atelid molecular phylogeny. The presence of a phylogenetic signal in facial morphology is particularly surprising, reflecting phylogeny and not diet and mastication, as otherwise one of *Alouatta-Brachyteles* or *Ateles-Lagothrix* would be the terminal clade rather than *Lagothrix-Brachyteles*. It will be especially interesting in future work to integrate facial data from *Lagothrix flavicauda* and from fossil taxa of *Protopithecus* and *Caipora* to test the affinities of those groups to the remaining atelid taxa. The exciting implication of these results is that they challenge the view that atelids provide an abundance of morphological support for sister relationships that the molecular data refute (Hartwig 2005). The perception of a clash between the morphology and molecular evolution in atelids may be, at least partially, due to the methods of past morphological analyses. However, the support for *Brachyteles* and *Lagothrix* as sister taxa in the phylogenetic analyses described in this chapter only come from facial morphology, so there is clearly a great deal of homoplasy and shape data that infer relationships that clash with molecularrelationships.

The modular approach, and its relative success in identifying different regions of the skull as inferring alternative phylogenetic hypotheses, may well inform us on how to better understand atelid evolution- namely with further concentration and study on the morphology of the face. Even if atelids are an unsual clade with peculiar facial evolution, the group are

prime candidates for further investigation and analysis of the connection between morphological and molecular evolution, as well as the effects of diet and mastication on cranial base evolution. If atelids turn out to be one of many groups that maintain phylogenetic information in the face, potentially modern humans, apes and guenons (e.g. Harvati & Weaver 2006a,b, Lockwood et al. 2004, Cardini & Elton 2008) are the exception to the primate and mammalian rule. It is possible that previous work, in a rush to dismiss facial similarity as plastic and vulnerable to homoplasy (e.g. Lieberman 1995, Wood & Lieberman 2001) have ignored a rich source of phylogenetic information.

It seems intuitive that different regions of the skull will retain different elements of phylogenetic information dependent on the taxa examined and the macroevolutionary or taxonomic level studied (Harvati & Weaver 2006a). Perhaps the temporal region of the skull is informative for inference of subspecies and species level relationships, but at the genus level, facial morphology becomes more reliable for understanding broader phylogenetic evolution. Resolution will only come from further work on all clades of the primate group. There is certainly an important lesson about utilising an experimental approach to the question of phylogenetic inference based on morphology, as for too long theoretical objections have stifled investigative analyses. It is clear that this clade of New World primates have managed to maintain a phylogenetic signal in the face with a derived facial morphotype even though the terminal taxa consist of two primates with very different diets.

5.4.3 Phylogenetic analyses considered

It is necessary to examine the phylogenetic relationships inferred by the diverse combination of outgroups and craniodental regions, and consider their relevance in light of past phylogenetic analyses. Phylogenetic analysis of the face (Table 16) reproduced the well-accepted atelid molecular phylogenetic relationships with many outgroups and outgroup combinations, and whilst *Macaca*, cercopithecine and Old World monkey combinations of outgroups inferred the molecular relationships, strepsirrhines were significantly more successful as all single strepsirrhine and strepsirrhine outgroup combinations inferred the same relationships as the molecular phylogeny from facial morphology. It seems likely that the strepsirrhine face is so distinct from the atelids that it provides adequate polarity for accurate phylogenetic inference. Considered in a broader context, this supports the use of more distantly related outgroups in phylogenetic analysis of morphology due to reduced homoplasy between ingroup and outgroup taxa.

Several of the female phylogenetic analyses with Old world monkey single or multiple outgroups inferred a dichotomous *Brachyteles-Lagothrix* and *Ateles-Alouatta* clade, indicating a role for sexual dimorphism in both promoting and obscuring the atelid phylogenetic signal. *Alouatta* females are definitely less robust in facial morphology, so potentially the *Ateles-Alouatta* connection is linked to decreased robusticity coupled with lower face projection shared by both groups. The *Ateles-Alouatta* sister relationship is also found in several analyses of Old World monkey single and combination outgroups, with *Lagothrix* sister. These tend to be male or separate sex analyses and are presumably linked to sexual dimorphism. When *Hylobates* is used as outgroup for facial shape, an *Alouatta-Ateles* clade sister to *Brachyteles* was inferred. The position of *Lagothrix* as the basal-most atelid strongly indicated shared homoplasy with *Hylobates* in the face, and a *Lagothrix-Hylobates* clade was inferred in analyses either using all Old World anthropoids or all outgroups. This is also interesting as Rosenberger et al. (2008) alluded to craniodental similarities shared by *Hylobates* and *Ateles*, whereas the phylogenetic analysis of shape data indicated *Lagothrix* and *Hylobates* share major homoplasy. *Hylobates* are mainly frugivorous, as are *Ateles* and *Lagothrix*, but *Lagothrix* and *Hylobates* also consume larger proportions of non-fruit, especially leaves, which could explain the morphological connection between the two.

Phylogenetic analysis of the entire skull (Table 15) and cranial base (Table 17) largely replicated the clade of *Ateles* and *Lagothrix* proposed by Kay (1990), also found in Horovitz & Meyer (1997) and Cole et al. (2002). For the whole skull, female, pooled sex and separate sex analyses from single and multiple outgroups of Old World monkeys and strepsirrhines consistently supported this relationship. For analyses of the cranial base, support for a sister relationship between *Ateles* and *Lagothrix* was overwhelming, with only the use of a *Hylobates* outgroup not inferring this sister relationship. The *Ateles-Lagothrix* sister relationship was also inferred by phenetic analysis of cranial base morphology, which will inevitably support the accusation of distance-based phylogenetic analysis being phenetic. On this point, whole skull and facial results from phenetic and phylogenetic analysis show a mixture of congruence, so that phylogenetic analyses sometimes infer phenetic relationships but just as often do not.

With the cranial base, it is abundantly clear that *Ateles* and *Lagothrix* share a morphological similarity, and a phylogenetic analysis that ignored that would be of questionable use. The cranial base phylogenetic results show that the original hypothesis of a *Lagothrix-Ateles* clade by Kay (1990) did not simply reflect dental traits, but measured shared divergent morphology

in the two taxa. The geometric morphometric approach coupled with distance-based phylogenetic methods clearly offers much support for the morphological similarity of *Lagothrix-Ateles* supported by Kay (1990) over the similarity of *Brachyteles-Ateles* from Rosenberger (1984) and Ford (1986). The primary treatment of atelid evolution by Rosenberger & Strier (1989) placed emphasis on the locomotor proximity and adaptations of *Ateles* and *Brachyteles*, yet facial, cranial base and overall skull shape strongly support either the molecular clade of *Brachyteles-Lagothrix* or the frugivorous clade of *Ateles-Lagothrix*. The time has come to revisit the key assumptions of atelid evolution and fundamentally rewrite them.

The inference of *Lagothrix-Ateles* from the cranial base shows that diversification in this region has been driven by diet and mastication rather than locomotion. The mandibles of *Brachyteles* and *Alouatta* are very large, and there is resulting robusticity in the cranial base, compared to the relatively gracile, narrow mandibles of *Ateles* and *Lagothrix*. Experiments in mice by Menegaz et al. (2010) have shown that masticatory loading can affect growth in areas of the skull not directly linked to masticatory forces, which the results from phylogenetic analysis certainly support. If mastication is the driving force of *Ateles-Lagothrix* similarity in the cranial base and whole skull, why does the atelid face not infer a similar relationship? In adults, mastication linked stress is exerted along a gradient and will be strongest in the lower face and weakest in the upper face, so chewing and dietary preference will likely shape lower-facial morphology more than mid and upper-facial morphology (Hylander et al. 1991, Ross & Hylander 1996, Ravosa et al. 2000, Ross 2001 , Ross & Metzger 2004, Paschetta et al. 2010). Most of the facial landmarks used in this thesis sample the mid and upper-face, so the shared mechanical stress linked to frugivory in *Lagothrix* and *Ateles* have limited impact in shaping facial phylogenetic analyses.

The phylogenetic analysis of the whole skull for male specimens with strepsirrhine outgroups also inferred the phylogenetic relationships supported by Rosenberger (1979, 1981, 1984) and Ford (1986), with *Ateles-Brachyteles* sister to *Lagothrix*. The presence of alternative results based on male morphology raises the question of how sexual dimorphism is interacting with phylogenetic analysis- especially because *Ateles* is monomorphic (Ford & Davis 1992). Potentially, the two taxa are drawn together because of their relative low levels of dimorphism, with *Alouatta* and *Lagothrix* drawn to the base of the phylogeny and exhibiting more pronounced dimorphism.

From the perspective of physical anthropology, phylogenetic analysis of the cranial base are especially intriguing, as this region has previously been strongly linked to maintaining a phylogenetic signal in the primate skull (e.g. Olson 1981, Lieberman et al. 1996a, Lieberman 1997, Lockwood et al. 2004, Harvati & Weaver 2006a,b, Cardini & Elton 2008). However, not a single phylogenetic tree derived from cranial base data was congruent with the atelid molecular phylogeny, and there are several ways to consider this lack of phylogenetic signal. On one hand, atelids may have experienced increased selection on cranial base morphology causing a complex pattern of divergence in certain lineages linked to mastication and the evolution of frugivory and folivory. The lack of phylogenetic signal in the cranial base compared to other anthropoids may also be linked to terrestriality and arboreality, as the atelids are strictly arboreal whilst other anthropoid clades that maintained a phylogenetic signal included terrestrial taxa.

Alternatively, the atelid cranial base may have experienced reduced selection and increased plasticity that has led to the loss of a phylogenetic signal, and allowed mastication to shape morphology to a greater degree than in other clades. Weakened selection is limited as an argument though because if true, you would expect some of the cranial base results to infer the molecular phylogenetic results by chance (which they do not), whereas an increased role for mastication and stress in shaping cranial morphology supports the strength of the *Ateles-Lagothrix* connection. Whether atelids are a relatively unusual clade and the cranial base is more, and the face less, plastic than in other primate clades remains to be seen and will require wider sampling of primates. The results presented in later chapters of this thesis suggest cranial base morphology is more plastic and variable, with a weaker phylogenetic signal as a result, than in guenons, apes and humans (e.g. Cardini & Elton 2008, Lockwood et al. 2004, Harvati & Weaver 2006a,b).

Of course, the atelid craniodental regions may be no more or less plastic than is true of other primate clades. Instead, the physical anthropology literature may have been overtly focused on pursuing a phylogenetic signal in regions that are heavily regulated, non-plastic and neutral in their evolution, whereas in fact more plastic areas maintain a phylogenetic signal when evolution proceeds along certain evolutionary trajectories. This latter point may concern evolutionary time frames and the extent or degree of evolution that has taken place over certain periods of time. Many of the previous studies that have found a strong phylogenetic signal in the cranial base such as Harvati & Weaver (2006a,b), Smith et al. (2007), von Cramon-Taubadel (2009) and Smith (2009), with the exceptions of Lockwood et

al. (2004) and Cardini & Elton (2008), have concentrated on clades that have recently evolved within one genus and often one single species. Potentially, other clades of multiple genera that have evolved over millions of years, that have experienced extensive craniodental evolution and diversification, will express stronger phylogenetic signals in an alternative region of the skull to the cranial base like the atelids have in the face.

Several methodological issues should also be considered. A large number of analyses were completed with different outgroups and outgroup combinations. This was partially justified, as outgroup choice did sometimes affect tree topology, but less so than expected. With phylogenetic analysis of the cranial base, outgroup choice made little difference except when a large number of outgroups was used which created atelid paraphyly. Phylogenetic analysis of the face and whole skull also had this problem with atelid paraphyly when multiple outgroups were used. Clearly, there appears to be a phenetic similarity shared between *Alouatta* and strepsirrhines on one hand, and the remaining atelids and Old World anthropoids on the other. The problems experienced when using large collections of outgroups was relatively surprising, as typically, the use of multiple outgroups has been viewed as beneficial to the accuracy of phylogenetic analysis (Nixon & Carpenter 1993, Sanderson & Shaffer 2002). The problem seems to be having a selection of outgroups where there is great diversity between outgroups, and it seems wiser to repeat phylogenetic analysis with each outgroup and draw a broad consensus of phylogenetic relationships inferred based on all analyses, rather than forcing all outgroups into one analysis and drawing all conclusions based on a single phylogenetic tree. Generally, altering outgroup composition allows for a much more thorough inference of phylogeny, and it is difficult to understand why so few phylogenetic analyses of morphology in the past have tested the effect of outgroups. Without full outgroup testing the peculiar results derived from use of *Hylobates* as outgroup, for example, would not have been observed.

There is also the issue of phylogenetic inference and sexual dimorphism. Generally, the four analyses (male, female, pooled sex, male and female as separate taxa) were congruent, and the presence of sexual dimorphism in platyrrhines did not seem to interfere with phylogenetic analysis, but there were several important exceptions. Phylogenetic analysis of the entire skull gave different results in male analyses with a strepsirrhine outgroup, as did female analyses of the face with several Old World monkey outgroup, although cranial base analyses very rarely registered such problems. The consistency of results from the cranial base may relate to there being reduced sexual dimorphism in the region (although this ought to be more

thoroughly tested before being accepted). Overall, in contrast to Gilbert & Rossie (2007), Gilbert et al. (2009) and Gilbert (2011), and much like Lockwood et al. (2004) and Bjarnason et al. (2011), sexual dimorphism did not appear to have a large effect on phylogenetic analysis. It appears that outgroup selection, and more importantly the craniodental region studied, has a much greater role in the accuracy of phylogenetic inference.

Chapter 6 Pitheciid phylogenetic analysis

6.1 Introduction

The acceptance of the sakis-uakaris *Cacajao*, *Chiropotes* and *Pithecia* as a natural, monophyletic group pre-dates the molecular revolution, making the group one of the more compelling due to the morphological consensus supported by modern molecular phylogenetic analyses (Rosenberger et al. 1996). However, there is dispute as to the sister taxa of saki-uakaris, as several morphological analyses supported a *Callicebus-Aotus* sister clade (Rosenberger 1984, Ford 1986) that is still supported by some (e.g. Rosenberger et al. 1996, Rosenberger et al. 2009). *Aotus* and *Callicebus* share a body size of around 1kg, relatively small brains for their body size, monogamous pair-bonded social systems, and similarity in craniodental morphology, particularly in cranial vault shape, but the morphological link between the taxa are most likely homoplasies as molecular phylogenetics firmly placed lone *Callicebus* as sister to the saki-uakaris and *Aotus* within the cebid clade (Fernandez-Duque 2011a, Kinzey 1997, Ford & Davis 1992, Wildman et al. 2009, Hodgson et al. 2009, Isler et al. 2008). The molecular genetic evidence is so strong that in the phylogenetic analysis described in this chapter *Aotus* is not included, and the pitheciid clade are recognised as including the titi monkeys (*Callicebus*), saki monkeys (*Pithecia*), bearded sakis (*Chiropotes*) and uakaris (*Cacajao*). Incorporating *Aotus* into the pitheciids due to major homoplasy with *Callicebus* would be a particularly odd methodological decision, much as including *Hylobates* in morphological analysis of the atelid postcranium due to homoplasy with *Ateles* would be difficult to justify.

Pitheciids display an evolutionary continuum of morphological adaptations for hard-fruit consumption and seed predation from *Callicebus* to *Pithecia* through to *Cacajao-Chiropotes* (Kinzey 1997). In seed predation, seeds within the fruit are the desired nutritional item, with hard fruits held between upper and lower canines with pressure exerted until the surface cracks, using canines that are robust, laterally divergent and separated from incisors by a diastema, with lower incisors compressed into a robust unit for gouging into opened fruits (Kinzey 1992, Kinzey & Norconk 1990, Norconk 2011). Whilst *Callicebus* are not seed predators, they consume small amounts of seeds, and have increased lower incisor height, which increases their efficiency in fruit feeding (Kinzey 1992). The dental adaptations can be considered morphoclines across the pitheciids, with lower molar reliefs and reduced canine

robusticity in *Pithecia* compared to *Cacajao* and *Chiropotes* that are specialised to exert greater force and pressure than *Pithecia* (Kinzey 1992).

Whilst all pitheciids primarily feed on fruit, there are differences in seed and fruit proportions; *Callicebus* consumes twice as much fruit as seeds, *Pithecia* and *Cacajao* consume around four times as much seeds as fruit, and *Chiropotes* consume significant amounts of both fruit and seeds with a higher proportion of the latter (Norconk et al. 2009). Seed predation increases at times of resource scarcity, with *Cacajao* groups observed descending to the ground and digging up seeds for consumption (Kinzey 1992). The seed-rich diet of saki-uakaris may prove advantageous in making the group less dependent on fruit seasonality, unlike other frugivorous platyrrhine taxa (Norconk 2011), and they also consume a plethora of secondary foods including leaves, flowers, bark, pith and insects (Norconk 2011).

Pitheciids also have a continuum in body size, with *Callicebus* the smallest pitheciid taxon, *Pithecia* larger than titi monkeys but smaller than the largest pitheciids, *Cacajao* and *Chiropotes* (Rosenberger et al. 1996). Relative brain size follows a similar pattern; *Callicebus* have a small brain size distinct from saki-uakaris that have experienced a relative size increase, and within saki-uakaris *Cacajao* and *Chiropotes* have a larger relative brain size than *Pithecia* (Isler et al. 2008). Pitheciids exhibit a range in group size, with very small groups of pair-bonded adults and offspring in *Callicebus*, larger groups in *Pithecia*, and very large groups in *Cacajao* and *Chiropotes* (Rosenberger et al. 1996, Norconk 2011). *Pithecia* and *Callicebus* share an ability to inhabit a wide range of habitats, whereas *Chiropotes* and *Cacajao* are generally restricted to undisturbed or flooded forests respectively, although these are general preferences and not absolute (Kinzey (1992). *Pithecia* are sympatric with both *Cacajao* and *Chiropotes*, although *Cacajao* and *Chiropotes* are allopatric with *Cacajao* in the western Amazon basin and *Chiropotes* in the eastern Amazon basin (Norconk 2011).

A comparative sample of photographs displaying craniodental morphology in each of the pitheciid genera are provided for frontal (Figure 41), lateral (Figure 42), and basal (Figure 43) views, and additional morphological, behavioural and ecological information is provided for each group below.

6.1.2 *Callicebus*

The titi monkeys are found in Brazil, Peru, Bolivia and Paraguay, spanning across the Atlantic and Parana forests of Brazil and Parana forests in Bolivia and Paraguay, occupying much of the Amazon and Orinoco basins (Kinzey 1997, Norconk 2011, Hershkovitz 1990). Ford (1986), Kinzey (1997) and Lawler et al. (2006) viewed *Callicebus* as a primitive lineage according to dental and postcranial morphology associated with ecological generalism (Lawler et al. 2006), whereas Hershkovitz (1990) viewed them as the most complex and diversified of platyrrhine genera. *Callicebus* are monomorphic and have an average body weight of around 1kg (Ford & Davis 1992). They are predominantly frugivores, favouring fleshy fruits, with a significant dietary contribution from seed predation, and some populations consume significant amounts of leaves particularly bamboo (Norconk et al. 2009, Kinzey 1997, Norconk 2011). *C. moloch* and *C. cupreus* have larger proportions of leaves in the diet than *C. personatus* and *C. torquatus* (Norconk 2011).

They are exclusively arboreal except for young animals, pair-bonded, monogamous and males are strongly paternalistic, with small groups of 2-5 occupying a small territory that they defend from other titi monkeys (Kinzey 1997, Norconk 2011). They are arboreal quadrupeds that used walking, running and leaping, with variation between groups in proportions of locomotor and postural behaviours linked to occupation of different forest levels (Kinzey 1997, Lawler et al. 2006). They have well defined supraorbital ridges, broad interorbital septum, v-shaped dental arcades, enlarged frontal, ethmoidal and maxillary sinuses, the cranial vault is dolichocephalic (long), and the premaxilla is non-projecting (Hershkovitz 1990). Diastema between canine and incisors are small or absent, canines are small and similar to premolars in morphology, and molars are larger than premolars, heavy and brachyodont with thick cusps (Hershkovitz 1990, Kinzey 1997).

6.1.3 *Pithecia*

Bearded sakis are found throughout Brazil, Peru, Bolivia, Guyana, French Guiana, Surinam and Venezuela, spanning highland, lowland and secondary forests, as well as flooded and disturbed habitats, and occupy low and middle canopy, and often come to the ground to collect food (Kinzey 1997). *Pithecia* weigh on average around 2kg, with the male average 2.4kg and female average 1.8kg, with quite high levels of sexual dimorphism (Ford & Davis 1992). They live in small family groups which appear to be socially monogamous and pair bonded, raising unresolved question of why such large sexual dimorphism has evolved

(Kinzey 1997). Group sizes can increase dependent on population density, resource availability and competition, so body size dimorphism may reflect more variable and complex interaction with other social groups and ecology than expected for a simple monogamous pair-bonded group (Kinzey 1997, Norconk 2011).

The diet is primarily seed-based with additional frugivory, although they are less capable at biting through the hard shells of fruits than *Cacajao* and *Chiropotes* (Norconk et al. 2009). They use extensive leaping in addition to quadrupedal walking, running and climbing; whilst at rest or when feeding they often cling vertically (Kinzey 1997). The *Pithecia* skull has a long, forward projecting premaxilla, concave dorsal part of the nasal bones, a low facial/nasal angle, low and depressed frontal region, non-inflated cranial vault, sagittal crest in older males, with the foramen magnum backwards compared to the Frankfurt plane (Hershkovitz 1987a). The nasal bone is also extended ventrally, with the nasal aperture smaller as a result and divergent from other pitheciids. The diastema separating the canine and incisors is equal to or greater than the mesio-distal length of the second incisor, the lower canines are smaller than upper canines, molars are larger than premolars, and enamel patterns are similar to *Cacajao* (Hershkovitz 1987a). The large-bodied *Pithecia monachus* and small-bodied *Pithecia pithecia* groups have significant size differences in the skull, but following principal component based size correction are phenetically very similar (Marroig & Cheverud 2004c). There is a consistent level of craniodental integration across *Pithecia* taxa, and genetic drift, rather than natural selection, is the primary mechanism of craniodental evolution (Marroig et al. 2004b).

6.1.4 *Chiropotes*

Bearded sakis are found in Brazil, Venezuela, Guyana, French Guiana and Surinam, and dwell in the upper canopies of high rainforests, terra firme forest, high mountain savannah forest and high moist forest (Kinzey 1997). *Chiropotes* weigh on average 2.8kg, males averaging around 3kg and females 2.5kg, with moderate sexual dimorphism (Ford & Davis 1992). The diet is around 50% seed and 40% fruit based (Norconk et al. 2009). They live in multimale groups of between 8-30, have large home ranges and a pattern of males caring little for infants (Kinzey 1997, Norconk 2011). They leap occasional from pronograde positions and pedal suspension to acquire food (Kinzey 1997). As with *Cacajao*, males have enlarged temporal muscles (Norconk 2011), which may affect craniodental morphology. Craniodental description by Hershkovitz (1985) notes similarity with *Pithecia* and *Cacajao* in

long, forward projecting premaxilla and with *Pithecia* in the concave shape of the dorsal part of nasal bones. Otherwise *Chiropotes* show a high facial/nasal angle, an inflated cranial vault with lateral expansion, a steeply vaulted frontal bone, continuous supraorbital and temporal ridges, sagittal cresting in older males, with a wide diastema between incisors and canine, and long, narrow and forward projecting incisors (Hershkovitz 1985).

6.1.5 *Cacajao*

Uakaris are found in Amazonian and upper Orinoco basins, including Brazil, Colombia, Venezuela and Peru, in flooded forests, which can be submerged by up to 20 metres of water and remain immersed for the majority of the year, but also inhabit terra firme and mixed forests (Kinzey 1997, Heymann & Aquino 2010, Norconk 2011). *Cacajao* are the largest pitheciids, on average weighing 3.1kg, the males on average 3.5kg and females 2.8kg, with moderate sexual dimorphism (Ford & Davis 1992).*Cacajao* diet is very similar to that of *Pithecia*, with large amounts of seed predation in addition to fruit consumption (Norconk et al. 2009). Uakaris live in large multimale-multifemale groups of 20-50 with evidence for fission-fusion social organisation, live within large home ranges of up to 550 hectares, and will travel up to 5km in a single day (Bowler & Bodmer 2009, Kinzey 1997). Although mainly viewed as quadrupeds, uakaris use leaping, clambering, dropping (from higher to lower levels) in significant proportions in addition to climbing, bridging and hopping (Kinzey 1997). With several traits also found in *Pithecia* and *Chiropotes*, *Cacajao* have long, projecting premaxilla, and thin, long incisors, with a diastema separating them from angular and divergent canines, low-crowned molars, a dorsal plane of the nasal that is slightly curved, a moderately high facial/nasal angle, and an inflated cranial vault with lateral expansion (Hershkovitz 1987b).

6.1.6 Pitheciid phylogenetic relationships

Rosenberger (1977) recognised a saki-uakari clade of *Chiropotes*, *Cacajao* and *Pithecia* with shared derived dental characteristics including narrow incisors, reduced cheek teeth, and enlarged hypocones. Rosenberger (1981) placed *Callicebus* as basal to *Chiropotes*, *Cacajao* and *Pithecia* with *Aotus* sister to the pitheciids. In contrast, Rosenberger (1984) included *Aotus* within the pitheciids, with *Aotus-Callicebus* basal, and *Pithecia* sister to *Cacajao-Chiropotes*. Ford (1986) supported a monophyletic saki-uakari group without a sister relationship to *Callicebus*. The three taxa shared 28 dental traits of which 5 were derived, and shared 10 postcranial traits, of which two related to femoral indices were derived. Ford

(1986) noted that traits present in *Pithecia*, *Chiropotes* and *Cacajao* were often present in *Callicebus* or atelids, which can be interpreted as representing phylogeny and homoplasy respectively. A sister relationship between *Cacajao* and *Chiropotes* was supported by sharing of 5 dental traits of which 3 were unique, including reduced occlusal relief for lower molars, and a reduced second incisor and enlarged canine on the upper dentition. Postcranial morphology provided a single unique derived trait with the deltopectoral crest on the humerus being rounded, along with 8 other shared traits and a shared body size increase.

Kay (1990) supported a pitheciid clade of *Pithecia*, *Cacajao* and *Chiropotes* based on several cranial traits from Rosenberger (1979), relating to the presence of paraoccipital processes, a narrow square-shaped dental arcade and large mandibular symphysis, and 30 derived dental traits including thin lower incisors, procumbent incisors, and first incisor larger than the second incisor. These dental traits were primarily linked to dietary adaptation for gouging or splitting the shells of tough/hard-shelled fruits (Kay 1990, Kinzey 1987, van Roosmalen et al. 1981, van Roosmalen et al. 1988). There was strong support for a sister relationship between *Cacajao* and *Chiropotes* based on multiple postcranial, crania and dental traits. Each saki-uakari taxon had postcranial (*Pithecia* 4, *Chiropotes* 7 and *Cacajao* 7) and dental autapomorphies (Ford 1986). Rosenberger (1992) also supported saki-uakari monophyly based on divergent incisor and canine morphology. Pitheciids have molars adapted to low relief, with molars forming a large surface area when teeth press together (Rosenberger 1992). Kinzey (1992) considered morphological evolution within living and fossil pitheciids, with multiple derived dental traits supported a saki-uakari clade and sister relationship between *Cacajao* and *Chiropotes* (Kinzey 1992). Horovitz & Meyer (1997), Horovitz et al. (1998) and Horovitz & MacPhee (1999) also supported a pitheciid clade, with *Pithecia* sister to *Chiropotes*-*Cacajao* and *Callicebus* basal. Kay et al. (2008) supported *Cacajao*-*Chiropotes* sister to *Pithecia* in parsimony analysis of craniodental data, with *Callicebus* sister to *Aotus* in a far removed clade.

Finally, there are two major pitheciid fossil taxa, *Soriacebus* and *Cebupithecia*, that both lack the low relief in molars found in saki-uakaris, but *Cebupithecia* in particular has robust canines and procumbent incisors (Kinzey 1992). It follows that the saki-uakari adaptation for opening hard fruits to access seeds would evolve the ability to open hard pericarps first, and then acquire molar adaptations for crushing seeds later, which supported the pitheciid affinity of these two groups. For *Cebupithecia sarmientoi* there is consensus that it is a pitheciid (Kay 1990), although the specific phylogenetic relationship with extant groups requires further

study, which future work could easily integrate considering there is a well preserved skull available. The pitheciid affinity of *Soriacebus* is more contentious as Rosenberger et al. (1990) viewed it as a pitheciid, but Kay (1990) rejected this interpretation on the basis of molar morphology. Whilst additional *Soriacebus* material has been discovered (Tejedor 2005a,b), only fragmentary mandible and dental specimens are available, and there is currently no possibility of extending the work described in this thesis to resolve the phylogenetic position of *Soriacebus*.

Figure 41 Frontal view of *Callicebus* (top left), *Pithecia* (top right), *Chiropotes* (bottom left) and *Cacajao* (bottom right)

Figure 42 Lateral view of *Callicebus* (top), *Pithecia* (second top), *Chiropotes* (second bottom) and *Cacajao* (bottom)

Figure 43 Basal view of *Callicebus* (top left), *Pithecia* (top right), *Chiropotes* (bottom left) and *Cacajao* (bottom right)

6.2 Methods & Materials

Geometric morphometric analysis was carried out in the MorphoJ program and phylogenetic analysis in the Phylip software package. Phylogenetic analysis of pitheciids included nine ingroup taxa and nine outgroup taxa that are listed in Table 19 with sample sizes for male, female and pooled sex specimens. As with analysis of atelids and cebids, all ingroup-outgroup combinations were completed for male-only, female-only, pooled-sex and treatment of males and females as separate taxa. Neighbor-joining with *Callicebus* as outgroup and UPGMA phenetic analysis were also carried out. Outgroup combinations included analysis with each single outgroup, two-taxa combinations of Cercopithecinae, Colobinae, Galagonidae and *Eulemur-Perodicticus*, all Old World monkeys (four taxa), all Old World anthropoids (five taxa), all strepsirrhines (four taxa) and all outgroups (nine taxa). Shape data were analysed for three datasets; the whole skull as described by 63 landmarks, 15 landmarks describing facial morphology and 24 landmarks describing cranial base morphology.

Tables 19 Pitheciid and outgroup sample sizes for male, female and pooled sex analyses

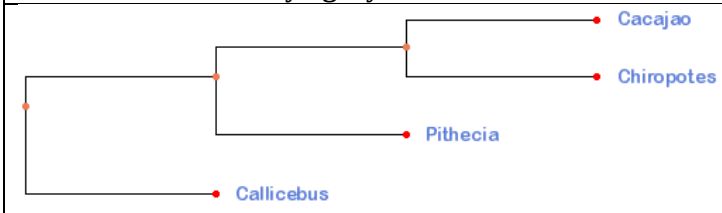
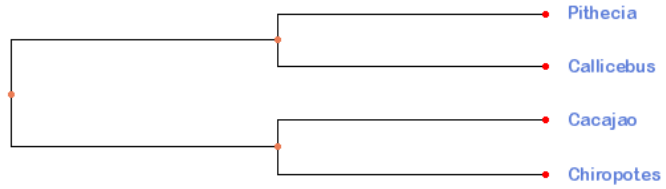
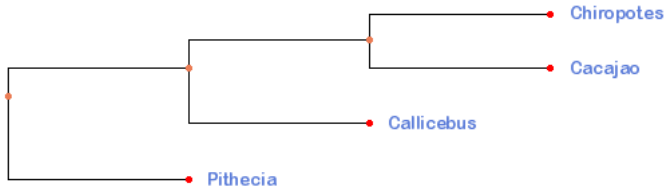
Genus	**Species**	**Male**	**Female**	**Pooled**
Callicebus	*moloch*	13	15	28
	torquatus	12	9	21
	cupreus	10	9	19
	hoffmannsi	9	10	19
Cacajao	*melanocephalus*	13	17	30
	calvus	13	10	23
Chiropotes	*satanas*	14	9	23
Pithecia	*pithecia*	12	10	22
	monachus	14	13	27
Outgroups				
Hylobates	*lar*	10	10	20
Macaca	*mulatta*	9	10	19
Perodicticius	*potto*	10	10	20
Colobus	*guereza*	11	10	21
Chlorocebus	*aethiops*	10	10	20
Trachypithecus	*obscura*	10	10	20
Otolemur	*garnetti*	10	9	19
Galago	*senegalensis*	10	11	21
Eulemur	*fulvus*	10	10	20

6.3 Results

6.3.1 Whole skull

Phenetic and phylogenetic results from analysis of whole skull morphology are shown in Table 20. Genus-level phylogenies of the pitheciid ingroup are shown on the left, with type of analysis and outgroup used listed on the right hand side. The well-supported pitheciid molecular phylogeny, with *Cacajao-Chiropotes* sister to *Pithecia* and *Callicebus* basal-most, was inferred by phenetic analysis and phylogenetic analysis using all nine outgroups, strepsirrhine, galagonid and *Perodicticus-Eulemur* combination of outgroups, and single outgroups of *Otolemur*, *Galago*, *Perodicticus*, *Callicebus* and female *Colobus*. A dichotomy was inferred for *Cacajao-Chiropotes* and *Pithecia-Callicebus* with outgroup combinations of Old World anthropoids, Old World monkeys, Cercopithecinae and Colobinae, and single outgroups of *Eulemur*, *Hylobates*, *Colobus* (except females), *Macaca* (except males) and *Trachypithecus*. Phylogenetic analysis of males with *Macaca* as outgroup inferred *Pithecia* as the basal-most taxa, with *Callicebus* sister to *Cacajao-Chiropotes*.

Table 20 Pitheciid phylogenetic relationships inferred from whole skull morphology

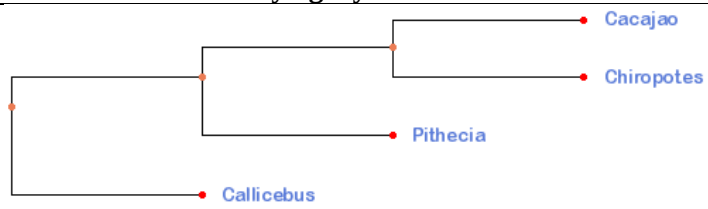
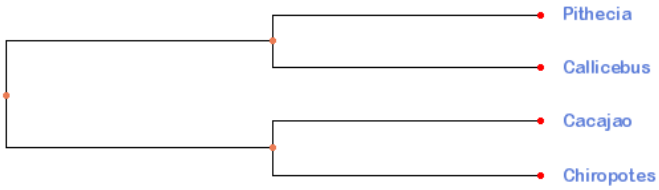
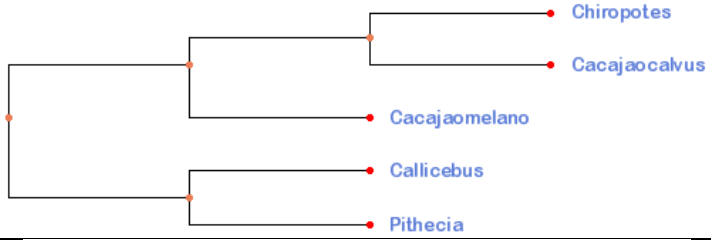
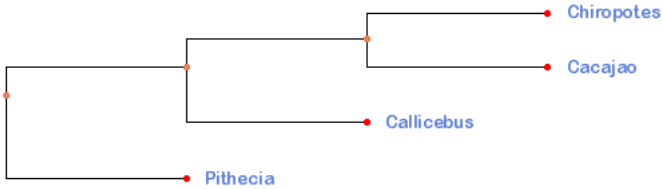
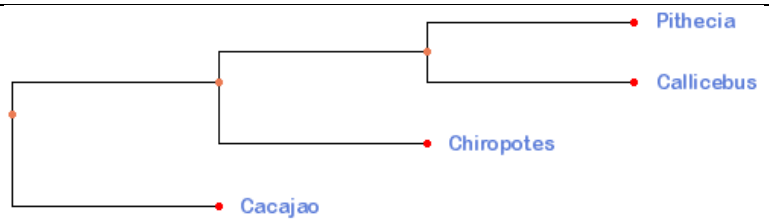
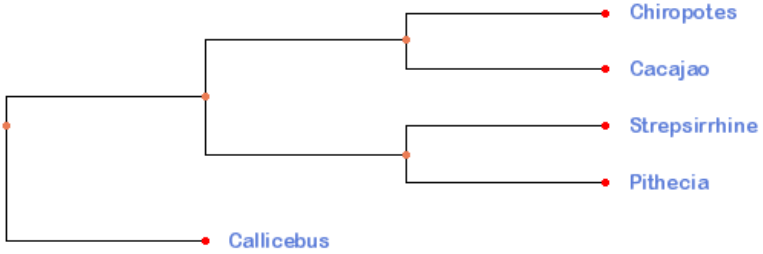
Phylogeny inferred	Outgroup(s) used
	UPGMA (all) *Callicebus* (all) *Colobus* (female) *Galago* (all) *Otolemur* (all) *Perodicticus* (all) Galagonids (all) *Perodicticus-Eulemur* (all) Strepsirrhine (all) All outgroups (all)
	*Chlorocebus* (all) *Colobus* (male, pooled, separate) *Hylobates* (all) *Eulemur* (all) *Macaca* (female, pooled, separate) *Trachypithecus* (all) Cercopithecinae (all) Colobinae (all) OWM (all) OW anthropoids (all)
	*Macaca* (male)

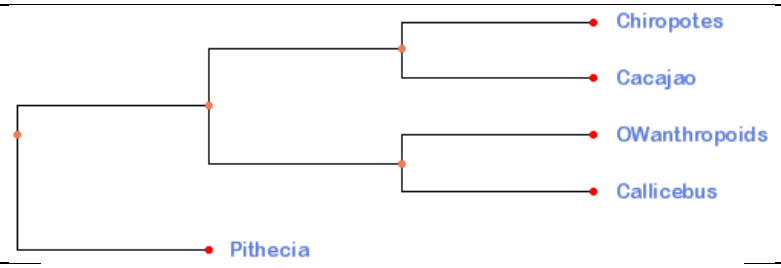
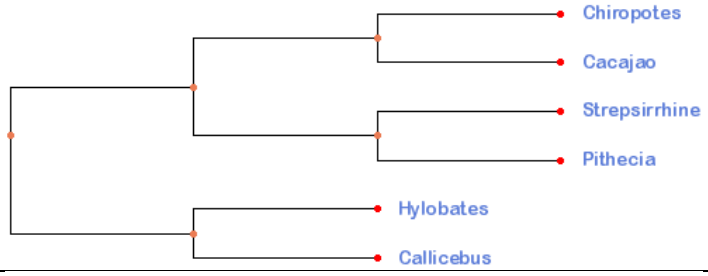
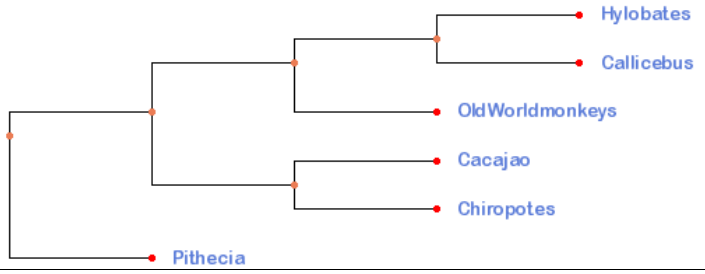
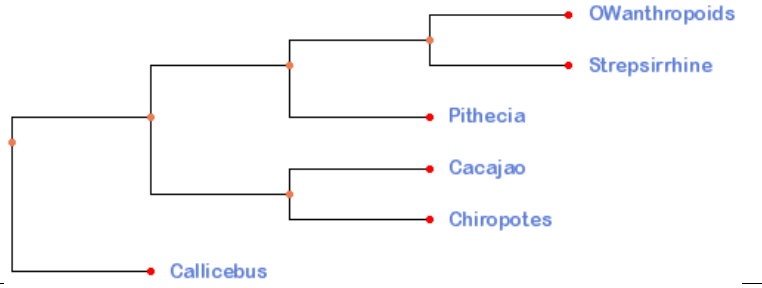
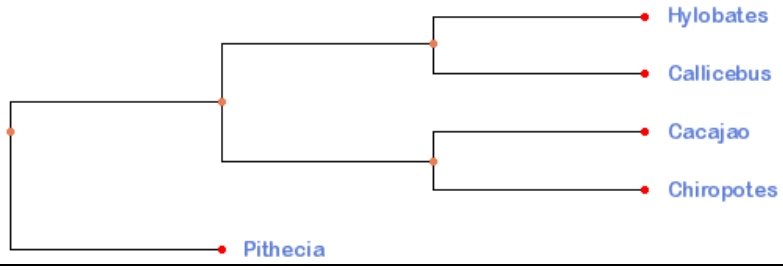
6.3.2 Face

Phenetic and phylogenetic results from analysis of facial morphology are shown in Table 21. Clearly the results of phylogenetic analysis are much more varied than those from the entire skull. Phenetic cluster analysis and phylogenetic analysis of all nine outgroups (male only), Old World anthropoids (except males) and Colobinae (except males) outgroup combinations, and single outgroups of *Hylobates*, *Colobus* (pooled sex and females), *Trachypithecus* (except males) and *Callicebus* all inferred the molecular phylogenetic relationships of pitheciids with *Cacajao-Chiropotes* sister to *Pithecia*. Phylogenetic analysis with outgroup combinations of Old World anthropoids (males only), Old World monkeys (separate sex and females), Cercopithecinae (males only) and Colobinae (males only), and single outgroups of *Colobus* (separate sex and males), *Trachypithecus* (males only) and *Chlorocebus* (except males), all inferred a dichotomy between *Pithecia-Callicebus* and *Cacajao-Chiropotes*. Five analyses inferred this same dichotomy, but with *Cacajao* paraphyletic and *Chiropotes* sister to *Cacajao calvus*, using Old World monkey (pooled and males), Cercopithecinae (pooled), *Chlorocebus* (males) and *Macaca* (males) outgroups. All phylogenetic analyses that used strepsirrhine combination or single outgroups inferred a *Chiropotes-Cacajao* clade sister to *Callicebus* with *Pithecia* basal-most. In the case of analysis with Cercopithecinae (separate sex and females) and *Macaca* (except males) outgroups, *Pithecia-Callicebus* was sister to *Chiropotes* with *Cacajao* basal-most.

When all nine outgroups were used in the same phylogenetic analysis, with the exception of male-only analyses, all phylogenies inferred paraphyletic pitheciid clades. *Cacajao-Chiropotes* was sister to strepsirrhines-*Pithecia* with outgroup rooting of *Hylobates* for pooled-sex and females and rooting with any Old World monkey outgroup for pooled-sex analysis. Rooting with a strepsirrhine outgroup for pooled sex inferred *Cacajao-Chiropotes* sister to a clade of *Callicebus* with Old World anthropoids. Females rooted using an Old World monkey outgroup inferred strepsirrhines and *Pithecia* as sister to *Cacajao-Chiropotes*, and *Callicebus-Hylobates* basal-most. Rooting with a strepsirrhine outgroup for females placed *Hylobates-Callicebus* sister to Old world monkeys, with *Cacajao-Chiropotes* basal. Females rooted with *Hylobates* had strepsirrhines in a clade with Old World anthropoids and sister to *Pithecia*, and *Cacajao-Chiropotes* basal. For separate sex analyses with a strepsirrhine or Old World monkey root, *Hylobates-Callicebus* was sister to *Cacajao-Chiropotes*.

