# RESOLVING THE XENARTHRAN PHYLOGENY USING NUCLEAR LOCI

# RESOLVING THE XENARTHRAN PHYLOGENY USING NUCLEAR LOCI

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## Lay Abstract

Xenarthrans – sloths, armadillos, and anteaters – have a long and complicated evolutionary history. In recent years a growth of new genetic information has made it easier to answer questions about their relation to each other and to other species. By examining many new gene sequences across all living Xenarthra, plus some extinct species, we aim to bolster our understanding of these relationships and the importance of particular traits.

**Abstract**

Xenarthra form the least diversified major clade of placental mammals, being comprised of 31 described species of sloth, armadillo, and anteater. The past decade has seen a growth in the amount of xenarthran genetic data available, including the recent publication of a phylogenetic framework based on mitochondrial genomes, but more is required to aid in conservation assessments and to elucidate the evolutionary history of this unique order. We aimed to expand upon this by generating a framework based on nuclear genes.

Using molecular baits, we enriched nuclear DNA from all extant and a selection of extinct ancient Xenarthrans for 74 phenotypically relevant genes. We aim to build phylogenetic trees based on each successfully enriched loci. Comparing trees against the previous mitochondrial framework will provide a measure of robustness. By seeing how estimated divergence times and rates vary across nuclear genes, we were able to examine how particular xenarthran phenotypes were selected upon in different points of their evolutionary history. While the sequencing of certain genes and species encountered mixed success rates, we have a solid framework for further study and can confirm that nuclear and mitochondrial information yields very similar evolutionary histories.

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# Contents

# List of Figures

# List of Tables

## Declaration of Academic Achievement

The following is a declaration that the content of the research in this document has been completed by Jonathan Hughes and recognizes the contributions of Dr. Hendrik Poinar, Dr. Brian Golding, Melanie Kuch, Dr. Ana Duggan, Jennifer Klunk, and Stephanie Marciniak in both the research process and the completion of the thesis. Jonathan Hughes contributed to the study design and was responsible for performing experiments, data collection, data analysis and writing of the manuscript. Dr. Hendrik Poinar and Dr. Brian Golding assisted with the study design, data analysis and manuscript review. Melanie Kuch prepared the original DNA extracts and many of the sequence libraries used in the analysis. Dr. Ana Duggan, Melanie Kuch, Jennifer Klunk, and Stephanie Marciniak provided insightful advice and experimental guidance.

# Chapter 1

# Introduction

## 1.1 Order Xenathra

The superorder Xenarthra is represented by 31 extant species divided into two orders; Cingulata and Pilosa. Cingulata contains the extant armadillos, whilst Pilosa is further divided into Vermilingua and Folivora, respectively anteaters and sloths. They are the oldest of the placental mammals (O'Leary *et al.*, 2013), with their origins being traced as far back as 59–65 million years ago in South America (Gibb *et al.*, 2016), where they were able to diversify and flourish during its separation from Central America, leaving behind over 200 extinct species (Möller-Krull *et al.*, 2007).

It was previously thought that pangolins and aardvarks were contained in the same superorder as Xenarthra, on account of their strikingly similar appearances. This group was named Edentata in reference to the unusual tooth morphology it displayed, with species possessing either no teeth whatsoever, or otherwise lacking any frontal incisors or apparent molars (Rose & Gaudin, 2001). There exist however a number of characteristics that distinguished Xenarthra from them, leading to pangolins and aardvarks to be classed separately amongst Ferae and Afrotheria. Firstly there is the presence of "xenarthry," the trait for which the superorder is named. This describes the presence of intervertebral articulations in the posterior thoracic and lumbar vertebrae (Gaudin & Biewener, 1992), thought to be used in defensive curling movements or, more probably, remnants from early adaptations for digging (Gaudin, 1999). Additional skeletal traits include a secondary scapular spine (Gaudin & Biewener, 1992) as well

as a fusion between in ischium and the sacrum (Delsuc *et al.*, 2001).

In terms of biological attributes, Xenarthra have the lowest basal metabolic rate of any mammal barring monotremes, likely as a result of low selection pressure from predation (Lovegrove, 2000). They have a long history of specialised feeding habits, which has reduced competition between them and other species occupying the same space, helping to account for their success; a lack of competition for a resource coupled with low energy requirements lowers the need for migration in times of resource scarcity (Lillegraven, 1983). Male Xenarthra possess internal testicles located between the rectum and bladder (Kleisner *et al.*, 2010). Female characteristics are somewhat variable; amongst the Cingulata, only *Tolypeutes* possess typical mammalian genital tracts, whereas in other armadillos a simple urogenital sinus is observed (Cetica *et al.*, 2005).

### 1.1.1 Pilosa

#### 1.1.1.1 Vermilingua

Vermilingua contains the four extant species of anteater across three genera; *Cyclopes*, *Tamandua*, and *Myrmecophaga*. As their name implies they are entire myrmecophagic, meaning they consume only ants and other small invertebrate insects. All Vermilingua possess powerful front legs with a single enlarged claw for burrowing into ant nests. This feeding behaviour is aided by the iconic long sticky tongue, covered in small hook–like structures, a trait that is coupled with the total absence of teeth. Instead the lining of the anteater's digestive system contains hard folds that are contracted in order to grind its food (Grzimek, 2004). The low calorie reward from their prey, as well as the possibility of reprisal when digging in an ants nest, has lead anteaters to be highly ambulatory as they must raid approximately 100 ant nests and consume 5000–30,000 ants in a single day, depending on their size (Redford, 1987).

The smallest of the anteaters, *Cyclopes didactylus* or the silky anteater, is both entirely arboreal and nocturnal. Slightly larger and partially terrestrial are the two *Tamandua* species, which have the northernmost territorial range and are the most common anteaters. Perhaps the most iconic and recognisable anteater is *Myrmecophaga tridactlya*, the Giant Anteater. Unlike the other three species it is entirely terrestrial,

being approximately 30kg in weight and 180cm long. Large mammals such as this are not typically considered to be insect specialists, but due to the characteristically low basal metabolism of Xenarthra, *Myrmecophaga* is still able to thrive (Carbone *et al.*, 1999; McNab, 2000). Its greatest threats instead come from the conversion of its habitat for farming practices, predominantly cattle pasture. Due to their need to compete for limited fragile resources, Giant Anteaters are highly solitary, and male aggression is common.

There are a few known examples of extinct ancestors to anteaters discovered at the turn of the 18th century, but there has been a limited amount of research into their relationships. *Palaeomyrmidon* was a close relative to the silky anteater and of similar size, though it appears to have been entirely terrestrial (Rovereto, 1914). Meanwhile the genera *Protamandua* and *Neotamandua* are much more closely related to the other groups of anteaters. *Protamandua* has been considered a common ancestor to both *Myrmecophaga* and *Tamandua* (Hirshcfield, 1976), while *Neotamandua* may be congeneric with *Myrmecophaga* (McDonald *et al.*, 2008). Genetic studies on any of these groups are lacking, and only scant morphological data is available.