Table 21 Pitheciid phylogenetic relationships inferred from facial morphology

Phylogeny inferred	Outgroup(s) used
	UPGMA (all) *Callicebus* (all) *Colobus* (pooled, female) *Hylobates* (all) *Trachypithecus* (pooled, separate, female) Colobinae (pooled, separate, female) OW anthropoid (pooled, separate, female) All outgroups (male)
	*Colobus* (separate, male) *Trachypithecus* (male) *Chlorocebus* (female, pooled, separate) Cercopithecinae (male) Colobinae (male) OWM (separate, female) OW anthropoid (male)
	*Chlorocebus* (male) *Macaca* (male) Cercopithecinae (pooled) OWM (pooled, male)
	*Otolemur* (all) *Galago* (all) *Eulemur* (all) *Perodicticus* (all) Galagonids (all) *Perodicticus-Euemur* (all) Strepsirrhine (all)
	Cercopithecinae (female, separate) *Macaca* (pooled,female, separate)
	All outgroups OWM root (pooled) All outgroups Hylobates root (pooled, female)

	All outgroups strepsirrhine root (pooled)
	All outgroups OWM root (female)
	All outgroups strepsirrhine root (female)
	All outgroups *Hylobates* root (separate sex)
	All outgroups OWM or Strepsirrhine root (separate sex)

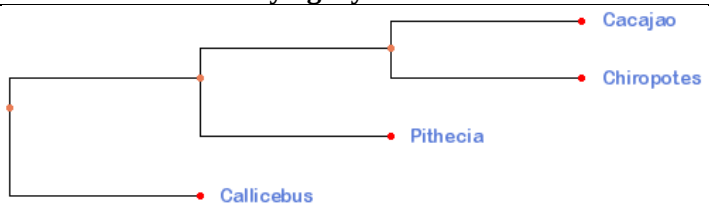
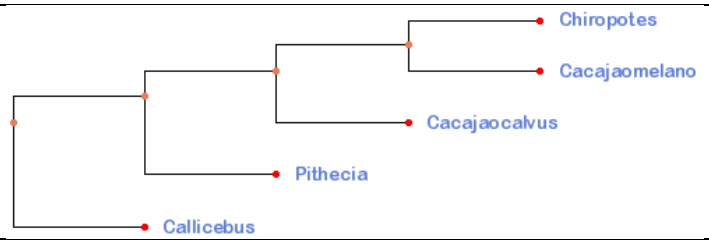
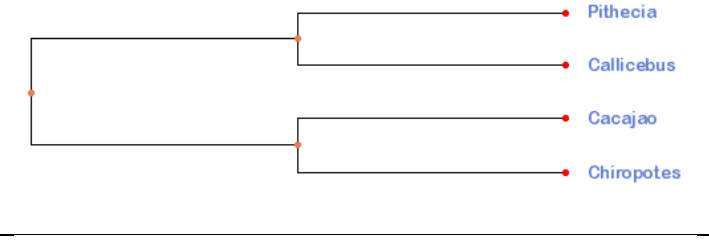
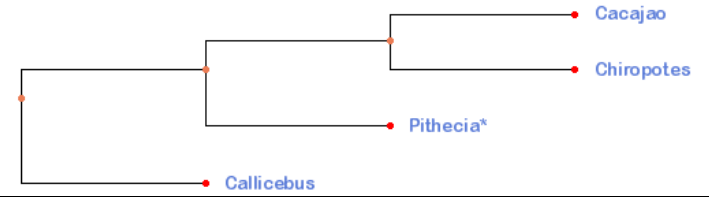
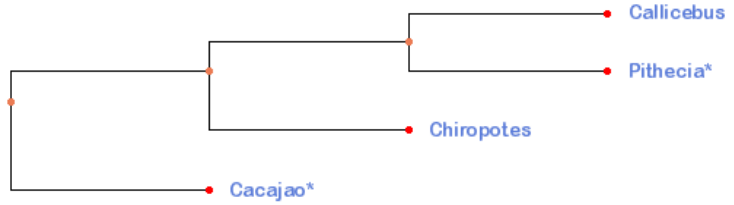
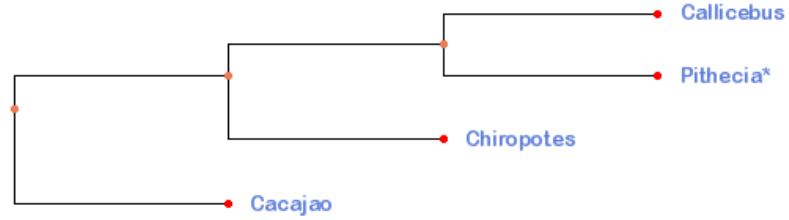
6.3.3 Cranial base

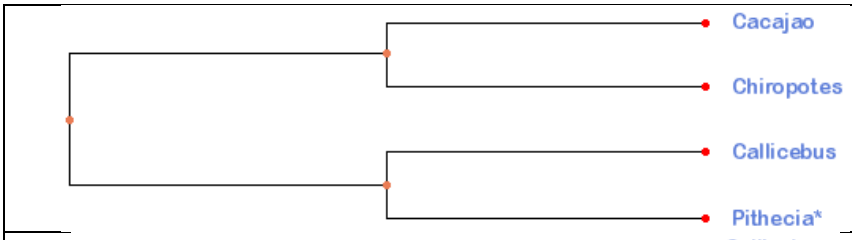
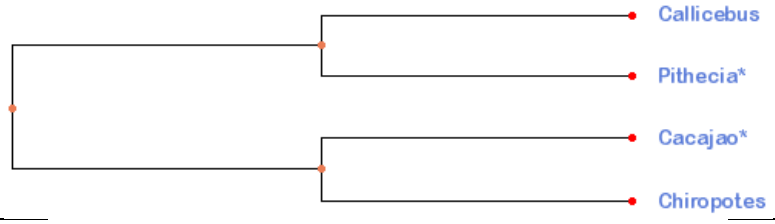
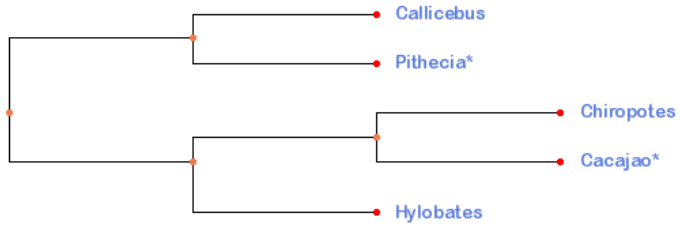
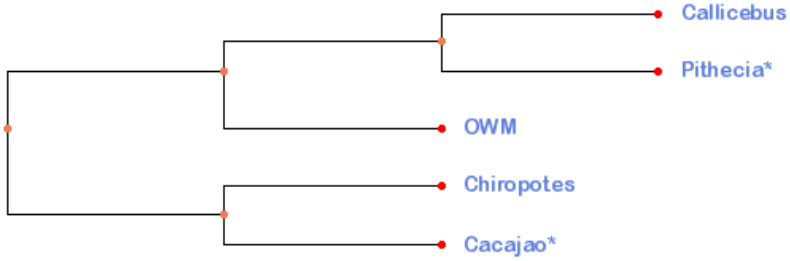
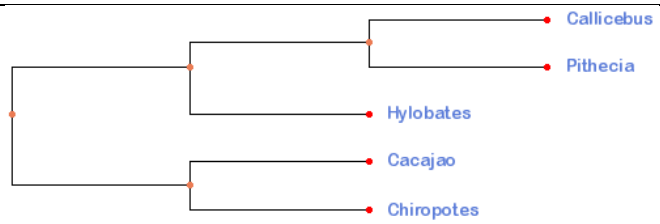
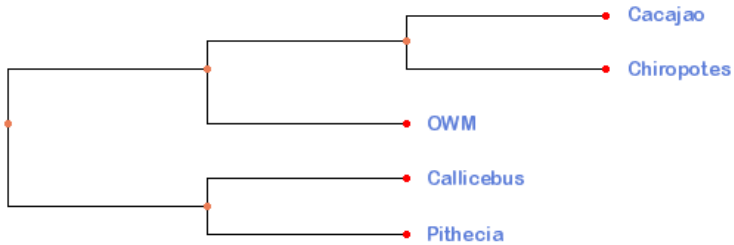
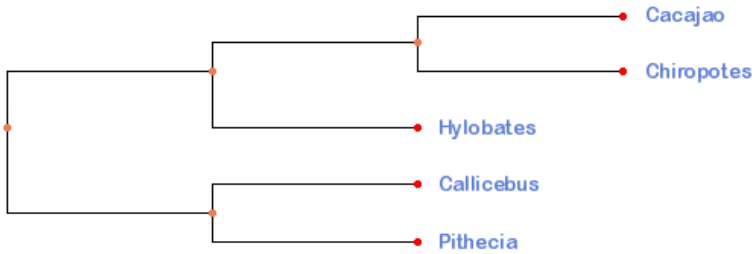
Phenetic and phylogenetic results from analysis of cranial base morphology are shown in Table 22. All phenetic (except females) and phylogenetic analyses using strepsirrhine, galagonids and *Perodicticus-Eulemur* outgroup combinations, and single outgroups of *Otolemur*, *Galago*, *Eulemur* (except females), *Perodicticus*, *Callicebus* and *Trachypithecus* (pooled only) inferred the pitheciid molecular phylogenetic relationships. The results of two analyses inferred molecular phylogenetic relationships except for paraphyly in *Cacajao* and *Pithecia* in females for UPGMA and *Eulemur* phylogenetic analyses respectively. A dichotomy between *Cacajao-Chiropotes* and *Pithecia-Callicebus* was inferred for phylogenetic analysis using Colobinae (except females), *Colobus*, *Hylobates* (except females) and *Trachypithecus* (males and separate sex) outgroups. This dichotomy was also inferred with *Pithecia* paraphyly using *Trachypithecus* (females) and Old World monkey (pooled) outgroups, or *Pithecia* and *Cacajao* paraphyly with an Old World monkey (females) outgroup. A *Callicebus-Pithecia* clade, with paraphyletic *Pithecia*, sister to *Chiropotes* and *Cacajao* basal-most was inferred with Cercopithecinae (males and pooled), Colobinae (females only), *Chlorocebus* (males only), *Macaca* (except females) and *Hylobates* (females only) outgroups. The same phylogeny, but with *Pithecia* and *Cacajao* paraphyletic, was inferred using Cercopithecinae (pooled and females), *Macaca* (females only) and *Chlorocebus* (except males) outgroups.

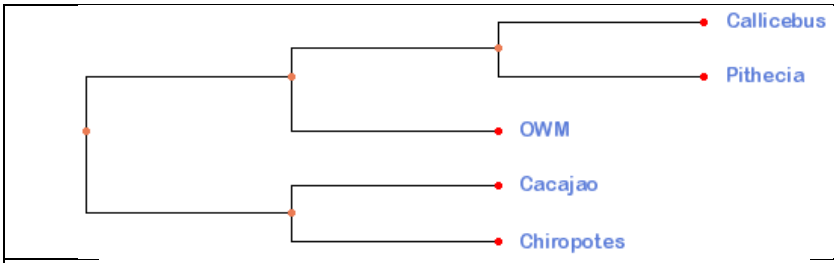
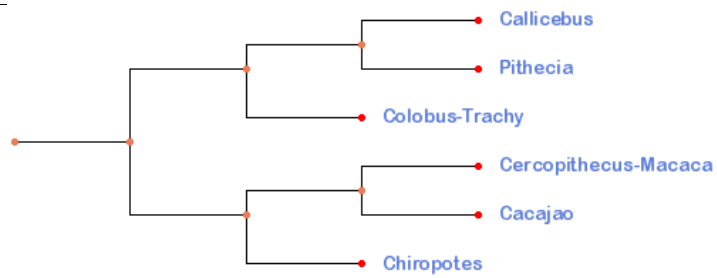
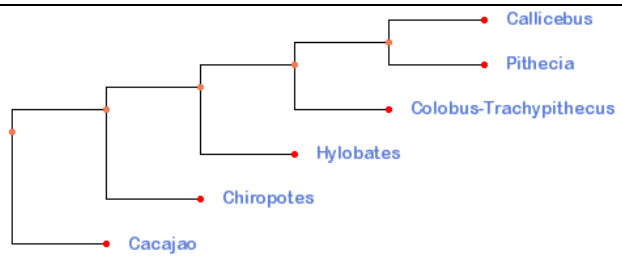
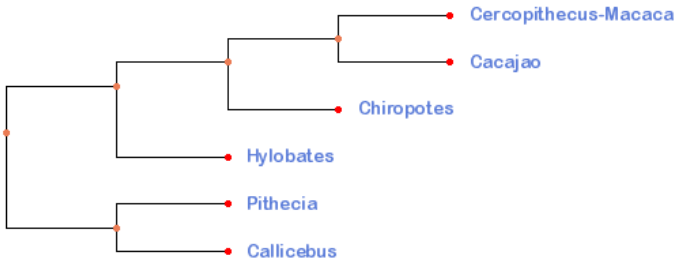
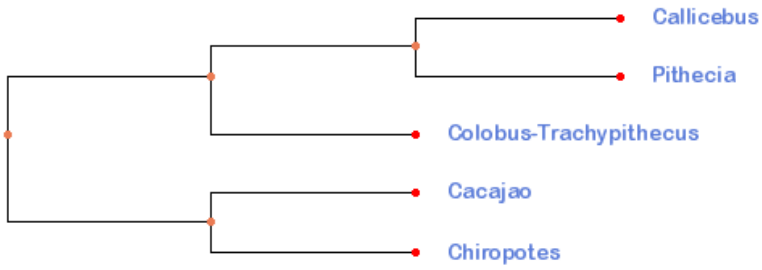
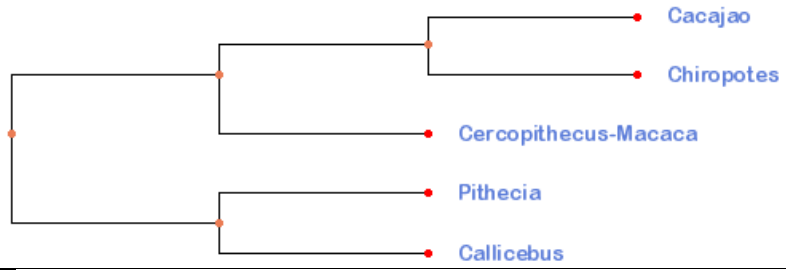
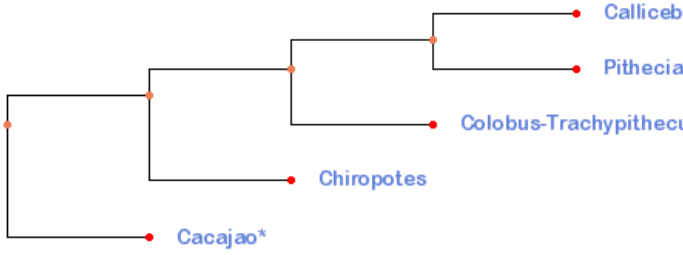
The use of Old World monkeys (males and separate sex analyses), Old World anthropoid and all nine outgroup combinations led to a very large number of alternative phylogenetic trees, all of which inferred pitheciid paraphyly with an outgroup taxon included within the pitheciids. Twenty-five trees were inferred, which is too great to individually describe, so general patterns are commented upon instead. With an Old World anthropoid outgroup *Cacajao-Chiropotes* and *Callicebus-Pithecia* clades are present in nearly all analyses. With females and pooled-sex there is an affinity between *Hylobates* and *Cacajao-Chiropotes*, and *Callicebus-Pithecia* and Old world monkeys, which is reversed in males. In separate sex analysis of the same outgroups Colobinae and Cercopithecinae split, with Cercopithecinae-*Cacajao* and Colobinae-*Callicebus-Pithecia* closely linked. These same affinities are inferred with male and separate sex analyses using an Old World monkey outgroup combination. For analyses that used all nine outgroups, there was a strong relationship shared by *Callicebus* and strepsirrhines in all analyses. *Hylobates* was closely linked to *Cacajao-Chiropotes* in phylogenetic analysis of females and pooled-sex, whereas Cercopithecinae were more closely

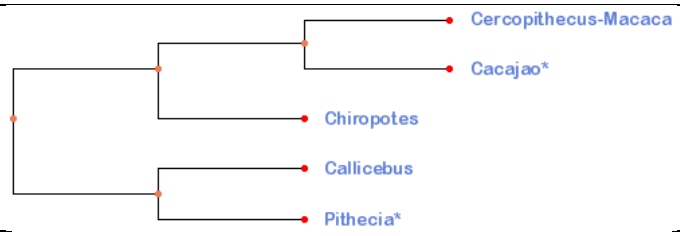
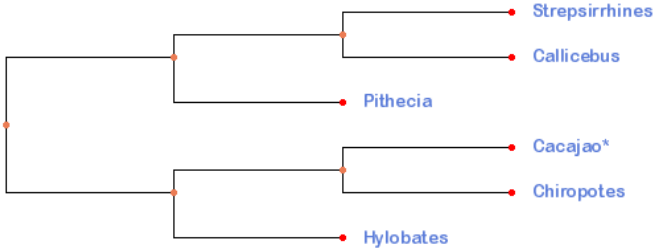
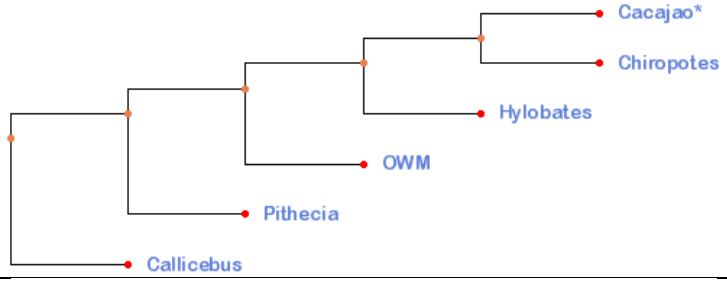
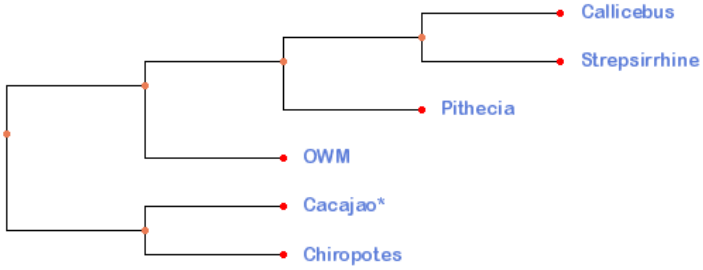
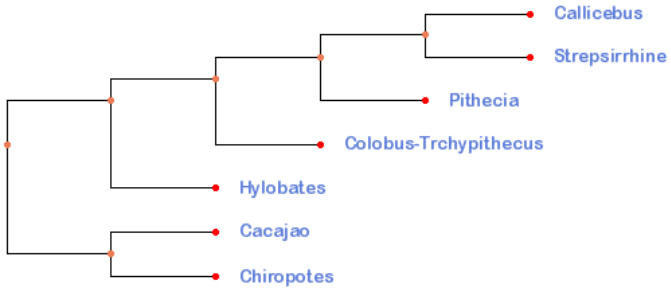
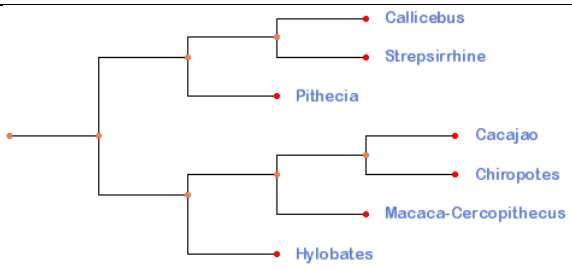
linked to *Cacajao-Chiropotes* with males. For separate-sex analyses, Old World anthropoids formed a monophyletic group linked to *Cacajao-Chiropotes*.

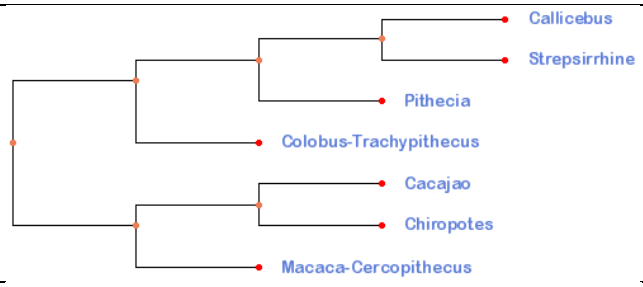
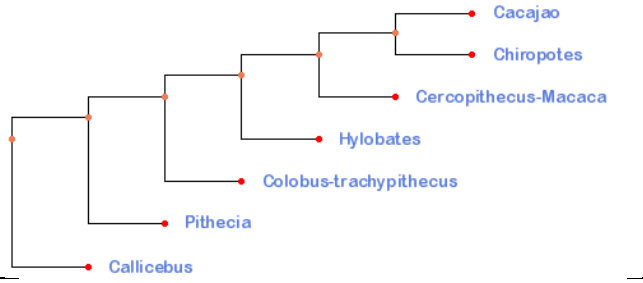
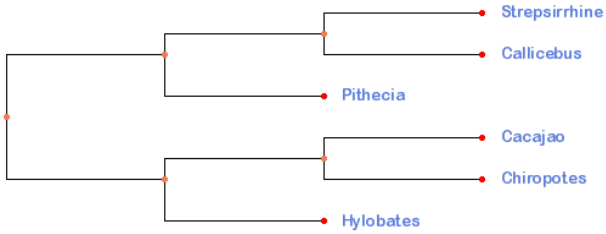
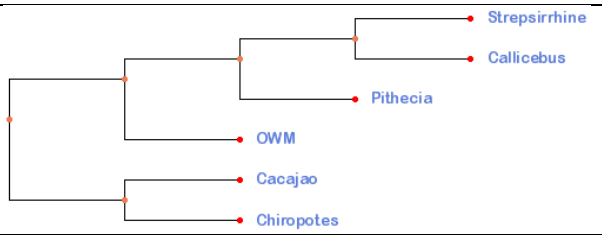
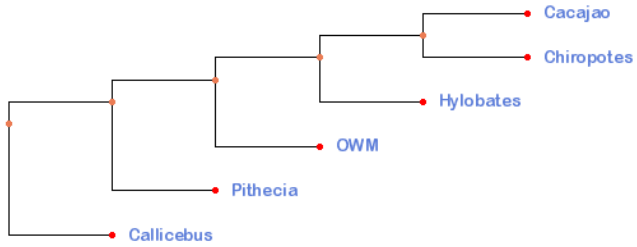
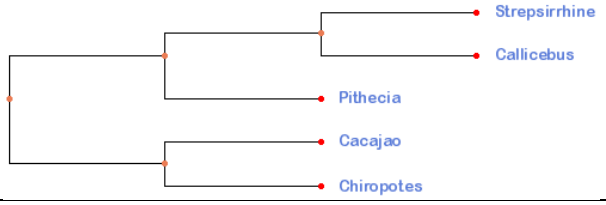
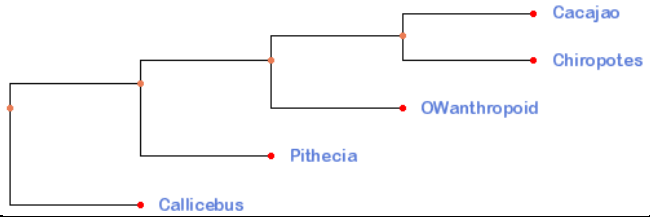
Table 22 Pitheciid phylogenetic relationships inferred from cranial base morphology (*asterisk denote genus paraphyly)

Phylogeny inferred	Outgroup(s) used
	UPGMA (pooled, male, separate) *Callicebus* (all) *Otolemur* (all) *Galago* (all) *Eulemur* (male, pooled, separate) *Perodicticus* (all) *Trachypithecus* (pooled) Galagonids (all) *Perodicticus-Eulemur* (all) Strepsirrhine (all)
	UPGMA (female)
	Colobinae (male, pooled, separate) *Colobus* (all) *Hylobates* (male, pooled, separate) *Trachypithecus* (male, separate)
	*Eulemur* (female)
	Cercopithecinae (pooled, female) *Macaca* (female) *Chlorocebus* (female, pooled, separate)
	Cercopithecinae (male, pooled) Colobinae (female) *Chlorocebus* (male) *Macaca* (male, pooled, separate) *Hylobates* (female)

	*Trachypithecus* (female) OWM (pooled)
	OWM (female)
	Old World anthropoids OWM root (female)
	Old World anthropoids *Hylobates* root (female)
	Old World anthropoids OWM root (male)
	Old World anthropoids *Hylobates* root (male)
	Old World anthropoids OWM root (pooled)

	Old World anthropoids *Hylobates* root (pooled)
	Old World anthropoids *Hylobates* root (separate)
	Old World anthropoids *Macaca* root (separate)
	Old World anthropoids *Colobus* root (separate)
	OWM *Macaca* rooted (male)
	OWM *Colobus* rooted (male)
	OWM *Macaca* rooted (separate)

	OWM *Colobus* rooted (separate)
	All outgroups OWM root (female)
	All outgroups Strepsirrhine root (female)
	All outgroups *Hylobates* root (female)
	All outgroups *Chlorocebus* root (male)
	All outgroups *Colobus* root (male)

	All outgroups *Hylobates* root (male)
	All outgroups Strepsirrhine root (male)
	All outgroups OWM root (pooled)
	All outgroups *Hylobates* root (pooled)
	All outgroups Strepsirrhine root (pooled)
	All outgroups OW anthropoid root (separate)
	All outgroups Strepsirrhine root (separate)

6.3.4 Summary of results

A summary of the results of phylogenetic analysis of pitheciids are provided in Table 23, with craniodental region in the first column, the inferred phylogenetic relationships (both molecular congruent and incongruent) in the second and third columns, the outgroup on the top row, and ticks showing which iterations of outgroup and craniodental region supported each phylogenetic relationship.

Table 23 Summary of pitheciid phylogenetic analyses

		Outgroup or Outgroup combination	UPGMA	*Chlorocebus*	*Colobus*	*Eulemur*	*Galago*	*Hylobates*	*Macaca*	*Otolemur*	*Perodicticus*	*Trachypithecus*	Cercopithecoidea	Colobinae	Galagonidae	*Eulemur-Perodicticus*	Old World anthropoid	Old World monkeys	Strepsirrhine	All outgroups
Whole skull	**Molecular clades**	*Cacajao-Chiropotes*	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓
		Cacajao-Chiropotes-Pithecia	✓		✓		✓			✓	✓				✓	✓			✓	✓
	Molecular incongruent clades	*Pithecia-Callicebus*		✓		✓		✓	✓			✓	✓	✓			✓	✓		
Face	**Molecular clades**	*Cacajao-Chiropotes*	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
		Cacajao-Chiropotes-Pithecia	✓	✓	✓			✓	✓			✓	✓	✓			✓	✓		
	Molecular incongruent clades	*Cacajao-Chiropotes Callicebus*				✓	✓			✓	✓				✓	✓			✓	
		Cacajao-Pithecia-Callicebus						✓	✓			✓					✓			
		Pithecia-Callicebus		✓				✓	✓				✓					✓		
		Chiropotes-C.calvus						✓				✓	✓					✓		
		Pitheciid paraphyly																✓		✓
Cranial base	**Molecular clades**	*Cacajao-Chiropotes*	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
		Cacajao-Chiropotes-Pithecia	✓			✓	✓			✓	✓	✓			✓	✓			✓	
	Molecular incongruent clades	*Chiropotes-Pithecia-Callicebus*		✓					✓				✓							
		Pithecia-Callicebus	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
		Pitheciid paraphyly															✓	✓	✓	✓

6.4 Discussion

6.4.1 The pitheciid phenetic-phylogenetic signal

Phenetic relationships based on craniodental shape (Tables 20-22) all supported the same relationships as the molecular phylogenies of pitheciids, with the exception of phenetic analysis of female cranial base data. The phylogenetic signal in phenetic data for pitheciids, also found in phenetic analysis of craniodental shape for atelids, highlights once again that phylogenetic relationships inferred with morphological data need not necessarily clash with those made from molecular data. That is, broad patterns of morphological similarity shared between closely related taxa can accurately reflect phylogenetic relationships. In the case of the pitheciids such a result should not be particularly surprising, as a sister relationship between *Pithecia* and a clade of *Cacajao* and *Chiropotes* has long been supported by cladistic morphological analyses (e.g. Rosenberger 1984, Ford 1986, Kay 1990). It seems inevitable that the inclusion of *Callicebus* into a pitheciid morphological analysis, on the basis of strong molecular evidence, will root the group to the base of the clade considering its problematic phylogenetic position is directly related to it being morphologically distinct from the saki-uakaris which have experienced an adaptive shift towards seed predation (Kinzey 1992). There is a clear size disparity between *Callicebus* and the saki-uakaris, but the titi monkeys are also distinct with smaller premaxilla and maxilla regions of the face, a less rounded, wider cranial base, a dolichocephalic cranial vault and more robust palate (Hershkovitz 1990). *Callicebus* have also experienced a possible brain size decrease, as their relative brain size is much smaller than in saki-uakaris, which will likely have an effect on shaping craniodental morphology (Isler et al. 2008).

In the discussion of atelid results, an important point was made that is worth reiterating here; when molecular evolutionary change proceeds according to a steady, balanced clock-like pattern then UPGMA cluster analysis will accurately infer phylogenetic relationships (Nei & Kumar 2000). The broad congruence between morphological phenetic and molecular phylogenetic relationships indicate that morphological evolution in this clade has proceeded in a constant, measured clock-like manner via a morphological clock. Considering platyrrhines are widely considered the product of an adaptive radiation with rapid diversification (e.g. Hodgson et al. 2009, Kay et al. 2008), such a result would be quite extraordinary. Potentially the adaptive radiation occurred during the initial divergence of the major clades, after which evolution within each of these clades returned to a slower, steadier

pace. This is of course very speculative, but the results presented do lend themselves to some creative thinking.

When speaking of the congruence between morphological phenetic and molecular phylogenetic analyses recall that the two were not in absolute agreement, as phenetic analysis of the female cranial base supported a closer relationship between *Cacajao melanocephalus* and *Chiropotes* than between the two *Cacajao* taxa. This could simply be due to sampling error, but it may also indicate that cranial base morphology, at least in female pitheciids, is more plastic, variable and less constrained as in the atelids and cebids. The specific cause of shape similarity shared by *Cacajao melanocephalus* and *Chiropotes*, whether linked to sexual dimorphism, social behaviour, diet, locomotion, or otherwise, remain unclear. As more fieldwork is carried out on pitheciid behaviour in the future, particularly relating to diet and locomotion, many of the results presented in this chapter that are currently difficult to explain will hopefully become easier to understand. The onus on future field studies will also be shared with morphological work to identify which particular landmarks or regions are most affected by homoplasy, and proposal of behavioural convergence on the basis of hypothesised relationships between form and function in morphology.

6.4.2 Pitheciid craniodental evolution

The presence of a phylogenetic signal in the pitheciid skull likely reflects the gradual specialisation for seed harvesting, which forms a continuum across the clade, with *Cacajao* and *Chiropotes* considered more highly derived in their harvesting ability than *Pithecia*, whilst all three are highly specialised compared to *Callicebus* (Rosenberger et al. 1996, Walker 1996). The differentiation between *Callicebus* and saki-uakaris, and *Pithecia* and *Cacajao-Chiropotes*, was reflected in the morphology of the face, cranial base and entire craniodental region of pitheciids. Clearly, the ability to exert pressure and open tough fruits using specialised canine and incisor morphology, greater in *Cacajao* and *Chiropotes* than in *Pithecia*, has helped to shape morphological evolution in the clade (Kinzey & Norconk 1990, Norconk 2011). In pitheciids, either mastication has a reduced role in shaping morphology or *Pithecia* and *Cacajao* have alternative strategies for chewing and grinding seeds, as these taxa are more similar in dietary proportions than either is to *Chiropotes* (Norconk et al. 2009), but support for a *Pithecia-Cacajao* sister relationship is absent throughout phylogenetic analysis.

This is not to say that allometry, locomotion, relative brain size, and numerous social variables are not also important, but considering the fundamental role of diet and mastication in other platyrrhine clades it seems likely that seed predation significantly contributed to shaping pitheciid craniodental morphology. There has been a clear shift in relative brain size between *Callicebus* and saki-uakaris with increased brain size in the latter, and further increases in *Cacajao* and *Chiropotes* (Isler et al. 2008). Brain size increases will not only effect cranial vault morphology, but can alter orbital orientation and basicranial flexion (Ross & Ravosa 1993), which helps to maintain a phylogenetic signal in pitheciids because the pattern of brain size evolution closely follows phylogeny (Isler et al. 2008).

Within the saki-uakaris, *Pithecia* is divergent from *Cacajao* and *Chiropotes* in using leaping more extensively and occupying lower forest strata (Walker 1996). Presumably, adaptations for alternative locomotor behaviours will have an effect on craniodental morphology and support a differentiation between *Pithecia* and *Cacajao-Chiropotes,* but more work is required to quantify locomotor behaviour and variation within and between taxa, as it is currently difficult to provide a more detailed picture of pitheciid locomotion and its effect on craniodental morphology. Social groups of *Cacajao* and *Chiropotes* are also much larger than those in *Pithecia,* although it is difficult to interpret the effect that could have in driving morphological evolution (Kinzey 1997).

The obvious connection is that between group size and body size- *Cacajao* and *Chiropotes* are larger than *Pithecia* and much larger than *Callicebus* in body size and allometric effects on morphology further support the pitheciid phylogenetic signal (Ford & Davis 1992, Kinzey 1997). Pitheciids may also maintain a strong phylogenetic signal because they lack major diversification that works against phylogeny, for example increased brain size in *Callicebus*, reduced seed predation in *Pithecia* or decreased body size in *Cacajao*, that could lead to disruption of the pitheciid phylogenetic signal. Due to the relatively straightforward evolution of craniodental morphology in pitheciids, homoplasy is reduced, and there is less need than in other chapters to consider how multiple disruptive variables have shaped morphology. The pitheciids clearly fall across a phylogenetic, morphological and behavioural range which supports the split between titi monkeys and saki-uakaris, and the close relationship shared by *Cacajao* and *Chiropotes*.

Alternatively, rather than a lack of diversification in pitheciids and a broadly conservative pattern of evolution, pitheciids may simply have diversified in a way that does not conflict

with phylogeny, so that *Callicebus* or saki-uakaris undergo diversification which accentuates legitimate phylogenetic similarities and differences. This latter point is important, because the presence of a more consistent phylogenetic signal in pitheciids could be viewed as pitheciids being relatively primitive and non-derived compared to more highly evolved clades that have been shaped strongly by natural selection.

The pitheciid results appear to contrast with those of the atelids in several important ways. Primarily, overall pitheciid craniodental shape can be used to accurately infer phylogenetic relationships whilst overall atelid craniodental shape cannot. The phylogenetic signal from the pitheciid face is weaker than that for the atelid face, but there is a phylogenetic signal nonetheless. The pitheciid cranial base had a strong phylogenetic signal, whereas the atelid cranial base had no phylogenetic signal. Morphological evolution in pitheciids should probably be considered more conservative than that of atelids. Pitheciids may have stronger genetic control of craniodental morphology or are perhaps less plastic in response to environmental variables, compared to atelids. What is clearly true, when considering both atelids and pitheciids, is that evolutionary processes have acted on two quite closely related groups to create a complex pattern of diversification mixed with phylogenetic signal in the atelid cranium and a quite consistent, strong phylogenetic signal across different regions of the pitheciid cranium. It will be important to consider and investigate what processes have given rise to these differences and when they occurred in platyrrhine evolution.

6.4.3 Phylogenetic analysis considered

Phylogenetic analysis of the whole skull (Table 20) produced pitheciid molecular relationships for outgroup combinations of all nine outgroups, strepsirrhines, galagonids and *Perodicticus-Eulemur*, and single outgroups of *Otolemur*, *Galago*, *Perodicticus*, *Callicebus* and *Colobus* (female only). *Macaca* (male only) inferred *Callicebus* sister to *Cacajao-Chiropotes*, whereas all other analyses including *Eulemur*, *Hylobates* and all Old World monkey outgroups supported a dichotomy between *Cacajao-Chiropotes* and *Pithecia-Callicebus*. There is clearly a strong phylogenetic signal in overall skull shape, and a sister relationship between *Cacajao* and *Chiropotes* was supported in all analyses.

It appears that, generally, a strepsirrhine outgroup proposed an accurate pitheciid topology whereas an Old World monkey or *Hylobates* outgroup supported a close relationship between *Callicebus* and *Pithecia*. It is possible that the strepsirrhine outgroup drew *Callicebus* to the base of the clade away from the saki-uakaris due to allometric similarity. In this case,

allometry helps rather than hinders phylogenetic analysis. Recall that Ford (1986), Kinzey (1997) and Lawler et al. (2006) viewed *Callicebus* as a platyrrhine primitive in dental and postcranial morphology; the connection between strepsirrhines and *Callicebus* could be interpreted as the latter displaying a primitive morphotype in cranial morphology. This may be a dangerous path to follow, as the designation of basal-lineages as primitive can lead to mistaken assumptions about taxa and evolutionary processes, but it is also true that some basal (and non-basal) lineages will retain primitive adaptations and patterns of shape variation similar to ancestral taxa, although they will most likely display different combinations of primitive traits to each other. *Callicebus* is either a platyrrhine that is genuinely primitive in multiple elements of its morphology, retaining elements of the platyrrhine, anthropoid or primate common ancestor, or the group have been shaped by evolutionary forces to develop homoplastic shape variation that converges upon an earlier primitive morphotype.

The support for a pitheciid dichotomy with Old World anthropoid and *Eulemur* outgroups is more difficult to explain. The morphological disparity between *Callicebus* and the saki-uakaris may not be as exaggerated as assumed, and when the allometric link between *Callicebus* and strepsirrhines is removed by using an anthropoid outgroup the geometric morphometric and distance data measured genuine, shared shape variation between titi and saki monkeys. However, there are no major adaptive reasons for shared similarity between *Callicebus* and *Pithecia*, for example neither share dietary specialisations and *Pithecia* are about twice the size of *Callicebus*.

Phylogenetic analysis of facial morphology (Table 21) replicated pitheciid molecular phylogenetic results for all outgroups (male only), OW anthropoid (except male), colobinae (except male), *Colobus* (pooled and female), *Trachypithecus*, *Hylobates* and *Callicebus* outgroups. There is therefore a phylogenetic signal in the pitheciid face with particular outgroups and sex of specimens, but the strength of the signal appears weaker compared to that of the whole skull. It also appears that trees proposed by male and female analyses with an Old World monkey outgroup tend to recover alternative phylogenetic trees, with males generally supporting a *Pithecia-Callicebus* clade and females placing *Pithecia* sister to *Cacajao-Chiropotes*. Therefore, the male morphotype interrupts the phylogenetic signal that female facial data more accurately measures. Several analyses of male or pooled-sex Old World monkey combinations and single outgroups inferred a paraphyletic *Cacajao* clade,

with *Cacajao calvus* sister to *Chiropotes*. Clearly the polarity implied by the outgroup is problematic.

Together these results could be interpreted as support for Gilbert & Rossie (2007), Gilbert et al. (2009) and Gilbert (2011), who found that sexual dimorphism can interfere with accurate phylogenetic analysis of morphology. However, considering this problem of sexual dimorphism was only present for Old World monkey and not strepsirrhines outgroups, sexual dimorphism was problematic for outgroups rather than ingroups. This suggestion is relatively novel, as sexual dimorphism is nearly always considered to be an issue for the ingroups, but are not discussed in relation to outgroups. There is also the possibility that levels of dimorphism in both outgroups and ingroups are combining and interacting to create the results presented. Either way, it appears clear that at least for facial morphology sexual dimorphism has an important role in influencing pitheciid phylogenetic analysis.

Phylogenetic analysis of facial morphology using strepsirrhine outgroups, whether as single outgroups or in combination, all proposed the same pitheciid phylogenetic relationships; *Cacajao-Chiropotes* sister to *Callicebus* with *Pithecia* basal-most. Rather than outgroup polarity connecting *Callicebus* with *Cacajao-Chiropotes*, it seems that a connection between strepsirrhines and *Pithecia* roots the latter to the base of the pitheciid clade. *Pithecia* have well-developed nasal bones, moving several landmarks of the face so that shape data links the group to strepsirrhines. For example, there is a greater distance between the landmarks of the piriform aperture and nasion in *Pithecia* as is seen in strepsirrhines. This shows how adaptation in a single aspect of morphology can have a wider effect on phylogenetic analysis, signifying that combined analysis of geometric morphometrics and distances are vulnerable to natural selection and diversification in morphology. This problem is exaggerated in a modular approach when fewer landmarks describe shape, as a shift in several landmarks has a much larger effect on distances between taxa.

For phylogenetic analysis that combined all nine outgroups, pitheciid monophyly was disrupted with clear affinity between *Callicebus* and *Hylobates*, and strepsirrhines with *Pithecia*. The connection between *Pithecia* and strepsirrhines supports the proposal above regarding shared similarity between the two groups. The link between *Callicebus* and *Hylobates* would explain why phylogenetic analysis of pitheciids using *Hylobates* inferred a molecular congruent phylogeny, as *Callicebus* is positioned basal-most due to a shared morphological connection with *Hylobates*. Facial morphological similarity shared between

gibbons and titi monkeys may relate to adaptations, and associated shape, related to frugivory as both groups obtain around 50% of their diet from fruit consumption (Norconk et al. 2009, Bartlett 2007). It is quite peculiar though that *Hylobates* has shown homoplasy with both atelid and pitheciid frugivorous genera. If shared diet is the common thread, it also raises questions as to why the relationship between diet and morphology varies so much depending on the taxa being studied. A morphological connection between *Hylobates* and *Callicebus* indicates a strong role for diet and mastication in shaping facial morphology, yet there is not a similar connection between the two pitheciids (*Cacajao* and *Pithecia*) that have the most similar diets. The type of forces generated seem to be more important in shaping facial morphology than the exact dietary proportions. Regardless, we are clearly dealing with a complex interaction between phylogeny, morphology and any number of extra variables.

6.4.4 Cranial base evolution

Phylogenetic analysis of the cranial base (Table 22) replicated the pitheciid molecular phylogeny for all strepsirrhine combination and single outgroups, except females with a *Eulemur* outgroup, in addition to *Callicebus* and *Trachypithecus* (pooled) analyses. There is a strong phylogenetic signal in the cranial base when analysed using a strepsirrhine outgroup, which supports the theoretical proposition that the basicranium is likely to hold a strong phylogenetic signal (e.g. Olson 1981, Lieberman et al. 1996a) and adds to the growing evidence from humans, apes and Old World monkeys for a strong phylogenetic signal in this region (e.g. Harvati & Weaver 2006b, Lockwood et al. 2004, Cardini & Elton 2008). The strepsirrhine outgroup provided the polarity required for separation of *Callicebus* from saki-uakaris, and then *Pithecia* from *Cacajao-Chiropotes*. As mentioned earlier, cranial base morphology of *Callicebus* is quite robust and wide, whilst *Pithecia* is less elongated and more compact compared to *Cacajao-Chiropotes* that seem to have a disparate spread of anatomical landmarks.

When phylogenetic analysis of cranial base shape used an Old World anthropoid outgroup, or included both strepsirrhines and Old World anthropoids as a combined collection of outgroups, the cranial base phylogenetic signal was largely lost. The use of Old World anthropoid outgroups (either singular or in combination) tended to support a form of the *Cacajao-Chiropotes* and *Pithecia-Callicebus* dichotomy or *Callicebus-Pithecia* with *Chiropotes* sister, and often supported paraphyletic *Pithecia* and *Cacajao* clades. When *Hylobates* and Old World monkeys were combined, there was no consistent pattern or strong

link between an outgroup and pitheciid ingroup. However, the use of outgroup combinations with Old World monkeys or all nine outgroups uncovered some underlying affinities. With an Old World monkey outgroup combination, female and pooled sex analyses supported pitheciid monophyly (although with *Pithecia* and *Cacajao* paraphyly) whilst male and separate sex analyses found colobine affinity with *Callicebus-Pithecia* and cercopithecine affinity with *Cacajao-Chiropotes*. The use of all nine outgroups consistently drew together *Callicebus* and strepsirrhines, with *Hylobates* and cercopithecines often linked to *Cacajao-Chiropotes*. It is difficult to draw many conclusions from such diverse results, but generally it appears broad similarities have been shared between pitheciids and Old World anthropoids in cranial base morphology. This morphological overlap between ingroup and outgroup has had a disruptive, and inconsistent, effect on phylogenetic analyses.

Two major trends were observed in the cranial base results. First, with the exception of outgroup combination of Old World monkeys, analyses of cranial base male and female datasets often inferred alternative phylogenetic relationships due to genus paraphyly rather than different genus-relationships. The results from males and females are therefore generally different, which can be viewed as evidence for sexual dimorphism distorting accurate phylogenetic analysis. Second, with use of all nine outgroups together, size appears to be driving results as the smallest pitheciids *Callicebus* are linked with the smaller strepsirrhine outgroups, and the largest pitheciids *Cacajao* and *Chiropotes* are linked to larger outgroups of *Hylobates* and cercopithecines.

The importance of size and allometry could explain why phylogenetic analysis of pitheciids with smaller strepsirrhine outgroups maintained a phylogenetic signal, drawing the smallest pitheciid *Callicebus* to the base of the tree and placing the two largest genera as sister taxa. The variation in size of Old World anthropoid outgroups could then be linked to the multitude of phylogenies and paraphyletic pitheciid genera inferred, with distances between ingroup and outgroup varying in different analyses due to the mix of allometric similarities and differences. This size/allometric factor would not completely explain the problem experienced with Old World anthropoids as outgroups for phylogenetic analysis of the cranial base, which may also be linked to a complex pattern of homoplasy and convergence shared between Old and New World anthropoids. As discussed later in chapter 8 of this thesis, current methods that control for allometry in geometric morphometric data are based on a principal components approach that are problematic for use in phylogenetic analysis (e.g.

Cardini et al. 2010, Adams et al. 2011), and development of new methods for allometric scaling are beyond the scope of this current project.

Considering the pitheciid phylogenetic results in their entirety, it is clear that a strong phylogenetic signal has been maintained in the pitheciid craniodental region. The saki-uakari clade of *Pithecia, Cacajao* and *Chiropotes,* and a sister relationship between the latter two genera, was supported from phylogenetic analysis of geometric morphometric shape data and provided additional quantitative evidence to the mostly cladistic, character state data that supported those phylogenetic relationships from numerous past morphological studies including Rosenberger (1977, 1981, 1984, 1992), Ford (1986) , Kay (1990), Horovitz et al. (1998), Horovitz & MacPhee (1999) and Kay et al. (2008). The congruence between molecular phylogenetic relationships and those based on morphology from both a character based approach, which mostly related to dental adaptations for seed based diets and postcranial adaptations, and a distance-based approach using skull shape is particularly interesting. Broad agreement from such contrasting types of data is a welcome addition in a field where the three approaches often disagree, and should position the pitheciids as a prime example of how morphological and molecular approaches can converge rather than clash. The success of pitheciid phylogenetic analysis outlined in this chapter also offers obvious hope for integrating pitheciid fossil taxa into a phylogenetic framework, especially with the well-preserved *Cebupithecia sarmientoi* skull.

Chapter 7 Cebid phylogenetic analysis

7.1 Introduction

The cebids include *Callithrix* (marmosets), *Callimico* (Goeldi's marmoset/monkey or callimicos), *Saguinus* (tamarins), *Leontopithecus* (lion tamarins), *Aotus* (owl or night monkeys), *Cebus* (capuchins) and *Saimiri* (squirrel monkeys). The cebids can be split into three major clades: callitrichines (*Callithrix*, *Callimico*, *Saguinus* and *Leontopithecus*), cebines (*Cebus* and *Saimiri*) and owl monkeys (lone *Aotus*). Callitrichines have small body sizes, third molar loss (but only a reduced third molar in *Callimico*), claw-like nails, relative small brain size, and have a high prevalence of twinning (except in *Callimico*), social suppression of reproduction, and mating systems that vary between and within taxa (Digby et al. 2011, Isler et al. 2008). Cebines include two sister taxa, *Cebus* and *Saimiri*, that are often sympatric, regularly forming mixed-species groups, and share short faces, large premolars, reduced third molars, a round cranial vault, narrow nasal bones and large brains, divergent cranial base morphology and complex, variable social systems (Jack 2011, Fedigan et al. 1996). *Aotus* are the only nocturnal anthropoid, share a strong morphological link with the basal-pitheciid *Callicebus*, and are one of the few monogamous, pair-bonded primates (Fernandez-Duque 2011a). Body size evolution in the cebid clade is extreme, as capuchins have trebled in body size compared to their sister taxa, whilst marmosets have experienced secondary size reduction in the pygmy and dwarf marmosets, and *Leontopithecus* have possibly experienced a size increase following phyletic dwarfing in the callitrichine common ancestor (Garber et al. 1996, Ford & Davis 1992, Rosenberger 1992). A comparative sample of photographs are provided at the end of the introduction that show cebid craniodental morphology for each genus from frontal (Figures 44-45), lateral (Figures 46-47), and basal (Figures 48-49) views.

7.1.1 Callitrichines

The callitrichines include *Callithrix*, *Callimico*, *Leontopithecus* and *Saguinus*. Ford (1980) proposed five callitrichine traits that confirmed their derived nature, challenging the central thesis of Hershkovitz (1977) that viewed the clade as primitive and characteristic of the platyrrhine common ancestor. Reproductive twinning is present in callitrichines, except *Callimico*, requiring complex uterus adaptations, but is absent in other platyrrhines and rare in primates suggesting a derived trait. Third molar loss is derived in callitrichines, as the anthropoid and primate common ancestors had a third molar, but *Callimico* has a reduced

third molar. Callitrichines have an absent hypocone on upper molars, which could be a retained primitive trait but for the ubiquitous presence of hypocones in non-callitrichine platyrrhines. On all digits except the hallux, callitrichines have claw-like nails, with redevelopment and convergent evolution of this trait more parsimonious than the repeated loss of claws and development of nails across the primates (Ford 1980, Soligo & Muller 1999). The primitive nature of callitrichine body size was a major argument from Hershkovitz (1977), but as small body size is relatively rare in primates its presence is likely derived (Ford 1980, Soligo & Martin 2006).

These five traits remain the major morphological traits linking callitrichine taxa together. Ford (1980) proposed these traits were interconnected and related to body size reduction (phyletic dwarfing). With *Callimico* exhibiting a mix of callitrichine and non-callitrichine traits, the narrative is untidy, but the argument holds that body size reduction has led to the evolution of an adaptive complex of highly derived traits. This was supported by comparative evidence from other mammalian groups, such as pygmy hippos and squirrels, where dwarfing had led to molar loss and reduced molar complexity (Ford 1980). Past climate and habitat change could have created islands of isolated groups (or a single ancestral group) due to a presence of arid regions separated by river systems, creating a selective pressure that led to dwarfism (Ford 1980).

Martin (1992) supported callitrichine phyletic dwarfing, further investigating many of the traits outlined in Ford (1980) with greater emphasis on life history and reproduction, and also clarified the position of *Callimico* within callitrichines from morphological and biochemical evidence, several years prior to molecular phylogenetic analyses (e.g. Pastorini et al. 1998, Canavez et al. 1999b). Callitrichine small body size is derived when using Old World monkeys as a comparative sample, as is third molar loss due to presence of three molars in primate and mammalian common ancestors. The loss of hypocone morphology was not an adjustment to small body size, as galagos and lorises of similar size have hypocones, and may be adaptations for insectivory. Data on molar area and body size indicated molar loss was an adaptation for reduction in tooth area upon body size decreases. The claw-like nails found in callitrichines are intermediate between primate nails and non-primate mammalian claws that are adaptations for maintaining grip, especially when feeding on gums that require clinging on tree trunks. Callitrichine reproductive adaptations included sharing of placental circulation by twins, except for callimicos, and extension of gestation by delaying embryonic growth after fertilisation. Another callitrichine trait related to dwarfing was proposed, with over-

scaling of eye size so that the diameter of the eye is greater than the diameter of the orbit (Martin 1992). Callitrichines also have relatively small brain sizes, which they share with *Callicebus* and *Aotus* (Isler et al. 2008).

Garber (1992) separated callitrichine feeding into four major strategies. The *Saguinus* feeding strategy involved opportunistic foraging of insects, fruit and nectar, using prehensile forelimb and hindlimb positional behaviour. They consumed exudates, but cannot gouge into bark like marmosets, and used their claw-like nails to cling onto tree trunks in a vertical clinging posture. *Saguinus fuscicollis* used an alternative strategy, with increased insectivory and foraging for large prey that involved vertical clinging and scansorial locomotion, with leaping between trunks. *Callimico* is similar to *Saguinus fuscicollis* with preference for undercanopy, leaping between trunks, and an emphasis on foraging and vertical clinging. *Leontopithecus* used a third strategy of specialist manipulative foraging, with dextrous long fingers used to probe and extract concealed prey unavailable to other callitrichines. The fourth strategy of tree gouging and exudate feeding in marmosets is probably the most specialised. Exudates include protein, minerals and carbohydrates, the latter of which can be complex and require hindgut specialisations for digestion. Gouging and exudate feeding are associated with clinging postures, mostly vertical clinging. Marmosets have elongated, chiselled incisors, thickened buccal and reduced lingual enamel, and a v-shaped jaw, allowing lower incisors to gouge into bark and stimulate exudate flow. The *argentata* marmosets have reduced exudativory and less specialised lower incisors for gouging, with a morphology that could be considered intermediate between non-gouging and gouging callitrichines. Considering the four strategies proposed by Garber (1992), it is intriguing that in the case of *Callithrix* and *Saguinus* there is variation in foraging and dietary preferences within each genus.

7.1.2 *Callithrix*- One Genus or Four?

Marmoset taxonomy is particularly controversial, as some (e.g. Rylands et al. 2000, Rylands et al. 2009) split *Callithrix* into four genera (*Callithrix*, *Mico*, *Cebuella* and *Callibella*) whilst others (e.g. Groves 2001) maintain a single *Callithrix* genus. The core argument for these and other groups should be how different do taxa need to be to belong to different genera. In addition, whether one source of data, molecular, morphological or behavioural, can be sufficient to elevate groups to a higher taxonomic level. In the case of marmosets, van Roosmalen & van Roosmalen (2003), Aguiar & Lacher jr (2009) and Ford & Davis (2009) suggest evidence from morphology, behaviour and molecules support the presence of four

marmoset genera. Deeper consideration of the morphological and molecular evidence challenges some of the key assumptions in elevating the four marmoset clades to genera.

Marmoset taxonomy is especially problematic because the pygmy marmoset has undergone secondary dwarfing that produces a distinct suite of morphological adaptations that led to the group being treated as a separate genus from *Callithrix* (e.g. Hershkovitz 1977), although some morphological and molecular analyses have identified the pygmy marmoset as a species of the genus *Callithrix* (e.g. Rosenberger 1981, Rosenberger 1984, Barroso et al. 1997, Canavez et al. 1999b). Rylands et al. (2000) chose to maintain *Cebuella* and split *Callithrix* into the two major clades that are geographically and phylogenetically distinct- the *Mico argentata* group of the Amazonian region and the *Callithrix jacchus* group of the Atlantic forests. The discovery of another marmoset, the dwarf marmoset, added another potential genus, that was originally classified as *Callithrix humilis* (van Roosmalen et al. 1998) and elevated later to *Callibella humilis* (van Roosmalen & van Roosmalen 2003). The body size of the dwarf marmoset is between 150-185g, not as small as the pygmy marmoset (110-130g) but closer to it in size than to the larger *jacchus* (250-430g) and *argentata* (340g) groups (Ford & Davis 2009). Dwarf marmosets are found in the Amazon, have increased gummivory, and a mix of shared traits with the pygmy and *argentata* marmosets (Aguiar & Lacher jr 2009).

The molecular phylogenetic analysis placed a monophyletic clade of *argentata* species as sister to the pygmy marmoset and the dwarf marmoset basal, with *jacchus* marmosets forming a separate monophyletic group. This supported the presence of four marmoset lineages, and a split between Atlantic and Amazonian marmosets, but the results are ambiguous regarding whether these differences relate to species- or genera-level differences. Aguiar & Lacher jr (2009) and Ford & Davis (2009) both studied the comparative anatomy of *C. humilis* and the callitrichines, and explored relationships mostly using discriminant function analysis (DFA) of linear measurements. Aguiar & Lacher jr (2009) recognised two marmoset clusters, one for small marmosets (pygmy and dwarf) and another for large marmosets (*jacchus* and *argentata*). This would seem to contradict the presence of four morphologically distinct marmoset groups, although even if DFA did find the groups were distinct, the justification for elevating the four groups to different genera would be contentious. There is clear similarity in the more gracile mandibular morphology of pygmy and dwarf marmosets, with divergence between *jacchus* and *argentata* morphology, but

considering the body size variation, it should be expected that morphological differences exist within the marmoset clade.

Ford & Davis (2009) made a case for postcranial morphological divergence within the marmosets. The sample size of one for *C. humilis* limits comparisons considering the wide range of intraspecific variation exhibited by all other taxa. It seems likely that increasing the sample size would show greater overlap between the dwarf marmoset and other taxa, making the group appear less divergent. Ford & Davis (2009) predicted, from the morphology studied, that the dwarf, pygmy and *argentata* marmosets were distinct from *jacchus* marmosets in behaviour and postcranial morphology. *Argentata* marmosets likely use the arm in a different way, pygmy marmosets use more scansorial behaviour with flexed hindlimb postures, and the dwarf marmoset potentially use clinging more than *jacchus* marmosets but are more quadrupedal than pygmy marmosets.

The fundamental problem with van Roosmalen & van Roosmalen (2003), Aguiar & Lacher jr (2009) and Ford & Davis (2009) is that, at best, they have only shown there are four distinct groups within the monophyletic marmoset clade. None of the evidence differentiates whether these four groups represent differences at the species or genus level. Whilst the size reduction of pygmy and dwarf marmosets is distinctive and divergent, that cannot be a basis for naming new genera, unless body size and morphological diversification (if present) corresponds to molecular change. Considering the abundant nature of molecular data, and likelihood that much of its evolution has been neutral, it seems pertinent to use a molecular approach to taxonomy of marmosets. Such a molecular taxonomic approach would need to look at much larger sequences of DNA, with multiple genes from different genomic regions, and must sample a wider comparative sample including *Saguinus*.

It is also necessary to ascertain whether the level of variation found in marmosets exceeds the variation in other speciose genera, although the trend towards taxonomic splitting may lead to other platyrrhine genera being further subdivided. The molecular, behavioural and morphological data produced thus far only confirms diversity within the group. None of these studies proves the presence of four genera, or disproves the presence of four species, and as a result, I treat the marmosets as a single, diverse genus. Goodman et al. (1998) and Groves (2004) have suggested standardising primate taxonomic ranks based on time, which I would support, but it relies upon accurate dating of divergence times, which is problematic because

of variation in the rates of evolution, and methods also have a major role in predicting divergence time (see Wilkinson et al. 2011 for example).

Under the following subheadings, information on each callitrichine genus are provided. Where possible this includes geographical distribution, habitat preference, diet, social grouping and behaviour, body size and sexual dimorphism, locomotor and postural behaviour, and a brief summary of craniodental morphology.

7.1.3 *Callithrix*

Callithrix can be subdivided into four groups, based on morphology, genetics and behaviour- the Atlantic forest *jacchus* group, and the Amazonian *argentata*, dwarf and pygmy marmosets (Kinzey 1997, van Roosmalen & van Roosmalen 2003). Body size for pygmy marmosets range from 110-130g, for dwarf marmosets 150-185g, and *jacchus* and *argentata* marmosets between 250-430g, with negligible sexual dimorphism (Ford & Davis 2009, Ford & Davis 1992). The marmosets have small faces that are quite gracile especially in the lower and mid-face, wide nasal bones, quite long and narrow cranial vaults, a very thin zygomatic arch and a robust basicranium. The nasal aperture varies from concave to slightly convex in shape, dental arcade varies between v-shaped and slightly u-shaped, and the foramen magnum is more central than posterior (Hershkovitz 1977). The pygmy marmoset has an even more gracile lower face, the orbits take up a larger proportion of the face, and the cranial vault appears slightly globular, but overall it looks much like a small marmoset.

Callithrix are distributed to the south of the Amazon river and the east of the Madeira river, with a distribution mostly in Brazil and additional populations in Bolivia, Paraguay, Colombia, Ecuador and Peru, inhabiting primary, secondary, savannah, white sand and disturbed forests, although pygmy marmosets are habitat specialists isolated to tropical lowland forests (Kinzey 1997, Digby et al. 2011). Diet varies across *Callithrix*, with reliance on a core resource dependent on availability within their environment. The *jacchus*, dwarf and pygmy marmosets share increased exudativory, with the pygmy marmoset consuming 60% exudates to 30% insects, whereas the *argentata* group have lower exudativory and higher frugivory despite being more closely related to pygmy and dwarf marmosets than the *jacchus* group (Kinzey 1997, Norconk et al. 2009, Ford & Davis 2009). Marmosets share chiselled lower incisors and enamel absent on the lingual side of lower incisors to sharpen teeth for effective gouging, with a large jaw gape that helps gouge into bark, anchoring upper dentition in the tree and using the lower dentition to gouge inwards and stimulate the flow of

exudates (Digby et al. 2011, Kinzey 1997, Taylor et al. 2009). The marmoset adaptations make exudates available throughout the year, giving marmosets an advantage over other callitrichines and allowing exploitation of habitats otherwise unavailable due to seasonal variation (Digby et al. 2011).

Locomotion is largely quadrupedal, but includes leaping, with climbing and clinging, linked to exudativory and feeding (Youlatos 1999). Youlatos (2009) observed increased use of large vertical supports and locomotion by vertical claw climbing, with only rare leaping, in pygmy marmosets. Social group size varies between 3-20; whilst pygmy marmosets have very small groups other marmosets have the largest groups of any callitrichine (Digby et al. 2011). Mating systems observed include monogamy, polyandry and polygyny, and usually only one female will breed at any one time, groups are stable, territorial, and ranging behaviour in the *jacchus* groups are much smaller than in *argentata* groups, which is likely linked to the differences in frugivory (Kinzey 1997, Youlatos 1999, Digby et al. 2011).

7.1.4 *Callimico*

The callimicos are a single-species genus that share single births and presence of a third molar with non-callitrichines, but share small body size (around 500g) and claw-like nails with callitrichines, whilst modern molecular phylogenetics place the taxon firmly within the callitrichine group as sister to *Callithrix* (Kinzey 1997, Horovitz & Meyer 1997, Pastorini et al. 1998, Chaves et al. 1999, Porter & Garber 2004). The callimicos' face is short, orbits are enlarged, dental arcade nearly u-shaped, and the foramen magnum central (Hershkovitz 1977). The lower face is quite robust especially with the canine roots well developed, and the nasal bones are extended ventrally, partially resembling *Pithecia*. The cranial vault is dolichocephalic, the palate is robust, and the posterior part of the cranial base past the foramen magnum is often extended.

Callimicos are found in the upper Amazon basin and western Amazon including populations in Brazil, Peru, Colombia, Ecuador and Bolivia, inhabiting mainly bamboo and secondary forests, and mostly dwell in the lower canopy (Kinzey 1997, Porter & Garber 2004). They are the only primate to consume fungi as a major dietary source, with an average diet of 30% fruit, 30% fungi and 40% insects, although study of a Bolivian population observed significant seasonal exudate feeding (Norconk et al. 2009, Garber & Porter 2011). The utilisation of fungi includes dental adaptations for very high molar shearing crests and requires a large home range due to wide dispersal (Porter & Garber 2004). Callimicos will

also frequently descend to the ground to prey on insects and climb to the upper canopy to acquire fruit (Porter & Garber 2004). *Callimico* have small group sizes between 4-12 with monogamy, polygyny and polyandry mating systems all observed, but polyandry likely the most common (Digby et al. 2011, Porter 2001, Porter & Garber 2009). Positional behaviour is linked to preference for understory/lower canopy, with a mixture of quadrupedal walking and leaping with climbing and vertical clinging (Kinzey 1997).

7.1.5 *Saguinus*

Tamarins are a highly-speciose group, and one of the most common and widely distributed of any platyrrhine genus (Cropp et al. 1999, Matauschek et al. 2011). Their body sizes range from 400-600g, with low levels of sexual dimorphism, and molecular phylogenetic methods support two clades for large-bodied and small-bodied tamarins (Kinzey 1997, Ford & Davis 1992, Cropp et al. 1999). The small-bodied *nigricollis* group include *S. nigricollis*, *S. fuscicollis* and *S. tripartitus* that may be one single super species, and the large-bodied *mystax* clade include all remaining species (Matauschek et al. 2011). These two clades have alternative feeding strategies, with increased insectivory and predation by smaller tamarins, requiring greater scansorial locomotion and leaping (Garber 1992).

The tamarins are distributed throughout the Amazon basin and north into central America, including Brazil, Colombia, Bolivia, Peru, Ecuador and Panama, with *S. oedipus* and *S. geoffroyi* inhabiting dry deciduous forest and all other tamarins found in humid tropical lowland forest, often in middle and lower canopy (Kinzey 1997). Tamarins are primarily mixed insectivore-frugivores with some exudate and leaf consumption (Norconk et al. 2009, Garber & Porter 2011). They are especially adept at vertical clinging whilst foraging for insects and exudates, yet they lack the gouging adaptations that *Callithrix* exhibit for gaining access to exudates (Kinzey 1997). Locomotion patterns are quadrupedal walking and running, with leaping between terminal branches (Kinzey 1997). Social groups consists of quite small multimale-multifemale groups of up to 13 individuals, with large home ranges, and mating systems including monogamy, polygyny, polyandry and polygynandry (Kinzey 1997).

The tamarin face is quite broad, especially across the wide zygomatic bones, with a well-developed infraorbital ridge, orbits are quite square, and the midface around the nasal bones projects more than in other callitrichines. The dental arcade is u-shaped (Hershkovitz 1977)

and wide at the posterior-end, the cranial vault is circular and basicranium dolichocephalic much like other callitrichines.

7.1.6 *Leontopithecus*

Leontopithecus are highly endangered and under-studied, and are present in four areas within lowland Atlantic Coastal forests of southeastern Brazil, inhabiting both primary and secondary forests associated with stream valleys or swamps (Kinzey 1997). Lion tamarins are the largest callitrichines, with an average weight just below 600g and low sexual dimorphism (Ford & Davis 1992). The *Leontopithecus* face is quite robust, the interorbital region is especially wide, the infraorbital ridge is often developed, the cranial vault is dolichocephalic, and the basicranium is wide in the regions next to the foramen magnum. The nasal aperture is concave, orbits are small and relatively square, the foramen magnum is positioned more posterior than in other callitrichines, the dental arcade is intermediate between v- and u-shaped and is broad at the posterior end (Hershkovitz 1977). They are manipulative, extractive foragers, using elongated fingers to probe and acquire insects and vertebrates, and have a mainly frugivorous diet, preferring soft fruits, with insects and exudates also consumed in large proportions (Kinzey 1997, Norconk et al. 2009). Socially there appears to be large variation, with group sizes ranging from 2-11, with a mix of monogamy, polyandry, polygyny and polygynandry, and dominance hierarchies also observed (Kinzey 1997, Digby et al. 2011). Locomotion is largely quadrupedal walking and running, with a mix of jumping, climbing and suspension (Kinzey 1997).

7.1.7 Cebines

As outlined by Janson & Boinski (1992), cebines contrast with similar-sized platyrrhines, with insectivory rather than folivory, share locomotor behaviour with quadrupedal running and walking predominant, and have a central foramen magnum, short nasal bones and a reduced pteroid-mastoid region of the temporal bone. *Cebus* are around three times larger in body size than *Saimiri*, and have associated differences in metabolic demand, leaping ability, strength and agility (Janson & Boinski 1992, Ford & Davis 1992). *Cebus* have thicker enamel and lower cusps lacking a lingual cingula that are adaptations for increased frugivory and processing of tough food material, and larger body size is linked to an increased exertion in bite force. In contrast, *Saimiri* are more insectivorous and have complex cingula on their teeth specialised for puncturing the exoskeleton of insects. Capuchins have a precision grip, long fingers and hands, with pseudo-opposable thumbs, and can move digits independently of each

other, none of which squirrel monkeys can do. In addition, *Cebus* have a prehensile tail that can anchor the whole body in suspension, although *Saimiri* have a large, strong tail used to help balance. There is also variation in social structure in both cebine genera, linked to an interaction with ecological and foraging factors. For example, a *S. sciureus* group in Peru have high fruit competition and a female dominance hierarchy, whereas an *S. oerstedi* population in Costa Rica have very little fruit competition and egalitarian, non-dominant female relationships (Janson & Boinski 1992).

Janson & Boinski (1992) considered foraging one of the key aspects of cebine evolution, with significant variation between the two groups. *Saimiri* use extraction of insects from within leaves or on the surface of branches, trunks and leaves, whilst *Cebus* favour either snatching mobile prey or extracting them from hidden and tough sources including termite nests, dead branches and bamboo. Capuchin foraging requires more time and often involves terrestriality, whereas *Saimiri* are faster and more successful in hunting insects. Unlike squirrel monkeys, capuchins prefer consuming social insects, such as termites and ants, involving a complex behavioural repertoire, such as caution when targeting wasps and ants. Due to their smaller body size, squirrel monkeys can survive exclusively feeding on insects in times of resource stress, an option unavailable for the much larger-bodied capuchins. *Cebus* will rapidly increase vertebrate consumption in response to seasonal change, mainly targeting birds and bats, and target larger fruits with tough skins or husks as well as palm seeds and other hard objects, which are unavailable to *Saimiri*, which tend to forage for small, soft fruits. The dietary preference for animal prey by both cebines requires longer periods of foraging than for any other platyrrhine, and the distribution of prey likely reduces competition and allows development of larger social groups with the benefits that entails.

Fedigan et al. (1996) noted several additional factors in cebine evolution. The size difference between *Cebus* and *Saimiri* leaves the squirrel monkeys much more susceptible to predation, which may contribute, in addition to dietary preference as detailed above, to much larger group size as an anti-predator strategy. Although cebines share relatively large brain sizes the ontogenetic trajectories are very different- *Cebus* have extensive postnatal growth and slow development of motor skill that increasingly becomes more complex, whereas *Saimiri* are born with relatively well developed brains and motor skills (Hartwig 1995, Hartwig 1996). Several studies have examined the patterns of growth and sexual dimorphism in *Cebus* (Corner & Richtsmeier 1991, O'Higgins et al. 2001, Flores & Casinos 2011) and *Saimiri*

(Corner & Richtsmeier 1992), and support these two alternative patterns of cranial growth and ontogeny in the two cebines.

Corner & Richtsmeier (1991) investigated growth in the *Cebus apella* skull using Euclidean distance matrix analysis (EDMA) and finite-element scaling analyses (FESA) methods. They found male and female crania had similar patterns of growth but males were larger in measurements taken at each developmental stage, with most growth in the lower and upper face, reduced size change associated with the cranial base and much less for the neurocranium. O'Higgins et al. (2001) applied a geometric morphometric approach to *C.apella* sexual dimorphism of the facial region, finding statistically significant sexual dimorphism, with increased prognathism around the nasal region, pronounced zygomatic roots, lateral expansion of the maxilla and contraction of the orbits in males. They also found that shape differences between sexes occurred in the later stages of development via an extended growth trajectory in males.

Matterson (1997) examined the patterns of sexual dimorphism in *C.apella* and *C.albifrons*, with a mix of congruence and incongruence between the two groups. Sexual dimorphism in males occurs earlier and is larger in *C.apella* than *C.albifrons*, but both groups share a pattern of faster growth and development in males. There are differences between the two groups, especially in traits related to mastication, which reflect the dietary specialisations of *C.apella* for hard foods. These differences reflect alterations to the same underlying pattern and process, rather than a completely different ontogenetic pattern as in *Saimiri*. Flores & Casinos (2011) examined ontogeny, allometry and dimorphism in *C.apella*, supporting significant levels of sexual dimorphism in males that were larger than females in all cranial variables examined, and extended growth in males that continued to grow longer into adulthood than females. Combined, these studies of ontogeny and dimorphism in capuchins broadly agree on the presence of significant sexual dimorphism, extended growth in males, and major growth in the face.

Corner & Richtsmeier (1992) used the same methods as Corner & Richtsmeier (1991) to investigate the extent and ontogeny of sexual dimorphism in *Saimiri sciureus*. They found only slight sexual dimorphism in the cranium expressed in later stages of development, with the cranial base exhibiting greatest sexual dimorphism and neurocranium the least. Overall, cranial growth is low throughout development, especially in the neurocranium, except for slight growth in the anterior part. Increased zygomatic growth in *Saimiri* and *Cebus* males are

one of the few shared cebine responses to dimorphism. The overall pattern of growth in *Saimiri* described by Corner & Richtsmeier (1992) quantified the same ontogenetic shift detailed by Hartwig (1995) and Hartwig (1996) that Fedigan et al. (1996) interpreted as an adaptation to predation and shift for squirrel monkeys to be born with well-developed brains and cognitive abilities. The low levels of sexual dimorphism in the *Saimiri* skull are interesting, as although body size dimorphism is reduced compared to *Cebus* it is still quite high (Ford & Davis 1992). These studies have outlined two alternative pathways for the development of sexual dimorphism, which are by themselves important examples of how two closely related groups can be shaped by alternative biological pressures to develop in very different ways. Further information on each of the cebine genera follows.

7.1.8 *Cebus*

Capuchins, after howler monkeys, have the widest distribution of any platyrrhine ranging from the south in Argentina as far north as Honduras, including distribution across Brazil, Bolivia, Colombia, Ecuador, Peru, Venezuela, Paraguay, Costa Rica, Panama, Nicaragua, French Guiana, Guyana and Suriname (Kinzey 1997). They are the largest cebids, with an average male around 3kg and female 2.3kg and sexual dimorphism of about 24%, although dimorphism appears to vary between populations and can rise much higher (Ford & Davis 1992, Kinzey 1997, Jack 2011). There is considerable variation in the morphology of capuchins, with differences arising from robusticity of the lower face and prominence of the orbits. Capuchins have small faces compared to non-cebids, but larger than callitrichines and owl monkeys, with wide maxilla and premaxilla but reduced zygomatics. The dental arcade is large and u-shaped, the foramen magnum is positioned quite far forward, the cranial base is quite broad, and the cranial vault is less dolichocephalic than in other cebids.

The capuchins are divided into two groups: the tufted capuchins, including either a single species, *C. apella*, or multiple species depending on taxonomy used, distributed east of the Andes, and the untufted capuchins, including *C. capucinus, C. albifrons* and C. *nigrivittatus*, which are parapatric and distributed in central America, western Amazonia, and north of the Amazon (Janson & Boinski 1992). *C. apella* are hard food specialists with a range of associated adaptations compared to other capuchins including larger, more robust faces and mandibles, thicker enamel, robust and flared zygomatic arches, flaring pterygoid plates, and sagittal cresting in the largest males (Cole 1992, Janson & Boinski 1992). Social groups are stable and multimale-multifemale ranging from 16-21 with variation in male to female ratio,

clear male dominance hierarchies with alpha males more reproductively successful, behavioural plasticity for inter-male behaviour ranging from despotic aggression to cooperation and affiliations, male dispersal, and mating systems that are polygamous, with home ranges dependent on fruit availability (Kinzey 1997, Digby et al. 2011).

The capuchin preference is for canopy-covered forest, generally operating in the middle strata, but they are highly adaptable habitat generalists present in primary and disturbed montane, dry tropical, swamp, seasonally flooded, semideciduous, gallery, young and old successional forests (Kinzey 1997, Jack 2011). Locomotion is mostly quadrupedal with leaping and climbing, and the prehensile tail is used as stabilisation for posture during foraging and feeding (Kinzey 1997). Capuchins are for the only platyrrhines with dextrous digits, with shortened, flexible fingers and semi-opposable thumbs, with large brain size relative to body size, and are known for their tool use including using rocks for nut cracking, probing tools to extract food, and using a club to attack a venomous snake (Visalberghi & Trinca 1989, Visalberghi 1990, Moura & Lee 2004, Jack 2011, Janson & Boinski 1992).

They are also the only non-atelids with presence of a prehensile tail, spend the most time terrestrially of any platyrrhine, have the thickest tooth enamel of any extant primate except humans, and have the longest lifespans of any primate outside the hominoids (Kinzey 1997, Jack 2011). Capuchins are mainly frugivores, with significant insectivory and additional feeding on seeds, leaves and vertebrates, are extractive foragers that are especially adept hunters of birds, lizards and squirrels, but can be considered opportunistic generalists and omnivores due to their dietary flexibility dependent on environmental availability (Kinzey 1997, Norconk et al. 2009, Jack 2011).

7.1.9 *Saimiri*

Squirrel monkeys are about one third the size of capuchins and have relatively high sexual dimorphism in body size: average males weigh around 900g and females 700g, with increased dimorphism in some populations (Ford & Davis 1992, Jack 2011). The face is slightly larger than in callitrichines and owl monkeys, with a broad lower face and thick canine roots, and the dental arcade is u-shaped and robust. The morphology of the squirrel monkey face, especially in large males, is reminiscent of the seed-harvesting pitheciids, especially with the prominence of the orbits and canines. The shape of the neurocranium is dolichocephalic, long and wide (Hartwig 1995), and the cranial base is more rectangular, whereas callitrichines and owl monkeys are more circular, and the cranial base is elongated

posteriorly past the foramen magnum which is itself central. The fetal brain is large at birth, as are the developing eyes, and constraints created by the large neurocranium interact with infraorbital regions to cause a large opening (fenestra) to develop (Hartwig 1995). The shift to large brains at birth have been linked to high predation and intraspecific food competition between infants, and life history evolves so that the neonate brain and behavioural repertoire is well developed and quickly develops further (Hartwig 1995).

Squirrel monkeys have a strict ecological niche and are distributed nearly exclusively in secondary, tropical lowland forests in the lower and middle canopy throughout South and Central America, particularly through the Amazon basin (Kinzey 1997). They have populations in Central America (Panama and Costa Rica) and a wider distribution in South America across Brazil, Ecuador, Colombia, Venezuela, Peru, Bolivia, Suriname, Guyana and French Guiana (Kinzey 1997, Jack 2011). As with several platyrrhines, squirrel monkeys are found in a range of altitudes from sea level to 2000 metres above sea level (Hershkovitz 1984).

Kinzey (1997) viewed *Saimiri* diet as frugivorous or insectivorous dependent on availability, but Norconk et al. (2009) reports 60% insect consumption compared to 25% fruit, making squirrel monkeys the most insectivorous platyrrhine (Zimbler-Delorenzo & Stone 2010). In pursuit of insects, they often unroll leaves to extract hidden prey, but they lack the manual dexterity and tool use of capuchins (Jack 2011, Janson & Boinski 1992). Locomotion is mostly quadrupedal walking and running but leaping is also common and the squirrel monkeys are quick and agile, using their tails for balancing, and mostly forage and feed on small branches (Kinzey 1997). Hershkovitz (1984) divided squirrel monkeys into two groups based on morphology and behaviour- *S. boliviensis* and *S. sciureus*, *S. oerstedii* and *S. ustus*. These two groups are known as gothic and roman types, *S. boliviensis* with a "roman" arch of rounded, shorter fur on the head, and *S.sciureus*, *S. oerstedii* and *S. ustus* have a "gothic" high arch of dark hair (Groves 2001). This taxonomic split has been supported by molecular genetic data in Boinski & Cropp (1999), Cropp & Boinski (2000), Lavergne et al. (2010) and Chiou et al. (2011).

Social groups in *Saimiri* are the largest of any platyrrhine, ranging between 20-75 with temporary unions of separate groups into a mass of up to 300 individuals observed (Digby et al. 2011). Patterns of dispersal, presence of dominance hierarchies, territoriality, and aggression are all population and taxa specific, although affiliate relationships between males

are consistent throughout all groups (Kinzey 1997, Digby et al. 2011). Boinski et al. (2002) linked variation in dispersal and social interactions to ecological factors of food distribution, availability and defence, for example with groups in Surinam able to monopolise areas due to the patchy distribution of fruits leading to dominance hierarchies (Digby et al. 2011). The squirrel monkey mating season is restricted to a two-month period during which males will increase body mass by up to 22%, with the largest male the preferred partner in mate choice, and male size is linked to length of time spent in the group rather than male dominance (Digby et al. 2011).

7.1.10 The owl monkeys

Early phylogenetic analyses supported a sister relationship between the owl monkey *Aotus* and the titi monkey *Callicebus* (e.g. Rosenberger 1984, Ford 1986), which Rosenberger et al. (1996) and Rosenberger et al. (2009) continue to support. It is clear that the two platyrrhines share a morphological similarity due to extensive homoplasy and convergent evolution, although the emphasis has often been on shared similarity between owl monkeys and pitheciids. In fact, homoplasy links *Callicebus* to the cebids, particularly with the dolichocephalic cranial vault and non-projecting midface similar to a scaled up callitrichine and quite unlike the other pitheciid taxa. Nonetheless, molecular phylogenetic evidence is overwhelming in placing the owl monkeys within the cebid clade and rejecting a link between *Aotus* and *Callicebus* (e.g. Wildman et al. 2009, Hodgson et al. 2009). As a result, the phenotypic link between these two groups is not considered further, although it is clearly one of the major primate examples of convergent evolution and requires further study.

Owl monkeys are the only nocturnal platyrrhines, although *Aotus azarai azarai* are cathemeral, and have very large eyes and associated orbits (Fernandez-Duque 2011a). The large orbits correspond with thin nasal bones and zygomatic bones that are rotated ventrally, although the lower face around the premaxilla and maxilla are much the same as a callitrichine, although less projecting. The cranial vault is dolichocephalic, and from a lateral perspective the owl monkey looks like a callitrichine with enlarged orbits, although the basicranium is more like a cebine with a well developed posterior region preceding the foramen magnum. The petrous portion of the temporal bone appears to be larger and more developed than in any other cebid. Owl monkeys have a relative small brain size, which they share with *Callicebus* and callitrichines (Isler et al. 2008).

Aotus average weight is around 1kg, although *A. azarai* are larger (average 1.25kg) and *A .trivirgatus* are smaller (average 0.7kg), and observed sexual dimorphism is minimal (Kinzey 1997, Ford & Davis 1992, Fernandez-Duque 2011a). They are distributed from as far north as Panama to as far south as Argentina, including populations in Brazil, Colombia, Ecuador, Peru, Venezuela, Bolivia and Paraguay, and are present in a range of altitudes from sea level to 3000m above sea, in both cold and warm regions, and inhabit primary, secondary, gallery, seasonally deciduous, subtropical dry and gallery forests (Kinzey 1997). *Aotus* were separated into grey-necked northern and red-necked southern groups in Hershkovitz (1983), and recent molecular phylogenetic analysis supports these two clades but for placement of *A. nancymaae* in the northern clade (Plautz et al. 2009, Fernandez-Duque 2011a, Menezes et al. 2010). Their diet is mixed frugivorous-folivorous, with slightly greater frugivory, and flowers and insects are also consumed (Norconk et al. 2009, Kinzey 1997). Owl monkeys are largely quadrupedal, but can leap (Kinzey 1997).

Aotus are primarily monogamous and pair bonded although this is not absolute and pairing often changes over time, with group size between 2 and 6 including two reproducing adults and offspring cared for by males, although not all individuals belong to a group and many are solitary (Kinzey 1997, Fernandez-Duque 2011a). They are also territorial, with confrontation upon overlap, rely on olfactory cues in communication, and activity patterns are linked to moonlight with greatest activity during a full moon. Fernandez-Duque (2011b) examined body size evolution and sexual dimorphism in the *A. azarai* group from the Chaco region of Argentina. The group have adapted to a difficult environment that is seasonal with large variation in rainfall, temperature and amount of daylight, with the development of cathemerality and a large increase in body mass. They found owl monkey body mass increased with latitude as predicted by Bergmann's effect, but Rensch's rule, that sexual dimorphism is greater in groups with larger body size, was rejected for body size with a negative scaling relationship between body mass and sexual dimorphism, but confirmed for the relationship between canine dimorphism and body mass. It is clear from this study, and the evolution of this population, that owl monkeys display greater variation and complexity than previously acknowledged.

Figure 44 Frontal view of *Callithrix* (top left), *Callimico* (top right), *Saguinus* (bottom left) and *Leontopithecus* (bottom right)

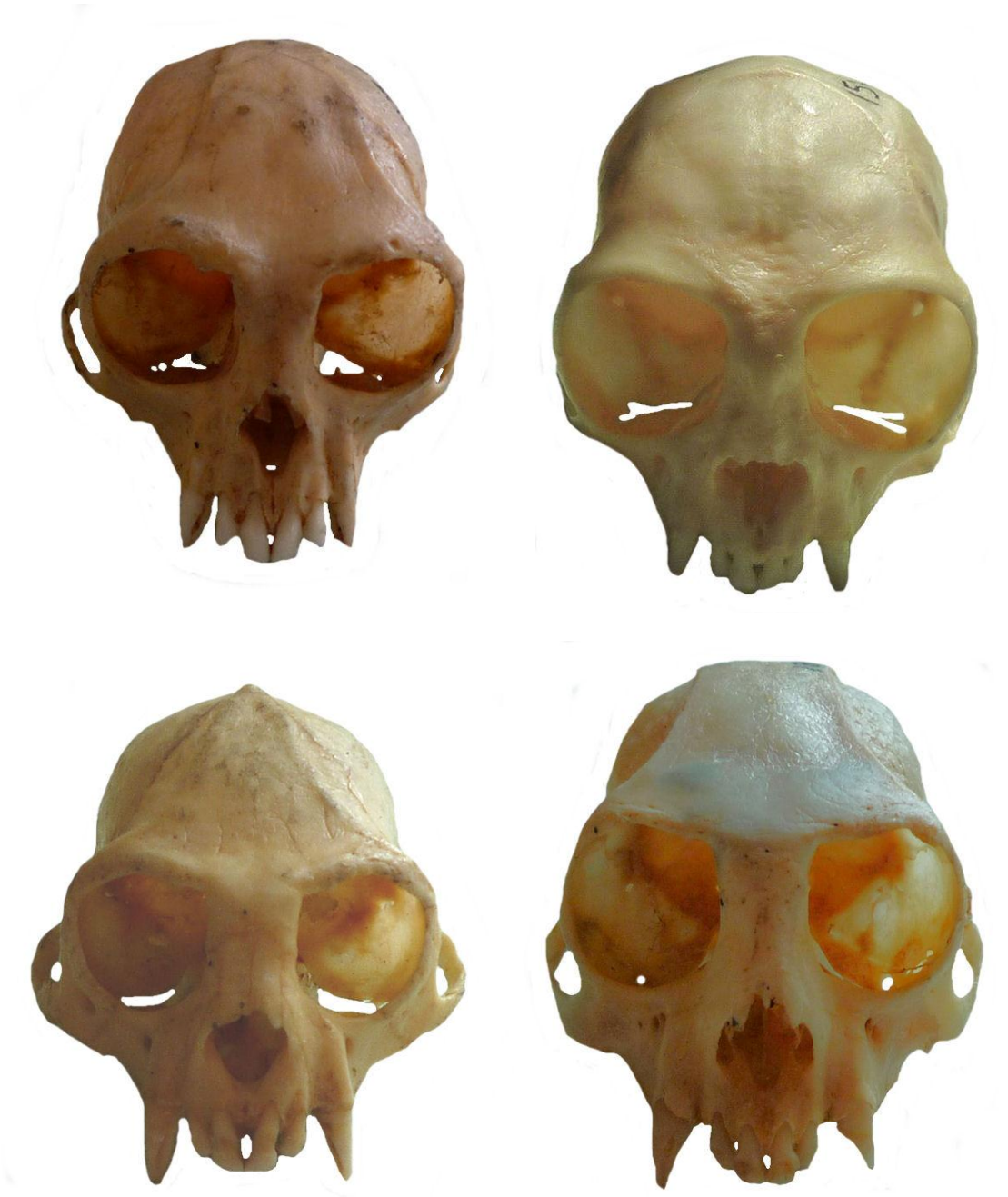


Figure 45 Frontal view of *Cebus* (top left), *Saimiri* (top right) and *Aotus* (bottom left)

Figure 46 Lateral view of *Callithrix* (top), *Callimico* (second top), *Saguinus* (second bottom) and *Leontopithecus* (bottom)

Figure 47 Lateral view of *Cebus* (top), *Saimiri* (second top) and *Aotus* (second bottom)

Figure 48 Basal view of *Callithrix* (top left), *Callimico* (top right), *Saguinus* (bottom left) and *Leontopithecus* (bottom right)

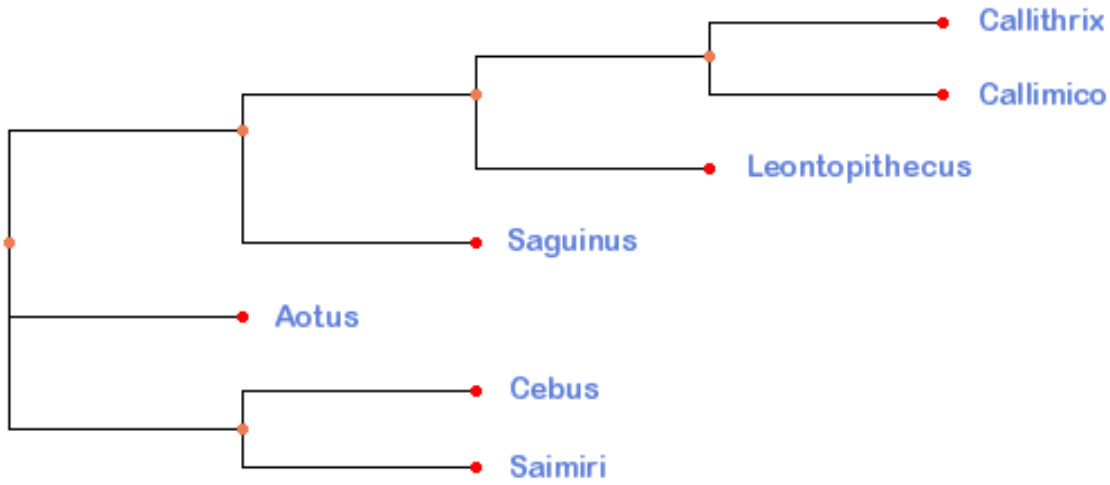
Figure 49 Basal view of *Cebus* (top left), *Saimiri* (top right) and *Aotus* (bottom left)

7.1.11 Cebid molecular phylogeny

Molecular phylogenetic analyses strongly support a sister relationship between *Cebus* and *Saimiri*, and a callitrichine clade with *Callithrix-Callimico* sister to *Leontopithecus* and *Saguinus* as the basal-most callitrichine (Barroso et al. 1997, Porter et al. 1997, Horovitz et al. 1998, von Dornum & Ruvolo 1999, Canavez et al. 1999a, Canavez et al. 1999b, Schneider 2000, Schneider et al. 2001, Opazo et al. 2006, Schrago 2007, Wildman et al. 2009, Perelman et al. 2011). The phylogenetic relationships between cebines, callitrichines and owl monkeys are unresolved. A sister relationship between callitrichines and *Aotus* was supported by Porter et al. (1997) and Horovitz et al. (1998), between cebines and callitrichines by Barroso et al. (1997), Porter et al. (1997), Porter et al. (1999), Schneider et al. (2001) and Schrago (2007), and between *Aotus* and cebines by Canavez et al. (1999a), Steiper & Ruvolo (2003), Ray et al. (2005) and Opazo et al. (2006).

Three more recent phylogenetic analyses have looked at much larger datasets, but have failed to resolve which of the three groups are most closely related. Phylogenetic analysis of complete mitochondrial genomes by Hodgson et al. (2009) placed callitrichines and owl monkeys as sister groups, as did the large phylogenetic analysis of Perelman et al. (2011), but Wildman et al. (2009) inferred a closer relationship between owl monkeys and cebines. These studies all agreed that the three lineages emerged in quick succession, and the two clades that share a more recent common ancestor would only have done so for a brief period of time. Considering both Perelman et al. (2011) and Wildman et al. (2009) used similar methods, sampling all platyrrhine genera and studying very large amounts of molecular data from multiple unlinked loci, the incongruence between the analyses means that for now the cebid trichotomy is unresolved and treated as such (see Figure 50 below).

Figure 50 Molecular phylogenetic relationships of cebids



7.1.12 Morphology-based phylogeny

Describing the morphology-based phylogenies of cebids is complicated because until the advent of molecular phylogenetic analysis, the group were not accepted as a monophyletic clade. The callitrichine and cebine clades however have been relatively well supported by morphological analyses. Rosenberger (1977) supported a sister-relationship between *Cebus* and *Saimiri* based on premolar enlargement, narrow inter-orbital distances and visual cortex specialisation. Rosenberger (1992) suggested *Saimiri* and *Cebus* shared molar proportions with the third molar reduced and broad premolars, but they also contrasted quite significantly, *Saimiri* having smaller molars and more distinct crests whilst *Cebus* molars are large with rounded cusps and thickened enamel. In Rosenberger (1977), cebines were inferred as more closely related to the large-bodied platyrrhines, whereas Rosenberger (1981) and Rosenberger (1984) suggested a sister relationship between the cebine and callitrichine clades. The cebine-callitrichine clade was supported by shallow, open glenoid fossa at the articulation with the mandibular condyle, gracile zygomatic arches, a foreshortened face, enlarged canines and mildly enlarged premolars, with absent or reduced third molars. Within the callitrichines, Rosenberger (1977) and Rosenberger (1984) placed *Callithrix-Leontopithecus* sister to *Saguinus* with *Callimico* basal-most. The phylogenetic position of *Aotus* has varied within these studies. Rosenberger (1977) created a clade of *Aotus*, *Callicebus* and atelids, while Rosenberger (1981) placed *Aotus* sister to pitheciids, and Rosenberger (1984) sister to *Callicebus*.

Ford (1986) supported the monophyly of the callitrichines with the traits Hershkovitz (1977) originally outlined: small body size, claw-like nails replacing nails, twinning, third molar loss and tritubercular molars with an absent hypocone. *Callimico* retained some of these traits and lost others. Within callitrichines, a close relationship between the pygmy and common marmosets was supported by 12 dental and 14 posctranial shared traits. Many of the shared dental traits related to the anterior dentition, linked to shared exudativorous feeding strategies. The inferred relationships between callitrichines were the same as in Rosenberger (1984): *Saguinus* sister to *Callithrix-Leontopithecus*, and *Callimico* as the basal-most callitrichine. *Callithrix, Leontopithecus* and *Saguinus* shared multiple traits to the exception of *Callimico*, alongside loss of the third molar and hypocone. The callitrichines share 7 dental traits and 18 postcranial traits. Ford (1986) did not link the callitrichines with *Aotus* or cebines, placing them as sister to an atelid-pitheciid clade instead. *Saimiri* and *Aotus* were placed in a group with *Callicebus*, whilst *Cebus* was a single basal lineage.