#### 1.1.1.2 Folivora

Modern extant sloths derive their name from their metabolic adaptations; idleness, slow movement, and low dietary requirements. As a result of their speed there are a number of symbiotic algae and insects that live in their fur, and some insects whose life cycle revolves around the sloth's fortnightly defecation. Their slow temperament even results in them mistaking their own arms for tree branches, causing them to fall from forest canopies. Despite this, they are in fact surprisingly fast when provoked, remarkably strong for their size, and are incredibly capable swimmers. As folivores their diet consists primarily of tender buds and leaves, though occasionally they have been observed consuming insects and faeces (Heymann *et al.*, 2010). The six extant species of sloth are divided across the genera *Choloepus* (two-toed sloths) and *Bradypus* (three-toed sloths); the divergence between them and the placement of the diverse array of extinct sloth species is however of some contention, and there is disagreement between morphological and genetic analyses (Gaudin, 2004; Farina *et al.*, 2013; Slater *et al.*, 2016). This disagreement can be largely attributed to lacking genetic data and the dearth of knowledge about the many extinct sloth relatives.

While extant sloths are predominantly arboreal, they are believed to be derived from terrestrial ancestors (Corbet, 1989). However, which taxon of ground–sloth *Bradypus* is thought to evolve from, or whether they in fact share an arboreal ancestor with *Choloepus*, is a matter of some discussion (White & MacPhee, 2001). The most anciently diverged of the sloths are Bradypodiae (Hoss *et al.*, 1996), represented only by the four extant species of three–toed *Bradypus*, two of which are at high risk of extinction. Given the long 7 million year divergence time between *Bradypus* and *Choloepus*, it would appear that their arboreal lifestyles and similar appearances are an example of convergent evolution, whereby the only surviving members of Folivora were those that could leave the ground behind them (Hoss *et al.*, 1996).

Both genera of modern sloths occupy the same regions of South America, and possess similar body plans. Their long forearms are for climbing and dragging but cannot be walked upon as a result of reduced musculature – a result of their low metabolism – and they are covered in a thick, algae–ridden fur that aids thermoregulation, as sloths cannot shiver. Beyond this however there are a large number of distinctions between the two. Both have simple dental structures, in keeping with other Xenarthra, but dentition between the two varies. Three–toed sloths are slightly smaller than their two–toed relatives, being about the size of small dog, and also possess a short tail. They are also the superior swimmers of the two species, which is perhaps unsurprising given their apparent close relationship to the extinct aquatic sloth, *Thalassocnus*. Furthermore, two–toed sloths are unique amongst mammals for possessing only five cervical vertebrae, in comparison to the six possessed by three–toed sloths and manatees, and the seven in all other mammals (Galis, 1999). The two also share a number of behavioural distinctions; Three–toed sloths are diurnal, versus the nocturnal two–toed, and when descending from trees *Bradypus* will go bottom–first while *Choloepus* will go head–first.

While the number of extant sloth species is low, Folivora was once a highly diverse suborder containing large numbers of the iconic giant sloth, until the last of them went extinct approximately 10,000 years ago. Beyond the early divergence of Bradypodiae, the positioning of the other Folivora families is somewhat disputed, but is comprised of Nothrotheriidae, Megatheriidae, Mylodontidae, and lastly Megalonychidae, which

contains *Choloepus*. These widely distributed ancient species are suggested to have occupied a much wider range of habits than their modern relatives, ranging from arboreal (Pujos *et al.*, 2007), aquatic (Canto *et al.*, 2008), and terrestrial environments; as well as possibly carnivorous (Farina & Blanco, 1996) and fossorial (Bargo *et al.*, 2000) behaviours.

Nothrotheriidae has been considered a sister taxon to Megatheriidae based on morphological data (Gaudin, 2004), but genetic evidence suggests it is a sister taxon to Bradypodiae (Greenwood *et al.*, 2001; Slater *et al.*, 2016). It contains the aforementioned aquatic *Thalassocnus*, a genus of giant sloths that resided either entirely or predominantly in water. The more recent the species, the more they appear to have adapted to an aquatic niche (Canto *et al.*, 2008). It is also the home clade of the reasonably well characterised Shasta ground sloth, *Nothrotheriops shastensis*. While one of the smaller ground sloths at around nine feet, *N. shastensis* was highly successful throughout western North America. Alongside other Nothrotheriidae, it was thought for a time to be closely related to the much larger *Megatherium* of the Megatheriidae family; they are now known to be distinct taxa (Muizon *et al.*, 2004).

Instead, Megatheriidae and Megalonychidae are thought themselves to be closely related to each other and distinct from Bradypodidae and Nothrotheridae (Slater *et al.*, 2016). At about six meters in height and four tonnes in weight (Bargo, 2001), members of the genus *Megatherium* may well be the most imposing and iconic of the ancient ground sloths with the largest, *M. americanum*, rivalled in size only by some mammoth species. Megalonychidae on the other hand exhibits some of the longest surviving and smallest species of sloth; while the older genera such as *Megalonyx* were still several meters tall, many of the island dwelling sloth populations became quite diminutive, as seen in the modern two–toed sloths; this is likely a result of the restrictions of island inhabitance (Steadman *et al.*, 2005).

Lastly there are the Mylodtonidae, typified by *Mylodon*. Their taxonomic position has been an element of contention, depending on the use of morphological (Gaudin, 1995; Gaudin, 2004) or genetic (Hoss *et al.*, 1996; Slater *et al.*, 2016) evidence, but they appear more closely related to Megalonychidae than to Bradypodidae or Megatheriidae. *Mylodon* had an expansive ecological tolerance (Brandoni *et al.*, 2010) and

no natural predators on account of its large size and thick fur lined with osteoderms, demonstrating the relation to armadillos.

### 1.1.2 Cingulata

The most successful and diverse of the Xenarthrans are the Cingulata, which are the only Xenarthrans still found in North America. They can be divided into three families. The first of these is the entirely extinct branch Pampatheriidae, containing the pampatheres or "pampas beasts." Then there are Dasypodidae, the long–nosed armadillos, and the Chlamyphoridae, which are the glyptodontids and other armadillos. While being similar in appearance, pampatheres differentiate themselves from armadillos in a number of ways. They were much less diverse, and had more powerful jaw musculature that suggests a diet of primarily vegetation, versus the predominantly insect based diet of armadillos (Vizcaíno *et al.*, 1998). Perhaps the most striking difference lies in the structure of their iconic osteodermal plates; their armoured shell was made flexible by the presence of three movable lateral bands of scutes – bony external plates overlaid with horn – as with three–banded armadillos. However, the osteoderms of pampatheres were covered by just one, whereas the osteoderms of armadillos each have multiple scutes (Vizcaíno *et al.*, 1998). Armadillos posses characteristic articulated dermal osteoderms, which are also found in the extinct ground sloth mylodon, indicating pleisomorphy (Hill, 2006). The last of the pampatheres went extinct approximately 12,000 years ago.