Kay (1990) supported *Saimiri* as sister to a callitrichine clade, with *Callithrix-Saguinus* sister to *Leontopithecus* and *Callimico* basal-most. *Aotus* was inferred as a sister taxon to a larger clade incorporating atelids, callitrichines and *Saimiri*, whilst *Cebus* was placed near the base of the tree as a lone lineage. Horovitz & Meyer (1997) placed *Saimiri* sister to the callitrichines in a cebid clade that had *Cebus* and *Aotus* at the base. Horovitz et al. (1998) and Horovitz & MacPhee (1999) supported cebid monophyly with several derived traits including reduced size in molars, loss of the lingual heel of the upper incisor and presence on two prominences in the middle ear bone. The callitrichine phylogenetic relationships inferred were in agreement with Rosenberger (1984) and Ford (1986) in having *Callithrix-Leontopithecus* sister to *Saguinus* and *Callimico* basal-most. Morphological analysis from Kay et al. (2008) inferred cebid paraphyly, with *Saimiri* sister to an atelid-pitheciid clade that included *Cebus* closely related to *Aotus*.

Having described cebid evolution , the adaptive radiation of three major clades of callitrichines, cebines and owl monkeys, their accepted molecular and past morphology-based phylogenetic relationships, phylogenetic analysis of cebid craniodental morphology is presented, and results are split into whole skull, facial and cranial base morphology. The aim of this chapter is to assess whether the alternative regions of the skull infer alternative phylogenetic relationships in cebids, and whether combining geometric morphometric and distance-based phylogenetic analysis support greater congruence between molecular and morphology than previous morphology-based analyses.

7.2 Materials and methods

Geometric morphometric analysis was carried out in the MorphoJ program and phylogenetic analysis in the Phylip software package. Three-dimensional anatomical data were analysed from nine cebine, four owl monkey, twelve callitrichine and nine outgroup species (listed in Table 24). Anatomical landmark data were subjected to geometric morphometric analysis in the MorphoJ software package (Klingenberg 2011) that used Generalized Procrustes Analysis to scale, translate and rotate all data (Adams et al. 2004, Gower 1975, Rohlf & Slice 1990, Goodall 1991). Mean shape of each taxa described by geometric morphometric data were used to infer Euclidean distances separating each taxon-combination. Euclidean distances were stored in distance matrices and were analysed using distance-based phylogenetic and phenetic methods to generate evolutionary trees in the Phylip software package (Felsenstein 2005).

Phylogenetic analyses were repeated for data that were male-only, female-only, pooled sex, and with male and female data treated as separate taxa but analysed together. The effect of outgroup selection on phylogenetic inference was tested, with phylogenies generated with each single outgroup, and combinations of outgroups including all nine outgroups, all strepsirrhines, all Old World anthropoids, all Old World monkeys, and two-taxon combinations for Cercopithecinae, Colobinae, Galagonidae and *Eulemur-Perodicticus*. To test whether separate modules of the skull inferred alternative phylogenetic relationships, all analyses were also completed for anatomy of the whole skull, and two modules for the face and cranial base.

Table 24 List of taxa sampled and sample sizes of male, female and pooled sex specimens used in phylogenetic analysis

Genus	Species	Male	Female	Pooled
Cebus	*capucinus*	10	10	20
	albifrons	10	10	20
	apella	92	60	152
	nigrivittatus	10	10	20
	libidinosus	11	10	21
Saimiri	*sciureus*	33	15	48
	oerstedii	11	9	20
	bolviensis	10	10	20
	ustus	10	6	16
Aotus	*trivirgatus*	13	11	24
	azarai	6	10	16

	lemurinus	10	10	20
	vociferans	10	10	20
Leontopithecus	*rosalia*	11	13	24
Callithrix	*jacchus*	8	7	15
	argentata	11	10	21
	humeralifer	11	9	20
	penicillata	18	14	32
	pygmaea	10	9	19
Callimico	*goeldii*	11	11	22
Saguinus	*midas*	12	10	22
	fuscicollis	27	11	38
	mystax	10	11	21
	leucopus	9	9	18
	geoffroyi	10	9	19
Outgroups				
Hylobates	*lar*	10	10	20
Macaca	*mulatta*	9	10	19
Perodicticius	*potto*	10	10	20
Colobus	*guerza*	11	10	21
Chlorocebus	*aethiopus*	10	10	20
Trachypithecus	*obscura*	10	10	20
Otolemur	*garnetti*	10	9	19
Galago	*senegalensis*	10	11	21
Eulemur	*fulvus*	10	10	20

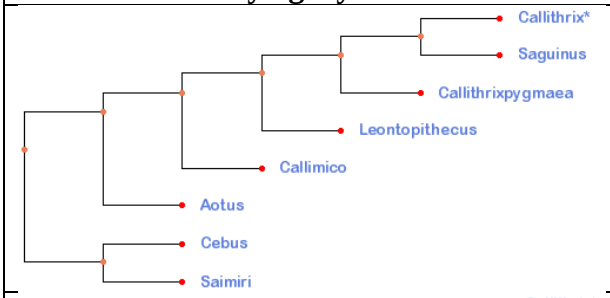
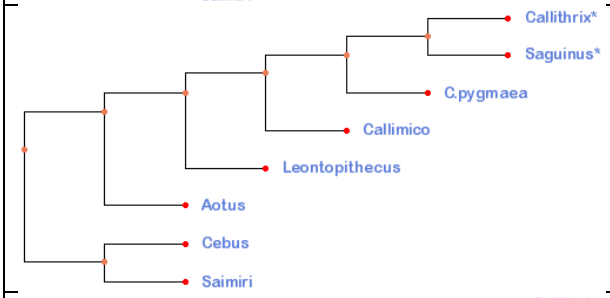
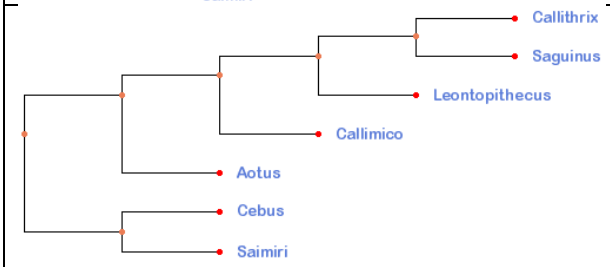
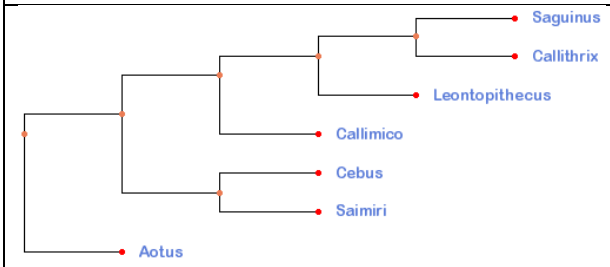
7.3 Results

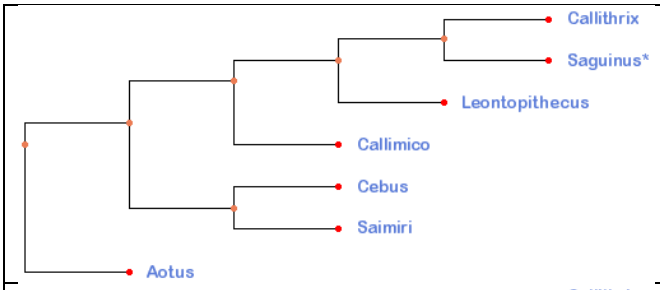
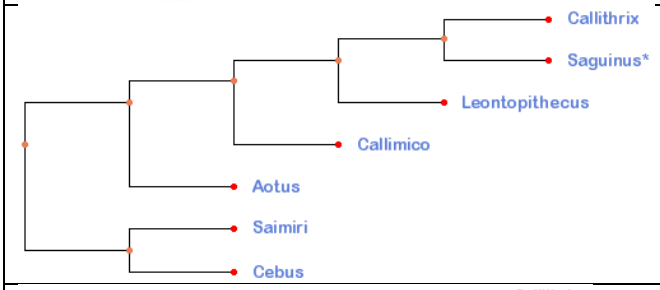
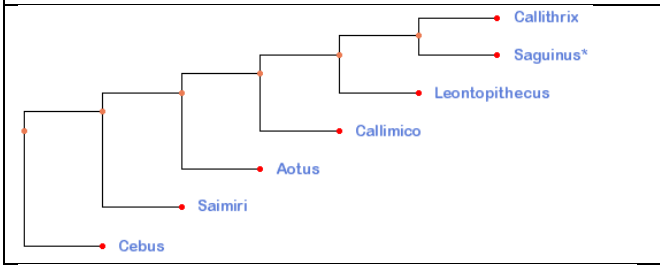
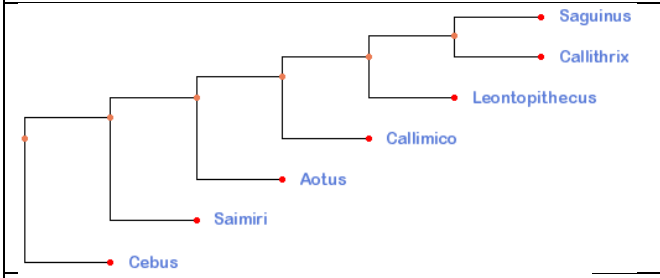
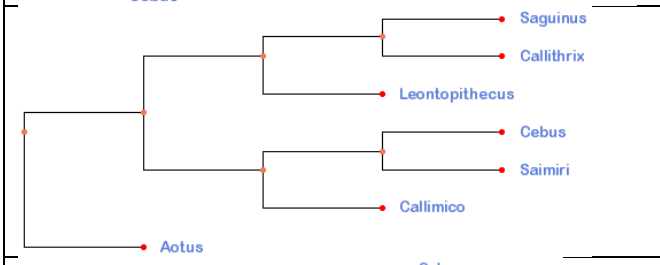
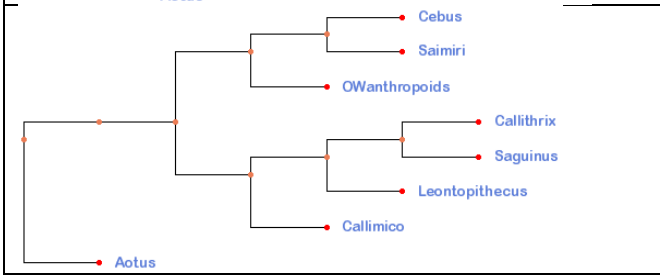
7.3.1 Whole skull

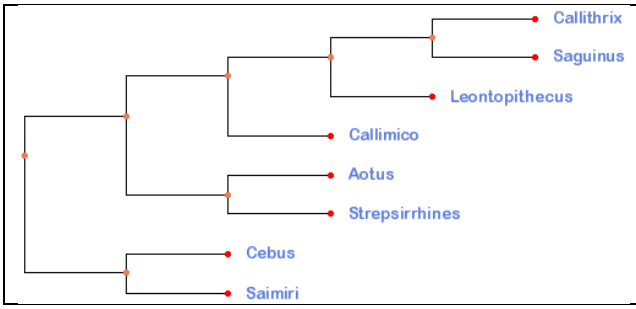
Phylogenies inferred by phenetic and phylogenetic analyses of whole skull morphology are displayed in Table 25. Phenetic analysis supported callitrichine, cebine and *Aotus* clades, with the owl monkey most similar to callitrichines in overall craniodental morphology. Within callitrichines, marmosets and tamarins were most similar with the pygmy marmoset falling outside the *Callithrix* group. Female, pooled, and separate sex analyses placed *Callimico* as the basal-most callitrichine, whereas male data placed *Leontopithecus* basal-most. The majority of phylogenetic analyses for pooled, female and separate sex data with a single Old World anthropoid, or combination of outgroups, inferred a phylogeny with cebines basal, owl monkeys sister to callitrichines, and a *Callithrix-Saguinus* clade sister to *Leontopithecus*. Several male-only analyses inferred a similar tree with *Saguinus* paraphyly, and *Cebus* as the basal-most cebid with *Saimiri* sister to *Aotus*-callitrichines. The results of phylogenetic analysis are much the same as the phenetic relationships.

Multiple analyses using a strepsirrhine single outgroup, or combination of outgroups, inferred a callitrichine clade with *Callithrix-Saguinus* sister to *Leontopithecus*, but with cebines sister to callitrichines. Many of the male-only analyses inferred a paraphyletic *Saguinus* clade. Female-only data with *Perodicticus* and *Eulemur* outgroups inferred a clade in which *Callimico* was sister to the cebines. The use of all nine outgroups, female-only and pooled sex data inferred close relationships between *Aotus* and strepsirrhines, and cebines and Old World anthropoids, although male-only and separate sex inferred trees with cebid monophyly.

Table 25 Cebid phylogenetic relationships inferred from whole skull morphology (*asterisk denotes genus paraphyly)

Phylogeny inferred	Outgroup used
	UPGMA (pooled, female, separate sex)
	UPGMA (male)
	*Chlorocebus* (pooled, female, separate sex) *Colobus* (pooled, female, separate sex) *Hylobates* (pooled, female, separate sex) *Macaca* (female) *Trachypithecus* (pooled, female, separate sex) Cercopithecinae (pooled, female) Colobinae (pooled, female, separate sex) OW anthropoid (pooled, female, separate sex) OWM (pooled, female, separate sex)
	*Eulemur* (pooled, separate sex) *Galago* (pooled, female, separate sex) *Otolemur* (pooled, female, separate sex) *Perodicticus* (male, separate sex) Galagonids (pooled, female, separate sex) *Perodicticus-Eulemur* (pooled, male, separate sex) Strepsirrhines (pooled, female, separate sex) All outgroups (separate sex)

	*Eulemur* (male) *Galago* (male) *Otolemur* (male) Galagonid (male) Strepsirrhines (male) All outgroups (male)
	*Colobus* (male) Colobinae (male)
	*Chlorocebus* (male) *Hylobates* (male) *Macaca* (male) *Trachypithecus* (male) Cercopithecinae (male) OW anthropoid (male) OWM (male)
	*Macaca* (pooled, separate sex) Cercopithecinae (separate sex)
	*Perodicticus* (pooled, female) *Eulemur* (female) *Perodicticus-Eulemur* (female)
	All outgroups *Galago* root (pooled, female)

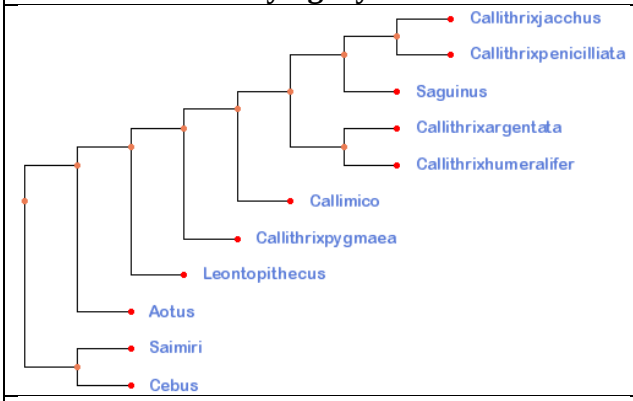
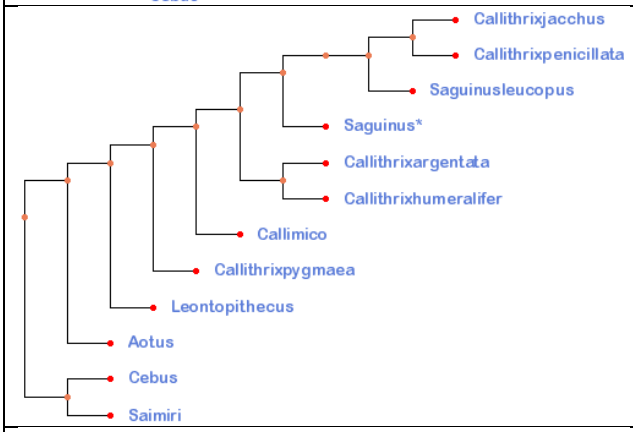
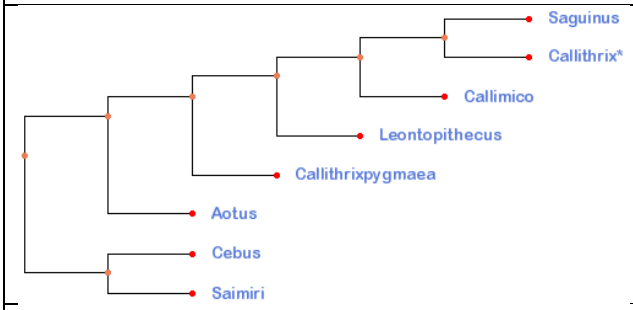
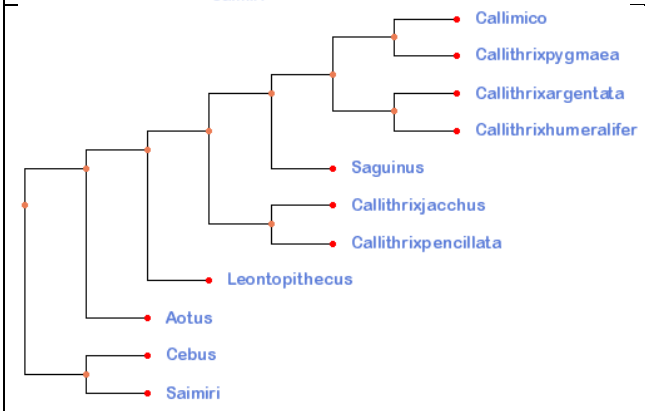
	All outgroups *Chlorocebus* root (pooled, female)

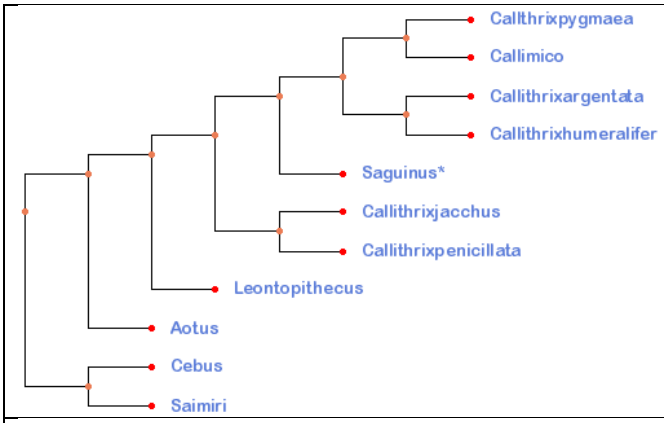
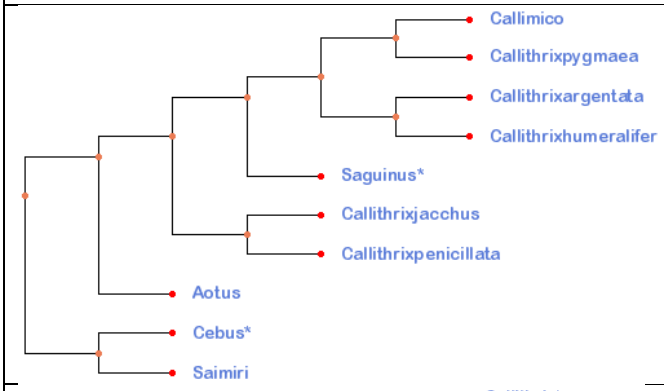
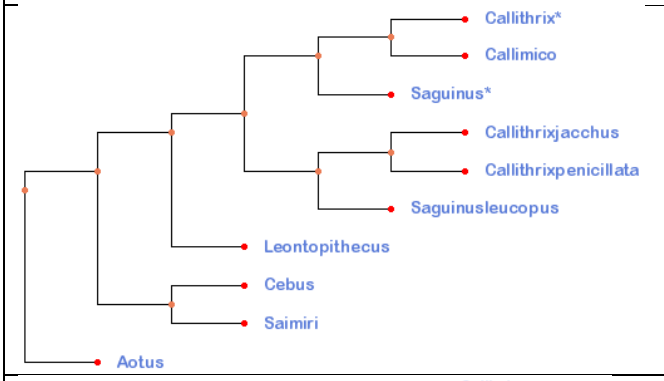
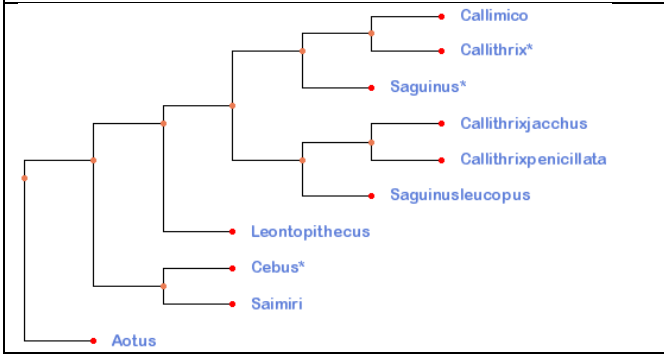
7.3.2 Face

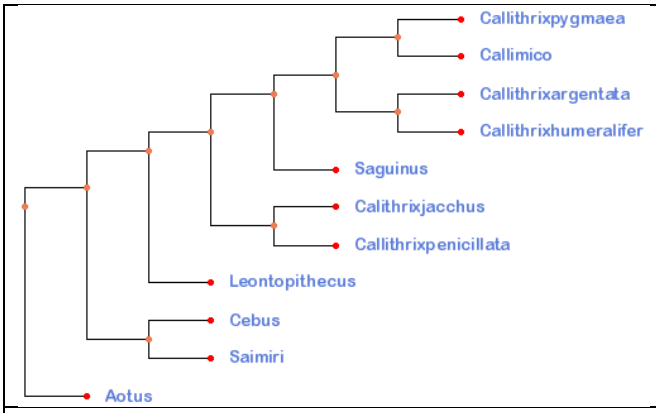
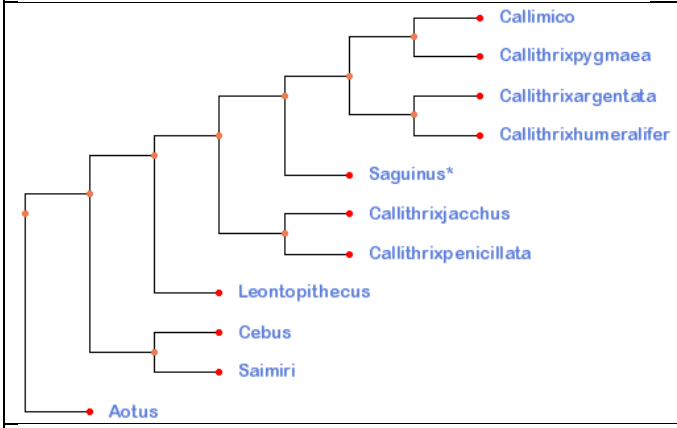
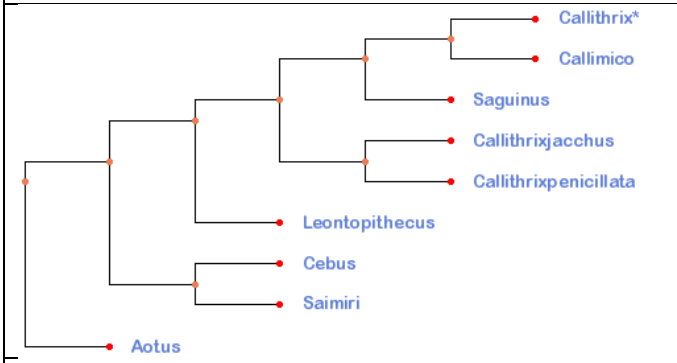
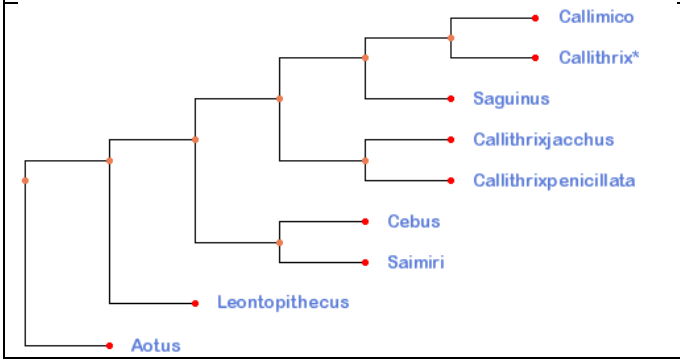
Results from phenetic and phylogenetic analysis of facial morphology are shown in Table 26. Phenetic results produce three clades for owl monkeys, cebines and callitrichines, but within callitrichines the pygmy marmoset is far removed from other marmosets and *jacchus* marmosets share a greater affinity with tamarins than *argentata* marmosets. The majority of phylogenetic analyses using an Old World anthropoid single or combination outgroup inferred a tree with *Aotus* sister to callitrichines and cebines basal-most. Within the callitrichines *Leontopithecus* was the basal-lineage, and *Callimico* and pygmy marmosets were sister to the *argentata* marmosets in a clade more closely related to *Saguinus* than the *jacchus* marmosets. Six analyses of female data inferred the same tree but with *Saguinus* paraphyly, and several other analyses with Old World anthropoid outgroups inferred a very similar tree but with *Aotus* switching places with *Leontopithecus* or joining the cebines.

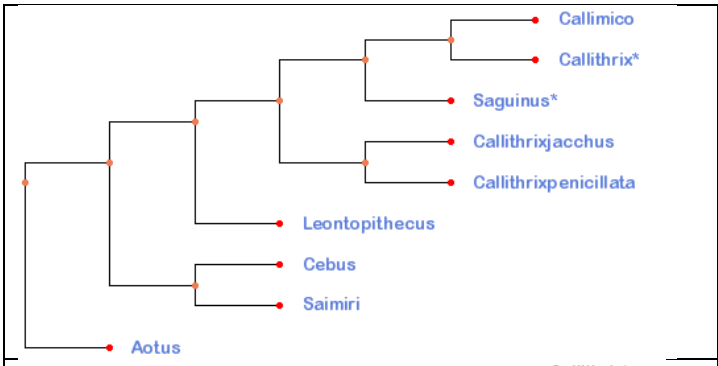
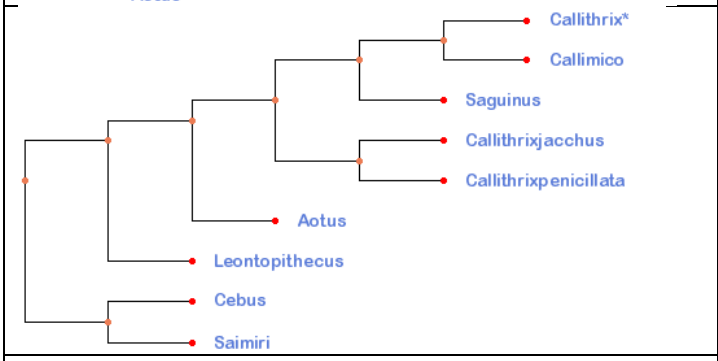
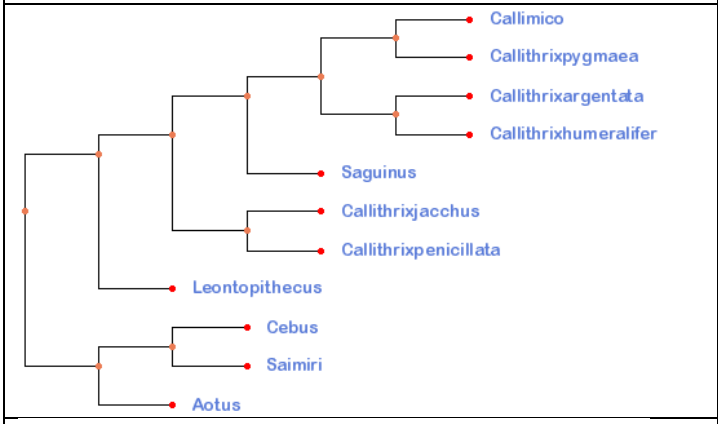
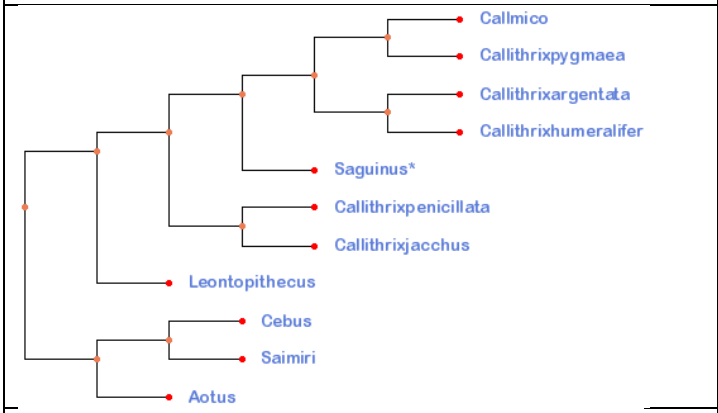
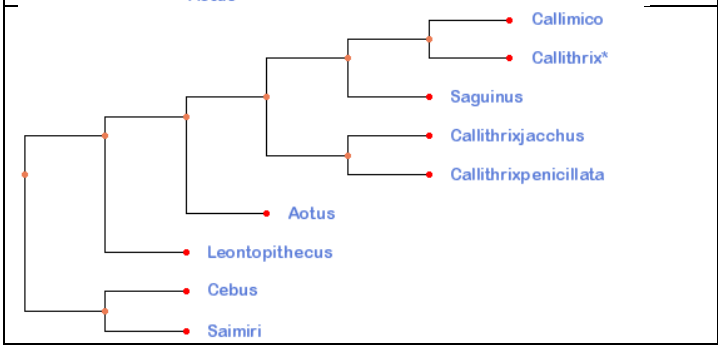
The use of a strepsirrhine outgroup drew *Aotus* to the base of the cebid tree, so that cebines and callitrichines were sister clades. Nearly all analyses inferred a close relationship between callimicos, pygmy marmosets and *argentata* marmosets. This clade was sister to *Saguinus*, with *jacchus* marmosets falling outside the clade, and *Leontopithecus* the basal-most callitrichine. There was some variation between analyses, mainly whether callimicos were sister to just the pygmy marmoset or both the *argentata* and pygmy marmosets, and around half the analyses placed *Saguinus leucopus* outside the tamarin clade as sister to *jacchus* marmosets. Phylogenetic analysis using all nine outgroups supported a closer relationship between owl monkeys and callitrichines, the connection between *Saguinus leucopus* and *jacchus* marmosets, a close relationship between callimicos, pygmy marmosets and *argentata* marmosets, and a link between cebines and Old World anthropoids and *Aotus* and strepsirrhines for female-only data.

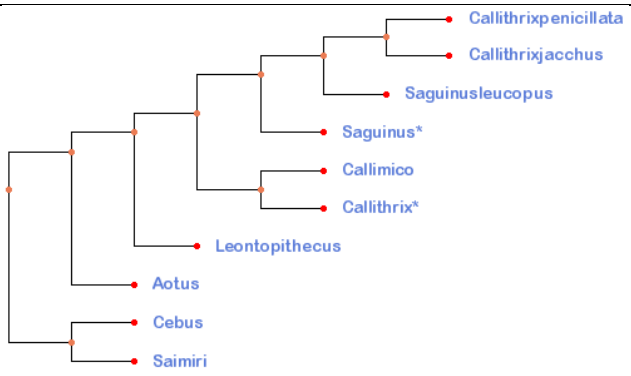
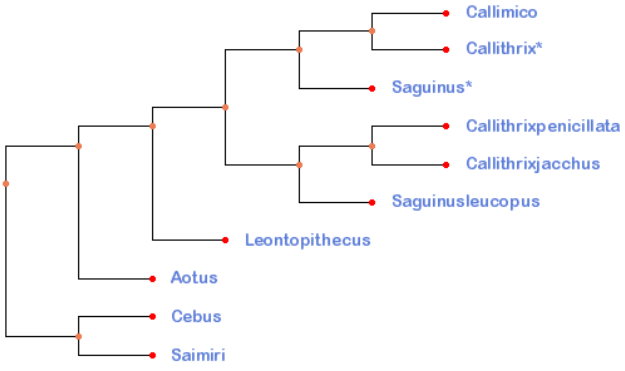
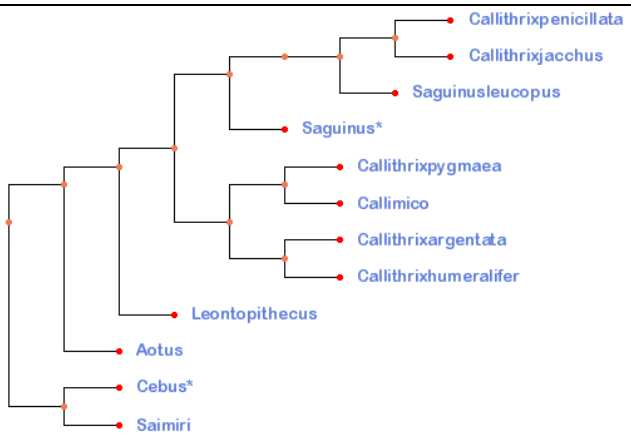
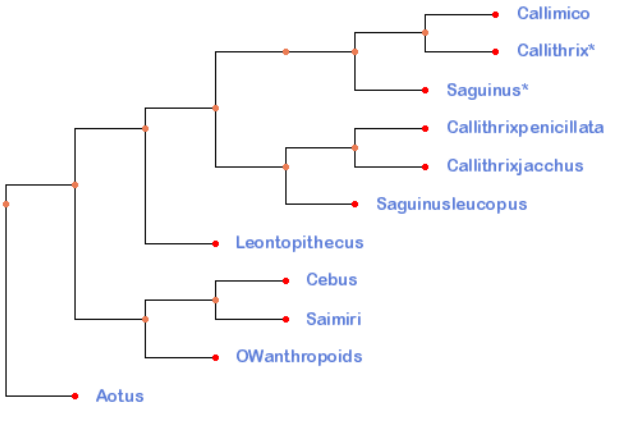
Table 26 Cebid phylogenetic relationship inferred from facial morphology (*asterisk denotes genus paraphyly)

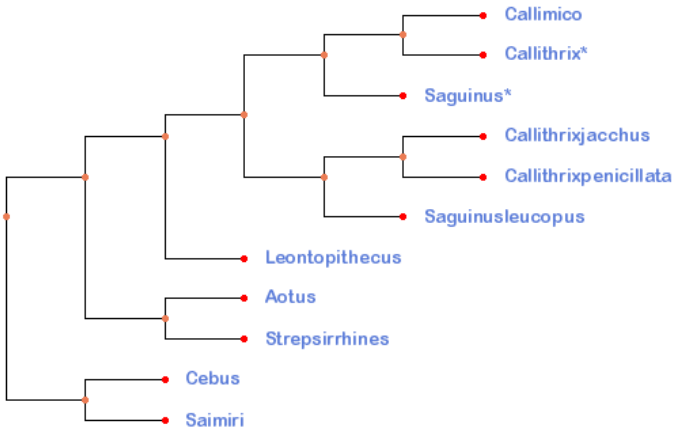
Phylogeny inferred	Outgroup used
	UPGMA (pooled, separate sex)
	UPGMA (female)
	UPGMA (male)
	*Chlorocebus* (male, separate sex) *Colobus* (male, separate sex) *Hylobates* (pooled, separate sex, male) *Macaca* (male, female, separate sex) *Trachypithecus* (pooled, male, separate sex) Colobinae (pooled, separate sex, male) Cercopithecinae (pooled, separate sex) OW anthropoid (pooled, male, separate sex) OWM (pooled, male, separate sex)

	*Hylobates* (female) *Trachypithecus* (female) Cercopithecinae (female) Colobinae (female) OW anthropoid (female) OWM (female)
	*Chlorocebus* (female)
	*Galago* (pooled, male, separate sex) *Otolemur* (all) Galagonid (pooled, separate sex) Strepsirrhines (pooled, male, separate sex)
	*Galago* (female) Galagonid (female) Strepsirrhines (female)

	*Eulemur* (separate sex) *Perodicticus* (separate sex, male) *Perodicticus-Eulemur* (pooled, male, separate sex)
	*Eulemur* (female) *Perodicticus-Eulemur* (female)
	*Eulemur* (pooled) *Perodicticus* (pooled)
	*Eulemur* (male)

	*Perodicticus* (female)
	*Macaca* (pooled)
	*Colobus* (pooled)
	*Colobus* (female)
	*Chlorocebus* (pooled)

	All outgroups (pooled)
	All outgroups (separate sex)
	All outgroups (male)
	All outgroups strepsirrhine root (female)

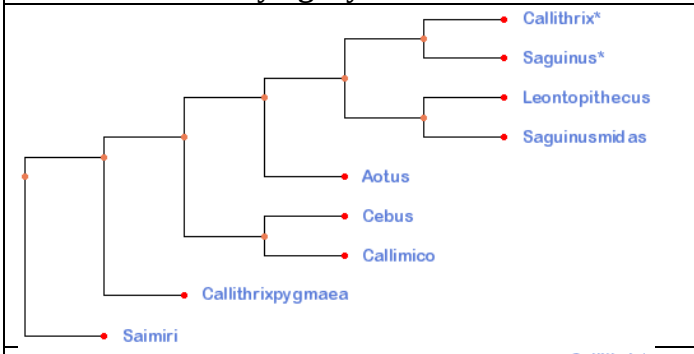
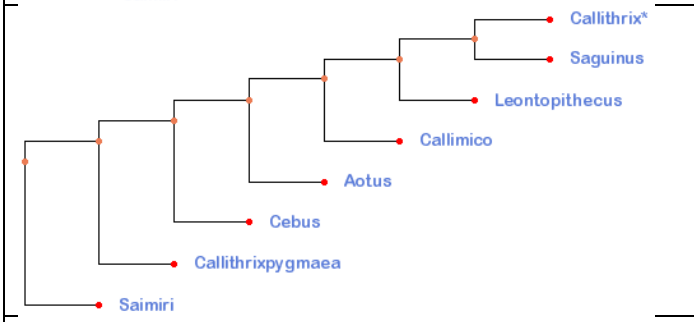
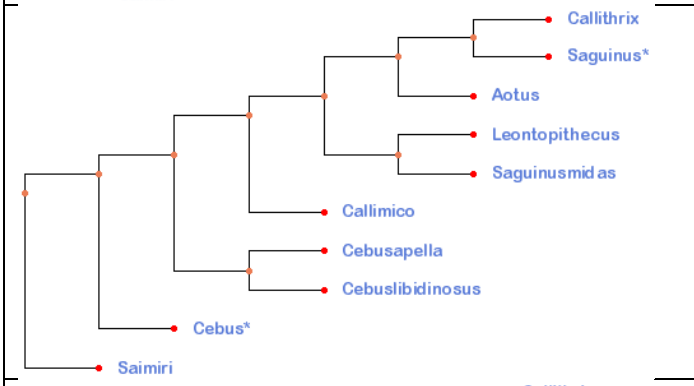
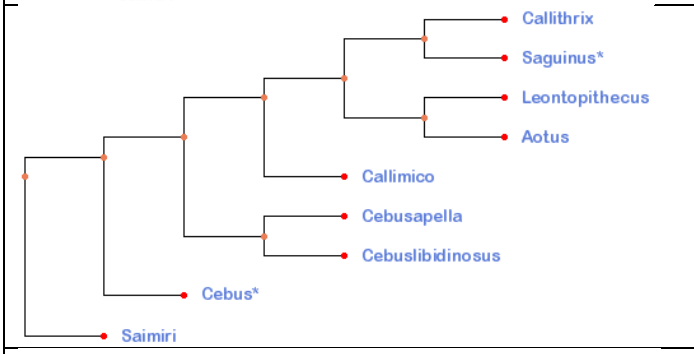
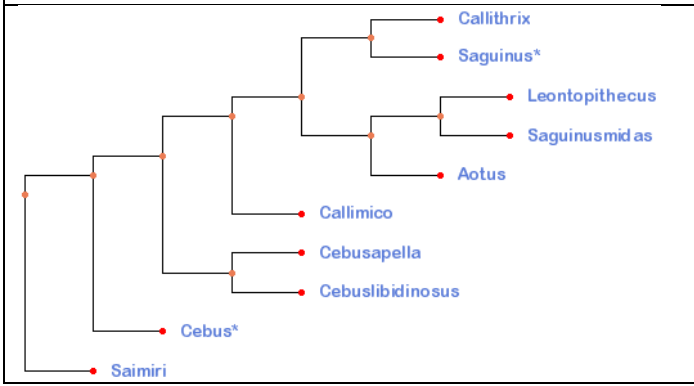
	All outgroups Old World anthropoid root (female)

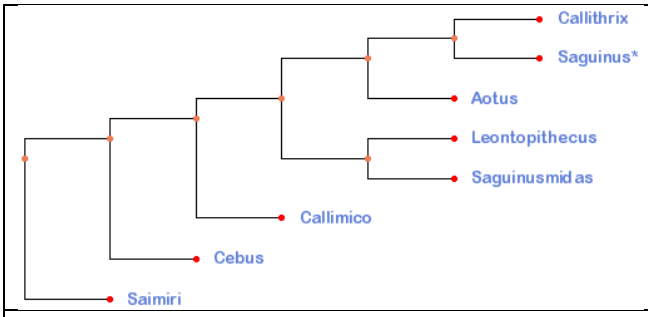
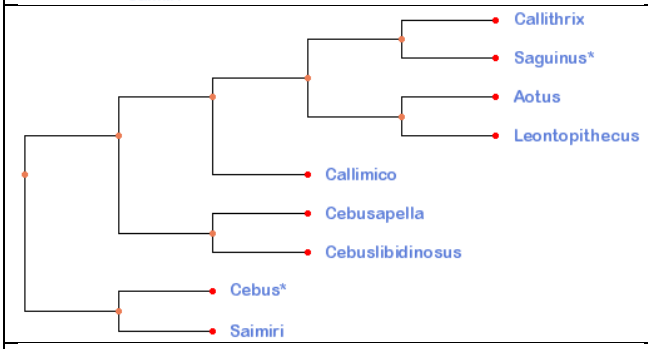
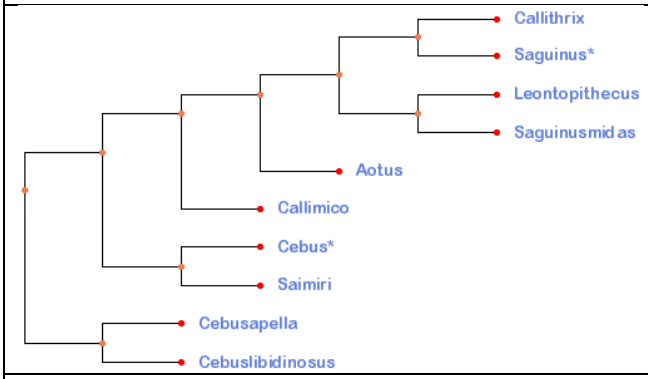
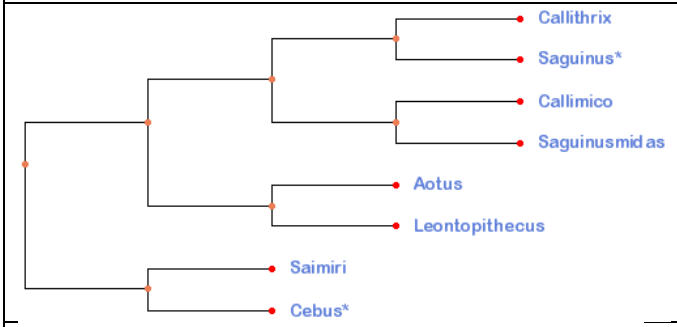
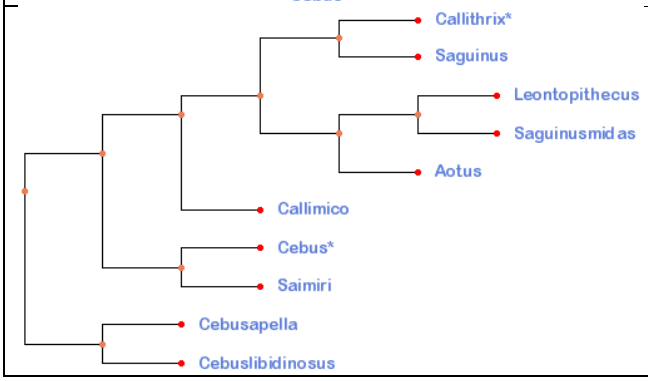
7.3.3 Cranial base

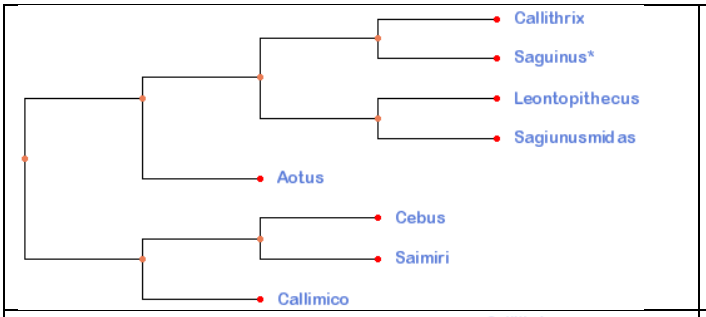
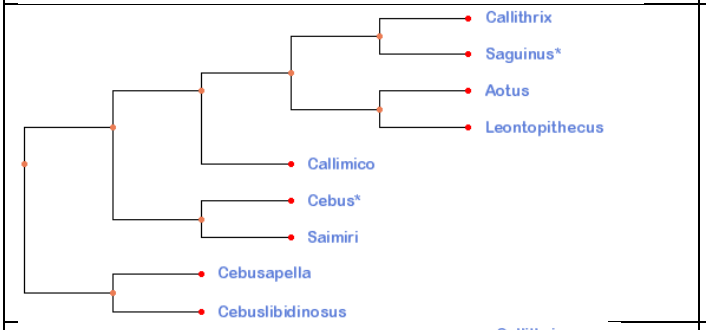
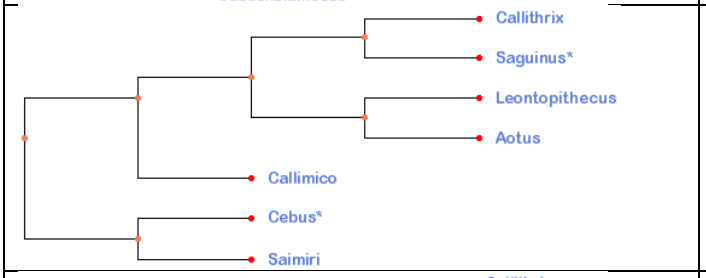
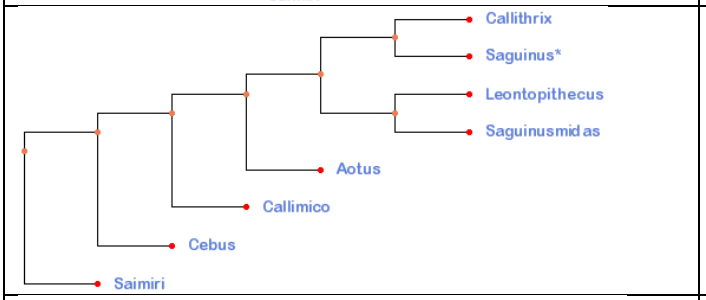
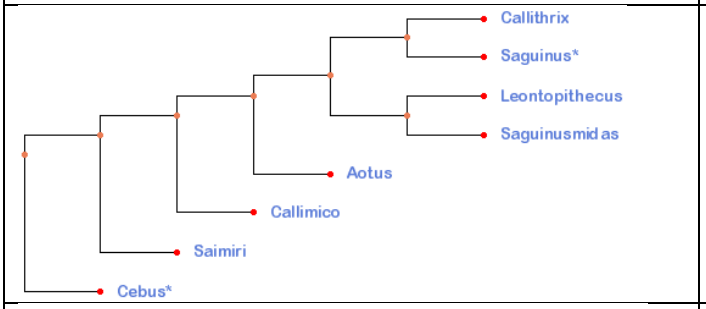
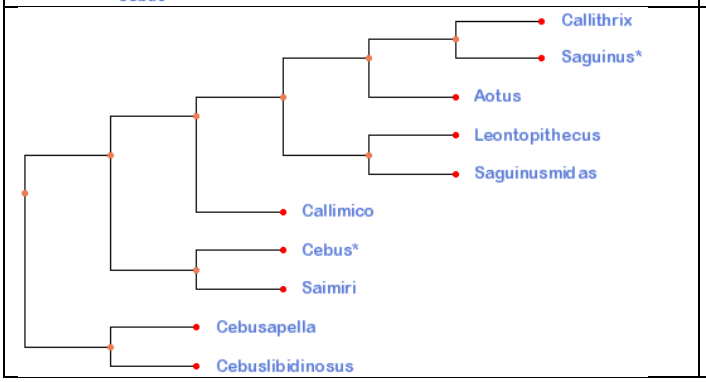
The phenetic and phylogenetic results from cranial base morphology are listed in Table 27. The phenetic relationships of male-only, pooled and separate sex found *Saimiri* to be the basal-most group, followed by the pygmy marmoset, then *Cebus*-*Callimico*. *Leontopithecus* and *Saguinus midas* had a sister relationship, as did remaining marmosets and tamarins. Female data did not support a close relationship between *Cebus* and *Callimico* or *Leontopithecus* and *Saguinus midas*. Phylogenetic analysis of cranial base morphology produces a very large number of alternative phylogenies, but there is a broad underlying consistency to the results. The use of Old World anthropoid outgroups often inferred trees with *Cebus* and *Saimiri* at the base of the tree, sometimes as a clade but not always, and *Cebus* paraphyly was quite common with *C. apella* and *C. libidinosus* falling outside the group. *Callimico* was nearly always basal to owl monkeys and the other callitrichines. *Aotus* appeared to have an affinity with *Leontopithecus*, although a sister relationship between *Leontopithecus* and *Saguinus midas* was inferred in multiple trees. *Callithrix* and *Saguinus* were nearly always sister clades, although *Saguinus* paraphyly was common.

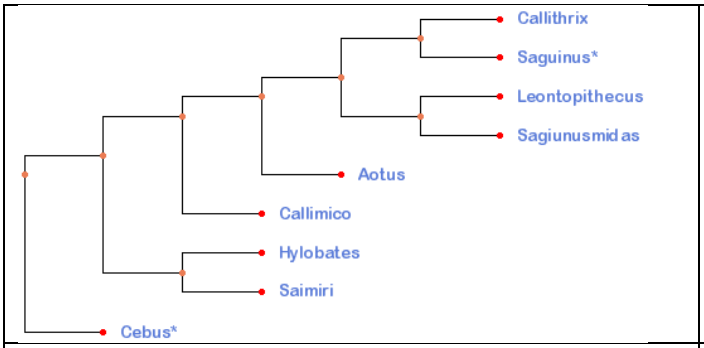
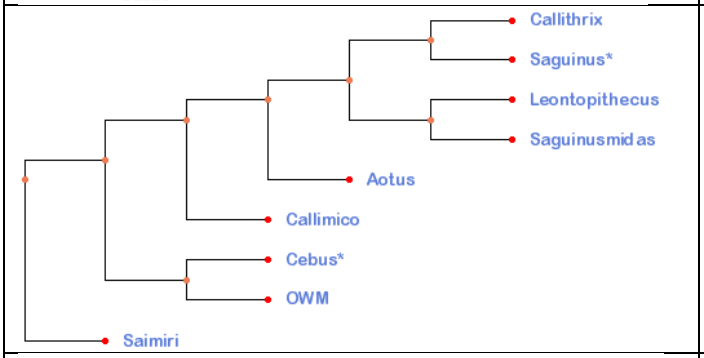
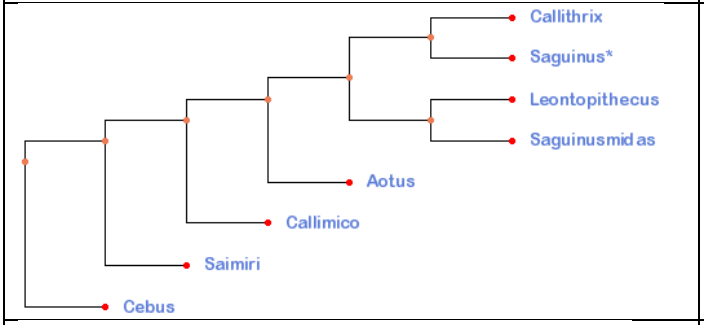
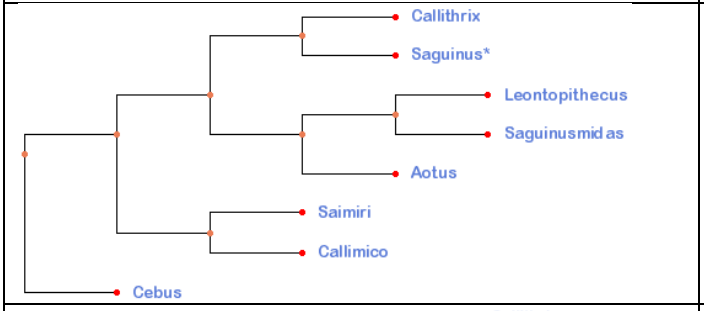
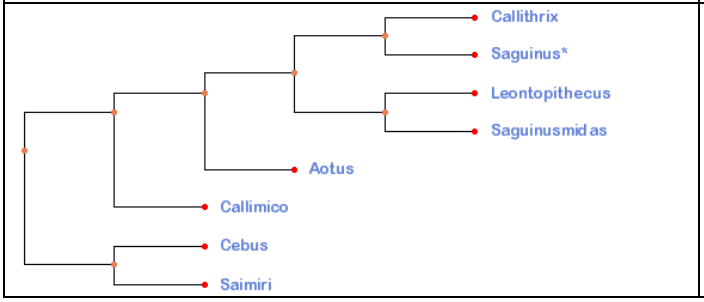
The use of a strepsirrhine outgroup inferred a basal position for *Callithrix* and *Saguinus*, occasionally replaced by *Aotus*, with a close relationship between *Callimico* and cebines sister to *Leontopithecus*. The *Eulemur* outgroup, whether as a single outgroup or in combination with *Perodicticus*, drew *Aotus* and the pygmy marmoset to a basal position, whereas the other strepsirrhines inferred *Callithrix*, and then *Saguinus*, as the basal-most cebids and placed *Aotus* close to *Callimico* and cebines. *Leontopithecus* and *Saguinus midas* were often inferred as sister taxa, and *Leontopithecus* was always inferred in a clade with *Callimico* and cebines. The use of all nine outgroups appeared to link cebines with Old World anthropoids, and strepsirrhines with the pygmy marmoset.

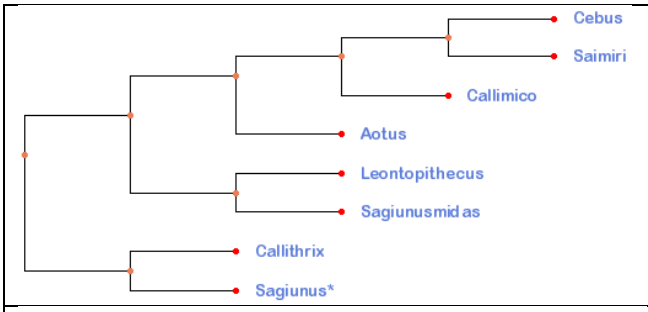
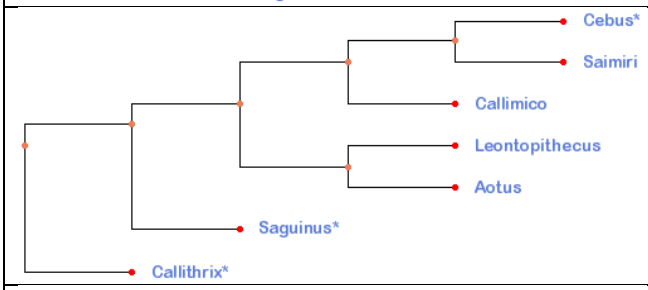
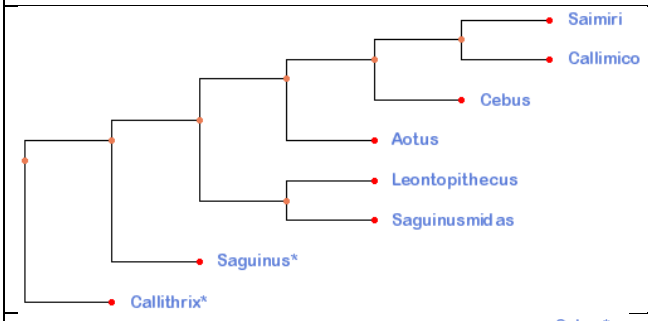
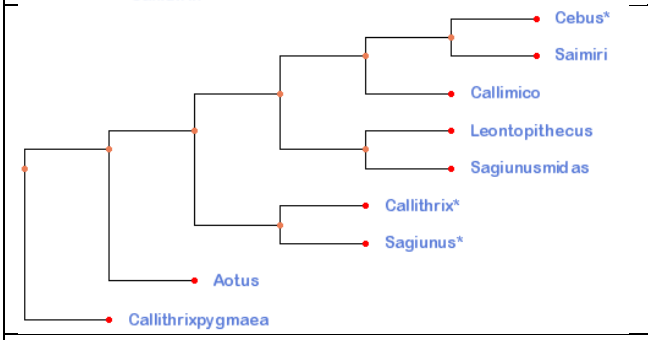
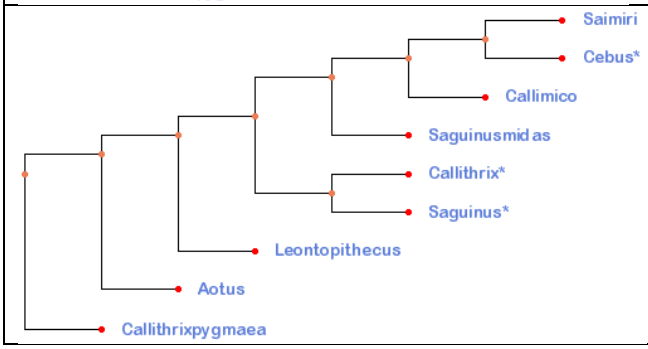
Table 27 Cebid phylogenetic relationship inferred from cranial base morphology

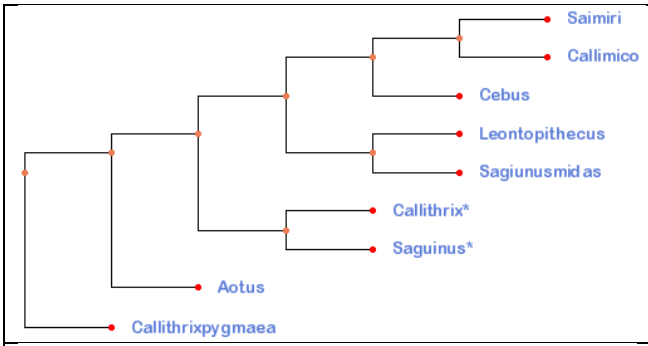
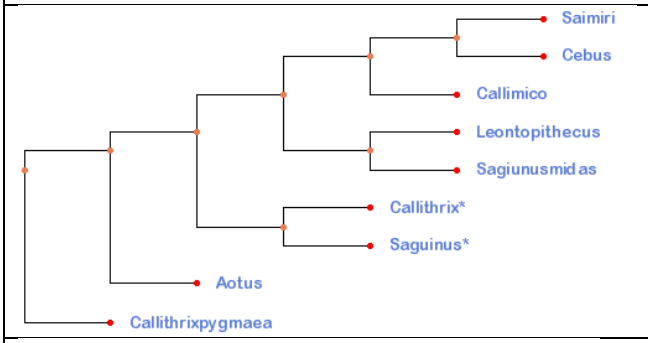
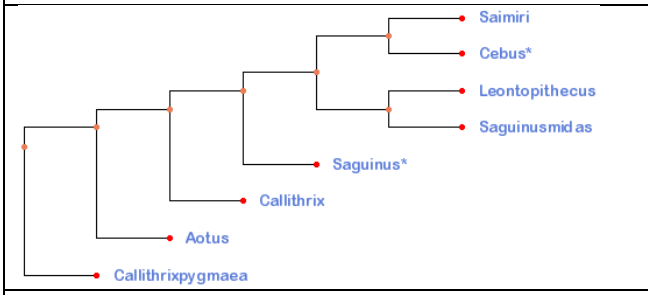
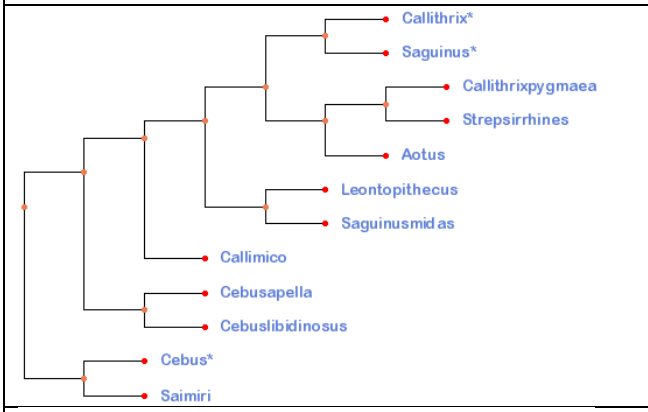
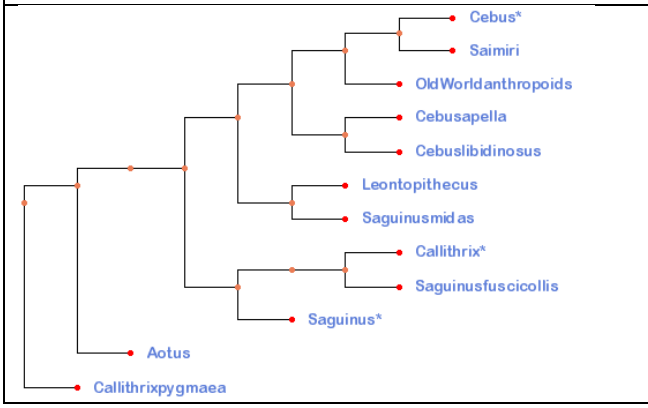
Phylogeny inferred	Outgroup used
	UPGMA (pooled, separate, male)
	UPGMA (female)
	*Chlorocebus* (pooled) Cercopithecinae (pooled)
	*Chlorocebus* (female)
	*Chlorocebus* (male) Cercopithecinae (male)

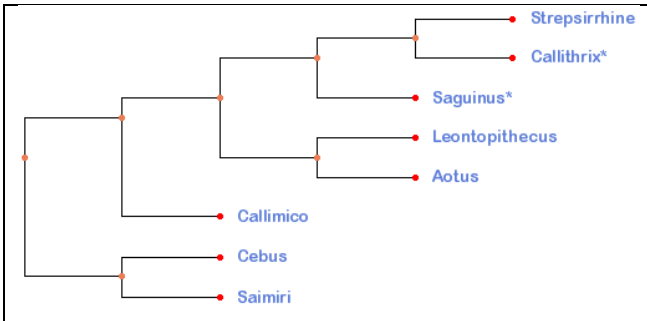
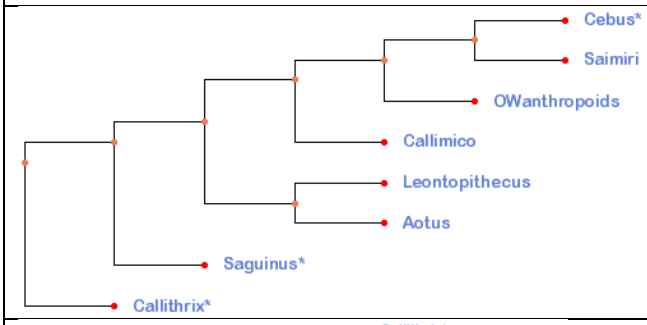
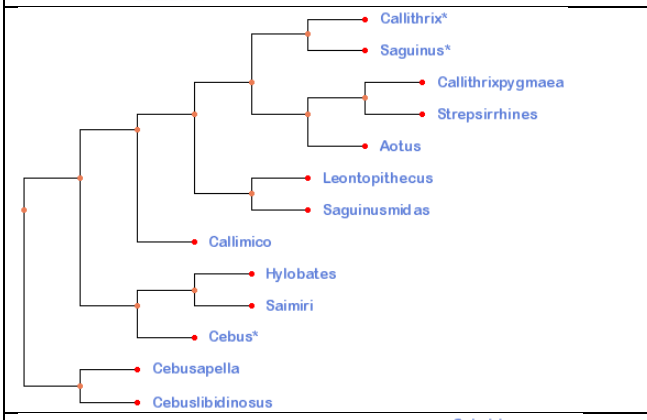
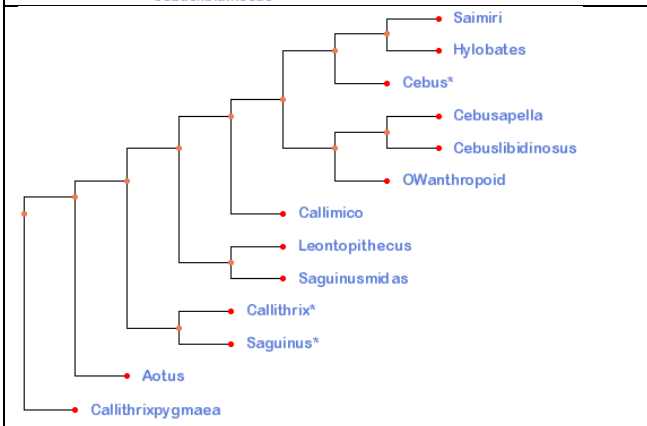
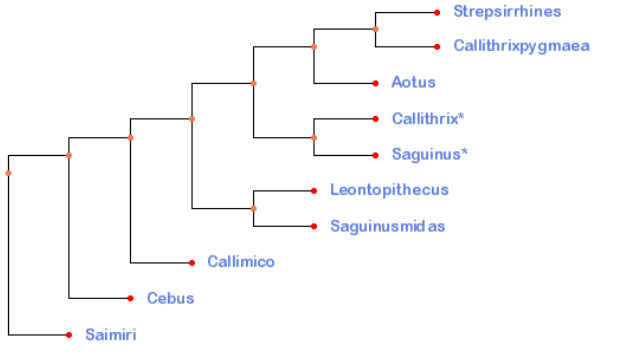
	*Chlorocebus* (separate) *Hylobates* (separate) Cercopithecinae (separate)
	*Hylobates* (pooled) Cercopithecinae (female) OW anthropoid (pooled)
	*Trachypithecus* (pooled) Colobinae (pooled) OWM (pooled)
	*Colobus* (female) Colobinae (female)
	*Colobus* (male) Colobinae (male) OWM (male)

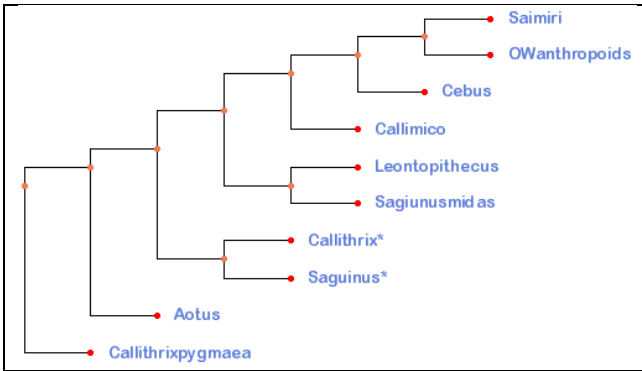
	Colobinae (separate)
	*Colobus* (pooled) *Macaca* (female)
	*Hylobates* (female) *Trachypithecus* (female) OW anthropoid (female) OWM (female)
	*Hylobates* (male) OW anthropoid (separate)
	*Macaca* (male, separate)
	*Macaca* (pooled)

	OW anthropoid *Chlorocebus* root (male)
	OW anthropoid *Hylobates* root (male)
	OWM (separate)
	*Trachypithecus* (male)
	*Trachypithecus* (separate)

	*Colobus* (separate)
	*Otolemur* (pooled, female) *Perodicticus* (pooled, female, separate) *Galago* (pooled, female) Galagonid (pooled,female) Strepsirrhine (female)
	*Otolemur* (male, separate) *Perodicticus* (male) *Galago* (male, seperate) Galagonid (male, separate)
	*Eulemur* (pooled) *Perodicticus-Eulemur* (separate) Strepsirrhine (pooled, separate)
	*Eulemur* (female)

	*Eulemur* (male) *Perodicticus-Eulemur* (male) Strepsirrhine (male)
	*Eulemur* (separate) *Perodicticus-Eulemur* (pooled)
	*Perodicticus-Eulemur* (female)
	All outgroups OW anthropoid root (pooled)
	All outgroups strepsirrhine root (pooled)

	All outgroups *Chlorocebus* root (female)
	All outgroups *Galago* root (female)
	All outgroups *Chlorocebus* root (male)
	All outgroups *Galago* root (male)
	All outgroups *Chlorocebus* root (separate)

	All outgroups *Galago* root (separate)

7.3.4 Summary of results

The results of phylogenetic analysis of whole skull, facial and cranial base morphology are summarised and presented below in Table 28.

Table 28 Summary of cebid phylogenetic analyses

		Outgroup or Outgroup combination	UPGMA	*Chlorocebus*	*Colobus*	*Eulemur*	*Galago*	*Hylobates*	*Macaca*	*Otolemur*	*Perodicticus*	*Trachypithecus*	Cercopithecine	Colobine	Galagonid	*Eulemur-Perodicticus*	Old World anthropoid	Old World monkeys	Strepsirrhine	All outgroups
Whole skull	**Molecular clades**	*Callitrichines*	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
		Owl monkeys	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
		Cebines	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	Molecular incongruent clades	*Leontopithecus-Saguinus-Callithrix*	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
		Saguinus-Callithrix	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
		Callimico-Cebus-Saimiri									✓									
		Callithrix paraphyly	✓								✓									
		Cebid paraphyly																		✓
Face	**Molecular clades**	*Callitrichines*	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
		Owl monkeys	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
		Cebines	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Molecular incongruent clades	*Callimico-Saguinus-Callithrix*	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
		Callithrix-Callimico-Saguinus-Aotus		✓					✓											
		Callithrix paraphyly	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
		Saguinus paraphyly					✓			✓					✓				✓	✓
Cranial base	**Molecular clades**	*Callitrichines*																		
		Owl monkeys	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
		Cebines				✓	✓			✓	✓				✓	✓			✓	
		Leontopithec-Callimico-Callithrix										✓		✓				✓		
	Molecular incongruent clades	*Callithrix-Leontopithecus-Saguinus-Aotus*		✓	✓			✓	✓			✓	✓	✓			✓	✓		
		Cebus-Callimico	✓																	
		Cebus-Saimiri-Callimico				✓	✓			✓	✓				✓	✓			✓	
		Aotus-Leontopithecus			✓		✓	✓		✓	✓				✓		✓			
		Callithrix paraphyly	✓			✓	✓			✓	✓				✓	✓			✓	✓
		Saguinus paraphyly	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓
		Cebus paraphyly	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
		Cebid paraphyly																		✓

7.4 Discussion

Phylogenetic analysis of the cebids did not recover a strong phylogenetic signal, largely due to incongruence between molecular and morphological relationships between callitrichine genera. However, craniodental morphology of the whole skull and face did strongly support monophyly of the three major cebid molecular clades for callitrichines, cebines and owl monkeys. Clearly, there is a form of phylogenetic signal present, but it is more difficult to measure than in atelids and pitheciids. Craniodental evolution in the clade does not simply reflect body-size or allometric similarity, and although these are important factors in cebid evolution, they are one of many influences on craniodental morphology. In particular, diet, and shape associated with processing similar foods, is strongly linked to inferred phylogenetic relationships.

7.4.1 Craniodental evolution in cebids

Phylogenetic analysis of the whole skull (Table 25) supported callitrichine, cebine and owl monkey monophyletic clades. This clearly reflects phylogeny and differentiation between the three major groups, but it could also reflect several factors that would help to maintain a phylogenetic signal. For example, the callitrichines have experienced phyletic dwarfing (Ford 1980, Martin 1992), which will inevitably affect craniodental morphology, as will the associated life history and reproductive specialisations linked to dwarfing. These factors act in concert to create craniodental diversification of callitrichines that separates them from owl monkeys and cebines in phylogenetic analyses, but whether the inference of a callitrichine clade was allometric or phylogenetic in origin is impossible to ascertain without further analysis. Monophyly of the owl monkeys likely relates to a combination of adaptations for nocturnality and folivory, as the very large orbits cause restructuring of associated anatomical landmarks, and dietary adaptations only found in the group as they are the only cebid that consumes significant amounts of leaves (Norconk et al. 2009, Kinzey 1997).

Small relative brain size in callitrichines and owl monkeys also contributes to differentiating these lineages from cebines (Isler et al. 2008), although the divergence between callitrichines, linked to allometry, and owl monkeys, linked to diet and nocturnality, probably masked shared morphological similarity linked to relative brain size. A monophyletic cebine clade was also strongly supported, but *Cebus* and *Saimiri* are unlikely to be connected by dietary adaptations, as squirrel monkeys are specialised for insectivory whilst capuchins are omnivorous and have particularly thick molar enamel as adaptations for a hard diet (Janson &

Boinski 1992). Rather, the two cebine genera share an increase in brain size associated with a rounded cranial vault and central positioning of the foramen magnum, increasingly complex foraging strategies and sophisticated social systems that are highly variable (Hartwig 1995, Hartwig 1996, Fedigan et al. 1996, Janson & Boinski 1992, Kinzey 1997, Digby et al. 2011, Jack 2011, Isler et al. 2008).

Results from phylogenetic analysis of the whole skull (Table 25) supported a basal position for *Callimico* and *Leontopithecus* sister to *Saguinus-Callithrix* within the callitrichines, the same topology as that proposed by Kay (1990). The inference from phylogenetic analysis of a basal position for *Callimico* within the callitrichines supports Rosenberger (1984), Ford (1986) and Kay (1990), and could be linked, at least partially, to the presence of an extra molar into the dental arcade and the effects that has on palate morphology. It seems likely that *Callimico* have followed a rare evolutionary trajectory, possibly linked to mycophagy and life in the understory, whilst *Leontopithecus* have experienced a size increase and associated life history and morphological changes including adaptations for extractive foraging (Garber 1992). The position of the largest callitrichine, *Leontopithecus*, as closer to *Callithrix-Saguinus* than *Callimico* also suggests the inferred phylogenetic trees did not simply reflect skull size and allometric factors. The marmosets have the most extreme cebid feeding adaptation for tree gouging, yet they share a morphological similarity with the tamarins. This shared morphology cannot simply reflect allometry, as callimicos and larger tamarin groups are more similar in size than marmosets and tamarins, but could reflect the ancestral callitrichine morphotype. Further work is obviously required, but the results presented strongly infer tamarins and marmoset as natural morphological sister taxa, and this must relate to either a shared biomechanical response to a common dietary stressor, or a social or behavioural convergence that shapes skull morphology.

It seems clear from the phenetic and phylogenetic analyses of whole skull morphology that the results from both are closely intertwined; inferred phylogenetic relationships largely reflected phenetic similarity between sister taxa, especially for the relationships between callitrichine genera. From one perspective, this could highlight a problem with distance-based phylogenetic methods, supporting the view that the methods are simply phenetic in nature. If this were true however, there would not have been seven alternative phylogenies proposed. A more plausible explanation is that, for cebids, both phenetic and phylogenetic analyses are measuring a biological reality, that certain groups share phenotypic similarity. For example, both phenetic and phylogenetic results strongly support the cebine clade of *Cebus* and

Saimiri, which relates to a genuine biological similarity shared by the two groups originating from their shared ancestry.

It is possible that for phylogenetic analysis of cebids the strepsirrhine and Old World anthropoid outgroups are not functioning as efficiently as they did for phylogenetic analyses of atelids and pitheciids. This could be a problem related to the size difference between the callitrichine ingroups and larger outgroups, although it may also relate to having three very distinct lineages incorporated into the same analyses, with an outgroup inevitably similar to at least one of the ingroup clades that are then positioned basal-most in the phylogenetic tree. The use of a strepsirrhine outgroup for phylogenetic analysis of whole skull morphology inferred a sister relationship between cebines and callitrichines, whereas Old World anthropoid outgroups and phenetic analyses inferred a closer relationship between owl monkeys and callitrichines. Considering the choice of outgroup affects the inferred relationships between callitrichines and a sister group, it is difficult to propose which two cebid clades are most closely related based on craniodental morphology. The phenetic evidence at least supports a closer relationship between owl monkeys and callitrichines that likely reflects shared small relative brain size and dolichocephalic cranial vault shape (Isler et al. 2008).

Size variation in cebids ranges from around 3kg in larger capuchins to 100g in the pygmy marmoset, and allometry is a major factor in phylogenetic analysis based on morphology. However, the results presented in this chapter do not appear to support allometry as the primary factor in cebid morphological evolution. The cebines are a prime example, as even though capuchins are about three times the size of squirrel monkeys, the size difference has not obscured the phylogenetic link between the two groups. From whole skull morphology, centroid size of *Saimiri* overlaps with *Aotus*, and the smallest squirrel monkeys are very close in size to *Leontopithecus*, yet no phylogenetic connection was inferred between these taxa. Within the callitrichines, *Callithrix* and *Saguinus* are sister taxa in phylogenetic analysis of whole skull morphology, and the largest marmosets and smallest tamarins are very close in centroid size, which could be interpreted as an allometric link. However, the larger *Saguinus* taxa are very close to *Callimico* in centroid size, yet *Callimico* was positioned as the basal-most callitrichine. There are many more examples from overall skull shape, as well as cranial base and facial morphology, where inferred phylogenetic relationships do not simply reflect similarities in size and allometry. It seems conceivable that allometry, like many biological

factors, interacts with the morphology of cebids and has an effect on phylogenetic inference, but is not the single, primary factor in morphological evolution of the group.

7.4.2 The cebid face

Phenetic analyses of facial data (Table 26) supported three clades for cebines, owl monkeys and callitrichines, with the cebines the basal-most clade. Observed divergence in facial morphology of the pygmy marmoset is likely due to a mix of phyletic dwarfing and the increased levels of exudativory. The major difference between phenetic and phylogenetic results is the placement of the pygmy marmoset. It appears that for facial morphology, as with whole skull morphology, the phenetic and phylogenetic relationships are similar, but not identical. Rather than suggesting that distance-based phylogenetic methods are simply phenetic, the congruence between phenetic and phylogenetic inference reflects the biological reality of both methods measuring clear phenotypic similarity, and possibly highlights the problem with the outgroups used. Phylogenetic analysis of facial morphology supported cebine, *Aotus* and callitrichine monophyly. Owl monkey monophyly was to be expected considering orbit size and its effect on the position of facial landmarks.

The connection between cebines is strong and reflects phylogeny, which may be supported by shared increased encephalization and the effects of brain size on craniodental morphology including orbital orientation (Isler et al. 2008, Ross & Ravosa 1993). Cebine monophyly supports the results from atelids and pitheciids that diet and mastication has a limited role in shaping facial morphology, as *Saimiri* is highly insectivorous and *Cebus* have a much tougher diet. There is a gradient of strain highest in the lower face and weakest in the upper face, so that masticatory stress has a greater effect on shaping lower than upper facial morphology, and the facial landmarks used to quantify facial morphology predominantly sampled the mid- and upper-face (Hylander et al. 1991, Ross & Hylander 1996, Ravosa et al. 2000, Ross 2001 , Ross & Metzger 2004, Paschetta et al. 2010). The callitrichines share exudate consumption, although considering only the marmosets tree gouge, it is questionable whether exudativory strongly moulds facial morphology. Using an Old World anthropoid outgroup supported callitrichines and *Aotus* as sister clades, whereas phylogenetic analysis with a strepsirrhine outgroup inferred cebines and callitrichines as sister clades. Once again, as the outgroup affected the relationships between cebid clades, craniodental morphology does not support any two cebid clades as more closely related.

Phylogenetic analysis of facial morphology came close to congruence for morphological and molecular phylogenies by inferring close relationships between callimicos and marmosets. The basal-position of *Leontopithecus* could reflect allometry as they are the largest callitrichines, or dietary adaptations, due to increased frugivory, but the dietary link between marmosets and callimicos is less clear considering pygmy marmosets have extensive exudativory whereas the callimicos have a mixed diet incorporating mycophagy. It is possible that the link relates to the use of captive, rather than wild, specimens for callimicos and the pygmy marmosets, although the effect of captivity on morphology, presumably linked to diet, would not necessarily localise solely around the face and has only limited power in explaining the link between the two. An alternative explanation could be linked to modularity, as callimicos and the pygmy marmoset are the only platyrrhines that lack integration in the anterior oral nasal region (Goswami 2006a). Possibly the lack of integration frees facial morphology of restraints, and both share an associated craniodental change. Within marmosets, although pygmy and *jacchus* marmosets share increased exudativory, and the *argentata* and *jacchus* sizes overlap, the *jacchus* group is far removed from pygmy and *argentata* marmosets in phylogenetic analysis of the face, so neither allometry or diet explain the divergence of jacchus marmosets.

7.4.3 Cebid variation in cranial base morphology

The results from phylogenetic analyses of the cranial base (Table 27) were particular variable, with a very large number of alternative phylogenies inferred. Paraphyly of the callitrichines was common, as was cebine paraphyly due to the divergence of the hard-food adapted capuchins. The apparent plasticity of cranial base morphology, and overlap between taxa belonging to separate genera, are only found in cebids and are one of the most striking results presented in this thesis. One explanation is that the morphology of the atelid cranial base was closely linked to diet and mastication, and the same could be true of cebids. If so, the phylogenetic results could indicate either greater overlap in dietary preference than previously acknowledged or shared adaptations between disparate taxa in the processing of food and the response of morphology to physical stress. The divergence of hard-food specialists within capuchins, and a clade comprising *Leontopithecus* and a tamarin taxon with increased frugivory, appear to support an important role for diet and mastication in cranial base morphology. Alternatively, cebids are relatively similar in their patterns of locomotion across all genera, unlike the atelids for example, and have reduced cranial base diversification as a result. This could be linked to either reduced selection on cranial base form and greater

overlap between taxa of separate genera, or an absence of alternative biomechanical stressors to create stronger distinctions between genera.

Phenetic analysis of cranial base morphology inferred a basal position for squirrel monkeys, undoubtedly linked to the almost rectangular shape of the cranial base, followed by the pygmy marmoset. The divergence of the pygmy marmoset from other marmosets in cranial base morphology is interesting, as owl monkeys and capuchins are more similar to *jacchus* and *argentata* marmosets than the pygmy marmosets are. The pygmy marmosets have experienced secondary dwarfing, engage in increased claw clinging and climbing (Youlatos 2009), and display increased exudativory. The cranial base diversification could relate to any of these factors, or a combination of all three, but it is clear that the pygmy marmoset has experienced its greatest diversification from other marmosets in cranial base morphology. A close phenetic and phylogenetic relationship between *Saguinus midas* and *Leontopithecus* was also inferred from phenetic and multiple phylogenetic analyses. The connection between the two likely relates to *Leontopithecus* being the most frugivorous callitrichine and the observation of increased frugivory in S*aguinus midas* (Pack et al. 1999). The shared diet of these groups presumably shapes the cranial base, in response to dietary forces and strain, so that morphometric data connects the two taxa.

Although the results from phylogenetic analysis of the cranial base were highly varied, there were some clear patterns. An Old World anthropoid outgroup placed the cebines as the basal-most cebids, although *Cebus* was often paraphyletic due to divergence of the more robust *Cebus apella* and *Cebus libidinosus* groups that have adapted to a dietary preference for tougher, harder foods (Cole 1992, Janson & Boinski 1992). Callitrichines were paraphyletic as *Callimico* was basal to a clade comprising *Aotus* and remaining callitrichines. The position of *Aotus* was variable, and the absence of a morphological connection between *Aotus* and a specific callitrichine indicate the owl monkeys do not share any great morphological connection to callitrichines. Rather, the basal-position of callimicos highlights its own divergence in cranial base morphology away from the callitrichines, with *Aotus* drawn into a clade with the remaining callitrichines as a result. Morphological divergence in callimicos was also present for whole skull morphology, and could be explained by the complex evolution of the clade incorporating a mix of callitrichine and non-callitrichine traits, derived traits linked to mycophagy, or the use of captive-bred specimens. The fracturing of callitrichine monophyly when using a strepsirrhine outgroup could also relate to size. Centroid size of the cranial base overlaps between strepsirrhines and callitrichines, likely

creating a complex pattern of size-related convergence between strepsirrhines and callitrichines. However, this size issue is only one of several factors influencing cranial base morphology and phylogenetic analysis in cebids, as it is clear that the connection between callimicos and cebines, and *Leontopithecus* and *Saguinus midas*, do not relate to size.

7.4.4 Taxonomy, modularity and dimorphism

Several additional issues are worthy of further comment. The question of marmoset taxonomy cannot be resolved with the phylogenetic analyses described in this chapter, as the taxonomic argument does not question the monophyly of the group, only that the level of divergence between marmosets warrants a higher taxonomic ranking. It is clear from facial morphology that there are distinct differences between the *jacchus* marmosets, that have reduced exudativory, and the *argentata* and pygmy marmosets, which have increased exudativory. But it is also true that marmoset monophyly was consistent throughout analyses of the whole skull and cranial base morphology, so the divergence in *jacchus* morphology may be restricted to facial changes linked to diet. Clearly much work remains to resolve marmoset taxonomy, and the morphometric data collected for this project could be used to measure within and between taxa variation for all cebid genera and compare them to the variation present for pygmy, *jacchus* and *argentata* marmosets. This would at least produce a more thorough, quantitative contribution from craniodental morphology to the debate on marmoset taxonomy.

The use of a modular approach, examining alternative phylogenetic signals in the face and cranial base compared to the skull as a whole, is justified once again as the results from each module clearly contrasted with each other. Quantification of the whole skull measures a very stable signal and genus paraphyly is rare, whereas facial and cranial base morphology is more variable and paraphyly frequent. This issue of paraphyly should not be considered a reason to discard a modular approach, as the variation is real and presence of alternative phylogenetic signals informs about morphological evolution within the cebids. For example, without a modular approach the overlap between separate genera in cranial base morphology or the link between callimicos and pygmy marmosets in facial morphology would have been unreported. Another issue from cebid phylogenetic analysis is outgroup selection, as the choice of either strepsirrhines or Old World anthropoids seemed to have most effect on whether cebines or owl monkeys were sister to callitrichines. The two outgroups rarely affected callitrichine relationships, which is an obvious area of improvement for future work. This could mean

incorporating alternative outgroups, particularly at smaller body-sizes similar to the callitrichines, although sample sizes are problematic as the smaller primates are often the least well-sampled specimens from museum collections.

The issue of sexual dimorphism is also interesting, because only *Cebus* and *Saimiri* show significant body-size dimorphism within the cebids (Ford & Davis 1992). The position of the cebines was relatively stable throughout phylogenetic analysis, except for several trees based on facial morphology that had *Cebus* paraphyly linked to diet, but sexual dimorphism in these two groups did not appear to play a significant role in the accuracy of phylogenetic analysis. In phylogenetic analyses of males for whole skull morphology *Saguinus geoffroyi* and *Saguinus leucopus* were more closely related to *Callithrix*, and for female facial morphology *Saguinus midas*, *Saguinus mystax* and *Saguinus fuscicollis* were more closely related to *argentata* and pygmy marmosets. Apart from *Saguinus* paraphyly, the phylogenies inferred from male and female data were the same. These results strongly indicate that there are shape differences (dimorphism) between the male and female tamarins even though there is not body-size dimorphism. The reason for this divergence within tamarins, and the shared similarity between marmosets and tamarins, does not have an obvious origin. It seems apparent that future work should look more deeply at evolution within tamarins, specifically the interaction between social groupings and morphology. With the exception of this issue in tamarins, sexual dimorphism does not appear to have a large effect on the phylogenetic analysis of cebids.

Chapter 8 Discussion

The extant South and Central American primates, the platyrrhines, are a monophyletic clade that diverged from Old World anthropoids somewhere between 35-59 million years ago, share a common ancestor around 14-33 million years ago, with the earliest platyrrhine fossil *Branisella boliviana* dated to around 26 million years ago (Steiper & Young 2006, Hodgson et al. 2009, Wilkinson et al. 2011, MacFadden 1990, Fleagle & Kay 1997, Kay et al. 1998, Kay et al. 2008). The timing of platyrrhine common ancestry and tempo of evolution is split between adherents of the deep-time (Rosenberger 1979, 1980, Rosenberger et al. 2009) and layered (Kay 1990, Kay et al. 2008) hypotheses. Today there is near consensus as to the molecular phylogenetic relationships of extant platyrrhines at the genus level (e.g. Wildman et al. 2009, Perelman et al. 2011), but most morphology-based phylogenetic analyses have been restricted to cladistic analyses of character state data (e.g. Rosenberger 1984, Ford 1986, Kay 1990) that have limited congruence with molecular phylogenetic relationships. In this thesis, I have combined geometric morphometric and distance-based phylogenetic methods to investigate the phylogenetic signal both within and between the three major molecular clades of atelids, pitheciids and cebids. Much like previous cladistic analyses, there was only mild congruence between the molecular and morphological relationships for analyses of the entire platyrrhine clade, but there was a stronger phylogenetic signal in analyses of the three major molecular clades of pitheciids, atelids and cebids.

8.1 Phylogenetic signal in the platyrrhine skull

The primary aim of this project was to examine the phylogenetic signal of the platyrrhine skull. Phylogenetic analysis of all 50 platyrrhine taxa found, at best, a mild phylogenetic signal, due to major homoplasy stemming from either the high level of morphological and size variation, relatively high rates of morphological evolution, or increased plasticity. Future work will seek to address which of these contributes to the phylogenetic results presented. Phylogenetic analysis of each of the three major platyrrhine clades found a strong phylogenetic signal in the pitheciids and atelids, and a partial signal in the cebids. The presence of a phylogenetic signal in these individual clades, but not in the wider platyrrhine group, relates to reduced morphological, size, ecological and behavioural variation, restricted levels of homoplasy, and the increased effectiveness of outgroups in applying and accurately inferring phylogenetic relationships.

The absence of a phylogenetic signal, and inference of sister relationships and clades not recognised by the well-supported molecular phylogenetic relationships of platyrrhines, are synonymous with homoplasy, where morphological similarity is either the result of reversal to an ancestral phenotype or independent evolution via the same (parallel) or different (convergent) developmental mechanisms (Wake et al. 2011). Homoplasy in craniodental morphology is common in platyrrhines, just as it is in postcranial morphology (Lockwood 1999), and is responsible for morphological similarity not inherited from a common ancestor. Good examples are craniodental morphology of *Callicebus* and *Aotus* or *Callithrix* and *Saguinus*. In this thesis the term homoplasy is used sparingly, but any reference to forces shaping craniodental morphology that are not linked to recent common ancestry and phylogeny is an attempt to explain the biological origins of homoplasy in the taxa studied. The origins of homoplasy lie in genetics, function, development, allometry and convergent adaptations, and it is natural that it plays an important role in shaping craniodental morphology and hence influencing phylogenetic analysis (Lockwood & Fleagle 1999, Kay & Fleagle 2010).

Considering the partial failure of both this, and previous (e.g. Rosenberger 1984, Ford 1986, Kay 1990), morphology-based phylogenetic analyses to find congruence with molecular data, it is clear that future morphological analyses ought to concentrate on clades that have evolved more recently, including a more restricted number of genera encompassing reduced levels of size and morphological variation. Whilst molecular phylogenetic analyses can escape the problem of increased homoplasy and convergent evolution associated with large number of ingroup taxa, morphological data are more finite and require an alternative solution: reduced taxon sampling, at least at the genus-level. The problems encountered in morphological analysis of the platyrrhine clade may be the case for other primate and mammalian clades.