#### 1.1.2.1 Dasypodidae

Of the armadillos there are 21 extant species, all sharing the common armour plating in bands across the shoulders, hips, legs and head. A majority of their diet is formed by invertebrates, though they also consume some small vertebrates alongside plant matter. The most successful group of armadillos are the Dasypodes, of which the most widespread is the Nine–Banded Armadillo, *Dasypus novemcinctus*. All Dasypodes have simple teeth that lack enamel, as well as poor vision (Vijayaraghavan, 2009). Fascinatingly, they are unique amongst Xenarthrans in being able to hold their breath and cross streams by walking along the stream bed (Vijayaraghavan, 2009).

While currently all extant Cingulata are experiencing some forms of habitat reduction,

*D. novemcinctus* proves the exception and is in fact exhibiting a dramatic increase in range size, being the only Xenarthran to remain as far north as the United States of America. They are non–territorial and have few natural predators, but their ranges are restricted by the absence of particular insect species and their inability to adapt to colder environments, a result of their slow metabolism (Vijayaraghavan, 2009). Further, they are the only Xenarthra to display jumping as a startle response; this can prove unfortunate when encountering vehicles. All Dasypus are also strangely unique for demonstrating polyembryony during reproduction, which is entirely uncharacteristic of the Xenarthran order and produces four genetically identical offspring at birth (Bagatto *et al.*, 2000). Data on many species of *Dasypus* is remarkably lacking, meaning that while many are considered to be under threat of extinction, there simply is not enough information to reach a definitive conclusion.

#### 1.1.2.2 Chlamyphoridae

The most diverse of the Cingulata are the Chlamyphoridae, containing four subfamilies including the extinct lineage of glyptodonts, which are much larger and more comprehensively armoured than modern armadillos. It was previously assumed that glyptodonts were a separate lineage from armadillos, but recent molecular evidence has suggested a much more recent divergence time, placing them within the Chlamyphoridae group (Delsuc *et al.*, 2016). They were for a time, along with other armadillos and sloths, able to successfully though temporarily colonise Northern America (Carlini *et al.*, 2008). As with many Xenarthra, the exact cause of their extinction is subject to debate.

Curiously the closest relative to the giant glyptodon is, it has been recently discovered, the Chlamyphorinae or fairy armadillos (Delsuc *et al.*, 2016). Contrary to being the size of small cars as per many glyptodon, fairy armadillos are often only about 15 centimeters in length. Very little is known about them as a result of their nocturnal and almost entirely subterranean lifestyle (Delsuc *et al.*, 2012), and individuals caught in the wild have almost universally died soon after capture, making them difficult to study (Superina, 2011). Sightings of them are becoming ever rarer, seemingly as a result of increased farming, and predation from cats and dogs (Borghi *et al.*, 2011; Superina, 2014), making them high priority targets for conservation efforts.

Sister to the Chlamyphorinae are the Tolypeutinae; the giant, three banded, and

naked–tailed armadillos. Many Tolypeutinae are specialist termite feeders and have remarkably low metabolism even in comparison to other Xenarthra (McNab, 1980), and the genera in this subfamily are rife with distinguishing characteristics. The giant *Priodontes*, of which *Priodontes maximus* is the largest, are the largest living Xenarthra at about a meter in length. In contrast to the giant anteater it possess a rather different strategy for ant – and termite – consumption, where it often digs burrows into active insect nests and slowly consumes the nest to exhaustion, as opposed to intentionally preserving the food source. Once incredibly widespread across the South American plains, most *Priodontes* are highly endangered as a result of overfarming.

As they have the least comprehensive armour plating of the Cingulata, the three banded *Tolypeutes* are the only ones that rely heavily on said armour for defence – no other armadillo can completely curl themselves into a ball as the two *Tolypeutes* can. Meanwhile the naked–tailed *Cabassous* armadillos, so named due to a lack or diminished quantity of the usual osteoderms on their tails, make up some of the smallest armadillos on average. The Northern naked–tailed armadillo, *Cabassous centralis* is the only other species of armadillo besides the nine–banded to be distributed outside of South America, with its range encompassing Central America and the southern tip of Mexico.

The most anciently diverged of all the Chlamyphoridae are the Euphractinae; five species across three genera. The dwarf armadillo *Zaedypus pichi* is the only member of its genus, as is the six–banded armadillo *Euphractus sexcinctus*. Dwarf armadillos are the only armadillo species to hibernate (Superina & Abba, 2014), and as their name implies they are some of the smallest armadillos. Despite their diminutive body size, they actually have one of the proportionally largest penises in the animal kingdom, with it occupying some 60% of their body length (Superina & Loughry, 2012). Six–banded armadillos conversely are one of the largest of the armadillo species. At one time they were considered to be in the same genera as dwarf armadillos, but karyotypical (Jorge *et al.*, 1978), morphological (Wetzel, 1985), and mitochondrial (Gibb *et al.*, 2016) studies have all since disputed this notion. Finally there are are last genera of Euphractinae; the three hairy armadillos, *Caetophractus*. They are so named for the prominent tufts of hair that emerge from between the osteodermal bands.

## 1.2 Evolutionary History

### 1.2.1 Genetic Data

Having established the key traits of the various members of Xenarthra, it is prudent to explore how each is related to one another. However given the incredibly broad range of phenotypic characteristic found throughout Xenarthra, determining their evolutionary relationships has proven difficult, a problem compounded by a lack of genetic and fossil evidence for many of the numerous extinct taxa (Slater *et al.*, 2016).

The reduced diversity of modern Xenarthra is the result of a mass extinction a mere 10,000 years ago. Before this point, Xenarthra experienced a large scale radiation after being isolated on the South American continent through most of the Cenozoic, with the Great American Interchange opening up vast new ecological niches for them (Patterson & Pascual, 1972). Across both extant and extinct Xenarthra there has been limited research aimed at elucidating the phylogenetic relationships between them (Delsuc & Douzery, 2009).

Early morphological assessments have proven contradictory and inconclusive, with the most prominent characteristics analysed being teeth (Ferigolo, 1985), ears (Segall, 1976), and crania (Bugge, 1979). Some would identify sloths and armadillos as the most closely related taxa (Bugge, 1979), while others gave evidence for the grouping of sloths and anteaters (Patterson *et al.*, 1992), in line with current thinking. Another common finding based on skull morphology was that anteaters were a basal group to other Xenarthra. Preliminary molecular analyses from the same time did little to improve our understanding, with studies of alpha crystallin–A (Van Dijk, 1999; McKenna, 1992) and serum albumin (Sarich, 1985) establishing little beyond the monophyly of Xenarthrans and supporting high taxonomic level divergences amongst them based on hairy (sloths and anteaters) and non–hairy phenotypes.