The analyses incorporating all platyrrhines strongly supported several clades and phylogenetic positions. Phenetic results from the whole skull supported partial atelid, pitheciid and cebid clades similar to molecular phylogenetic relationships, so a form of phylogenetic signal was reflected in phenetic relationships, although the phenetic tree also appeared to broadly reflect size similarity. Phylogenetic analysis of the skull supported several molecular clades (*Cacajao-Chiropotes*, *Cebus-Saimiri*, and *Lagothrix-Brachyteles*), but pitheciid and callitrichine paraphyly was common, with strong support for major homoplasy between *Aotus* and *Callicebus* as found in Rosenberger (1984) and Ford (1986). Whilst the two groups overlap in size and may have shared convergent responses to body

size, *Saimiri* is also similar to both groups in body size but is not drawn to either taxon. There were a number of other cases of convergence and clades unlinked to allometry or size, with facial morphology inferring a clade of cebines, *Chiropotes* and *Cacajao*, which may reflect encephalization and orbital orientation (Isler et al. 2008, Ross & Ravosa 1993).

8.2 Atelids

The presence of a strong phylogenetic signal in the face of atelids, but not the cranial base, are surprising as the face has previously been considered vulnerable to homoplasy, developmentally plastic, and shaped by non-genetic factors, whilst the cranial base is considered more conservative due to its role in multiple functional systems (Lieberman 1995, Wood & Lieberman 2001, Lockwood et al. 2004, Smith et al. 2007). Essentially, phylogeny and common ancestry shape atelid facial morphology. The basal lineage, *Alouatta*, has a very large face that is curved and robust, linked to both extensive folivory and chewing, but also restructuring linked to an enlarged vocal tract and reduced brain size (Rosenberger & Strier 1989, Hartwig et al. 1996, Kinzey 1997, Isler et al. 2008). Reduced brain size and increased facial robusticity linked to mastication have also been found in marsupials (Wroe & Milne 2007), and howler monkeys provide a convergent primate example.

The face of *Ateles* is more gracile compared to *Brachyteles* and *Lagothrix*, which share a relatively large, robust face without the extreme specialisation of howler monkeys (Rosenberger & Strier 1989, Rosenberger et al. 2008). Possibly, the wider atelid skull and cranial base would maintain a phylogenetic signal but for diversification due to diet and mastication. There is no doubt that overall skull and cranial base morphology supports divergence of the frugivorous taxa *Ateles* and *Lagothrix*, predominantly shaped by diet and forces related to mastication. Menegaz et al. (2010) have shown that diet and mastication can indirectly shape cranial regions including the cranial base and vault, which phylogenetic analysis of the whole skull and cranial base supports.

8.3 Pitheciids

For pitheciids, phylogenetic analysis of whole skull, facial and cranial base morphology all strongly supported the accepted molecular phylogeny. The phylogenetic results clearly reflect the differentiation of *Callicebus* from saki-uakaris, and *Cacajao-Chiropotes* from *Pithecia*. The divergence of these four taxa reflects diet, allometry, relative brain size and social behaviour, as the basal-positioned *Callicebus* are significantly smaller, non-seed harvesting,

monogamous with smaller relative brain size, whereas saki-uakaris are larger in body and relative brain size, seed predators, and have larger social groups (Kinzey 1997, Ford & Davis 1992, Kinzey 1992). Within the saki-uakaris, *Cacajao* and *Chiropotes* are larger than *Pithecia*, with increased force generation and seed-harvesting abilities, and have larger and more complex social groups (Kinzey 1997).

The pitheciids are one of the few primate clades where morphological, molecular and behavioural data support the same differentiation between groups (*Callicebus* and saki-uakari, *Pithecia* and *Cacajao-Chiropotes*), and homoplasy is especially low. This could make them an ideal group within which to study the interaction between genotype and phenotype. More specifically, it seems rational to examine the molecular genetic basis of morphological variation in the group, linking morphological change with specific areas of the genome, individual genes or changes in gene expression. Another obvious avenue of research would be to examine patterns of craniodental integration within the group, as the proposed craniodental modules of the face and cranial base inferred the same phylogenetic relationships to each other and overall craniodental morphology. Although Marroig & Cheverud (2001) have studied platyrrhine modularity and integration, they used a restricted number of anatomical landmarks compared to those collected in this thesis, which may affect the results of integration work.

8.4 Cebids

In the cebids, the phylogenetic signal is only moderate, as phylogenetic analysis recognised cebine, owl monkey and callitrichine lineages, but the phylogenetic relationships within the callitrichines were incongruent with the molecular phylogenetic relationships. Cebids have greater size and morphological variation than seen in atelids or pitheciids, supporting the suggestion of a detrimental effect of variation on accurate phylogenetic inference. Variation across the cebids could mean the polarity introduced by the use of an outgroup is concentrated on differentiating between the three major cebid clades, and loses resolution when inferring callitrichine relationships. In this thesis, the two sets of phylogenetic analyses that measured a strong phylogenetic signal were those that sampled fewer genera, and many past phylogenetic analyses that have found a strong phylogenetic signal in morphological data have examined either a single genus (e.g. Harvati & Weaver 2006a,b, Smith et al. 2007) or only a few genera (Lockwood et al. 2004, Cardini & Elton 2008). It seems clear that

restricting the number of genera sampled will help to produce more accurate phylogenetic analyses, possibly because the number of possible trees that can be inferred are more limited.

If the number of genera sampled is the problem, phylogenetic analysis of just callitrichines or cebines (when a more detailed molecular phylogeny is available for capuchins) should find greater congruence between molecular and morphological analyses. However, callitrichine morphological evolution may not follow phylogeny, irrespective of the number of genera included and outgroups used. There is clearly a complex relationship between morphology, size, diet and phylogenetic inference difficult to tease apart in this clade. Marroig & Cheverud (2001) found that craniodental integration was reduced in callitrichines and owl monkeys compared to the other major platyrrhine clades, and the reduced integration could allow greater evolutionary change and overlap between taxa from separate genera. Within callitrichines a sister relationship between *Saguinus* and *Callithrix* is strongly supported, and the smaller *Saguinus* taxa are very close in size to larger *Callithrix* taxa, suggesting smaller callitrichines look like each other and phylogenetic analysis is complicated by size similarities. However, larger *Saguinus* taxa are closer in size to *Callimico* than *Callithrix*, yet a sister relationship is not inferred from any of the cebid phylogenetic analyses, so phylogenetic relationships within callitrichines does not simply reflect allometry. The morphological link is strong between these two taxa, and studies concentrating on social group and behaviour and shared life history variables may explain the homoplasy. There is a big question as to how social behaviour could affect craniodental morphology, which should be addressed in the future.

8.5 Craniodental evolution in platyrrhines

Phylogenetic analysis of platyrrhine craniodental morphology does not simply separate platyrrhines according to size (Hershkovitz 1977), adaptive radiations of frugivorous-insectivorous and frugivorous-folivorous clades (Rosenberger 1980), or strict phylogenetic-diet clades for atelids, cebines, callitrichines and pitheciids (Rosenberger 1992). Aspects of diet shape platyrrhine craniodental morphology to an extent, but there is a clear interaction between multiple elements relating to phylogeny, function and adaptive evolution including allometry, diet and encephalization (Kay 1975, Rosenberger 1992). The connection between various life history variables and social systems is poorly understood in platyrrhines, but as the behavioural ecology of the clade becomes clearer (particularly diversity within and

between species and populations), it will become apparent that additional factors have helped shape platyrrhine morphology.

The advocacy of important roles for phylogeny, size and diet in shaping craniodental morphology supports Marroig & Cheverud (2001), who found significant correlations between morphology, diet and size. Perez et al. (2011) examined the relationship between platyrrhine craniodental morphology and phylogeny, diet and size, and found a strong correlation between morphology and phylogeny, but not with diet or size. These results are incongruent with the work detailed in this thesis, as such a strong connection between morphological and molecular distances would allow reliable phylogenetic inference of morphology in platyrrhines. Instead, homoplasy between platyrrhine taxa was high, as suggested by the platyrrhine phylogenetic analyses presented and past cladistic analyses, and the difference in results of Marroig & Cheverud (2001) and Perez et al. (2011) require further testing. The methodological issue raised by Klingenberg & Gidaszewski (2010), that a strong phylogenetic signal can be measured but inferred phylogenies based on morphological and molecular distances can be incongruent, likely explains the difference in my results compared to Perez et al. (2011).

Phenetic analyses of the platyrrhine skull identified *Alouatta* and *Saimiri* as two off-shoot divergent lineages, and two additional clades split into large and small platyrrhines, that also correspond with large and small relative brain sizes (Isler et al. 2008). The howler monkeys have massive restructuring of the skull linked to the huge hyoid bone, large face and reduction in brain size, and squirrel monkeys are born practically fully developed, with a central foramen magnum and large brain, and social groups that are highly flexible in response to ecological change (Kinzey 1997, Rosenberger & Strier 1989, Hartwig et al. 1996). Phylogenetic analysis placed *Saimiri* with its sister taxon *Cebus* and *Alouatta* with atelids, but the phylogeny did not just reflect allometry or encephalization, placing cebines and pitheciids closer to the callitrichines. The atelid clade is connected by phylogeny, size and diet, and the latter may be key to supporting the clade, as the group are specialised for frugivory-folivory and have both an overall size increase and larger faces than found in other platyrrhines (Rosenberger & Strier 1989). Diet and mastication are linked to size and robusticity increases in the whole skull and cranial base, cranial vault and temporal fossa, and could support the atelid clade (Larsen 1995, Sardi et al. 2006, Lieberman et al. 2004, Menegaz et al. 2010, Paschetta et al. 2010).

Craniodental evolution of the atelid skull followed the molecular phylogeny for phenetic analyses, but strongly supported a *Lagothrix-Ateles* clade sister to *Brachyteles* in phylogenetic analyses. There is a question about why the phenetic shape differences reflect phylogeny but phylogenetic analyses reflect diet and mastication in *Ateles*, *Lagothrix* and *Brachyteles*. Phenetic and phylogenetic analyses of pitheciids both support the molecular phylogenetic relationships, and offer rare congruence. Phenetic and phylogenetic analyses of the cebid skull supported cebine, owl monkey and callitrichine lineages, with *Callithrix-Saguinus* sister to *Leontopithecus* and *Callimico* basal-most. Although much is made of the homoplasy that connects *Aotus* and *Callicebus*, the morphological overlap between *Callithrix* and *Saguinus* may actually be greater, and future work will further investigate this convergence.

8.6 Evolution of facial morphology

In adults, the gradient of stress across the face, greatest in the lower faces and heavily reduced in the upper face, is particularly important when considering facial morphology as quantified in this thesis, as most of the facial landmarks used sample middle and upper face morphology (Hylander et al. 1991, Ross & Hylander 1996, Ravosa et al. 2000, Ross 2001 , Ross & Metzger 2004, Paschetta et al. 2010). Strain related to dietary properties and mastication ought to have a limited effect on the quantified region and subsequent phylogenetic analyses, explaining for example the apparent disjuncture in atelids between diet and facial morphology. Ross & Metzger (2004) found the gradient of strain in primates had a strong positive allometric relationship, so larger primates have an exaggerated strain gradient and the facial morphology of smaller primates will be shaped more by resistance to feeding. This may explain why analyses of the face that incorporated the callitrichines were less phylogenetically informative, as strain has a more prominent role in shaping facial morphology in the clade, although this is one of multiple factors contributing to platyrrhine facial morphology.

Phylogenetic analyses of atelids and pitheciids recovered a strong phylogenetic signal. Whereas pitheciid facial evolution follows the pattern of the cranial base and overall skull morphology, the phylogenetic signal maintained in the atelid face is the only region that is phylogenetically informative. The atelids have a clear dichotomy between frugivorous *Ateles-Lagothrix* and folivorous *Alouatta-Brachyteles*, yet facial morphology strongly supports a *Lagothrix-Brachyteles* clade sister to *Ateles* (Kinzey 1997, Rosenberger & Strier 1989). This

highlights the limited role of diet and mastication in shaping mid and upper-facial morphology as proposed by the gradient of strain across the face (e.g. Hylander et al. 1991, Ross & Hylander 1996). The strong phylogenetic signal indicates that facial morphology, at least in atelids and pitheciids, is less plastic and vulnerable to homoplasy, with a greater functional importance and conserved morphology than previously acknowledged (e.g. Lieberman 1995, Wood & Lieberman 2001).

Platyrrhine phylogenetic trees supported a clade with *Cacajao*, *Chiropotes* and cebines, that could relate to brain size and orbital orientation. Ross & Ravosa (1993) found a correlation between orbital orientation and basicranial flexion, and the cebines and *Cacajao-Chiropotes* have experienced a relative increase in encephalization that would affect orbital orientation and support morphological similarity in the face (Isler et al. 2008). Platyrrhine facial morphology is predominantly shaped by a combination of allometry, encephalization and increased strain on smaller taxa, with a high level of homoplasy and presence of a weak phylogenetic signal. Phylogenetic analyses of all platyrrhines were hindered by apparent convergence between atelids and Old World anthropoids, and *Aotus* and *Pithecia* with strepsirrhines. Due to these ineffective outgroups, little can be concluded about the inferred phylogenetic relationships beyond those inferred by phenetic analysis. The polarity inferred by the outgroup appears to be especially problematic, and once again raises a question of whether variation and the number of genera sampled, with the inevitable homoplasy and convergence between disparate groups, is too great when analysing so many taxa. Alternatively, the use of a non-primate outgroup may be the solution, but this seems unlikely.

Phylogenetic analysis of the cebid face supported *Aotus*, cebine and callitrichine monophyly, but the phylogenetic relationships within callitrichines were less straightforward. The callimicos and pygmy marmosets form a clade, whilst the *jacchus* marmosets diverge away from pygmy and *argentata* marmosets and are even basal to *Callimico* and *Saguinus*. The *jacchus* and pygmy marmosets share increased exudativory, yet are far removed, indicating diet and mastication have not shaped facial morphology in callitrichines, at least not for marmosets (Ford & Davis 2009). Allometry has a mixed role in shaping cebid facial morphology, as the callitrichine clade shares derived small size and the largest callitrichine *Leontopithecus* is basal-most (Ford & Davis 1992). But *Leontopithecus* is also highly frugivorous which could account for its phylogenetic position (Norconk et al. 2009). The lack of sister relationship between *Saimiri* and *Aotus*, the divergence of *jacchus* marmosets away from *argentata* marmosets (same sizes), and the sister relationship between pygmy

marmosets and callimicos (large size gap), indicate a limited role for size in shaping facial morphology.

In the case of the pygmy marmoset-*Callimico* clade, the use of captive specimens may have drawn the two taxa together, although *Leontopithecus* also included captive specimens and a similar relationship was not inferred. The owl monkey and cebine lineages have also diverged from callitrichines, with *Aotus* monophyly due to huge orbits and the effect that has on the position of facial landmarks, and the cebine clade could reflect encephalization and its effect on orbital orientation, increased body size or dietary flexibility (Kinzey 1997, Isler et al. 2008, Ross & Ravosa 1993). It is clear that cebid facial morphology and phylogenetic analysis is not simply shaped by a single factor, and it shares the same problem of platyrrhine phylogenetic analysis- facial morphology in cebids incorporates too many genera, and too much variation and homoplasy to reliably infer phylogenetic relationships.

8.7 Cranial base evolution

Cranial base morphology is shaped by a multitude of factors due to its role in multiple functional systems including cognition and brain size, posture and locomotion, diet and mastication (Lockwood et al. 2004, Olson 1981). Its morphology is hypothesised to be strongly controlled by genetics, with reduced plasticity and vulnerability to dietary and mechanical stresses (Lockwood et al. 2004, Olson 1981). As a result, theoretical and experimental research has supported the presence of a strong phylogenetic signal in primate cranial base morphology (Olson 1981, Lieberman et al. 1996a, Lieberman 1997, Strait et al. 1997, Lockwood et al. 2004, Harvati & Weaver 2006a,b, Cardini & Elton 2008). Phylogenetic analysis of cranial base in pitheciids recovered a strong phylogenetic signal, as did overall skull and facial morphology, so the phylogenetic signal in the pitheciid cranial base was no stronger than in the other regions of the skull. Phylogenetic analyses of all platyrrhines, atelids and cebids failed to recover molecular phylogenetic relationships for those clades. It is clear that in platyrrhines, with the exception of one clade, cranial base morphology did not conserve a phylogenetic signal: either evolutionary processes have created diversification that removes a phylogenetic signal, or cranial base morphology is more variable and plastic than previously acknowledged, with reduced genetic control and an increased role for dietary and mechanical factors.

The role of brain size and shape in cranial base morphology, and its effect on phylogenetic analysis, is an important factor worth considering. Relative brain size is small in

callitrichines, *Aotus*, *Callicebus* and *Alouatta*, with increased encephalization and relative larger brains in cebines, *Cacajao* and *Chiropotes*, and atelids bar *Alouatta* (Isler et al. 2008). These brain size changes are important in shaping cranial base morphology as there is a strong relationship between encephalization and cranial base flexion/angle and morphology (Ross & Ravosa 1993, Spoor 1997, Lieberman et al. 2000a, Bastir et al. 2010). In pitheciids the polarity of brain size evolution, with a split between the small-brained basal *Callicebus* and more encephalized *Cacajao-Chiropotes*, helps to maintain a phylogenetic signal in addition to multiple additional factors discussed earlier (Isler et al. 2008). In atelids, where basal *Alouatta* experienced a brain size decrease and the other atelids increased encephalization, *Brachyteles* are positioned sister to *Ateles-Lagothrix* rather than with *Alouatta*, even though the two share folivory and large mandibles, which requires restructuring of cranial base morphology (Isler et al. 2008, Kinzey 1997).

In the case of cebids, cranial base results can be partially linked to brain size reduction (Isler et al. 2008). Several of the phylogenetic relationships inferred from cebid analyses make it clear dietary divergence is also important, such as the frugivorous link between *Leontopithecus* and *Saguinus midas* or divergence between soft and hard food adapted capuchins (Kinzey 1997, Norconk et al. 2009, Janson & Boinski 1992, Pack et al. 1999). It seems reasonable that brain size changes, dietary adaptations, allometry and phylogeny interact to produce the cebid cranial base results. For phylogenetic analysis of all platyrrhines the role of brain size in shaping cranial base morphology is more complex, as outgroup selection affects the inferred phylogeny, and several phylogenetic relationships do not appear to have a link to encephalization, such as a sister relationship between *Leontopithecus* and *Pithecia* or divergence in *Cebus* linked to diet (Janson & Boinski 1992). There are some clades repeatedly inferred for atelids, cebines and *Cacajao-Chiropotes*, which all experienced encephalization increases but are also larger-bodied, and a group of small brained callitrichines plus *Aotus* and *Callicebus*, although the larger brained *Pithecia* also falls within this group (Ford & Davis 1992, Isler et al. 2008).

Gilbert (2011) found allometric variation severely inhibited accurate phylogenetic analysis of cranial base morphology in papionins, which may be responsible for the lack of phylogenetic signal in the cranial base of platyrrhines. However, considering that allometric corrections did not improve congruence of morphology-based phylogenies with molecular relationships (Gilbert 2011), the diversification in the clade may relate to non-allometric factors. For example, the phylogenetic analysis of Gilbert (2011) used distances derived from principal

components, which may be problematic (see Adams et al. 2011), and phylogenetic relationships were only tested with a single outgroup.

The apparent platyrrhine plasticity, and general absence of a phylogenetic signal, contrasts with the theoretical and experimental support for a strong phylogenetic signal in the cranial base morphology of humans, hominoids and guenons (e.g. Lockwood et al. 2004, Harvati & Weaver 2006a,b, Cardini & Elton 2008). With the exception of the hominoid study by Lockwood et al. (2004), these studies have concentrated on a restricted number of genera that have evolved over a smaller period of time than the phylogenetic analyses of platyrrhine clades presented in this thesis. The number of genera sampled, the variation introduced in analyses with multiple genera, and the scales of time involved might inhibit accurate phylogenetic analysis. Harvati & Weaver (2006a) hypothesised that cranial base morphology reflected evolutionary history across longer periods of time and cranial vault morphology more recent periods of time. The phylogenetic information in the cranial base may be phylogenetically informative in platyrrhines, but not for the time scales investigated. For example, cranial base morphology may accurately reflect phylogenetic relationships at the species level within individual genera, or the time scale involved in diversification between the cebines, but not at the family or parvorder level.

The lack of phylogenetic signal could relate to platyrrhines being strictly arboreal, that would implicate posture and locomotion as important in shaping cranial base morphology and maintaining a phylogenetic signal in other clades, with reduced diversification or increased plasticity in platyrrhines due to arboreality and relative lack of locomotory diversification outside the atelids. There may also be a shift in genetic control of cranial base morphology linked to body or brain size evolution in platyrrhines, and comparative work on other primate and mammalian groups with similar size variation could test this further.

8.8 Phylogenetic analysis of morphology

In this project geometric morphometric and distance-based phylogenetic methods were combined to infer phylogenetic relationships from quantitative morphological data derived from craniodental morphology. The use of distance-based methods contrasts with previous analyses that have used cladistic methods (e.g. Rosenberger 1984, Ford 1986, Kay 1990, MacPhee et al. 1995, Horovitz & Meyer 1997, Horovitz & MacPhee 1999). Phylogenetic analysis of all platyrrhine genera using distance-based phylogenetic methods was no more successful than those cladistic analyses, but phylogenetic analysis of the major platyrrhine

clades inferred high congruence between molecular and morphological phylogenetic relationships. The presence of a phylogenetic signal in atelid, pitheciid and cebid data using geometric morphometric and distance-based phylogenetic methods further justifies the use of these combined methods for inferring phylogenetic relationships based on morphology.

The relative success of these methods compared to previous cladistic analyses of platyrrhines, may relate to the type of data used (geometric morphometric rather than character states), the phylogenetic method used (distance-based versus cladistic), or reduced genus-sampling (encompassing less variation and homoplasy). The beneficial role of reduced genera sampling in accurate phylogenetic analysis cannot be ruled out and further cladistic analysis of atelid, pitheciid, and cebid clades are required to further investigate the ability of cladistic methods to measure a phylogenetic signal. Each of these factors probably contributes to congruence between morphological and molecular data as detailed in this thesis, although I place particular emphasis on the combination of geometric morphometric analysis and distance-based phylogenetic methods, which has also met with success in the primate phylogenetic studies of Lockwood et al. (2004), Cardini & Elton (2008) and Bjarnason et al. (2011). In particular, phylogenetic analysis of hominoid morphology using both cladistic and distance-based phylogenetic methods on two datasets, one geometric morphometric quantification of temporal bone morphology and another based on linear craniodental measurements, measured the strongest phylogenetic signal combining geometric morphometric and distance-based phylogenetic methods (Bjarnason et al. 2011).

The use of geometric morphometric data in phylogenetic inference based on morphological distances can use dissimilarity in mean shape, used here and by Lockwood et al. (2004), Cardini & Elton (2008) and Bjarnason et al. (2011), distances derived from partial warps (e.g. Monteiro & Abe 1999) or distances based on principle components (e.g. Viguier 2002). Matrix correlations between morphological and molecular distances (e.g. Polly 2001), mapping morphological data onto a molecular phylogeny (Klingenberg & Gidaszewski 2010) or use of a parsimony method based on inferring hypothetical ancestors (Catalano et al. 2010) are also viable alternatives.

The phylogenetic analyses based on partial warps were unsuccessful in measuring a phylogenetic signal, and distances based on principal components have some major methodological problems that makes their use inappropriate for phylogenetic analysis (Adams et al. 2011). The Catalano et al. (2010) method is so computationally intensive that

for a project such as this it is unusable, whilst many of the methodological details are unclear and ambiguous. Matrix correlations between morphological and molecular distances have one major problem acknowledged by Klingenberg & Gidaszewski (2010); the morphological and molecular distances can have high correlations, but the phylogenetic trees inferred by the strongly correlated data may be incongruent with each other. This is a major problem as the aim of phylogenetic analysis based on morphology is to reliably infer phylogenetic relationships that agree with the molecular phylogenetic relationships so that fossil taxa can be placed with some certainty and accuracy alongside living groups. In contrast, phylogenetic analysis of distances separating taxa have been used in this thesis and in previous analyses to infer phylogenetic relationships that agree with the accepted molecular phylogeny. This strongly justifies the methodological decision to use distances between taxa to infer phylogenetic relationships, and should provide support for the use of these phylogenetic methods in future analyses of geometric morphometric data.

Another issue that should be mentioned is the methodological decision to test the consistency of phylogenetic inference by using a range of outgroups and outgroup combinations rather than a statistical measure of tree support and node repeatability, such as bootstrapping used in Lockwood et al. (2004) and Bjarnason et al. (2011) with geometric morphometric data. The primary argument against the use of bootstrapping in this thesis was simply that presenting all phylogenies inferred from phylogenetic analysis for all outgroups and outgroup combinations, modules and different sex-combinations, with bootstrap support (or an alternative statistical measure of node repeatability) would have been overwhelming. The benefit of providing statistical support for each clade would have been lost to the detrimental effects of huge amounts of results data and associated problems with analysing the results within a coherent framework. There is also a serious methodological issue raised by Caumal & Polly (2005) and Cardini & Elton (2008) as to how node repeatability should be inferred using morphometric data. In cladistic analyses node support is tested by sampling and replacement to build new datasets, whereas with morphometric data the resampled variables are dependent on mean shape which is not repeated, so the bootstrap procedure is statistically problematic (Caumal & Polly 2005, Cardini & Elton 2008).

8.9 Outgroups and phenetics

The phylogenetic analyses presented in this thesis took an experimental approach to the use of outgroups, with all analyses repeated using one of nine single outgroups or combination of

outgroups. The effect of outgroup selection on phylogenetic analysis appears to be one of the major factors in measuring a phylogenetic signal, and future morphology-based systematic work should incorporate extensive outgroup testing. Previous work combining geometric morphometric and distance-based phylogenetic methods have rarely tested the effect of outgroup selection on phylogenetic inference, although Bjarnason et al. (2011) highlighted the potential for variation in outgroups to infer alternative phylogenetic relationships in morphology-based work. Outgroup selection in morphology-based analyses tend to use the closest relative that falls outside the group of interest, but the results from outgroup testing in platyrrhines conducted as part of this thesis suggest using a more distantly related outgroup may be more successful in establishing correct polarities of morphological change (Maddison et al. 1984, Nixon & Carpenter 1993, Sanderson & Shaffer 2002).

In the successful phylogenetic analyses of atelids and pitheciids, strepsirrhine outgroups were more often responsible for inferring the same relationships as a molecular phylogeny, with the exception of phylogenetic analysis of the pitheciid face. There is clearly homoplasy between New and Old World anthropoids, the effects of which is avoided by using a strepsirrhine outgroup, strepsirrhines being more distinct from platyrrhines than Old World apes or monkeys. Using a single outgroup was preferable to outgroup combinations encompassing the variation of strepsirrhines and anthropoids, or Old World monkeys and *Hylobates*, which were ineffective and often inferred paraphyletic ingroups with one or several outgroups closely related to ingroup taxa. This problem of monophyly/paraphyly due to outgroup insertion was particularly common in analyses of all platyrrhines and of cebid clades, further supporting the suggestion that inaccurate inference of phylogenetic relationships in those groups relates to the large variation incorporated in sampling relatively high numbers of taxa.

A common criticism of distance-based phylogenetic methods is that they are phenetic and reflect overall similarity, which ignores the use of an outgroup that introduces evolutionary polarity and groups taxa by derived similarity (Felsenstein 1984, Lockwood et al. 2004). Comparing the results of phenetic and phylogenetic analysis shows that applying a root via the outgroup often infers alternative evolutionary trees for phenetic and phylogenetic methods, and the number of alternative phylogenetic relationships inferred in phylogenetic analysis proves inferred relationships are not merely phenetic. There are times when the phenetic relationships are congruent with multiple phylogenetic trees, which is inevitable considering the number of phylogenetic analyses carried out. Nonetheless, congruence

between phenetic and phylogenetic analysis are more than chance, and reflect the measurement of the same biological reality: underlying similarity shared by groups, both overall and derived. Molecular genetic analyses where phenetic and phylogenetic analyses are congruent are not evidence of phylogenetic methods being phenetic in nature, but indicate a steady molecular clock that allows phenetic analysis to measure accurate phylogenetic relationships (Nei & Kumar 2000). The same may be true for morphological data, and requires further study.

8.10 Modularity

Although the skull of platyrrhines is clearly a single morphological structure, it is conceivable that there are semi-autonomous regions, within which there are strong interactions between integrated traits, that are distinct and partially independent from each other in structure or function (Wagner et al. 2007, Klingenberg 2008). Cheverud (1995) and Marroig & Cheverud (2001) supported the presence of oral, nasal, orbit, zygomatic, cranial vault and cranial base functional modules in the skull of platyrrhines, broadly supported by patterns of modularity in mice (Hallgrimsson et al. 2004), guenons (Cardini & Elton 2008) and the wider mammalian group (Goswami 2006a). The justification for three major craniodental modules for the face, cranial base and cranial vault are particularly strong, as the developing cranium can be clearly split into the face and neurocranium, with earlier completed development of the neurocranium and extended growth in the face, and separation of the neurocranium into the cranial vault and cranial base according to alternative patterns of ossification (Cheverud 1982, 1995, 1996).

A modular approach to platyrrhine phylogenetic analysis was also supported by the results presented in this thesis. With the exception of the pitheciid clade, the phylogenetic relationships inferred by the whole skull, face and cranial base did not reach consensus, supporting the presence of alternative phylogenetic signals and semi-autonomous modules. The phylogenetic signal was not stronger in any particular craniodental module, but varied depending on the clade examined. The modular approach had no benefit for phylogenetic analysis of all platyrrhines or cebids, but the facial module of atelids measured a strong phylogenetic signal not present in the cranial base or whole skull. The absence of modularity, or at least alternative phylogenetic signals, in pitheciids, but presence in atelids and cebids, indicates patterns of modularity are evolvable and can shift. Whilst the quantification of facial and cranial base morphology by a lower number of landmarks will increase associated

error (Cardini & Elton 2008), it seems unlikely that this would account for the presence of alternative phylogenetic signals. If error was a major problem, the phylogenetic signal in pitheciids would not be supported from cranial base and facial morphology, and the presence of a phylogenetic signal in the atelid face would most likely be eroded rather than supported by increased error.

8.11 Sexual dimorphism and phylogenetic analysis

Sexual dimorphism of body size in platyrrhines ranges from a ratio of 0.99 in *Ateles* and *Callicebus* up to 1.39 in *Alouatta*, with quite large variation in levels of dimorphism between populations and species of the same genera, especially in *Alouatta*, *Saimiri* and *Lagothrix* (Ford & Davis 1992, Plavcan & van Schaik 1998). Due to the presence of sexual dimorphism in platyrrhines, phylogenetic analyses were repeated for data of pooled-sex, male-only, female-only, and designation of male and females of the same taxa as separate taxonomic units, the latter based on a suggestion by Gilbert et al. (2009) and Gilbert (2011). Phylogenetic analysis of male and female data separately have been used in other geometric morphometric analyses (Lockwood et al. 2004, Cardini & Elton 2008, Bjarnason et al. 2011and Gilbert 2011), and character states analyses of Gilbert & Rossie (2007) and Gilbert et al. (2009).

Overall, sexual dimorphism had a limited effect on phylogenetic analysis. For atelids, the clade with the greatest amount of sexual dimorphism, female analyses of overall craniodental morphology, with mostly strepsirrhine outgroups, inferred slightly different phylogenetic relationships. However, most phylogenetic analyses of the whole skull, face and cranial base showed no variation between male, female, pooled sex and separate sex analyses. In pitheciids, phylogenetic analysis of the whole skull found all sex-based data were congruent, but male and female data inferred alternative relationships for facial and cranial base modules using an Old World anthropoid outgroup. Phylogenetic analysis of pitheciid facial morphology in female and pooled-sex analyses inferred molecular phylogenetic relationships, whilst male data favoured partially molecular incongruent relationships. Phylogenetic analysis of the pitheciid cranial base also found differences in male and female phylogenies using Old World anthropoid outgroups, although neither retained a phylogenetic signal. Clearly, sexual dimorphism in pitheciids hindered accurate phylogenetic analysis of facial morphology, but the problem may lie with the outgroup. Strepsirrhines, which have little

dimorphism, inferred the same phylogenetic relationships for male, female, pooled and separate sex taxa, and only analyses with Old World anthropoids were variable.

Cebid sexual dimorphism is interesting because cebines have quite large sexual dimorphism, but owl monkeys and the callitrichines have low, negligible levels (Ford & Davis 1992). Phylogenetic analysis of whole skull morphology generally inferred the same phylogeny for male, female, pooled and separate sex analyses, except for instances of paraphyly in *Saguinus* and cebines for several male analyses with both strepsirrhine and Old World anthropoid outgroups. For phylogenetic analysis of the face, female data inferred *Saguinus* and cebine paraphyly with several Old World anthropoid outgroups. For phylogenetic analysis of the cebid cranial base, results were so variable that there was little coherent relationship between dimorphism and the phylogenies inferred. Although there are two examples listed where cebine dimorphism has eroded support for the clade, the vast majority of analyses supported their monophyly, and their increased dimorphism did not have a large effect on phylogenetic analysis (Corner & Richtsmeier 1991, O'Higgins et al. 2001, Flores & Casinos 2011). More interesting is the link between dimorphism and *Saguinus* paraphyly, which occurs with male and female data in different regions of the skull, which is particularly strange.

Overall, sexual dimorphism occasionally affects phylogenetic analysis, but neither male or female data appear to be any more or less reliable for phylogenetic inference. Dimorphism has an especially restricted impact on phylogenetic analysis of the atelids, the largest platyrrhine clade with highest levels of sexual dimorphism, which offers hope for accurate phylogenetic analysis of other clades that sample taxa of large body size and high levels of dimorphism (Ford & Davis 1992). One observation that has previously received little attention, is the effect of sexual dimorphism on outgroups, as strepsirrhines generally found greater congruence between analyses based on the different sex-data than did analyses using an Old World anthropoid outgroup. To reiterate, sexual dimorphism in both the outgroups and platyrrhines has not had a major effect on phylogenetic analysis, but it does bear consideration in subsequent phylogenetic work. Regarding the suggestion by Gilbert & Rossie (2007) and Gilbert et al. (2009) that male and female specimens of the same taxa could be treated as separate taxa and both included in phylogenetic analysis, the platyrrhine results indicate little benefit to this, as they nearly always support the same phylogeny as pooled sex analyses. As a result, the recommendation for future phylogenetic analyses would be to repeat analyses for male, female and pooled sex analyses, but not for separate sexes treated as alternative taxa.

8.12 Allometry & size

A project that concentrates on a clade with such a large amount of size variation needs to seriously consider allometry, the effect size has on morphology, physiology, behaviour, and ecology amongst other variables, and the relationship between allometry and morphological similarity (Gould 1966, Martin 1990, Fleagle 1999). Although allometric and size similarities make a significant contribution to phenotypic similarity in the platyrrhine skull, the results from this thesis indicate size is one of several factors that shape craniodental morphology-phylogenetic results do not simply reflect size. Allometry is clearly important for platyrrhine morphological evolution (e.g. Marroig & Cheverud 2001,2005), and if allometry is detrimental to accurate phylogenetic analysis, methods may be required to control for it. There is a counter argument that allometry is a biological factor important in shaping morphology and its removal or control is no more justifiable than removing variation linked to diet and mastication, locomotion, encephalization or any number of biological variables. If all morphological variation linked to explicit variables were removed, there would be little left to analyse.

The geometric morphometric methods used in this thesis use Procrustes superimposition to scale for the isometric effects of size (Adams et al. 2004, Gower 1975, Rohlf & Slice 1990, Goodall 1991), but do not control for allometric variation. There are not any accepted methods for controlling for allometry in geometric morphometric data, except for those based on the use of principal component scores (e.g. Cardini et al. 2010, Elton et al. 2010, Gilbert 2011), but the use of principal components as the basis for phylogenetic analysis is disputed and controversial (see Adams et al. 2011). One method to control for size differences using Procrustes residuals was regression against centroid, and log centroid size, but the results of phylogenetic analysis were practically identical when compared to non-regressed analyses (these results are not presented). Whilst the development of new methods to integrate the effects of allometry into geometric morphometric analysis are of interest and something to be welcomed, this was beyond the scope of the current project.

8.13 The platyrrhine fossil record

Future work will seek to incorporate several relatively well preserved fossil platyrrhines into phylogenetic analysis. Of the earliest platyrrhine fossils, *Homunculus patagonicus* (CORD-PZ 1.130), *Tremacebus harringtoni* (FLM 619) and *Dolichocebus gaimanensis* (MACN 14128) are quite distorted and fragmentary, and may not be suitable for geometric

morphometric analysis. *Chilecebus carrascoensis* (SGOPV 3213) is better preserved, and it would be interesting to see if it shares an affinity with the extant cebines (Fleagle & Tejedor 2002). A very well preserved Antillean craniodental specimen of *Antillothrix bernensis* (PN-09-01) was recently discovered and described by Kay et al. (2010) and Rosenberger et al. (2010). Geometric morphometric analysis could certainly make an important contribution, as Rosenberger et al. (2010) view *Antillothrix* as a cebine, whereas Kay et al. (2010) suggest the taxa are most likely a stem platyrrhine, but they appear to have several howler monkey traits such as frontal-sphenoid contact (Fleagle 1999). The partial palate of *Xenothrix mcgregori* (AMNHM 268006) and *Paralouatta varonai* (MNHNH V 194) are also Antillean specimens that could be sampled in future platyrrhine work and compared with the comparative extant dataset. Two well preserved Pleistocene atelids, *Protopithecus brasiliensis* (IGC-UFMG 06) and *Caipora bambuiorum* (IGC-UFMG 05), should be sampled for future phylogenetic analysis.

8.14 A comparative view from carnivores

There is a lack of comparable phylogenetic analyses to those described in this thesis, that have used geometric morphometric data from a clade with as many genera or such high levels of morphological variation. However, carnivores in particular present an interesting comparative group, as they include a large number of genera with extensive diversity in craniodental morphology, incorporating a range of body sizes and dietary specialisations, and relatively high levels of homoplasy. This is at best a brief review and far from exhaustive, but several of the studies discussed can help to interpret the results presented in this thesis and better understand platyrrhine evolution.

Two very different relationships between carnivore morphology and diet have been proposed. Figueirido et al. (2011) used eigenshape analysis to analyse carnivore craniodental morphology, finding a strong phylogenetic signal and role for phylogeny in shaping morphology, much like Perez et al. (2011) found for the platyrrhines. They viewed phylogeny, function and natural selection as exerting a constraint within clades to restrict morphological variation and major homoplasy between hypercarnivores in separate families. The alternative view (e.g Wroe & Milne 2007, Goswami et al. 2011) is that diet and feeding ecology have directed carnivore evolution, and convergence is more common, although phylogeny does contribute to shaping craniodental morphology. Such a view is similar to that promoted throughout this chapter and by Marroig & Cheverud (2001), that multiple

biological factors are responsible for shaping platyrrhine craniodental evolution. Principal component analysis of morphometric data showed clear divergence along principal component one from hypercarnivores to omnivorous and insectivorous taxa (Goswami et al. 2011). There is often a correlation between size and PC1, so the results may detail a joint link between size, allometry and diet in carnivore morphological evolution. There is an underlying phylogenetic signal present, as most families clustered together, even within groups where taxa specialised in herbivory, omnivory and hypercarnivory (Goswami et al. 2011).

Carnivores and marsupials share a fundamental relationship between morphology, diet and bite force For example predators of large prey have a shortened skull and snout, although the relationship between form and diet is stronger in marsupials, and carnivore evolution is more closely related to, and constrained by, phylogeny (Wroe & Milne 2007). The carnivore connection between diet and phylogeny is similar to platyrrhines, although allometry and encephalization have increased importance in the latter. Marsupial taxa separate along the first principal component according to diet (Wroe & Milne 2007). Marsupials are especially interesting because they have reduced brain size compared to carnivores, and increased masseter muscles and more robust zygomatic arches linked to mastication and feeding behaviour (Wroe & Milne 2007). Such diversification is reminiscent of the howler monkeys, which have extremely robust zygomatic arches, and have experienced a major reduction in brain size (Hartwig et al. 1996, Kinzey 1997, Isler et al. 2008).

The carnivores include several clades of interest, such as the cat family (Felidae) that have a clear relationship between morphology, size and diet, and include several hypercarnivore taxa, that consume only vertebrate flesh. Larger felids consume larger prey, requiring wider jaw gapes that involves lengthening the palate at the cost of reduced bite force, a similar relationship between increased jaw gape and reduced bite force occurs in marmosets (Taylor et al. 2009), but due to the prolonged stress involved in killing larger prey they also have stronger, thicker skulls that respond more effectively to stress (Slater & van Valkenburgh 2009). The sabertooth fossil taxa, thought to be phylogenetically distinct from felids, displayed a pattern of "repeated parallel convergence" (Slater & Van Valkenburgh 2008:p414) both with extant and other fossil groups, that is at least partially influenced by allometric variation, much like the pattern of convergence shared by *Aotus* and *Callicebus*, *Callithrix* and *Saguinus*, and potentially extant and fossil platyrrhines. The dog family (Canidae) are also predators of vertebrates, although they exhibit greater variation in predator strategies (Slater et al. 2009). Canids repeatedly evolved convergent change in jaw

morphology in response to the type of prey hunted, with short robust jaws that exert greater bite force adapted for a diet of large prey, and long, narrow jaws that close faster adapted for smaller prey that are faster and more mobile (Slater et al. 2009). Although not predators, the folivore atelids *Brachyteles* and *Alouatta* exhibit a large mandible whereas the frugivorous *Ateles* has a much more narrow, gracile mandible. Homoplasy and convergence is clearly a common thread across mammalian groups, where mastication, diet and allometry help shape craniodental morphology.

Homoplasy in carnivores is not restricted to hypercarnivores, and are as common in herbivores. Figueirido et al. (2010) quantified extensive convergence linked to herbivory and exertion of high bite forces, including a robust mandible and mandibular corpus, well-developed zygomatic arches, a brachycephalic cranium and short neurocranium, with multiple traits similar to those found in howler monkeys. Within bears (Ursidae), there is a clear morphological distinction between herbivory and carnivory, underlying the clear relationship between diet and morphology (Figueirido et al. 2009). Herbivorous bears share short vaulted skulls and robust zygomatic arches, whereas carnivorous bears share large, flattened crania and changes in orbit and zygomatic orientation. In the case of the polar bear there is also rapid diversification, with an increased rate of morphological change, upon exploitation of a carnivorous diet, which could also occur in the platyrrhine adaptive radiations linked to exploitation of insectivory, exudativory, folivory or frugivory (Slater et al. 2010).

These case studies from carnivores show that craniodental morphological variation within large groups such as platyrrhines are synonymous with homoplasy and convergence (Wroe & Milne 2007, Goswami et al. 2011, Slater & Van Valkenburgh 2008, Slater et al. 2009). However, the carnivore groups appear to have a much stronger link between morphology and diet, in particular bite force and strain (Figueirido et al. 2010, Slater et al. 2009). This could relate to primate evolution being an exception to the mammalian rule due the greater significance of brain size and encephalization in shaping craniodental morphology, possibly placing greater constraints on morphological variation and promoting greater resistance to the divergent forces of mastication and diet (Martin 1990, Fleagle 1999). Possibly, the carnivore skull is shaped by bite force and strain because the levels of force exerted are so much greater, with platyrrhines placing much less strain on the skull. Irrespective of these differences, the carnivore example shows that morphological divergence and variation naturally leads to high levels of homoplasy, such as found in the platyrrhine skull.

Chapter 9 Conclusion

The aim of this thesis was to collect a unique morphological dataset from platyrrhine primates and investigate the phylogenetic relationships inferred by morphometric data from different craniodental regions. Phylogenetic analysis of all platyrrhines, including 16 genera and 50 species, supported several molecular clades, but the overall phylogenetic signal was relatively weak. The results are not straight forward, but it seems that there is a phylogenetic signal in craniodental region for the major platyrrhine clades of atelids, pitheciids and cebids. However, the phylogenetic signal is mostly lost when sampling all 16 platyrrhine genera and including them in one single analysis, although several molecular clades are supported. The combination of multiple genera with large amounts of size, morphological, ecological and behavioural variation, and associated homoplasy, may explain why this and past morphology-based cladistic analyses failed to reach consensus with the platyrrhine molecular phylogeny (Rosenberger 1984, Ford 1986, Kay 1990, Wildman et al. 2009, Perelman et al. 2011).

The pitheciids had a strong phylogenetic signal from whole skull, facial and cranial base morphology, whereas only the atelid face maintained a strong phylogenetic signal. In cebids the whole skull and facial morphology supported the three major clades for callitrichines, cebines and owl monkeys, but phylogenetic relationships within callitrichines were incongruent with current molecular phylogenies. The results presented support a major role for modularity in the platyrrhine skull, with facial and cranial base morphology often inferring alternative phylogenetic relationships. The strong phylogenetic signal in facial morphology is a surprising result, as the region is considered vulnerable to homoplasy and more plastic than other craniodental regions (Lieberman 1995, Wood & Lieberman 2001, Smith et al. 2007). In platyrrhines, facial morphology may be more conserved, less plastic and vulnerable to homoplasy, with a greater number of functional roles and importance in functional systems than previously acknowledged. Cranial base morphology had a weaker phylogenetic signal than found in other primate clades (e.g. Harvati & Weaver 2006a, Lockwood et al. 2004, Cardini & Elton 2008), and the role of mastication was quite strong in shaping cranial base morphology. Overall skull shape maintained a strong phylogenetic signal in pitheciids and cebids, but it was weak in atelids due to adaptations for frugivory in *Ateles* and *Lagothrix*.

More generally, the results presented in this thesis indicate that platyrrhine craniodental morphology has been shaped by an interaction between phylogeny, diet and mastication,

allometry, encephalization, with social and ecological factors also important. Testing of various outgroups and outgroup combinations indicated the appropriate outgroup is specific to the clade and module examined, although strepsirrhines did appear to perform better overall, probably because they set a clear polarity between ingroup and outgroup taxa, whereas Old World anthropoids share homoplasy to varying degrees with platyrrhines. Although some platyrrhine taxa do have significant and large levels of body size dimorphism (Ford & Davis 1992), sexual dimorphism had a limited role in the accuracy of phylogenetic inference, and neither male, female, or pooled sex data was more reliable than the other.