However, since the turn of the century great progress has been made in the use of molecular markers to resolve the Xenarthran phylogeny across the core five families; Bradypodidae and Megalonychidae forming sloths, Cyclopedidae and Myrmecophagidae forming anteaters, and Dasypodidae representing armadillos. The first study to

give strong evidence for this arrangement examined eight of the extant Xenarthran genera and utilised both mitochondiral 12S and 16S rRNAs, as well as Von Willebrand Factor (VWF) exon 28 (Delsuc *et al.*, 2001). While attempts had been made to examine the relationships between Xenarthra and other mammalian groups (Huchon *et al.*, 1999), this represented a huge leap in the amount of available genetic information for Xenarthrans specifically and was swiftly followed by an expanded phylogenetic analysis (Delsuc *et al.*, 2002). This time twelve genera were included and three nuclear genes assessed; VWF exon 28, alpha–2B adrenergy receptor gene (ADRA2B) and breast cancer susceptibility (BRCA1) exon 11.

While this study was unable to fully resolve the phylogeny of armadillos, it did successfully corroborate morphological evidence suggesting three major subfamilies; Dasypodinae), Tolypeutinae, and Euphractinae (McKenna & Bell, 1997). The inclusion of mitochondrial genes into these analyses only gave further credence to this division (Delsuc *et al.*, 2003), as did wider scale studies of mammalian phylogenies that utilised large nuclear genetic data sets in extant species (Madsen *et al.*, 2001; Murphy *et al.*, 2001b; Murphy *et al.*, 2001a). These studies were also useful in confirming earlier works (Meyer & Kircher, 2015; Shoshani & McKenna, 1998) that pointed to Xenarthra as the earliest offshoot of placental mammals. These findings were in direct contradiction to the earlier morphological studies suggesting anteaters as basal to Xenarthrans (Segall, 1976; Bugge, 1979; Ferigolo, 1985), likely as a result of adaptations in anteater skulls suited for myrmecophagy (Delsuc & Douzery, 2009).

### 1.2.2 Establishing A Phylogenetic Framework

The succession of studies from Delsuc et al (2001, 2002, 2003) was able to cement the relationships between anteaters. Molecular analyses all suggested that both Tamandua and Myrmecophaga shared a close relationship. This is in line with morphological assessments (Gaudin & Branham, 1998; Reiss, 1997) that described the lone *Cyclopes* species as divergent from other Vermilingua, with one ancestral genus in the form of *Palaeomyrmidon* (Hayssen *et al.*, 2012).

Regarding sloths, while the fossil record is comparatively plentiful for the many ancient and diverse ground sloths there have only been very few historical remains found of the two extant genera. Based primarily on cranial evidence they are assumed to be

diphyletic (Gaudin, 2004), and a number of molecular studies on a wide range of extinct sloth species (Hoss *et al.*, 1996; Poinar *et al.*, 1998; Greenwood *et al.*, 2001; Poinar *et al.*, 2003) seem to confirm this with varying degrees of certainty. These studies are predominantly focused on 12S and 16S rRNA, and imply a close relationship between Nothrotheriidae and the two–toed Bradypodidae; the three–toed Megalonychidae meanwhile are more closely related to the Megatheriidae and Mylodontidae. A study of retrovirus and mitochondrial DNA suggests that *C. didactylus* and *C. hoffmani* diverged between six and seven million years ago (Slater *et al.*, 2016). Prior to this a similar date of divergence between the two extant *Choloepus* species was suggested based on cytochrome c oxidase subunit I sequences (Moraes-Barros & Arteaga, 2015), all of which agree with the suggestions of Delsuc et al (2002).

Analyses of armadillo phylogeny has been formed to a large degree by studies of their shells, which have impacted the evolution of many of their other traits (Superina & Loughry, 2012). Positioning Pampatheres amongst other Cingulata has proven challenging, as it has thus far been difficult to recover DNA from any samples (Shapiro *et al.*, 2014), and there is disagreement over placement of particular species, particularly fairy armadillos, as a result of conflicting morphological and genetic data (Delsuc *et al.*, 2012; Billet *et al.*, 2011). Analyses of retrosposons from representatives of each of the 13 major genera suggested for the first time that *Cheatophractus* and *Zaedyus* formed a sister group to *Euphractus* (Möller-Krull *et al.*, 2007), a grouping that has been confirmed in subsequent studies (Gibb *et al.*, 2016), see Figure 1.1.

With the gradual increase in available data, as well as an increase in available methods, it has been possible to derive a time scale for Xenarthran evolution and make more precise estimates of their phylogeny. In the Delsuc et al (2001) paper described in Section 1.2.1, maximum likelihood methods were used to calculate divergence rates amongst key lineages, based upon a local molecular clock. Over the following two years with the refinement of a Bayesian relaxed clock method (Thorne *et al.*, 1998; Kishino *et al.*, 2001; Thorne & Kishino, 2002), a large set of both mitochondrial and nuclear genes (Murphy *et al.*, 2001a) were analysed across a large number of mammalian clades, including a small selection of Xenarthra (Springer *et al.*, 2003). These works suggested placing the root of the Xenarthran clade at 60–90 mya, with the order undergoing

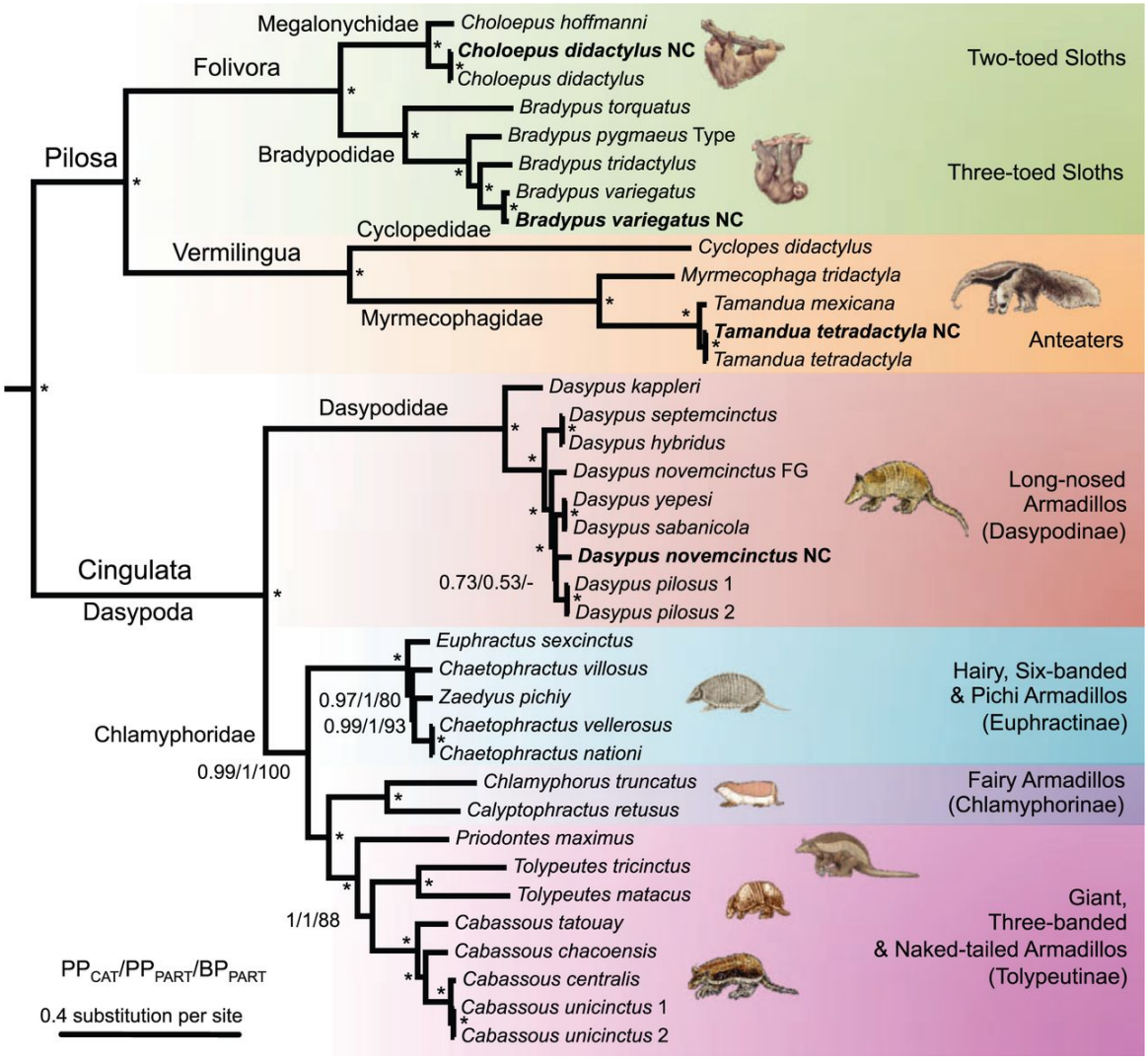


Figure 1.1: Taken from Gibb et al 2015. Tree showing the relationships between all extant Xenarthra based on mitochondrial data. Asterisks (*) mark branches with maximal support. Values at nodes indicate Bayesian posterior probabilities (PP) obtained under the CAT-GTR-G4 mixture model and maximum-likelihood bootstrap percentages (BP) obtained under the optimal partitioned model, respectively. Afrotheria was used as an outgroup. This represents the first time that phylogenetic inferences have been made using genetic data from every extant Xenarthran.

intraordinal diversification about 12 million years before the boundary between the Cretaceous and Paleocene periods.

This approach has been applied more specifically to Xenarthra, with studies across twelve extant genera approximating the divergence between Cingulata and Pilosa, representing the Xenarthran MRCA, to have occurred 70.5 mya (Delsuc *et al.*, 2004). The same three nuclear genes in the 2002 Delsuc et al paper were used here; ADRA2B, BRCA1, and VWF. While this conflicts with earlier molecular studies that suggested divergence occurred about ten million years earlier (Hoss *et al.*, 1996), it aligns better with morphological evidence (Bergqvist *et al.*, 2004) that notes the earliest armadillo scutes in the fossil record being dated to approximately 58 mya in Brazil. In either instance, the next major split, between Folivora and Vermilingua, appears to have followed shortly after, around 60 mya.

There are certain difficulties in calibrating the lineages of the sloth genera. The oldest fossil remains that are undoubtedly sloth are approximately forty million years old, but thus far they have not been placed within any particular lineage (Vizcaíno *et al.*, 1998). This trend persists with a number of more recent extinct sloths, especially in the numerous Caribbean species (Anderson & Handley Jr, 2001; White & MacPhee, 2001). Amongst these sloth, which outlived giant sloths on the mainland by a few thousand years until the arrival of humans (Steadman *et al.*, 2005), many are representatives of Megalonychidae and comprise a contested set of phylogenetic placements. That said, there is little doubt that divergence between the two– and three–toed sloths runs deep at around 20 mya(Delsuc *et al.*, 2004), supporting their distinction.

Molecular dating studies that explore anteaters show that the two genera, Cyclopedidae and Myrmecophagidae, have an ancient split between them, though corroborating this with fossil evidence has proven a little difficult. The fossil record for anteaters is thought to be very incomplete on account of their low diversity and difficult environmental conditions for fossilisation. The earliest unquestioned anteater fossil can be dated to approximately 20 mya in Patagonia (Carlini *et al.*, 1992), while molecular estimates have consistently placed the Cyclopode/Myrmecophage split at around 40 mya (Delsuc *et al.*, 2004; Meredith *et al.*, 2011), supporting *C. didactylus* as being

distinct from other anteaters (Barros *et al.*, 2008).

The dates obtained in these analyses indicate that environmental shifts in South and Central America over the past 65 million years influenced Xenarthran evolution (Delsuc *et al.*, 2004). More specifically, the aforementioned divergences appear to correlate with a series of tectonic events whereby the North Andes plate experienced major uplift (Marshall & Sempere, 1993). The split between anteater lineages seems to have occurred shortly after a period of heavy uplift in the Andes around 43 mya (Marshall & Sempere, 1993). Likewise the divergence between sloth families 20 mya coincides with another period of geological upheaval associated with widespread climate change and rapid shifts in fossil communities (Marshall & Sempere, 1993).

## 1.3 Research Aims

As has been noted numerous times, Xenarthrans possess numerous unique characteristics that make them particularly peculiar mammals, and the evolution and significance of these traits is of importance. After all, Xenarthra are a basal clade of placental mammals, and events in their early life history may be reflective of early mammal evolution (Superina & Loughry, 2015). Studies into both the earliest mammals (O'Leary *et al.*, 2013) and the earliest Xenarthrans(Gaudin & Croft, 2015) have suggested that Xenarthrans began as myrmecophages and transitioned to omnivory and herbivory, and also make predictions about the development of their preferences for habitat and ambulation. Their formerly diverse clade is now represented by just 31 extant species, marking them as important models for conservation.Broadening our knowledge of Xenarthrans requires a firm understanding of the relationships between them, an understanding which is necessarily coming more and more from genetic data.

Despite difficulties in acquiring genetic information in Xenarthra on account of their scarcity and the poor environmental conditions for preservation (Superina, 2014), there has been a steady growth in the amount of available information. This has culminated in the development of a phylogenetic framework based on full mitochondrial genomes (Gibb *et al.*, 2016), the first to cover all extant species. However, there are two intuitive ways in which it can be further expanded upon. Firstly, one can bolster the mitochondrial information with nuclear genes. Nuclear genes are typically more

efficient at recovering benchmark clades (Springer *et al.*, 2001) and can give conflicting phylogenetic accounts to mtDNA (Shaw, 2002), though nuclear DNA has worse long term survivability *in situ*, hence the prevalence of mtDNA in studies with ancient specimens (Hunter, 2006). Secondly, while the Gibb et al (2015) paper accounts for all extant Xenarthra, the use of extinct fossil species will allow more accurate phylogenetic calibration.