The methodological approach used in this thesis, combining geometric morphometrics and distance-based phylogenetic analysis, should encourage the use of these same methods in other primate and mammalian groups. Future phylogenetic analyses will seek to integrate the platyrrhine fossil record, including *Protopithecus*, *Caipora*, *Antillothrix* and *Dolichocebus*, but phylogenetic analysis including all extant platyrrhine genera will need to consider how to achieve greater congruence between morphological and molecular analyses when the entire platyrrhine clade are used in the same single phylogenetic analysis.

Appendix

Table 29: Taxa sampled, location, museum collection specimens belonged to, and sample sizes

Genus	Species	Subspecies	Location	Museum	Male	Female	Pooled
Alouatta	*belzebul*	*belzebul* *nigerrima* *ululata*	Brazil	Field Museum of Natural History, Chicago Natural History Museum, London	10	10	20
	caraya		Argentina Bolivia Brazil Paraguay	Field Museum of Natural History, Chicago Natural History Museum, London Smithsonian National Museum of Natural History, Washington DC	9	11	20
	fusca	*clamitans*	Brazil	Museum fur Naturkunde, Berlin Natural History Museum, London Naturhistorische Museum Wien	9	9	18
	palliata	*palliata*	Costa Rica Ecuador Nicaragua Panama	Natural History Museum, London Naturhistorische Museum Wien	18	13	31
	seniculus	*seniculus*	Bolivia Brazil Colombia Dutch Guiana Ecuador Guyana Trinidad Venezuela	Natural History Museum, London	22	10	32
	pigra		Mexico	Smithsonian National Museum of Natural History, Washington DC	8	10	18
	coibensis	*coibensis*	Panama	Smithsonian National Museum of Natural History, Washington DC	8	9	17

Ateles	*paniscus*	*paniscus*	Brazil French Guiana Peru	Field Museum of Natural History, Chicago Natural History Museum, London	7	12	19
	belzebuth	*belzebuth* *hybridus* *marginatus* *unknown*	Brazil Peru Venezuela	Field Museum of Natural History, Chicago Natural History Museum, London Naturhistoriska Riksmuseet, Stockholm	11	10	21
	fusciceps	*fusciceps* *robustus*	Colombia Ecuador	Field Museum of Natural History, Chicago Smithsonian National Museum of Natural History, Washington DC	10	10	20
	geoffroyi	*vellerosus*	Mexico Nicaragua	Field Museum of Natural History, Chicago Naturhistorische Museum Wien Anthropological Institue & Museum, University of Zurich	10	10	20
Lagothrix	*lagothrica*		Colombia	Field Museum of Natural History, Chicago Smithsonian National Museum of Natural History, Washington DC	10	10	20
	lugens		Colombia	Field Museum of Natural History, Chicago	8	10	18
	poeppigii		Ecuador Peru	Field Museum of Natural History, Chicago Natural History Museum, London Smithsonian National Museum of Natural History, Washington DC	10	10	20

	cana		Brazil Ecuador Peru	Field Museum of Natural History, Chicago Natural History Museum, London Smithsonian National Museum of Natural History, Washington DC	10	11	21
Brachyteles	*arachnoides*	*geoffroy* *hypoxanthus*	Brazil	Museum fur Naturkunde, Berlin Field Museum of Natural History, Chicago Natural History Museum, London Smithsonian National Museum of Natural History, Washington DC	7	5	12
Callicebus	*moloch*		Bolivia Brazil Colombia Peru	Natural History Museum, London	13	15	28
	torquatus	*lucifer* *lugens* *medemi*	Brazil Colombia Peru Venezuela	Museum fur Naturkunde, Berlin Field Museum of Natural History, Chicago Natural History Museum, London Naturhistoriska Riksmuseet, Stockholm Smithsonian National Museum of Natural History, Washington DC	12	9	21
	cupreus	*discolour* *ornatus*	Colombia Ecuador Peru	Field Museum of Natural History, Chicago	10	9	19
	hoffmannsi	*baptista* *hoffmannsi*	Brazil	Field Museum of Natural History, Chicago Naturhistoriska Riksmuseet, Stockholm	9	10	19

Cacajao	*melanocephalus*	*ouakary*	Brazil Colombia Venezuela	Museum fur Naturkunde, Berlin Field Museum of Natural History, Chicago Natural History Museum, London Naturhistoriska Riksmuseet, Stockholm	13	17	30
	calvus	*ucayalii*	Brazil Peru	Museum fur Naturkunde, Berlin Field Museum of Natural History, Chicago Natural History Museum, London	13	10	23
Chiropotes	*satanas*		Brazil Guyana Surinam	Museum fur Naturkunde, Berlin Field Museum of Natural History, Chicago Natural History Museum, London Naturhistoriska Riksmuseet, Stockholm Anthropological Institue & Museum, University of Zurich	14	9	23
Pithecia	*pithecia*		Brazil Guyana Surinam	Museum fur Naturkunde, Berlin Field Museum of Natural History, Chicago Natural History Museum, London	12	10	22
	monachus		Brazil Ecuador Peru	Natural History Museum, London	14	13	27
Cebus	*capucinus*	*capucinus*	Colombia Panama Venezuela	Field Museum of Natural History, Chicago Natural History Museum, London	10	10	20

	albifrons	*adustus* *leucocephalus* *unicolor*	Brazil Colombia Ecuador Peru Trinidad Venezuela	Field Museum of Natural History, Chicago Natural History Museum, London	10	10	20
	apella		Bolivia Brazil Dutch Guiana French Guiana Guyana Peru Surinam	Museum fur Naturkunde, Berlin Natural History Museum, London Naturhistoriska Riksmuseet, Stockholm Naturhistorische Museum Wien Anthropological Institue & Museum, University of Zurich	92	60	152
	nigrivittatus	*castaneus* *nigrivittatus*	Guyana Suriname Venezuela	Field Museum of Natural History, Chicago Natural History Museum, London Smithsonian National Museum of Natural History, Washington DC	10	10	20
	libidinosus		Brazil	Smithsonian National Museum of Natural History, Washington DC	11	10	21
Saimiri	*sciureus*		Bolivia Brazil Dutch Guiana Ecuador Guyana Peru	Natural History Museum, London	33	15	48

	oerstedii	*citrinellus* *oerstedii*	Costa Rica Panama	Field Museum of Natural History, Chicago Natural History Museum, London Naturhistorische Museum Wien Smithsonian National Museum of Natural History, Washington DC Anthropological Institue & Museum, University of Zurich	11	9	20
	bolviensis	*boliviensis* *peruviensis*	Peru	Field Museum of Natural History, Chicago	10	10	20
	ustus		Brazil	Field Museum of Natural History, Chicago Naturhistoriska Riksmuseet, Stockholm Smithsonian National Museum of Natural History, Washington DC	10	6	16
Aotus	*trivirgatus*		Bolivia Brazil Colombia Ecuador Paraguay Peru	Natural History Museum, London	13	11	24
	azarai	*azarai* *boliviensis*	Bolivia Brazil Paraguay Peru	Field Museum of Natural History, Chicago Naturhistoriska Riksmuseet, Stockholm	6	10	16
	lemurinus	*griseimembra* *lemurinus*	Venezuela	Field Museum of Natural History, Chicago Smithsonian National Museum of Natural History, Washington DC	10	10	20

	vociferans		Colombia Ecuador Peru	Field Museum of Natural History, Chicago	10	10	20
Leontopithecus	*rosalia*	*rosalia*	Brazil Captive	Museum fur Naturkunde, Berlin Field Museum of Natural History, Chicago Natural History Museum, London Naturhistoriska Riksmuseet, Stockholm Smithsonian National Museum of Natural History, Washington DC	11	13	24
Callithrix	*jacchus*	*flaviceps* *jacchus*	Brazil	Natural History Museum, London	8	7	15
	argentata	*argentata* *leucippe* *melanura*	Bolivia Brazil	Museum fur Naturkunde, Berlin Field Museum of Natural History, Chicago Natural History Museum, London Naturhistoriska Riksmuseet, Stockholm Smithsonian National Museum of Natural History, Washington DC	11	10	21
	humeralifer	*chrysoleuca* *humeralifer*	Brazil	Field Museum of Natural History, Chicago Naturhistoriska Riksmuseet, Stockholm	11	9	20
	penicillata		Brazil	Museum fur Naturkunde, Berlin Natural History Museum, London Smithsonian National Museum of Natural History, Washington DC Anthropological Institue & Museum, University of Zurich	18	14	32

	pygmaea	*pygmaea*	Brazil Ecuador Peru Captive	Field Museum of Natural History, Chicago Natural History Museum, London Smithsonian National Museum of Natural History, Washington DC Anthropological Institue & Museum, University of Zurich	10	9	19
Callimico	*goeldii*		Captive	Field Museum of Natural History, Chicago Naturhistorische Museum Wien Anthropological Institue & Museum, University of Zurich	11	11	22
Saguinus	*midas*	*midas* *tamarin*	Brazil Guyana Surinam	Museum fur Naturkunde, Berlin Natural History Museum, London Naturhistoriska Riksmuseet, Stockholm	12	10	22
	fuscicollis	*lagonatus* *leucogenys* *melanoleucus* *nigrifrons* *tripartitus* *weddelli*	Brazil Ecuador Peru	Natural History Museum, London	27	11	38
	mystax	*mystax* *pileatus* *pluto*	Brazil Peru	Field Museum of Natural History, Chicago Natural History Museum, London Naturhistoriska Riksmuseet, Stockholm Smithsonian National Museum of Natural History, Washington DC	10	11	21

	leucopus		Colombia	Field Museum of Natural History, Chicago Natural History Museum, London Smithsonian National Museum of Natural History, Washington DC	9	9	18
	geoffroyi		Colombia	Field Museum of Natural History, Chicago	10	9	19
Outgroups							
Hylobates	*lar*	*entelloides*	Malaysia Thailand	Natural History Museum, London	10	10	20
Macaca	*mulatta*	*mulatta* *villosa*	India	Natural History Museum, London	9	10	19
Perodicticius	*potto*		Equatorial Guinea	Anthropological Institue & Museum, University of Zurich	10	10	20
Colobus	*guerza*	*guerza* *dodingae* *matschiei* *occidentalis*	Ehtiopia Sudan Kenya Uganda Chad	Natural History Museum, London	11	10	21
Cercopithecus	*aethiops*	*aethiops* *sabreus* *arenarius* *zavattarii* *matschiei*	Sudan Sierra Leone Kenya Ethoipia Senegal Ghana	Natural History Museum, London	10	10	20
Trachypithecus	*obscura*	*obscura* *flavicauda* *seimundi* *halonifer*	Malaysia Thailand Burma	Natural History Museum, London	10	10	20

Otolemur	*garnetti*	*garnetti* *panganiensis* *lasiotis* *kikuyuensis*	Tanzania Kenya	Natural History Museum, London	10	9	19
Galago	*senegalensis*	*alpipes*	Uganda	Natural History Museum, London	10	11	21
Eulemur	*fulvus*	*fulvusa* *albifrons* *albocollaris* *mayotiensis* *rufus*	Madagascar	Natural History Museum, London	10	10	20

References

Ackermann, R. R. and Cheverud, J. M. (2000). "Phenotypic covariance structure in tamarins (genus *Saguinus*): A comparison of variation patterns using matrix correlation and common principal component analysis." *American Journal of Physical Anthropology* **111**: 489-501.

Adams, D. C., Cardini, A., Monteiro, L. R., O'Higgins, P. and Rohlf, F. J. (2011). "Morphometrics and phylogenetics: principal components of shape from cranial modules are neither appropriate nor effective cladistic characters." *Journal of Human Evolution* **60**: 240-243.

Adams, D. C., Rohlf, F. J. and Slice, D. E. (2004). "Geometric morphometrics: Ten years of progress following the 'revolution'." *Italian Journal of Zoology* **71**: 5-16.

Aguiar, J. M. and Lacher jr, T. E. (2009). Cranial morphology of the dwarf marmoset *Callibella* in the context of callitrichid variability. The Smallest Anthropoids: The Marmoset/Callimico Radiation. Ford, S. M., Porter, C. A. and Davis, L. C. New York, Springer**:** 355-380.

Allman, E. S. and Rhodes, J. A. (2010). "Trees, fast and accurate." *Science* **327**: 1334-1335.

Andrews, P. and Martin, L. (1987). "Cladistic relationships of extant and fossil hominoids." *Journal of Human Evolution* **16**: 101-118.

Archie, J. W. (1985). "Methods for coding variable morphological features for numerical taxonomic analysis." *Systematic Biology* **34**: 326-345.

Baba, M., Darga, L. and Goodman, M. (1979). "Immunodiffusion systematics of the primates. Part V. The platyrhini." *Folia Primatologica* **32**: 207-238.

Baba, M., Darga, L. and Goodman, M. (1980). Biochemical evidence on the phylogeny of Anthropoidea. Evolutionary Biology of the New World Monkeys and Continental Drift. Ciochon, R. L., Chiarelli, A.B. New York, Plenum Press**:** 423-444.

Bailey, R. C. and Byrnes, J. (1990). "A new, old method for assessing measurement error in both univariate and multivariate morphometric studies." *Systematic Zoology* **39**: 124-130.

Barroso, C. M. L., Schneider, H., Schneider, M. P. C., Sampaio, I., Harada, M. L., Czelusniak, J. and Goodman, M. (1997). "Update on the phylogenetic systematics of New World monkeys: Further DNA evidence for placing the pygmy marmoset (*Cebuella*) within the genus *Callithrix*." *International Journal of Primatology* **18**: 651-674.

Bartlett, T. Q. (2007). The hylobatidae: small apes of Asia. Primates In Perspective. Campbell, C. J., Fuentes, A., MacKinnon, K.C., Panger, M., Beader, S.K. Oxford, Oxford University Press**:** 274-289.

Bastir, M., Rosas, A., Stringer, C., Cuetara, J. M., Kruszynski, R., Weber, G. W., Ross, C. F. and Ravosa, M. (2010). "Effects of brain and facial size on basicranial form in human and primate evolution." *Journal of Human Evolution* **58**: 424-431.

Bastir, M., Rosas, A., Tabernero, A. G., Pena-Melian, A., Estalrrich, A., de la Rasilla, M. and Fortea, J. (2010). "Comparative morphology and morphometric assessment of the Neandertal occipital remains from the El Sidrón site (Asturias, Spain: years 2000–2008)." *Journal of Human Evolution* **58**: 68-78.

Baum, D. A., Smith, S. D. and Donovan, S. S. S. (2005). "The tree-thinking challenge." *Science* **310**: 979-980.

Begun, D. R. (1992). "Miocene fossil hominoids and the chimp-human clade." *Science* **257**: 1929-1933.

Begun, D. R. (1994). "Relationships among the great apes and humans: New interpretations base on the fossil great ape *Dryopithecus*." *Yearbook of Physical Anthropology* **37**: 11-63.

Bjarnason, A., Chamberlain, A. T. and Lockwood, C. A. (2011). "A methodological investigation of hominoid craniodental morphology and phylogenetics." *Journal of Human Evolution* **60**: 47-57.

Boinski, S. and Cropp, S. (1999). "Disparate datasets resolve squirrel monkey (*Saimiri*) taxonomy: Implications for behavioral ecology and biomedical usage." *International Journal of Primatology* **20**: 237-256.

Boinski, S., Sughrue, K., Selvaggi, L., Quatrone, R., Henry, M. and Cropp, S. (2002). "An expanded test of the ecological model of primate social evolution: competitive regimes and female bonding in three species of squirrel monkeys (*Saimiri oerstedii, S. boliviensis* and *S. sciureus*)." *Behaviour* **139**: 227-261.

Bookstein, F. L. (1991). Morphometric tools for landmark data: Geometry and biology. Cambridge, Cambridge University Press.

Bowler, M. and Bodmer, R. (2009). "Social behaviour in fission–fusion groups of red uakari monkeys (*Cacajao calvus ucayalii*)." *American Journal of Primatology* **71**: 976-987.

Bromham, L. and Penny, D. (2003). "The modern molecular clock." *Nature Reviews Genetics* **4**: 216-224.

Brown, J. H., West, G. B. and Enquist, B. J. (2000). Scaling in biology: Patterns and processes, causes and consequences.Oxford, Oxford University Press.

Cameron, D. W. and Groves, C. P. (2004). Bones, stones and molecules: "Out of Africa" and human origins. London, Elsevier Academic Press.

Canavez, F. C., Moreira, M. A. M., Ladasky, J. L., Pissinatti, A., Parham, P. and Seuanez, H. N. (1999a). "Molecular phylogeny of New World primates (Platyrrhini) based on β2-microglobulin DNA sequences." *Molecular Phylogenetics and Evolution* **12**: 74-82.

Canavez, F. C., Moreira, M. A. M., Simon, F., Parham, P. and Seuanez, H. N. (1999b). "Phylogenetic relationships of the callitrichinae (Platyrrhini, Primates) based on B2-microglobulin DNA sequences." *American Journal of Primatology* **48**: 225-236.

Cardini, A., Diniz Filho, J. A. F., Polly, P. D. and Elton, S. (2010). Biogeographic analysis using geometric morphometrics: Clines in skull size and shape in a widespread African arboreal monkey. Morphometrics for Nonmorphometricians, Lecture Notes in Earth Sciences 124. Elewa, A. M. T. Heidelberg, Springer-Verlag Publishers.

Cardini, A. and Elton, S. (2008). "Does the skull carry a phylogenetic signal? Evolution and modularity in the guenons." *Biological Journal of the Linnean Society* **93**: 813-834.

Cardini, A., Jansson, A. U. and Elton, S. (2007). "A geometric morphometric approach to the study of ecogeographical and clinal variation in vervet monkeys." *Journal of Biogeography* **34**: 1663-1678.

Catalano, S. A., Goloboff, P. A. and Giannini, N. P. (2010). "Phylogenetic morphometrics (I): The use of landmark data in a phylogenetic framework." *Cladistics* **26**: 539-549.

Caumal, R. and Polly, P. D. (2005). "Phylogenetic and environmental components of morphological variation: Skull, mandible, and molar shape in marmots (Marmota, Rodentia)." *Evolution* **59**: 2460-2472.

Cavalli-Sforza, L. L. and Edwards, A. W. F. (1967). "Phylogenetic analysis: models and estimation procedures." *American Journal of Human Genetics* **19**: 233-257.

Chamberlain, A. T. and Wood, B. A. (1987). "Early hominid phylogeny." *Journal of Human Evolution* **16**: 119-133.

Chatterjee, H. J., Ho, S. Y. W., Barnes, I. and Groves, C. (2009). "Estimating the phylogeny and divergence times of primates using a supermatrix approach." *BMC Evolutionary Biology* **9**: 1-19.

Chaves, F. C., Moreira, M. A., Ladasky, J. J., Pissinatti, A., Parham, P. and Seuanez, H. N. (1999). "Molecular phylogeny of New World primates (Platyrrhini) based on beta2-microglobulin DNA sequences." *Molecular Phylogenetics and Evolution* **12**: 74-82.

Cheverud, J. (1982). "Relationship among ontogenetic, static, and evolutionary allometry." *American Journal of Physical Anthropology* **59**: 139-149.

Cheverud, J. M. (1982). "Phenotypic, genetic, and environmental morphological integration in the cranium." *Evolution* **36**: 499-516.

Cheverud, J. M. (1995). "Morphological integration in the saddle-back tamarin (*Saguinus fuscicollis*) cranium." *The American Naturalist* **145**: 63-89.

Cheverud, J. M. (1996a). "Quantitative genetic analysis of cranial morphology in the cotton-top (*Saguinus oedipus*) and saddle-back (*S. fuscicollis*) tamarins." *Journal of Evolutionary Biology* **9**: 5-42.

Cheverud, J. M. (1996b). "Developmental integration and the evolution of pleiotropy." *American Zoologist* **36**: 44-50.

Chiou, K. L., Pozzi, L., Lynch Alfaro, J. W. and Di Fiore, A. (2011). "Pleistocene diversification of living squirrel monkeys (*Saimiri* spp.) inferred from complete mitochondrial genome sequences." *Molecular Phylogenetics and Evolution* **59**: 736-745.

Ciochon, R. L. and Chiarelli, A. B. (1980). Paleobiographical perspectives on the origin of the platyrrhini. Evolutionary biology of New World monkeys and continental drift. Ciochon, R. L. and Chiarelli, A. B. New York, Plenum Press**:** 459-493.

Cole, T. M. I. (1992). "Postnatal heterochrony of the masticatory apparatus in *Cebus apella* and *Cebus albifrons*." *Journal of Human Evolution* **23**: 253-282.

Cole, T. M. I. (1995). Comparative craniometry of the Atelinae (Platyrrhini, Primates): Function, development, and evolution. New York, SUNY Stony Brook. **PhD dissertation**.

Cole, T. M. I., Lele, S. and Richtsmeier, J. T. (2002). A parametic bootstrap approach to the detection of phylogenetic signals in landmark data. Morphology, Shape, and Phylogenetics. Macleod, N. F., P. London, Taylor & Francis**:** 194-219.

Collard, M. and Wood, B. (2000). "How reliable are human phylogenetic hypotheses?" *PNAS* **97**: 5003-5006.

Colless, D. H. (1995). "On the status of outgroups in phylogenetics." *Systematic Zoology* **34**: 364-366.

Corner, B. D. and Richtsmeier, J. T. (1991). "Morphometric analysis of craniofacial growth in *Cebus apella*." *American Journal of Physical Anthropology* **84**: 323-342.

Corner, B. D. and Richtsmeier, J. T. (1992). "Cranial growth in the squirrel monkey (*Saimiri sciureus*): A quantitative analysis using three dimensional coordinate data." *American Journal of Physical Anthropology* **87**: 67-81.

Couette, S., Escarguel, G. and Montuire, S. (2005). "Constructing, bootstrapping, and comparing morphometric and phylogenetic trees: A case study of New World monkeys (Platyrrhini, Primates)." *Journal of Mammalogy* **86**: 773-781.

Creel, N. (1986). "Size and phylogeny in hominoid primates." *Systematic Zoology* **18**: 81-99.

Cronin, J. E. and Sarich, V. M. (1975). "Molecular systematics of the New World monkeys." *Journal of Human Evolution* **4**: 357-375.

Cronin, J. E. and Sarich, V. M. (1978). "Marmoset evolution: The molecular evidence." *Primates Med.* **10**: 12-19.

Cropp, S. and Boinski, S. (2000). "The central american squirrel monkey (*Saimiri oerstedii*): Introduced hybrid or endemic species?" *Molecular Phylogenetics and Evolution* **16**: 350-365.

Cropp, S. J., Larson, A. and Cheverud, J. (1999). "Historical biogeography if tamarins, genus Saguinus: The molecular phylogenetic evidence." *American Journal of Physical Anthropology* **108**: 65-89.

de Oliveira, F. B., Molina, E. C. and Marroig, G. (2009). Paleogeography of the South Atlantic: A route for primates and rodents into the New World? South American Primates Comparative Perspectives in the Study of Behaviour, Ecology, and Conservation. Garber, P. A., Estrada, A., Bicca-Marques, J. C., Heymann, E. W. and Strier, K. B. New York, Springer.

Delson, E., Eldredge, N. and Tattersall, I. (1977). "Reconstruction of hominid phylogeny: A testable framework based on cladistic analysis." *Journal of Human Evolution* **6**: 263-278.

Delson, E. and Rosenberger, A. L. (1984). Are there any anthropoid primate "living fossils"? Casebook on Living Fossils. Eldredge, N. and Stanley, S. New York, Fischer Publishing.

Desper, R. and Gascuel, O. (2005). The Minimum-evolution distance-based approach to phylogenetic inference. Mathematics of Evolution and Phylogeny. Gascuel, O.**:** 1-32.

Di Fiore, A. (2009). Genetic approaches to the study of dispersal and kinship in New World primates. South American Primates: Comparative Perspectives in the Study of Behaviour, Ecology, and Conservation. Garber, P. A., Estrada, A., Bicca-Marques, J. C., Heymann, E. W. and Strier, K. B. New York, Springer**:** 211-250.

Di Fiore, A., Link, A. and Campbell, C. J. (2011). The atelines: Behaioural and socioecological diversity in a New World monkey radiation. Primates in Perspective. Campbell, C. J., Fuentes, A., MacKinnon, K. C., Bearder, S. K. and Stumpf, R. M. Oxford, Oxford University Press. **2nd:** 155-188.

Dial, K. P., Greene, E. and Irschick, D. J. (2008). "Allometry of behaviour." *Trends in Ecology and Evolution* **23**: 394-401.

Digby, L. J., Ferrari, S. F. and Saltzman, W. (2011). Callitrichines the role of competition in cooperatively breeding species. Primates In Perspective. Campbell, C. J., Fuentes, A., MacKinnon, K. C., Bearder, S. K. and Stumpf, R. M. Oxford, Oxford University Press. **2nd**.

Disotell, T. R. (1994). "Generic level relationships of the Papionini (Cercopithecoidea)." *American Journal of Physical Anthropology* **94**: 47-57.

Disotell, T. R., Honeycutt, R. L. and Ruvolo, M. (1992). "Mitochondrial DNA phylogeny of the Old-World monkey tribe Papionini." *Molecular Biology and Evolution* **9**: 1-13.

Dryden, I. L. and Mardia, K. V. (1998). Statistical Shape Analysis. Chichester, John Wiley & Sons.

Eaglen, R. H. (1980). The systematics of living strepsirrhini with special reference to the Lemuridae, Duke University. **PhD dissertation**.

Eaglen, R. H. (1983). "Parallelism, parsimony, and the phylogeny of the Lemuridae." *International Journal of Primatology* **4**: 249-273.

Edwards, A. W. F. and Cavalli-Sforza, L. L. (1963). "A method for cluster analysis." *Biometrics* **21**: 362-375.

Edwards, S. V. (2009). "Is a new and general theory of molecular systematics emerging?" *Evolution* **63**: 1-19.

Elton, S., Dunn, J. and Cardini, A. (2010). "Size variation facilitates population divergence but does not explain it all: An example study from a widespread African monkey." *Biological Journal of the Linnean Society* **101**: 823-843.

Enstam, K. L. and Isbell, L. A. (2007). The guenons (genus *Cercopithecus*) and their allies: Behavioural ecology of polyspecific associations. Primates In Perspective. Campbell, C. J., Fuentes, A., MacKinnon, K.C., Panger, M., Beader, S.K. Oxford, Oxford University Press**:** 252-273.

Erikson, G. E. (1963). "Brachiation in New World monkeys and in anthropoid apes." *Symposum of the Zoologcal Society London* **10**: 135-164.

Farris, J. S. (1970). "Methods for computing wagner trees." *Systematic Zoology* **19**: 83-92.

Farris, J. S. (1981). Distance data in phylogenetic analysis. Advances In Cladistics. Funk, V. A., Brooks, D.R. New York, New York Botanical Garden.

Fashing, P. J. (2007). African colobine monkeys: Patterns of between-group interaction. Primates In Perspective. Campbell, C. J., Fuentes, A., MacKinnon, K.C., Panger, M., Beader, S.K. Oxford, Oxford University Press**:** 201-223.

Fedigan, L. M., Rosenberger, A. L., Boinski, S., Norconk, M. A. and Garber, P. A. (1996). Critical issues in cebine evolution and behaviour. Adaptive Radiations of Neotropical Primates. Norconk, M. A., Rosenberger, A. L. and Garber, P. A. New Tork, Plenum Press.

Felsenstein, J. (1984). "Distance methods for inferring phylogenies: A justification." *Evolution* **38**: 16-24.

Felsenstein, J. (1997). "An alternating least squares approach to inferring phylogenies from pairwise distances." *Systematic Biology* **46**: 101-111.

Felsenstein, J. (2004). Inferring Phylogenies. Sunderland, MA, Sinauer Associates, Inc.

Felsenstein, J. (2005). PHYLIP (Phylogeny Inference Package), Department of Genome Sciences, University of Washington, Seattle.

Fernandez-Duque, E. (2011a). Aotinae social monogamy in the only nocturnal anthropoid. Primates In Perspective. Campbell, C. J., Fuentes, A., MacKinnon, K. C., Bearder, S. K. and Stumpf, R. M. Oxford, Oxford University Press.

Fernandez-Duque, E. (2011b). "Rensch's rule, bergmann's effect and adult sexual dimorphism in wild monogamous owl monkeys (*Aotus azarai*) of argentina." *American Journal of Physical Anthropology* **146**: 38-48.

Figueirido, B., Palmqvist, P. and Perez-Claros, J. A. (2009). "Ecomorphological correlates of craniodental variation in bears and paleobiological implications for extinct taxa: An approach based on geometric morphometrics." *Journal of Zoology* **277**: 70-80.

Figueirido, B., Serrano-Alarcon, F. J., Slater, G. J. and Palmqvist, P. (2010). "Shape at the cross-roads: Homoplasy and history in the evolution of the carnivoran skull towards herbivory." *Journal of Evolutionary Biology* **23**: 2579-2594.

Fitch, W. M. and Margoliash, E. (1967). "Construction of phyogenetic trees." Science **155**: 279-284.

Fleagle, J. G. (1984). Size and adaptation in primates. Size and Scaling in Primate Biology. Jungers, W. L. New York, Plenum Press.

Fleagle, J. G. (1999). Primate Adaptation and Evolution. San Diego, Academic Press.

Fleagle, J. G. and Kay, R. F. (1997). Platyrrhines, catarrhines and the fossil record. New World Primates: Ecology, Evolution and Behaviour. Kinzey, W. G. New York, Aldine**:** 3-24.

Fleagle, J. G. and Tejedor, M. F. (2002). Early platyrrhines of southern South America. The Primate Fossil Record. Hartwig, W. C. Cambridge, Cambridge University Press**:** 161-174.

Flores, D. and Casinos, A. (2011). "Cranial ontogeny and sexual dimorphism in two New World monkeys: *Alouatta caraya* (Atelidae) and *Cebus apella* (Cebidae)." *Journal of Morphology* **272**: 744-757.

Flynn, J. J. and Wyss, A. R. (1998). "Recent advances in South American mammalian paleontology." *Trends in Ecology and Evolution* **13**: 449-454.

Ford, S. M. (1980). "Callitrichids as phyletic dwarfs, and the place of the Callitrichidae in Platyrrhini." *Primates* **21**: 31-43.

Ford, S. M. (1980). A systematic revision of the Platyrrhini based on features of the postcranium. Pittsburgh, University of Pittsburgh. **PhD dissertation**.

Ford, S. M. (1982). A systematic revision of the Platyrrhini based on features of the postcranium.

Ford, S. M. (1986). Systematics of the New World monkeys. Comparative Primate Biology Volume 1: Systematics, Evolution, and Anatomy. Swindler, D. R., Erwin, J. New York, Alan R. Liss, Inc. **:** 73-135.

Ford, S. M. and Davis, L. C. (1992). "Systematics and body size: Implications for feeding adaptations in New World monkeys." *American Journal of Physical Anthropology* **88**: 415-468.

Ford, S. M. and Davis, L. C. (2009). Marmoset postcrania and the skeleton of the dwarf marmoset, *Callibella humilis*. The Smallest Anthropoids: The Marmoset/Callimico Radiation. Ford, S. M., Porter, C. A. and Davis, L. C. New York, Springer.

Fredslund, J. (2006). "Phyfi." from http://www.cs.au.dk/~chili/Phyfi/about.html.

Futuyma, D. J. (2005). Evolution. Sundreland, MA, Sinauer Associates.

Garber, P. A. (1992). "Vertical climbing, small body size, and the evolution of adaptations in the callitrichinae " *American Journal of Physical Anthropology* **88**(4): 469-482.

Garber, P. A. and Porter, C. A. (2011). The ecology of exudate production and exudate feeding in *Saguinus* and *Callimico*. The Evolution of Exudativory in Primates, Developments in Primatology: Progress and Prospects. Burrows, A. M. and Nash, L. T. New York, Springer**:** 89-108.

Garber, P. A., Rosenberger, A. L. and Norconk, M. A. (1996). Marmoset misconceptions. Adaptive radiations of neotropical primates. Norconk, M. A., Rosenberger, A.L., Garber, P.A. New York, Plenum Press**:** 87-95.

Gilbert, C. C. (2011). "Phylogenetic analysis of the African papionin basicranium using 3-D geometric morphometrics: The need for improved methods to account for allometric effects." *American Journal of Physical Anthropology* **144**: 60-71.

Gilbert, C. C., Frost, S. R. and Strait, D. S. (2009). "Allometry, sexual dimorphism, and phylogeny: A cladistic analysis of extant African papionins using craniodental data." *Journal of Human Evolution* **57**: 298-320.

Gilbert, C. C. and Rossie, J. B. (2007). "Congruence of molecules and morphology using a narrow allometric approach." *PNAS* **104**: 11910-11914.

Gingerich, P. D. (1980). Eocene adapidae, paleobiogeography, and the origin of South American Platyrrhini. Evolutionary Biology of the New World Monkeys and Continental Drift. Ciochon, R. L. and Chiarelli, A. B. New York, Plenum Press.

Gonzalez-Jose, R., Escapa, I., Neves, W. A., Cuneo, R. and Pucciarelli, H. M. (2008). "Cladistic analysis of continuous modularized traits provides phylogenetic signals in *Homo* evolution." *Nature* **453**: 775-778.

Gonzalez-Jose, R., Escapa, I., Neves, W. A., Cuneo, R. and Pucciarelli, H. M. (2011). "Morphometric variables can be analyzed using cladistic methods: A reply to Adams et al." *Journal of Human Evolution* **60**: 244-245.

Gonzalez-Jose, R., van der Molen, S., Gonzalez-Perez, E. and Hernandez, H. (2004). "Patterns of phenotypic covariation and correlation in modern humans as viewed from morphological integration." *American Journal of Physical Anthropology* **123**: 69-77.

Goodall, C. (1991). "Procrustes methods in the statistical analysis of shape." *Journal of the Royal Stastical Society. Series B (Methodological)* **53**: 285-339.

Goodman, M., Porter, C. A., Czelusniak, J., Page, S. L., Schneider, H., Shoshani, J., Gunnell, G. F. and Groves, C. P. (1998). "Toward a phylogenetic classification of Primates based on DNA evidence complemented by fossil evidence." *Molecular Phylogenetics and Evolution* **9**: 585-598.

Goswami, A. (2006a). "Cranial modularity shifts during mammalian evolution." *The American Naturalist* **168**: 270-280.

Goswami, A., Milne, B. S. and Wroe, S. (2011). "Biting through constraints: Cranial morphology, disparity and convergence across living and fossil carnivorous mammals." *Proceedings of the Royal Society B Biological Sciences* **278**: 1831-1839.

Gould, L. and Sauther, M. (2007). Lemuriformes. Primates In Perspective. Campbell, C. J., Fuentes, A., MacKinnon, K.C., Panger, M., Beader, S.K. Oxford, Oxford University Press**:** 46-72.

Gould, S. J. (1966). "Allometry and size in ontogeny and phylogeny." *Biological Reviews* **41**: 587-640.

Gower, J. C. (1975). "Generalized procrustes analysis." *Psychometrika* **40**: 33-51.

Gregory, T. R. (2008). "Understanding evolutionary trees." *Evolution Education & Outreach* **1**: 121-137.

Groves, C. (2001). Primate Taxonomy. Washington, Smithsonian Institution.

Groves, C. (2004). "The what, why and how of primate taxonomy." *International Journal of Primatology* **25**: 1105-1128.

Groves, C. P. (1986). Systematics of the great apes. Comparative Primate Biology: Systematics, Evolution and Anatomy. Swindler, D. R., Erwin, J. New York, Alan R. Liss. **1:** 187-217.

Groves, C. P. and Eaglen, R. H. (1988). "Systematics of the Lemuridae (Primates, Strepsirrhini)." *Journal of Human Evolution* **17**: 513-538.

Groves, C. P. and Paterson, J. D. (1991). "Testing hominoid phylogeny with the PHYLIP programs." *Journal of Human Evolution* **20**(2): 167-183.

Groves, C. P. and Trueman, J. W. H. (1995). "Lemurid systematics revisited." *Journal of Human Evolution* **28**: 427-437.

Haimoff, E. H., Chivers, D. J., Gittins, S. P. and Whitten, T. (1982). "A phylogeny of gibbons (*Hylobates* spp.) based on morphological and behavioural characters." *Folia Primatologica* **39**: 213-237.

Hallgrimsson, B., Lieberman, D. E., Liu, W., Hutchinson, A. F. and Jirik, F. R. (2007). "Epigenetic interactions and the structure of phenotypic variation in the cranium." *Evolution and Development* **9**: 76-91.

Hallgrimsson, B., Willmore, K., Dorval, C. and Cooper, D. M. L. (2004). "Craniofacial variability and modularity in macaques and mice." *Journal of Experimental Zoology. Part B. Molecular and Developmental Evolution* **302**: 207-225.

Hallgrimsson, B., Zelditch, M. L., Parsons, T. E., Kristensen, E., Young, N. M. and Boyd, S. K. (2008). Morphometrics and biological anthropology in the postgenomic age. Biological Anthropology of the Human Skeleton. Katzenberg, M. A. and Saunders, S. R. Hoboken, New Jersey, Wiley-Siss**:** 207-235.

Harada, M. L., Schneider, H., Schneider, M. P. C., Sampaio, I., Czeluzniak, J. and Goodman, M. (1995). "DNA evidence on the phylogenetic systematics of New World monkeys: Support for the sister-grouping of *Cebus* and *Saimiri* from two unlinked nuclear genes." *Molecular Phylogenetics and Evolution* **4**: 331-349.

Harcourt, C. (1986). "Seasonal variation in the diet of South African Galagos." *International Journal of Primatology* **7**: 491-506.

Hartwig-Scherer, S. (1993). Allometry in hominoids: A comparative study of skeletal growth trends, University of Zurich. **PhD dissertation**.

Hartwig, W. C. (1993). Comparative morphology, ontogeny and phylogeny of the platyrrhine cranium. Berkeley, University of California. **PhD dissertation**.

Hartwig, W. C. (1995). "Effect of life history on the squirrel monkey (Platyrrhini, *Saimiri*) cranium." *American Journal of Physical Anthropology* **97**: 435-449.

Hartwig, W. C. (1996). "Perinatal life history traits in New World monkeys." *American Journal of Primatology* **6**: 257-312.

Hartwig, W. C. (2005). "Implications of molecular and morphological data for understanding ateline phylogeny." *International Journal of Primatology* **26**: 999-1015.

Hartwig, W. C. (2011). Primate evolution. Primates in Perspective. Campbell, C. J., Fuentes, A., MacKinnon, K. C., Bearder, S. K. and Stumpf, R. M. Oxford, Oxford University Press. **2**.

Hartwig, W. C., Rosenberger, A. L., Garber, P. A. and Norconk, M. A. (1996). On Atelines. Adaptive Radiations of Neotropical Primates. Norconk, M. A., Rosenberger, A. L. and Garber, P. A. New York, Plenum Press**:** 427-431.

Harvati, K. and Weaver, T. (2006b). "Human cranial anatomy and the differential preservation of population history and climate signatures." *The Anatomical Record. Part A, Discoveries in molecular, cellular, and evolutionary biology* **288**: 1225-1233.

Harvati, K. and Weaver, T. D. (2006a). Reliability of cranial morphology in reconstructing neanderthal phylogeny. Neanderthals Revisited: New Approaches and Perspectives. Harvati, K., Harrison, T.L. Dordrecht, Springer**:** 239-254.

Hennig, W. (1966). Phylogenetic Systematics. Urbana, University of Illinois Press.

Hershkovitz, P. (1977). Living New World monkeys (Platyrrhini) with an introduction to primates. Chigaco, The University of Chicago Press.

Hershkovitz, P. (1983). "Two new species of night monkeys, genus *Aotus* (Cebidae, platyrrhini): A preliminary report on Aotus taxonomy." *American Journal of Primatology* **4**: 209-243.

Hershkovitz, P. (1984). "Taxonomy of squirrel monkeys genus *Saimiri* (Cebidae, Platyrrhini): A preliminary report with description of a hitherto unnamed form." *American Journal of Primatology* **6**: 257-312.

Hershkovitz, P. (1985). "A preliminary taxonomic review of the South American bearded saki monkeys genus *Chiropotes* (Cebidae, Platyrrhini), with the description of a new subspecies." *Fieldiana Zoology* **27**: 1-46.

Hershkovitz, P. (1987a). "The taxonomy of South American sakis, genus *Pithecia* (Cebidae, Platyrrhini): A preliminary report and critical review with the description of a new species and a new subspecies." *American Journal of Primatology* **12**: 387-468.

Hershkovitz, P. (1987b). "Uacaries, New World monkeys of the genus *Cacajao* (Cebidae, Platyrrhini): A preliminary taxonomic review with the description of a new subspecies." *American Journal of Primatology* **12**: 1-53.

Hershkovitz, P. (1990). "Titis, New World monkeys of the genus *Callicebus* (Cebidae, Platyrrhini): A preliminary raxonomic review." *Fieldiana, Zoology* **55**: 1-109.

Heymann, E. W. and Aquino, R. (2010). "Peruvian red uakaris (*Cacajao calvus ucayalii*) are not flooded-forest specialists." *International Journal of Primatology* **31**: 751-758.

Hodgson, J. A., Sterner, K. N., Matthews, L. J., Burrell, A. S., Jani, R. A., Raaum, R. L., Stewart, C. B. and Disotell, T. R. (2009). "Successive radiations, not statis, in the South American primate fauna." *PNAS* **106**: 5534-5539.

Horovitz, I. (1999). "A phylogenetic study of living and fossil platyrrhines." *American Museum Novitates* **3269**: 1-40.

Horovitz, I. and MacPhee, R. D. E. (1999). "The quaternary Cuban platyrrhine *Paralouatta varonai* and the origin of Antillean monkeys." *Journal of Human Evolution* **36**: 33-68.

Horovitz, I. and Meyer, A. (1995). "Systematics of the New World monkeys (Platyrrhini, Primates) based on 16s mitochondrial DNA sequences: A comparative analysis of different weighting methods in cladistic analysis." *Molecular Phylogenetics and Evolution* **4**: 448-456.

Horovitz, I. and Meyer, A. (1997). Evolutionary trends in the ecology of New World monkeys inferred from a combined phylogenetic analysis of nuclear, mitochondrial, and morphological data. Molecular evolution and Adaptive Radiation. Givnish, T. J., Sytsma, K.J. New York, Cambridge University Press**:** 189-224.

Horovitz, I., Zardoya, R. and Meyer, A. (1998). "Platyrrhine systematics: A simultaneous analysis of molecular and morphological Data." *American Journal of Physical Anthropology* **106**: 261-281.

Houle, A. (1999). "The origin of platyrrhines: An evaluation of the Antarctic scenario and the floating island model." *American Journal of Physical Anthropology* **109**: 541-559.

Huelsenbeck, J. P. (1995a). "Performance of phylogenetic methods in simulation." *Systematic Biology* **44**: 17-48.

Huelsenbeck, J. P. (1995b). "The robustness of two phylogenetic methods: Four-taxon simulations reveal a slight superiority of Maximum Likelihood over neighbor-joining." *Molecular Biology and Evolution* **12**(5): 843-849.

Hylander, W. L., Picq, P. C. and Johnson, K. R. (1991). "Masticatory-stress hypotheses and the supraorbital region of primates." *American Journal of Physical Anthropology* **86**: 1-36.

Isler, K., Kirk, E. C., Miller, J. M. A., Albrecht, G. A., Gelvin, B. R. and Martin, R. D. (2008). "Endocranial volume of primate species: Scaling analyses using a comprehensive and reliable dataset." *Journal of Human Evolution* **55**: 967-978.

Jack, K. (2011). The cebines toward an explanation of variable social structure. Primates In Perspective. Campbell, C. J., Fuentes, A., MacKinnon, K. C., Bearder, S. K. and Stumpf, R. M. Oxford, Oxford University Press.

Janson, C. H. and Boinski, S. (1992). "Morphological and behavioral adaptations for foraging in generalist primates: The case of the cebines." *American Journal of Physical Anthropology* **88**: 483-498.

Jardine, N. (1967). "The concept of homology in biology." *British Journal For The Philosophy of Science* **18**: 125-139.

Jenner, R. A. (2004). "Accepting partnership by submission? Morphological phylogenetics in a molecular millenium." *Systematic Biology* **53**: 333-359.

Jin, L. and Nei, M. (1990). "Limitations of the evolutionary parsimony method of phylogenetic analysis." *Molecular Biology and Evolution* **7**: 82-102.

Kay, R. F. (1975). "The functional adaptations of primate molar teeth." *American Journal of Physical Anthropology* **43**: 195-216.

Kay, R. F. (1990). "The phyletic relationships of extant and fossil Pitheciinae (Platyrrhini, Anthropoidea)." *Journal of Human Evolution* **19**: 175-208.

Kay, R. F. and Fleagle, J. G. (2010). "Stem taxa, homoplasy, long lineages, and the phylogenetic position of *Dolichocebus*." *Journal of Human Evolution* **59**: 218-222.

Kay, R. F., Fleagle, J. G., Mitchell, T. R. T., Colbert, M., Brown, T. and Powers, D. W. (2008). "The anatomy of *Dolichocebus gaimanensis*, a stem platyrrhine monkey from Argentina." *Journal of Human Evolution* **54**: 323-382.

Kay, R. F., Hunt, K. D., Beeker, C. D., Conrad, G. W., Johnson, C. C. and Keller, J. (2010). "Preliminary notes on a newly discovered skull of the extinct monkey *Antillothrix* from Hispaniola and the origin of the Greater Antillean monkeys." *Journal of Human Evolution* **60**: 124-128.

Kay, R. F., MacFadden, B. J., Madden, R. H., Sandeman, H. and Anaya, F. (1998). "Revised age of the Salla Beds, Bolivia, and its bearing on the age of the Deseadan South American land mammal 'Age.'." *Journal of Vertebrate Palaeontology* **18**: 189-199.