Using well established methods of DNA enrichment (Horn, 2011; Enk *et al.*, 2014), we intend to fulfil both of these aims. From the samples used in the Gibb et al (2015) paper we intend to enrich 74 nuclear genes. From the genes that can be most successfully enriched across all species, we can create a framework based on nuclear genes. Furthermore, using mitochondrial genomes previously sequenced from extinct Xenarthran species, we can further calibrate the mitochondrial framework.

# Chapter 2

# Methods

## 2.1 Samples

The taxa of all samples used can be found in tables 2.1 and 2.2. All modern samples were previously used in a Xenarthran mitochondrial genome study (Gibb *et al.*, 2016). Ancient samples were previously sequenced but most are unpublished, with the exception of An28 (Delsuc *et al.*, 2016). In this context, "modern" will refer to samples from extant Xenarthra, while "ancient" refers exclusively to extinct species.

| ID | Species | Collection No. | Origin | Sample Type |
|---|---|---|---|---|
| An16 | Mylodon darwinii | MNHN 1905–4 | Unknown | Derm & osteoderm |
| An28 | Glyptodon doedicurus | MACN 6744 | Rio Salado, Arizona | Carapace |
| An54 | Megatherium parodii | UF 75452 | Unknown | Long bone |
| An58 | Acratocnus ye | UF76365 | Unknown | Mandible with molar |
| An67 | Mylodon darwinii | MPI SP57 | Mylodon Cave | Bone |
| An69 | Megalonyx jeffersonii | PMA P98.6.28 | Unknown | Bone |
| AnX18 | Mylodon | 2 C.2c | Penas de las Trampas | Coprolite |
| AnX22 | Mylodon | #1 Capa 1.C16-17/H1 | Penas de las Trampas | Coprolite |
| AnX23 | Mylodon | C.2E_Layer 4 | Penas de las Trampas | Coprolite |
| AnX25 | Mylodon | 3 C.2E. Layer 4 | Penas de las Trampas | Coprolite |
| AnX32 | Nothrotheriops shastensis | RC L12 #1 | Rampart Cave | Coprolite |

Table 2.1: Table showing species, type, and location of ancient samples used in this study.

| ID | Species | Collection No. | Origin | Year | Sample Type |
|---|---|---|---|---|---|
| Mod1 | Dasypus novemcinctus | T-1863 | French Guiana | 1995 | Ear |
| Mod2 | Dasypus kappleri | T-3365 | French Guiana | 2001 | Internal organ |
| Mod3 | Dasypus pilosus | T-1246 | Bolivia | 1993 | Internal organ or skin |
| Mod4 | Dasypus septemcinctus | T-3002 | Rosario, Argentina | 2000 | Ear |
| Mod5 | Chlamyphorus truncatus | T-CT1 | Mendoza, Argentina | 2005 | Internal organ |
| Mod6 | Chaetophractus villosus | T-NP390 | Argentina | 2001 | Ear |
| Mod7 | Chaetophractus vellerosus | T-CV1 | Mendoza, Argentina | 2005 | Internal organ |
| Mod8 | Euphractus sexcinctus | T-ES1 | Santa Fe Zoo, Argentina | 2000 | Blood in 95% EtOH |
| Mod9 | Zaedyus pichiy | T-ZP67 | Mendoza, Argentina | 2005 | Internal organ |
| Mod10 | Cabassous unicinctus | T-1641 | French Guiana | 1995 | Internal organ |
| Mod11 | Cabassous unicinctus | T-2291 | French Guiana | 2000 | Internal organ |
| Mod12 | Cabassous chacoensis | T-2350 | Argentina | 2000 | Skin from tail |
| Mod13 | Priodontes maximus | T-2353 | Argentina | 2000 | Muscle or skin |
| Mod14 | Tolypeutes matacus | T-2348 | Argentina | 2000 | Ear |
| Mod15 | Cyclopes didactylus | T-1631 | French Guiana | 1995 | Internal organ |
| Mod16 | Myrmecophaga tridactyla | T-5150 | French Guiana | 2007 | Internal organ |
| Mod17 | Tamandua mexicana | T-3000 | Chiapas, Mexico | 1977 | Internal organ |
| Mod18 | Bradypus tridactylus | T-5013 | French Guiana | 2006 | Ear |
| Mod19 | Bradypus variegatus | T-2999 | Amazonas, Peru | 1978 | Internal organ |
| Mod20 | Choloepus hoffmanni | T-33694 | Philadelphia Zoo, USA | 2001 | Kidney |
| Mod21 | Choloepus didactylus | T-1722 | French Guiana | 1997 | Internal organ |
| Mod22 | Myrmecophaga tridactyla | T-2862 | French Guiana | 2001 | Internal organ |
| Mod23 | Chaetophractus nationi | T-LP1 | Bolivia | 2008 | Blood |
| Mod24 | Dasypus yepesi | T-Dyep | Argentina | 1988 | bone |
| Mod25 | Calyptophractus retusus | T-Bret | Bolivia | 1974 | Internal organ |
| Mod26 | Dasypus pilosus | T-1246 | Bolivia | 1993 | Internal organ |
| MoL27 | Dasypus hybridus | 205722 | Uruguay | Unknown | Vertebral disk |
| MoL28 | Cabassous centralis | 10752 | Costa Rica | Unknown | Bone fragments from skull |
| MoL29 | Dasypus pilosus | MSB49990 | Huanuco, Peru | 1980 | Skin |
| MoL30 | Dasypus pilosus | LSUMZ19240 | Huanuco, Peru | 1974 | Skin |
| MoL31 | Dasypus pilosus | LSUMZ21888 | Amazonas, Peru | 1978 | Skin |
| MoL32 | Bradypus pygmaeus | USNM579179 | Panama | 1991 | Tissue sample in DMSO |
| MoL33 | Dasypus sabanicola | USNM372834 | Venezuela | 1966 | Skin |
| MoL34 | Bradypus torquatus | USNM259473 | Brazil | 1919 | Skin |
| MoL35 | Dasypus hybridus | ZVC-M843 | Uruguay | Unknown | Dried skin |
| MoL36 | Dasypus hybridus | ZVC-M844 | Uruguay | Unknown | Dried skin |
| MoL37 | Dasypus hybridus | ZVC-M1118 | Uruguay | Unknown | Dried skin |
| MoL38 | Dasypus hybridus | ZVC-M2010 | Uruguay | 1976 | Dried skin + Tail |
| MoL39 | Cabassous tatouay | ZVC-M365 | Uruguay | Unknown | Dried skin |
| MoL40 | Tamandua tetradactyla | (PK44) T-6054 | French Guiana | Unknown | Ear biopsy in 95% EtOH |
| MoL41 | Bradypus torquatus | Tube 449/11 | Unknown | Unknown | DNA |
| MoL42 | Tolypeutes tricinctus | Tube 21 | Unknown | Unknown | DNA |

Table 2.2: Table showing species, type, and location of modern extant Xenarthran samples used in this study.

## 2.2 DNA Extraction

### 2.2.1 Ancient DNA Laboratory Set–up

All laboratory experiments were performed under appropriate conditions for aDNA (Fulton, 2011). Modern and ancient samples were stored separately and were processed in entirely different buildings. All work was performed in clean rooms which were separated across sample preparation, pre–PCR, and PCR, with a separate DNA free room for preparing reagents. All rooms maintained a positive–pressure air flow and contained dead air workstations. In the PCR room were two separate dead air workspaces; one for totally DNA free reagent preparation and the other for DNA extraction and manipulation. All work spaces and large pieces of equipment were regularly sterilised with bleach and water; smaller tools were placed in a UV crosslinker for30 minutes after every use. Water used in reactions and for cleaning was UV filtered.

Movement in and between clean rooms was strictly regulated. Entry into pre–PCr environments was contingent on the entrant having showered and put on clothing that had not been in a post–PCR room. All persons therein were required to wear surgical masks, control their hair with a hairnet, and don a whole–body boiler suit with a hood, as well as shoe covers and easy to clean shoes that were used only in the clean rooms. Protective clothing was stored and equipped in an atrium adjacent to the clean rooms. The outermost of the two pairs of gloves was changed after interacting with each sample or between each step of a given protocol.

### 2.2.2 Ancient Paleofeces

Paleofecal extractions were adapted from a well established protocol utilising N-phenacyl thiazolium bromide (PTB) (Poinar *et al.*, 1998; Kuch, 2011; **?**). 1.75ml of GuSCN extration buffer was added to each 100–200 mg subsample of paleofeces. Samples were then left overnight at 37°C while shaking at 1000 rpm. The next day the samples were centrifuged at maximum speed for 5 minutes, with the resulting supernatant being transferred to a 2 ml tube.

This was followed by part of an ultra–filtration step. The membrane of an Amicon Ultra 0.5ml 30 kDa filter was primed with 450 $\mu$l of 0.1X TE, which was passed

through the membrane via centrifuge. Raw DNA extract was applied to the membrane and spun through 450$\mu$l at a time. However, due to difficulties with the columns that resulted in some breakage, the ultra–filtration step was disregarded and the flowthrough poured back into the raw extract. Finally a MinElute (Qiagen) purification was conducted in accordance with the manufacturer's protocol, eluting into 25$\mu$l 0.1X TE and 0.05% Tween.

### 2.2.3 Ancient Bones and Modern Tissues

DNA extraction of modern samples was performed as described in Gibb et al's 2015 paper. Ancient bones underwent identical extraction but were first pulverised with a hammer, resulting in a crumbly powder. Samples were split into small pieces weighing a combined 50mg, and bone samples were demineralized in 0.75ml of 0.5M EDTA by being agitated overnight at room temperature. A digestion buffer was then prepared, containing per reaction; 10$\mu$l 20mM Tris, 25$\mu$l of 0.5% Sarcosyl, 1$\mu$l of 5 mM $CaCl_2$, 5mg of 1% PVP, 38.55mg of 50mM DTT, 3.55mg of 2.5 mM PTB, and 6.25$\mu$l of 250$\mu$g/ml Proteinase K. Samples were digested using this buffer by being agitated within it at 55°C for two hours, after which the resulting supernatants were removed. Organics left in the supernatant were extracted using pheonl:chloroform:isoamyl alcohol (25:24:1), and the aqueous phase was passed through a 30 kDA Amicon Ultra 0.5 ml centrifugal filter (Millipore) at 14,000 × g. Washes for the filter utilised 0.1X TE buffer and 0.05% Tween–20. The final extraction had a volume of 50$\mu$l.

Samples Mod1 through Mod26 also underwent DNA fragmentation using NEBNext dsDNA Fragmentase (New England Biolabs). Reactions were purified with the MinElute PCR Purification kit (Qiagen) and eluted in 20 µl buffer EB. Samples ModL27 through MoL42 were sonicated using a Covaris S220 according to manufacturer's protocol to yield a median fragment length of 200 bp.

## 2.3 Library Preparation and Indexing

### 2.3.1 Library Preparation

All libraries were prepared in approximate accordance with, and using the primers from, a well established protocol (Meyer & Kircher, 2010). For each reaction a blunt–end

repair mix was prepared, containing 4$\mu$l of 10X Buffer Tango (Fermentas), 0.16$\mu$l 25nM dNTPs, 0.40$\mu$l 100mM dATPs, 2$\mu$l of 10U/$\mu$l T4 polynucleotide kinase (Fermentas), 0.8$\mu$l of 5 U/$\mu$l T4 DNA polymerase (Fermentas), 12.64$\mu$l water, and 20$\mu$l of template DNA. These were processed in an MJ thermocycler (BioRad) at 25°C for 15 minutes, 12°C for 5 minutes, and then held at 4°C. The resultant template was purified through a MinElute column, eluted in 20$\mu$l EB buffer, and maintained on ice.

Next, the template was combined with an Adapter Ligation mix containing 4$\mu$l 10X T4 DNA ligase buffer, 4$\mu$l 50% PEG-4000, 1$\mu$l 100 $\mu$M Adapter mix, 1$\mu$l 5U/$\mu$l T4 DNA ligase (Fermentas), and 10$\mu$l of water, which was then held at 16°C for a minimum of 15 hours. Again, this was then purified over a MinElute column and eluted in 20$\mu$l of EB buffer. Finally, to complete the library preparation, the template was combined with an Adapter Fill–In Mix containing 4$\mu$l 10X ThermoPol rxn buffer, 4$\mu$l 2.5mM dNTPs, 1.50 8U/$\mu$l Bst polymerase. 10.5$\mu$l of water and 20$\mu$l of template. The mixture was heated to 37° for 20 minutes, then once more eluted in 20$\mu$l EB buffer through a MinElute column.

### 2.3.2 Indexing of Ancient Samples

Indexing methods were adapted from the 2010 Meyer & Kircher paper, with all libraries being double indexed with unique forward/reverse primer combinations. For each library, the Indexing Mix contained 10$\mu$l of 1X Herculase II Reaction buffer, 1.25$\mu$l 0.5X EvaGreen, 5$\mu$l 0.25mM dNTP mix, 2$\mu$l each of the forward and reverse primers, 0.5$\mu$l Herculase II Fusion DNA Polymerase, 22.50$\mu$l water, and 10$\mu$l of template. Primer combinations can be found in Appendix C.

Amplification cycles were as follows: 95°C for 2 minutes; cycle 10 times through 95°C for 15 seconds, 60°C for 20 seconds, 72°C for 30 seconds; after 10 cycles, hold at 72°C.

### 2.3.3 Indexing of Modern Samples

Fragmented modern samples (Mod1 through Mod26) were single indexed with a common forward primer (P5) and a unique reverse primer (P7). The Indexing Mix contained 5$\mu$l of 10X PCR Buffer II, 5$\mu$l 25mM $MgCl_2$, 5$\mu$l 0.25mM dNTP mix, 1$\mu$l

each of the forward and reverse primers, 0.5$\mu$l Amplitaq Gold, 22.50$\mu$l water, and 10$\mu$l of template. Primer combinations can again be found in Appendix C. The sonicated samples (ModL27 through ModL42) were indexed with both a unique P5 and a unique P7, based on Kircher et al's 2012 paper. For double indexing, the Indexing Mix contained 5$\mu$l 10X Accuprime Pfx Reaction mix, 1.25$\mu$l 20X EvaGreen, 0.5$\mu$l 2.5U/$\mu$l AccuPrime Pfx DNA polymerase, 2.5$\mu$l each of the forward and reverse primers, and 5$\mu$l of template.