Kay, R. F., Ross, C. F. and Williams, B. A. (1997). "Anthropoid origins." Science **275**: 797-804.

Kay, R. F., Williams, B. A., Ross, C. F., Takai, M. and Shigehara, N. (2004). Anthropoid origins: A phylogenetic analysis. Anthropoid Origins New Visions. Ross, C. F., Kay, R.F. New York, Plenum Publishers.

Kim, J., Rohlf, F. J. and Sokal, R. R. (1993). "The accuracy of phylogenetic estimation using the neighbor-joining method." *Evolution* **47**: 471-486.

Kinzey, W. G. (1987). "Comparative functional morphology of the dentition of bearded saki and spider monkeys." *Anat Rec* **218**: 72a.

Kinzey, W. G. (1992). "Dietary and dental adaptations in the Pitheciinae." *American Journal of Physical Anthropology* **88**: 499-514.

Kinzey, W. G. (1997). New World Primates: Ecology, Evolution, and Behavior. New York, Aldine de Gruyter.

Kinzey, W. G. and Norconk, M. A. (1990). "Hardness as a basis of fruit choice in two sympatric primates." *American Journal of Physical Anthropology* **81**: 5-15.

Kirkpatrick, R. C. (2007). The Asian colobines: Diversity among leaf-eating monkeys. Primates In Perspective. Campbell, C. J., Fuentes, A., MacKinnon, K.C., Panger, M., Beader, S.K. Oxford, Oxford University Press**:** 186-200.

Kitching, I. J., Forey, P. L., Humphries, C. J. and Williams, D. M. (1998). Cladistics: The Theory and Practice of Parsimony. Oxford, Oxford University Press.

Klingenberg, C. P. (1998). "Heterochrony and allometry: The analysis of evolutionary change in ontogeny." *Biological Reviews* **73**: 79-123.

Klingenberg, C. P. (2008). "Morphological integration and developmental modularity." *Annual Review of Ecology and Systematics* **39**: 115-132.

Klingenberg, C. P. (2008). "Novelty and "homology-free" morphometrics." *Evolutionary Biology* **35**: 186-190.

Klingenberg, C. P. (2010). "Evolution and development of shape: Integrating quantitative approaches." *Nature Reviews Genetics* **11**: 623-635.

Klingenberg, C. P. (2011). "MorphoJ: An integrated software package for geometric morphometrics." *Molecular Ecology Resources* **11**: 353-357.

Klingenberg, C. P. and Gidaszewski, N. A. (2010). "Testing and quantifying phylogenetic signals and homoplasy in morphometric data." *Systematic Biology* **59**: 245-261.

Kluge, A. G. (1983). Cladistic and the classification of the great apes. New Interpretations of Ape and Human Ancestry. Ciochon, R. L., Corrucini, R. New York, Plenum Press**:** 151-177.

Kuhner, M. K. and Felsenstein, J. (1994). "A simulation comparison of phylogeny algorithms under equal and unequal evolutionary rates." *Molecular Biology and Evolution* **11**: 459-468.

Larsen, C. (1995). "Biological changes in human populations with agriculture." *Annual Review of Anthropology* **24**: 185-213.

Lavergne, A., Ruiz-Garcia, M., Catzeflis, F., Lacote, S., Contamin, H., Mercereau-Puijalon, O., Lacoste, V. and de Thposy, B. (2010). "Phylogeny and phylogeography of squirrel monkeys (Genus *Saimiri*) based on cytochrome B genetic analysis." *American Journal of Primatology* **72**: 242-253.

Lawing, A. M. and Polly, P. D. (2010). "Geometric morphometrics: Recent applications to the study of evolution and development." *Journal of Zoology* **280**: 1-7.

Lawler, R. R., Ford, S. M., Wright, P. C. and Easley, S. P. (2006). "The locomotor dehaviour of *Callicebus brunneus* and *Callicebus torquatus*." *Folia Primatologica* **77**: 228-239.

Lele, S. (1993). "Euclidean distance matrix analysis of landmark data: Estimation of mean form and mean form difference." *Mathematical Geology* **25**: 573-602.

Lele, S. and Cole, T. M. I. (1996). "Testing for shape differences when the covariance matrices are unequal." *Journal of Human Evolution* **31**: 193-212.

Lele, S. and Richtsmeier, J. T. (1991). "Euclidean distance matrix analysis: A coordinate-free approach for comparing biological shapes using landmark data." *American Journal of Physical Anthropology* **86**: 415-427.

Lieberman, D. E. (1995). "Testing hypotheses about recent human evolution from skulls: Integrating morphology, function, development and phylogeny." *Current Anthropology* **36**: 159-197.

Lieberman, D. E. (1997). "Making behavioural and phylogenetic inferences from hominid fossils: Considering the developmental influence of mechanical forces." *Annual Review of Anthropology* **26**: 185-210.

Lieberman, D. E. (1999). "Homology and hominid phylogeny: Problems and potential solutions." *Evolutionary Anthropology* **7**: 142-151.

Lieberman, D. E., Krovitz, G. E., Yates, F. W., Devlin, M. and St. Clair, M. (2004). "Effects of food processing on masticatory strain and craniofacial growth in a retrognathic face." *Journal of Human Evolution* **46**: 655-677.

Lieberman, D. E., Pearson, O. M. and Mowbray, K. M. (2000a). "Basicranial influence on overall cranial shape." *Journal of Human Evolution* **38**: 291-315.

Lieberman, D. E., Ross, C. F. and Ravosa, M. (2000b). "The primate cranial base: ontogeny, function and integration." *Yearbook of Physical Anthropology* **43**: 117-169.

Lieberman, D. E., Wood, B. A. and Pilbeam, D. R. (1996a). "Homoplasy and darly *Homo*: An analysis of the evolutionary relationships of *H. habilis sensu stricto* and *H. rudolfensis*." *Journal of Human Evolution* **30**: 97-120.

Lockwood, C. A. (1999). "Homoplasy and adaptation in the atelid postcranium." *American Journal of Physical Anthropology* **108**: 459-482.

Lockwood, C. A. (2007). "Adaptation and functional integration In primate phylogenetics." *Journal of Human Evolution* **52**: 490-503.

Lockwood, C. A. and Fleagle, J. G. (1999). "The recognition and evaluation of homoplasy in primate and human evolution." *American Journal of Physical Anthropology* **110**: 189-232.

Lockwood, C. A., Kimbel, W. H. and Lynch, J. M. (2004). "Morphometrics and hominoid phylogeny: Support for a chimpanzee-human clade and differentiation among great ape subspecies." *PNAS* **101**: 4356-4360.

Lockwood, C. A., Lynch, J. M. and C.A., K. (2002). "Quantifying temporal bone morphology of great apes and humans: An approach using geometric morphometrics." *Journal of Anatomy* **201**: 447-464.

MacFadden, B. J. (1990). "Chronology of cenozoic primate localities in South America." *Journal of Human Evolution* **19**: 7-21.

Macholan, M. (2006). "A geometric morphometric analysis of the shape of the first upper molar in mice of the genus *Mus* (Muridae, Rodentia)." *Journal of Zoology* **270**: 672-681.

MacPhee, R. D. E. and Horovitz, I. (2002). Extinct quaternary platyrrhines of the Greater Antilles and Brazil. The Primate Fossil Record. Hartwig, W. C. Cambridge, Cambridge University Press**:** 189-200.

MacPhee, R. D. E., Horovitz, I., Arredondo, O. and Vasquez, O. J. (1995). "A new genus for the extinct Hispaniolan monkey *Saimiri bernensis* Rimoli, 1977, with notes on its systematic position." *American Museum Novitates* **3134**: 1-21.

Maddison, W. P., Donoghue, M. J. and Maddison, D. R. (1984). "Outgroup analysis and parsimony." *Systematic Zoology* **33**: 83-103.

Manzi, G., Bruner, E. and Passarello, P. (2003). "The one-million-year-old *Homo* cranium from Bouri (Ethiopia): A reconsideration of its *H. erectus* affinities." *Journal of Human Evolution* **44**: 731-736.

Marcus, L. F., Hingst-Zaher, E. and Zaher, H. (2000). "Application of landmark morphometrics to skulls representing the orders of living mammals." *Hystrix* **11**: 27-47.

Marroig, G. and Cheverud, J. (2001). "A comparison of phenotypic variation and covariation patterns and the role of phylogeny, ecology and ontogeny during cranial evolution of New World monkeys." *Evolution* **55**: 2576-2600

Marroig, G. and Cheverud, J. (2005). "Size as a line of least evolutionary resistence: Diet and adaptive morphological radiation in New World monkeys." *Evolution* **59**: 1128-1142.

Marroig, G., de Vivo, M. and Cheverud, J. M. (2004b). "Cranial evolution in sakis (*Pithecia*, Platyrrhini) II: Evolutionary processes and morphological integration." *Journal of Evolutionary Biology* **17**: 144-155.

Marroig, G., Shirai, L. T., Porto, A., de Oliveira, F. B. and De Conto, V. (2009). "The evolution of modularity in the mammalian skull II: Evolutionary consequences." *Evolutionary Biology* **36**: 136-148.

Martin, L. (1986). Relationships among extant and extinct great apes and humans. Major Topics in Primate and Human Evolution. Wood, B., Martin, L., Andrews, P. Cambridge, Cambridge University Press**:** 161-187.

Martin, R. D. (1990). Primate Origins and Evolution: A Phylogenetic Reconstruction. London, Chapman Hall.

Martin, R. D. (1992). "Goeldi and the dwarfs: The evolutionary biology of the small New World monkeys." *Journal of Human Evolution* **22**: 367-393.

Martinez-Abadias, N., Esparza, M., Sjovold, T., Gonzalez-Jose, R., Santos, M. and Hernandez, M. (2009). "Heritability of human cranial dimensions: Comparing the evolvability of different cranial regions." *Journal of Anatomy* **214**: 19-35.

Matauschek, C., Roos, C. and Heymann, E. W. (2011). "Mitochondrial phylogeny of tamarins (Saguinus, Hoffmannsegg 1807) with taxonomic and biogeographic implications for the *S.nigricollis* species group." *American Journal of Physical Anthropology* **144**: 564-574.

Matterson, T. J. (1997). "Sexual dimorphism and interspecific cranial form in two capuchin species: *Cebus albifrons* and *C.apella*." *American Journal of Physical Anthropology* **104**: 487-511.

Matthews, L. J. and Rosenberger, A. L. (2008). "Taxon combinations, parsimony analysis (PAUP*), and the taxonomy of the yellow-tailed woolly monkey, *Lagothrix flavicauda*." *American Journal of Physical Anthropology* **137**: 245-255.

McCollum, M. A. (1999). "The robust australopithecine face: A morphogenetic perspective." *Science* **284**: 301-305.

McFarland Symington, M. (1990). "Fission-fusion social organization in *Ateles* and *Pan*." *International Journal of Primatology* **11**: 47-61.

Menegaz, R. A., Sublett, S. V., Figueroa, S. D., Hoffman, T. J., Ravosa, M. J. and Aldridge, K. (2010). "Evidence for the influence of diet on cranial form and robusticity." The Anatomical Record. Advances in Integrative Anatomy and Evolutionary Biology **293**: 630-641.

Menezes, A. N., Bonvicino, C. R. and Seuanez, H. N. (2010). "Identification, classification and evolution of owl monkeys (*Aotus*, Illiger 1811)." *BMC Evolutionary Biology* **10**: 1-15.

Mickevich, M. F. and Johnson, M. S. (1975). "Congruence between morphological and allozyme data in evolutionary inference and character evolution." *Systematic Biology* **25**: 260-270.

Mihaescu, R., Levy, D. and Pachter, L. (2009). "Why neighbor-joining works." *Algorithmica* **54**: 1-24.

Milton, K. and May, M. L. (1976). "Body weight, diet and home rangearea in primates." *Nature* **259**: 459-462.

Minelli, A. (1993). Biological Systemtics: The State of the Art. London, Chapman & Hall.

Mitteroecker, P. and Gunz, P. (2009). "Advances in geometric morphometrics." *Evolutionary Biology* **36**: 235-247.

Monteiro, L. R. and Abe, A. S. (1999). "Functional and historical determinants of shape in the scapula of Xenarthran mammals: Evolution of a complex morphological structure." *Journal of Morphology* **241**: 251-263.

Monteiro, L. R., Bordin, B. and dos Reis, S. F. (2000). "Shape distances, shape spaces, and the comparison of morphometric methods." *Trends in Ecology and Evolution* **15**: 217-220.

Monteiro, L. R. and Dos Reis, S. F. (2005). "Evolutionary integration and morphological diversification in complex morphological structures: Mandible shape divergence in spiny rats (Rodentia, Echimyidae)." *Evolution & Development* **7**: 429-439.

Moreira, M. A. M. and Seuanez, H. N. (1999). "Mitochondrial pseudogenes and phyletic relationships of *Cebuella* and *Callithrix* (Platyrrhini, Primates)." *Primates* **40**: 353-364.

Mounier, A., Marchal, F. and Condemi, S. (2009). "Is *Homo heidelbergensis* a distinct species? New insight on the Mauer mandible." *Journal of Human Evolution* **56**: 219-246.

Moura, A. C. and Lee, P. C. (2004). "Capuchin stone tool use in Caatinga dry forest." *Science* **306**: 1909.

Napier, J. R. and Napier, P. H. (1967). A Handbook of Living Primates. New York, Academic Press.

Napier, J. R. and Napier, P. H., Eds. (1985). The Natural History of the Primates. Cambridge, Massachusetts, MIT Press.

Nei, M. (1972). "Genetic distances between populations." *The American Naturalist* **106**: 283-292.

Nei, M. and Kumar, S. (2000). Molecular Evolution and Phylogenetics. Oxford, Oxford University Press.

Nekaris, A. and Bearder, S. K. (2007). The lorisiform primates of Asia and mainland Africa: Diversity shrouded in darkness. Primates In Perspective. Campbell, C. J., Fuentes, A., MacKinnon, K.C., Panger, M., Beader, S.K. Oxford, Oxford University Press**:** 24-45.

Nicola, P. A., Monteiro, L. R., Pessoa, L. M., von Zuben, F. J., Rohlf, F. J. and Dos Reis, S. F. (2003). "Congruence of hierarchical, localized variation in cranial shape and molecular phylogenetic structure in spiny rats, genus *Trinomys* (Rodentia: Echimyidae)." *Biological Journal of the Linnean Society* **80**: 385-396.

Nixon, K. C. and Carpenter, J. M. (1993). "On outgroups." *Cladistics* **9**: 413-426.

Norconk, M. A. (2011). Sakis, uakaris, and titi monkeys. Primates in Perspective. Campbell, C. J., Fuentes, A., MacKinnon, K. C., Bearder, S. K. and Stumpf, R. M. Oxford, Oxford University Press.

Norconk, M. A., Wright, B. W., Conklin-Brittain, N. L. and Vinyard, C. J. (2009). Mechanical and nutritional properties of food as factors in platyrrhine dietary adaptations. South American Primates Comparative Perspectives in the Study of Behaviour, Ecology, and Conservation. Garber, P. A., Estrada, A., Bicca-Marques, J. C., Heymann, E. W. and strier, K. B. New York, Springer.

O'Higgins, P. (2000). "Advances in approaches to the study of morphological variation in the fossil hominid record: Biology, landmarks and geometry." *Journal of Anatomy* **197**: 103-120.

O'Higgins, P., Chadfield, P. and Jones, N. (2001). "Facial growth and the ontogeny of morphological variation within and between the primates *Cebus apella* and *Cercocebus torquatus*." *Journal of Zoology* **254**: 337-357.

O'Higgins, P. and Jones, N. (2006). "Tools for statistical shape analysis.", from http://hyms.fme.googlepages.com/resources.

Olson, T. R. (1981). Basicrania and evolution of the pliocene hominids. Aspects of Human Evolution. Stringer, C. B. London, Taylor and Francis**:** 99-128.

Opazo, J. C., Wildman, D. E., Prychitko, T., Johnson, R. M. and Goodman, M. (2006). "Phylogenetic relationships and divergence times among New World monkeys (Platyrrhini, Primates)." *Molecular Phylogenetics and Evolution* **40**: 274-280.

Orlosky, F. J. (1973). Comparative dental morphology of extant and extinct Cebidae. Washington, University of Washington. **PhD dissertation**.

Owen, R. (1843). Lectures on the comparative anatomy and physiology of the invertebrate animals. London, Longman, Brown, Green, and Longman.

Pack, K. S., Henry, O. and Sabatier, D. (1999). "The insectivorous-frugivorous diet of the golden-handed tamarin (*Saguinus midas midas*) in French Guiana." *Folia Primatologica* **70**: 1-7.

Page, R. D. M. (1996). "TREEVIEW: An application to display phylogenetic trees on personal computers." *Computer Applications in the Biosciences* **12**: 357-358.

Panchen, A. L. (1992). Classification evolution and the nature of biology. Cambridge, Cambridge University Press.

Paschetta, C., de Azevedo, S., Castillo, L., Martinez-Abadias, N., Hernandez, M., Lieberman, D. E. and Gonzalez-Jose, R. (2010). "The influence of masticatory loading on craniofacial morphology: A test case across technological transitions in the Ohio Valley." *American Journal of Physical Anthropology* **141**: 297-314.

Pastorini, J., Forstner, M. R. J., Martin, R. D. and Melnick, D. J. (1998). "A reexamination of the phylogenetic position of *Callimico* (Primates) incorporating new mitochondrial DNA sequence data." *Journal of Molecular Evolution* **47**: 32-41.

Patterson, C. (1987). Molecules and Morphology in Evolution: Conflict or Compromise? Cambridge, Cambridge University Press.

Perelman, P., Johnson, W. E., Roos, C., Seuanez, H. N., Horvath, J. E., Moreira, M. A., Kessing, B., Pontius, J., Roelke, M., Rumpler, Y., Schneider, M. P., Silva, A., O'Brien, S. J. and Pecon-Slattery, J. (2011). "A molecular phylogeny of living primates." *PLoS Genet* **7**: e1001342.

Perez, S. I., Klaczko, J., Rocatti, G. and Dos Reis, S. F. (2011). "Patterns of cranial shape diversification during the branching process of New World monkeys (Primates: Platyrrhini)." *Journal of Evolutionary Biology* **24**: 1826–1835.

Philippe, H. and Telford, M. J. (2006). "Large-scale sequencing and the new animal phylogeny." *Trends in Ecology and Evolution* **21**: 614-620.

Plautz, H. L., Goncalves, E. C., Ferrari, S. F., Schneider, M. P. C. and Silva, A. (2009). "Evolutionary inferences on the diversity of the genus Aotus (Platyrrhini, Cebidae) from mitochondrial cytochrome c oxidase subunit II gene sequences." *Molecular Phylogenetics and Evolution* **51**: 383-387.

Plavcan, J. M. and van Schaik, C. P. (1998). "Intrasexual competition and body weight dimorphism in anthropoid primates." *American Journal of Physical Anthropology* **103**: 37-68.

Polly, P. D. (2001). "On morphological clocks and paleogeography: Towards a timescale for *Sorex* hybrid zones." *Genetica* **112-113**: 339-357.

Polly, P. D. (2003). "Paleophylogeography of *Sorex araneus*: Molar shape as a morphological marker for fossil shrews." *Mammalia* **68**: 233-243.

Polly, P. D. (2008). "Developmental dynamic and G-matrices: Can morphometric spaces be used to model phenotypic evolution." *Evolutionary Biology* **35**: 83-96.

Porter, C. A. (2001). "Social organization, reproduction and rearing strategies of *Callimico goeldii*: New clues from the wild." *Folia Primatologica* **72**: 69-79.

Porter, C. A., Czelusniak, J., Schneider, H., Schneider, M. P. C., Sampaio, I. and Goodman, M. (1997). "Sequences of the primate Ɛ-globin gene: Implications for systematics of the marmosets and other New World primates." *Gene* **205**: 59-71.

Porter, C. A., Czelusniak, J., Schneider, H., Schneider, M. P. C., Sampaio, I. and Goodman, M. (1999). "Sequence from the 5' flanking region of the Ɛ-globin gene support the relationship of *Callicebus* with the Pitheciines." *American Journal of Physical Anthropology* **48**: 69-75.

Porter, C. A. and Garber, P. A. (2009). Social behaviour of callimicos: Mating strategies and infant care. The Smallest Anthropoid: The Marmoset/Callimico Radiation. Ford, S. M., Porter, C. A. and Davis, L. C. New York, Springer**:** 87-101.

Porter, L. M. and Garber, P. A. (2004). "Goeldi's monkeys: A primate paradox?" *Evolutionary Anthropology* **13**: 104-115.

Porto, A., de Oliveira, F. B., Shirai, L. T., De Conto, V. and Marroig, G. (2009). "The evolution of modularity in the mammalian skull I: Morphological integration patterns and magnitudes." *Evolutionary Biology* **36**: 118-135.

Prychitko, T., Johnson, R. M., Wildman, D. E., Gumucio, D. and Goodman, M. (2005). "The phylogenetic history of New World monkey β globin reveals a platyrrhine β to δ gene conversion in the atelid ancestry." *Molecular Phylogenetics and Evolution* **35**: 225-234.

Purvis, A. (1995). "A composite estimate of primate phylogeny." *Philosophical Transactions of the Royal Society Biological Sciences* **348**: 405-421.

Ramirez (1988). The woolly monkey, genus *Lagothrix*. Ecology and Behaviour of Neotropical Primates. Mittermeier, R. A., Rylands, A. B., Coimbra-Filho, A. F. and Fonseca, G. A. B. Washington DC, World Wildlife Fund**:** 539-575.

Rao, C. R. and Suryawanshi, S. (1996). "Statistical analysis of shape of objects based on landmark data." *PNAS* **93**: 12132-12136.

Rao, C. R. and Suryawanshi, S. (1998). "Statistical Analysis of Shape Through Triangulation of Landmarks: A Study of Sexual Dimorphism in Hominids." *PNAS* **95**: 4121-4125.

Ravosa, M., Johnson, K. R. and Hylander, W. (2000). "Strain in the galago facial skull." *Journal of Morphology* **245**: 51-66.

Ray, D. A., Xing, J., Hedges, D. J., Hall, M. A., Laborde, M. E., Anders, B. A., White, B. R., Stoilova, N., Fowlkes, J. D., Landry, K. E., Chemnick, L. G., Ryder, O. A. and Batzer, M. A. (2005). "Alu insertion loci and platyrrhine primate phylogeny." *Molecular Phylogenetics and Evolution* **35**: 117-126.

Richtsmeier, J. T., Cheverud, J. and Lele, S. (1992). "Advances in anthropological morphometrics." *Annual Review of Anthropology* **21**: 283-305.

Richtsmeier, J. T., DeLeon, V. B. and Lele, S. (2002). "The promise of geometric morphometrics." *Yearbook of Physical Anthropology* **45**: 63-91.

Richtsmeier, J. T. and Lele, S. (1993). "A coordinate-free approach to the analysis of growth patterns: models and theoretical considerations." *Biological Reviews* **68**: 381-411.

Roch (2010). "Toward extracting all phylogenetic information from matrices of evolutionary distances." *Science* **327**: 1376-1379.

Rohlf, F. J. (1990). "Morphometrics." *Annual Review of Ecology and Systematics* **21**: 299-316.

Rohlf, F. J. (2000a). "On the use of shapes spaces to compare morphometric methods." *Hystrix* **11**: 1-17.

Rohlf, F. J. (2000b). "Statistical power comparisons among alternative morphometric methods." *American Journal of Physical Anthropology* **111**: 463-478.

Rohlf, F. J. (2003). "Bias and error in estimates of mean shape in geometric morphometrics." *Journal of Human Evolution* **44**: 665-683.

Rohlf, F. J. and Marcus, L. F. (1993). "A revolution in morphometrics." *Trends in Ecology and Evolution* **8**: 129-132.

Rohlf, F. J. and Slice, D. (1990). "Extensions of the Procrustes method for the optimal superimposition of landmarks." *Systematic Zoology* **39**: 40-59.

Ronquist, F., van der Mark, P. and Huelsenbeck, J. P. (2009). Bayesian phylogenetic analysis using MrBayes. The Phylogenetic Handbook: A Practical Approach to Phylogenetic Analysis & Hypothesis Testing. Lemey, P., Salemi, M., Vandamme, A. Cambridge, Cambridge University Press. **2nd**.

Rosenberger, A. L. (1977). "*Xenothrix* and ceboid phylogeny." *Journal of Human Evolution* **6**: 541-561.

Rosenberger, A. L. (1979). Phylogeny, evolution and classification of New World monkeys (Platyrrhini, Primates). New York, City University of New York **PhD Dissertation.**

Rosenberger, A. L. (1980). "Gradistic views and adaptive radiation of platyrrhine primates." *Zeitschrift fur Morphologie und Anthropologie* **71**: 157-163.

Rosenberger, A. L. (1981). Systematics: the higher taxa. Ecology and Behaviour of Neotropical Primates. Coimbra-Filho, A. F., Mittermeier, R.A. Rio de janeiro, Academia Brasilia de Ciencias.

Rosenberger, A. L. (1984). "Fossil New World monkeys dispute the molecular clock." *Journal of Human Evolution* **13**: 737-742.

Rosenberger, A. L. (1992). "Evolution of feeding niches in New World monkeys." *American Journal of Physical Anthropology* **88**: 525-562.

Rosenberger, A. L. (2002). Platyrrhine paleontology and systematics: The paradigm shifts. The Primate Fossil Record. Hartwig, W. C. Cambridge, Cambridge University Press.

Rosenberger, A. L. (2010). "Platyrrhines, PAUP, parallelism, and the long lineage hypothesis: A reply to Kay et al. (2008)." *Journal of Human Evolution* **59**: 214-217.

Rosenberger, A. L., Cooke, S. B., Rimoli, R., Ni, X. and Cardoso, L. (2010). "First skull of *Antillothrix bernensis*, an extinct relict monkey from the Dominican Republic." *Proceedings of the Royal Society B Biological Sciences* **278**: 67-74.

Rosenberger, A. L., Halenar, L., Cooke, S. and Hartwig, W. C. (2008). Morphology and evolution of the spider monkey, genus *Ateles*. Spider Monkeys: Behaviour, Ecology and Evolution of the Genus *Ateles*. Campbell, C. J. Cambridge, Cambridge University Press.

Rosenberger, A. L., Norconk, M. A. and Garber, P. A. (1996). New perspectives on the Pitheciines. Adaptive Radiations of Neotropical Primates. Norconk, M. A., Rosenberger, A. L. and Garber, P. A. New York, Plenum Press.

Rosenberger, A. L., Setoguchi, T. and Shigehara, N. (1990). "The fossil record of callitrichine primates." *Journal of Human Evolution* **19**: 209-236.

Rosenberger, A. L. and Strier, K. B. (1989). "Adaptive radiation of the ateline primates." *Journal of Human Evolution* **18**: 717-750.

Rosenberger, A. L., Tejedor, M. F., Cooke, S., Halenar, L. and Pekkar, S. (2009). Platyrrhine ecophylogenetics, past and present. South American Primates Comparative Perspectives in the Study of Behaviour, Ecology and Conservation. Garber, P. A., Estrada, A., Bicca-Marques, J. C., Heymann, E. W. and Strier, K. B.

Ross, C. F. (2001). "In vivo function of the craniofacial gaft: The interorbital "pillar"." *American Journal of Physical Anthropology* **116**: 108-139.

Ross, C. F. and Hylander, W. (1996). "In vivo and in vitro bone strain in the owl monkey Circumorbital region and the function of the postorbital septum." *American Journal of Physical Anthropology* **101**: 183-215.

Ross, C. F. and Metzger, K. A. (2004). "Bone strain gradients and optimization in vertebrate skulls." *Annals of Anatomy* **186**: 387-396.

Ross, C. F. and Ravosa, M. (1993). "Basicranial flexion, relative brain size, and facial kyphosis in nonhuman primates." *American Journal of Physical Anthropology* **91**: 305-324.

Ross, C. F., Williams, B. and Kay, R. F. (1998). "Phylogenetic analysis of anthropoid relationships." *Journal of Human Evolution* **35**: 221-306.

Ruiz-Garcia, M. and Pinedo-Castro, M. O. (2010). "Molecular systematics and phylogeography of the genus *Lagothrix* (Atelidae, Primates) by means of the mitochondrial COII gene." *Folia Primatologica* **81**: 109-128.

Rylands, A. B., Coimbra-Filho, A. F. and Mittermeier, R. A. (2009). The systematics and distributions of the marmosets (*Callithrix*, *Callibella*, *Cebuella*, and *Mico*) and callimicos (*Callimico*) (Callitrichidae, Primates). The Smallest Anthropoids: The Marmoset/Callimico Radiation. Ford, S. M., Porter, C. A. and Davis, L. C. New York, Springer.

Rylands, A. B., Schneider, H., Langguth, A., Mittermeier, R. A., Groves, C. P. and Rodriguez-Luna, E. (2000). "An assessment of the diversity of New World primates." *Neotropical Primates* **8**: 61-93.

Saitou, N. and Imanishi, T. (1989). "Relative efficiences of the fitch-margoliash, maximum-parsimony, maximum-likelihood, minimum-evolution, and neighbor-joining methods of phylogenetic tree construction in obtaining the correct tree." *Molecular biology and evolution* **4**: 406-425.

Saitou, N. and Nei, M. (1987). "The neighbor-joining method: a new method for reconstructing phylogenetic trees." *Molecular Biology and Evolution* **4**: 406-425.

Sanderson, M. J. and Shaffer, H. B. (2002). "Troubleshooting molecular phylogenetic analyses." *Annual Review of Ecology and Systematics* **33**: 49-72.

Sardi, M. L., Novellino, P. S. and Pucciarelli, H. M. (2006). "Craniofacial morphology in the Argentine center-west: Consequences of the transition to food production." *American Journal of Physical Anthropology* **130**: 333-343.

Sarich, V. M. and Cronin, J. R. (1976). Molecular systematics of the primates. Molecular Anthropology. Goodman, M., Tashian, R. New York, Plenum Press**:** 141-170.

Sarich, V. M. and Cronin, J. R. (1980). South America mammal molecular systematics, evolutionary clocks, and continental drift. Evolutionary Biology of New World Monkeys and Continental Drift. Napier, J. R., Napier, P.H. New York, Academic Press**:** 399-421.

Schillaci, M. A. (2008). "Human cranial diversity and evidence for an ancient lineage of modern humans." *Journal of Human Evolution* **54**: 814-826.

Schluter, D. (1996). "Adaptive radiation along genetic lines of least resistance." *Evolution* **50**: 1766-1774.

Schneider, H. (2000). "The current status of the New World monkey phylogeny." *Anais da Academia Brasileira de Ciências* **72**: 165-172.

Schneider, H., Canavez, F. C., Sampaio, I., Moreira, M. A. M., Tagliaro, C. H. and Seuanez, H. N. (2001). "Can molecular data place each neotropical monkey in its own branch?" *Chromosoma Focus* **109**: 515-523.

Schneider, H. and Rosenberger, A. L. (1996). Molecules, morphology, and platyrrhine systematics Adaptive Radiations of Neotropical Primates. Norconk, M. A., Rosenberger, A.L., Garber, P.A. New York, Plenum Press**:** 3-19.

Schneider, H., Sampaio, I., Harada, M. L., Barroso, C. M. L., Schneider, M. P. C., Czelusniak, J. and Goodman, M. (1996). "Molecular phylogeny of the New World monkeys (Platyrrhini, Primates) based on two unlinked nuclear genes: IRBP Intron 1 and E-globin sequences." *American Journal of Physical Anthropology* **100**: 153-179.

Schneider, H., Schneider, M. P. C., Sampaio, I., Harada, M. L., Stanhope, M., Czelusniak, J. and Goodman, M. (1993). "Molecular phylogeny of the New World monkeys (Platyrrhini, Primates)." *Molecular Phylogenetics and Evolution* **2**: 225-242.

Schrago, C. G. (2007). "On the time scale of New World primate diversification." *American Journal of Physical Anthropology* **132**: 344-354.

Schuh, R. T. and Brower, A. V. Z. (2009). Biological Systematics Principles and Applications. Ithaca, Cornell University Press.

Schwartz, J. H. (1984). "The evolutionary relationships of man and orang-utans." *Nature* **308** (5959): 501-505.

Schwartz, J. H. and Tattersall, I. (1985). "Evolutionary relationships of living lemurs and lorises (Mammalia, Primates) and their potential affinites with European Eocene Adapidae." *Anthropological Papers of the American Museum of Natural History* **60**: 139-192.

Scotland, R. W., Olmstead, R. G. and Bennet, J. R. (2003). "Phylogeny reconstruction: The role of morphology." *Systematic Biology* **52**: 539-548.

Shirai, L. T. and Marroig, G. (2010). "Skull modularity in neotropical marsupials and monkeys: Size variation and evolutionary constraint and flexibility." *Journal of Experimental Zoology. Part B. Molecular and Developmental Evolution*. **314**: 663-683.

Shoshani, J., Groves, C. P., Simons, E. J. and Gunnell, G. F. (1996). "Primate phylogeny: Morphological vs. molecular results." *Molecular Phylogenetics and Evolution* **5**: 102-154.

Siebert, J. R. and Swindler, D. R. (2002). Evolutionary changes in the midface and mandible: Establishing the primate form. Understanding Craniofacial Anomalies.The Ethiopathogenesis of Craniosynostoses and Facial Clefting. Mooney, M. P., Siegel, M.I. New York, Wiley-Liss.

Simons, E. (1972). Primate Evolution. New York, Macmillan.

Singer, S. S., Schmitz, J., Schwiegk, C. and Zischler, H. (2005). "Molecular cladistic markers in New World monkey phylogeny (platyrrhini, Primates)." *Molecular Phylogenetics and Evolution* **26**: 490-501.

Skelton, R. R. and McHenry, H. M. (1992). "Evolutionary relationships among early hominids." *Journal of Human Evolution* **23**: 309-349.

Skelton, R. R., McHenry, H. M. and Drawhorn, G. M. (1986). "Phylogenetic analysis of early hominids." *Current Anthropology* **27**: 329-340.

Slater, G. J., Dumont, E. R. and Van Valkenburgh, B. (2009). "Implications of predatory specialization for cranial form and function in Canids." *Journal of Zoology* **278**: 181-188.

Slater, G. J., Figueirido, B., Louis, L., Yang, P. and Van Valkenburgh, B. (2010). "Biomechanical consequences of rapid evolution in the polar bear lineage." *PloS ONE* **5**: 1-7.

Slater, G. J. and Van Valkenburgh, B. (2008). "Long in the tooth: Evolution of sabretooth cat cranial shape." *Paleobiology* **34**: 403-419.

Slater, G. J. and van Valkenburgh, B. (2009). "Allometry and performance: The evolution of skull form and function in Felids." *Journal of Evolutionary Biology* **22**: 2278-2287.

Slice, D. E. (2005). Modern Morphometrics in Physical Anthropology, Kluwer Academic Publishers.

Slice, D. E. (2007). "Geometric morphometrics." *Annual Review of Anthropology* **36**: 261-281.

Smith, H. F. (2009). "Which cranial regions reflect molecular distances reliably in humans? Evidence from three-dimensional morpohology." *American Journal of Human Biology* **21**: 36-47.

Smith, H. F., Terhune, C. E. and Lockwood, C. A. (2007). "Genetic, geographic, and environmental correlates of human temporal bone variation." *American Journal of Physical Anthropology* **134**: 312-322.

Sneath, P. H. A. and Sokal, R. R. (1973). Numerical Taxonomy. San Fransisco, W.H. Freeman.

Sokal, R. R. and Michener, C. D. (1958). "A statistical method for evaluating systematic relationships." *The University of Kansas Scientific Bulletin* **38**: 1409-1438.

Sokal, R. R. and Sneath, P. H. A. (1963). Principles of Numerical Taxonomy. San Fransisco, W.H. Freeman.

Soligo, C. and Martin, R. D. (2006). "Adaptive origins of primates revisited." *Journal of Human Evolution* **50**: 414-430.

Soligo, C. and Muller, A. E. (1999). "Nails and claws in primate evolution." *Journal of Human Evolution* **36**: 97-114.

Sourdis, J. and Nei, M. (1988). "Relative rfficiencies of the maximum parsimony and distance-matrix methods in obtaining the correct phylogenetic tree." *Molecular Biology and Evolution* **5**: 298-311.

Spoor, F. (1997). "Basicranial architecture and relative brain size of Sts 5 (*Australopithecus africanus*) and other plio-pleistocene hominids." *South African Journal of Science* **93**: 182-186.

Stanger-Hall, K. F. (1997). "Phylogenetic affinities among the extant malagasy lemurs (Lemuriformes) based on morphology and behaviour." *Journal of Mammalian Evolution* **4**: 163-194.

Steiper, M. E. and Ruvolo, M. (2003). "New World monkey phylogeny based on X-linked G6PD DNA sequences." *Molecular Phylogenetics and Evolution* **27**: 121-130.

Steiper, M. E. and Young, N. M. (2006). "Primate molecular divergence dates." *Journal of Human Evolution* **41**: 384-394.

Stevens, P. F. (1991). "Character states, morphological variation and phylogenetic analysis: A review." *Systematic Botany* **16**: 553-583.

Strait, D. S. (2001). "Integration, phylogeny, and the hominid cranial base." *American Journal of Physical Anthropology* **114**: 273-297.

Strait, D. S. and Grine, F. E. (2004). "Inferring hominoid and early hominid phylogeny using craniodental characters: The role of fossil taxa." *Journal of Human Evolution* **47**: 399-452.

Strait, D. S., Grine, F. E. and Moniz, M. A. (1997). "A reappraisal of early hominid phylogeny." *Journal of Human Evolution* **32**: 17-82.

Strasser, E. and Delson, E. (1987). "Cladistic analysis of cercopithecid relationships." *Journal of Human Evolution* **16**(1): 81-99.

Strier, K. B. (1987). "Activity budgets of woolly spider monkeys, or muriquis (*Brachyteles arachnoides*)." *American Journal of Primatology* **13**: 385-395.

Strier, K. B. (1992). "Atelinae adaptations: Behavioural strategies and ecological constraints." *American Journal of Physical Anthropology* **88**: 515-524.

Stringer, C. B. (1987). "A numerical cladistic analysis for the Genus *Homo*." *Journal of Human Evolution* **16**: 135-146.

Sussman, R. W. (2005). Primate Ecology and Social Structure. MA, Pearson Custom Publishing.

Szalay, F. S. and Delson, E. (1979). Evolutionary History of Primates. New York, Academic Press.

Takahashi, K. and Nei, M. (2000). "Efficiences of fast algorithms of phylogenetic inference under the criteria of maximum parsimony, minimum evolution, and maximum likelihood when a large number of sequences are used." *Molecular Biology and Evolution* **17**: 1251-1258.

Tamura, K., Nei, M. and Kumar, S. (2004). "Prospects for inferring very large phylogenies by using the neighbor-joining method." *PNAS* **30**: 11030-11035.

Tattersall, I. and Schwartz, J. H. (1974). "Craniodental morphology and the systematics of the malagasy lemurs (Primates, Prosimii)." *Anthropological Papers of the American Museum of Natural History* **52**: 139-192.

Tattersall, I. and Schwartz, J. H. (1975). Relationships among the malagasy lemurs: The craniodental evidence. Phylogeny of the Primates: A Multidisciplinary Approach. Luckett, W. P., Szalay, F.S. New York, Plenum Press.

Tattersall, I. and Schwartz, J. H. (1991). "Phylogeny and nomenclature in the "lemur-group" of Malagasy strepsirrhine primates." *Anthropological Papers of the American Museum of Natural History* **69**: 3-18.

Taylor, A. B., Eng, C. M., Anapol, F. C. and Vinyard, C. J. (2009). The functional significance of jaw-muscle fiber architecture in tree-gouging marmosets. The Smallest Anthropoids: The Marmoset/Callimico Radiation. Ford, S. M., Porter, C. A. and Davis, L. C. New York, Springer**:** 381-394.

Tejedor, M. F. (2005). "New fossil platyrrhine from Argentina." *Folia Primatologica* **76**: 146-150.

Tejedor, M. F. (2005). "New specimens of *Soriacebus adrianae* Fleagle, 1990, with comments on pitheciin primates from the Miocene of Patagonia." *Ameghiniana* **42**: 249-251.

Thierry, B. (2007). The macaques: A double-layered social organization. Primates In Perspective. Campbell, C. J., Fuentes, A., MacKinnon, K.C., Panger, M., Beader, S.K. Oxford, Oxford University Press**:** 224-239.

Thorpe, R. S. (1984). "Coding morphometric characters for constructing distance wagner networks." *Evolution* **38**: 244-255.

van de Peer, Y. (2009). Phylogenetic inference based on distance methods. The Phylogenetic Handbook: A Practical Approach. Lemey, P., Salemi, M., Vandamme, A. Cambridge, Cambridge University Press. **2nd**.

van Roosmalen, G. M., Mittermeier, R. A. and Fleagle, J. G. (1988). "Diet of the northern bearded saki (*Chiropotes*)." *American Journal of Primatology* **14**: 11-35.

van Roosmalen, G. M., Mittermeier, R. A. and Milton, K. (1981). The bearded saki, genus *Chiropotes*. Ecology and Behaviour of Neotropical Primates. Ciombra-Filho, A. F. and Mittermeier, R. A. Rio de Janeiro, Academia Brasileira de Ciencias**:** 419-442.

van Roosmalen, M. G. M. and van Roosmalen, T. (2003). "The description of a new marmoset genus, *Callibella* (Callitrichinae, Primates), including its molecular phylogenetic status." *Neotropical Primates* **11**: 1-10.

van Roosmalen, M. G. M., van Roosmalen, T., Mittermeier, R. A. and fonseca, G. A. B. (1998). "A new and distinctive species of marmoset (Callitrichidae, Primates), from the lower Rio Aripuana, state of Amazonas, central Brazilian Amazonia." *Goeldiana Zoology* **22**: 1-27.

Viguier, B. (2002). "Is the morphological disparity of lemur skulls (Primates) controlled by phylogeny and/or environmental constraints." *Biological Journal of the Linnean Society* **76**: 577-590.

Visalberghi, E. (1990). "Tool use in *Cebus*." *Primates* **54**: 146-154.

Visalberghi, E. and Trinca, L. (1989). "Tool use in capuchin monkeys: Distinguishing between performing and understanding." *Primates* **30**: 511-521.

von Dornum, M. and Ruvolo, M. (1999). "Phylogenetic relationships of the New World monkeys (Primates, Platyrrhini) based on nuclear G6PD DNA sequences." *Molecular Phylogenetics and Evolution* **11**: 459-476.

Wagner, G. P., Pavlicev, M. and Cheverud, J. M. (2007). "The road to modularity." *Nature Reviews Genetics* **8**: 921-931.

Wake, D. B., Wake, M. H. and Specht, C. D. (2011). "Homoplasy: From detecting pattern to determining process and mechanism of evolution." *Science* **25**(331).

Walker, S. E. (1996). The evolution of positional behaviour in the saki-uakaris (*Pitheica*, *Chiropotes*, and *Cacajao*). Adaptive Radiations of Neotropical Primates. Norconk, M. A., Rosenberger, A. L. and Garber, P. A. New York, Plenum Press**:** 335-368.

Whelan, S., Lid, P. and Goldman, N. (2001). "Molecular phylogenetics: State-of-the-art methods for looking into the past." *Trends in Genetics* **17**: 262-272.

Wiens, J. J. (2004). "The role of morphological data in phylogeny reconstruction." *Systematic Biology* **53**: 653-661.

Wildman, D. E., Jameson, N. M., Opazo, J. C. and Yi, S. V. (2009). "A fully resolved genus level phylogeny of Neotropical primates (Platyrrhini)." *Molecular Phylogenetics and Evolution* **53**: 694-702.

Wilkinson, R. D., Steiper, M. E., Soligo, C., Martin, R. D., Yang, Z. and Tavare, S. (2011). "Dating primate divergences through an integrated analysis of palaeontological and molecular data." *Systematic Biology* **60**: 16-31.

Wood, A. E. (1993). The history of the problem. The Africa-South America Connection. George, W., Lavocat, R. Oxford, Clarendon Press**:** 1-7.

Wood, B. A. and Lieberman, D. E. (2001). "Craniodental variation in *Paranthropus boisei*: A developmental and functional perspective." *American Journal of Physical Anthropology* **116**: 13-25.

Wright, K. A. (2007). "The relationship between locomotor behavior and limb morphology in brown (*Cebus apella*) and weeper (*Cebus olivaceus*) capuchins." *American Journal of Primatology* **69**: 736-756.

Wroe, S. and Milne, N. (2007). "Convergence and remarkably consistent constraint in the evolution of carnivore skull shape." *Evolution* **61**: 1251-1260.

Xing, J., Witherspoon, D. J., Ray, D. A., Batzer, M. A. and Jorde, L. B. (2007). "Mobile DNA elements in primate and human evolution." *American Journal of Physical Anthropology* **134**: 2-19.

Yang, Z. (2006). Computational Molecular Evolution. Oxford, Oxford University Press.

Yoder, A. D. and Irwin, J. A. (1999). "Phylogeny of the Lemuridae: Effects of character and taxon sampling on resolution of species relationships within *Eulmeur*." *Cladistics* **15**: 351-361.

Youlatos, D. (1999). "Positional behaviour of *Cebuella pygmaea* in Yasuni National Park, Ecuador." *Primates* **40**: 543-550.

Youlatos, D. (2009). Locomotion, postures, and habitat use by pygmy marmosets (*Cebuella pygmaea*). The Smallest Anthropoids: The Marmoset/Callimico Radiation. Ford, S. M., Porter, C. A. and Davis, L. C. New York, Springer**:** 279-297.

Zelditch, M. L., Swiderski, D. L., Sheets, H. D. and Fink, W. L. (2004). Geometric Morphometrics For Biologists: A Primer. London, Elsevier Academic Press.

Zimbler-Delorenzo, H. S. and Stone, A. I. (2010). "Integration of field and captive studies for understanding the behavioral ecology of the squirrel monkey (*Saimiri* Sp.)." *American Journal of Primatology* **73**: 607-622.