Both methods used almost identical amplification cycles, as follows: 95°C for 4 minutes; cycle 12 times through 95°C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds; hold at 8°C. However, for the double indexed variant, temperature of 72°C were changed to 68°C.

High protocol variability comes as a result of the extractions, library preparations, and indexing, being performed by Melanie Kuch over a period of four years between 2010 and 2014. As such, reagents, concentrations, and PCR cycles were adjusted for performance between modern and ancient samples, as well as to account for improved understanding of and experience with the underlying chemistry.

### 2.3.4 Re–indexing of Exhausted Libraries

Due to the fact that some indexed libraries were exhausted, a number of both ancient and modern libraries required re–indexing; specifically, all AnX samples, and modern samples Mod5, 11, 13, 19, L28, L29, L31, L33 & L38. Samples that were previously double indexed were re–indexed with the same combination of primers, while those that were single indexed were re–indexed with entirely new combinations. The Indexing Mix contained 25$\mu$l of 2X KAPA SYBR FAST qPCR Master Mix, 3.50$\mu$l water, 0.75$\mu$l each of the forward and indexing primers, and 20$\mu$l of template. The amplification cycle was performed on an MJ thermocycler (BioRad), and applied the following scheme: 95°C for 5 minutes; 10 cycles of 95°C for 30 seconds, 60°C for 45 seconds; 60°C for 3 minutes.

## 2.4 Bait Design

The methods in sections 2.4 through 2.7 describe only work performed in aid of enriching the nuclear loci; mitochondrial sequences were generated previously by other persons.

Each nuclear loci and their respective variants were repeat masked using RepeatMasker (Smit *et al.*, 2013–2015), so that repetitive regions were substituted with an N. This was done to limit non–specific binding of baits to non–targets. From the repeat masked sequences, 80mer baits were generated with a 4x tiling density. This yielded approximately 20bp probe spacing, or 60bp probe overlap Where regions were too short to land 80mer baits, the minimum permitted length was 50bp; these baits were brought up to 80bp by padding with Ts. Any stretches of unknown base pairs (N) that were 10bp or fewer were replaced with lowercase t's. Tiling was flexible to ensure even distribution of baits across the loci, as most loci were not perfect multiples of 20.

All baits were then BLASTed against the two–toed sloth, *Choloepus hoffmanni* (NCBI assembly GCA_000164785.2), and nine–banded armadillo, *Dasypus novemcinctus* (NCBI assembly GCA_000208655.2), reference sequences. Baits with one or no hits are described as specific or unique, while non–specific and non–unique baits have more than one hits returned. Then the melting temperature (Tm's) of all the hits were tallied and a judgment made about whether or not to drop that individual bait from the baitset, depending on the number of hits, the Tm composition of the hits, and the performance of the neighboring baits. Ideally baits should only have one hit and Tm of 35–40°C.

It may be worth noting that the two reference sequences are sequenced to a high coverage depth, but not yet well curated and organised; in particular the *Choloepus hoffmanni* genome is still in 260,000 scaffolds not organised by chromosome. The approach used here to detect non–specific probes is only as reliable as the reference genome used for the analysis. Where a reference genome has a large degree of incomplete contig assembly, unique probes will appear to be non–specific. If the genome used does not contain a homologous locus, or there is too much divergence, there will be no BLAST hits at the appropriate Tm.

At this stage there were 22,495 raw unfiltered baits. In order to fit the smallest baitset scale available, which allows up to 20,020 baits, baits with a similarity of up to 3 mismatches were collapsed, leaving 19,659 unfiltered collapsed baits. The vast majority of probes had 1 or 0 hits across the two genomes, and there are only a small number of probes with 2–3 hits and Tm's greater than 40°C. There were two exceptions to this: firstly, CNGB1partcdsSLOTH, when blasted to the *Choloepus hoffmanni* genome, possessed a section (baits 2909–3035) that have 5–6 genome hits with a high Tm. The second exception was GJA10exon1ARMADILLO, which when blasted to the *Dasypus novemcinctus* genome, has anywhere from a few tens to several hundreds of hits at low Tms for over half of its' probes. Therefore the baits for these two loci were removed entirely from the baitset, leaving a final set of 19,435 baits.

## 2.5 Targeted Enrichment

All libraries were enriched with the baitset in order to selectively capture sequences representing the loci of interest. Five enrichments were performed in total across two "sets." The first set contained one enrichment on ancient samples, and three on modern samples. The second set contained one enrichment on both ancient and modern samples. All enrichments were performed in two rounds following the same basic methodology.

### 2.5.1 Hybridisation

5$\mu$l of indexed libraries were used for hybridisation. Where there was less than 5$\mu$l available, the library was exhausted and the full remaining amount was used. Each volume was brought to 10$\mu$l by adding water. 1.94$\mu$l of Human Cot1 DNA (Life Sciences, 1$\mu$g/$\mu$l), 0.19$\mu$l of Salmon sperm DNA (Life Sciences, 10$\mu$g/$\mu$l) and 0.40$\mu$l of Illumina bloligos (48$\mu$M) were added to create a Library Mix.

Alongside this, a Hybrid–Capture Mix was produced containing for each library; 8.23$\mu$l 20X SSPE, 0.33$\mu$l 0.5M EDTA, 3.29$\mu$l 50X Denhardt's, 0.33$\mu$l10% SDS, 1.16$\mu$l SUPERase–In, and 1.16$\mu$l of the Xenarthran nuclear baits, equivalent to 100ng. The Lib Mix was heated to 95°C for five minutes then cooled to 55°C – the Hybrid–

Capture Mix was also heated to 55° for four minutes. Once both mixes were at 55°, 12.72$\mu$l of the Hybrid–Capture Mix was added to each Library Mix. The resulting Library–Hybrid–Capture Mix was kept at 55°C for 20–24 hours.

### 2.5.2 Reagent Removal

For each reaction, 20$\mu$l of Dynabeads MyOne Streptavidin C1 beads (Life Technologies) were washed with 80$\mu$l of Binding Buffer (MYcroarray). Washing was performed by adding Binding Buffer to beads in 1.7$\mu$l tubes, each tube containing beads for 18 reactions, then vortexing. Beads were then magnetically pelleted and the supernatant removed; this was repeated twice more before resuspending the beads in 360$\mu$l of Binding Buffer per tube. The now washed beads were transfered in 20$\mu$l quantities to high–profile PCR strip tubes and incubated at 55°C.

Next the bead mixtures were added to the Library–Hybrid–Capture Mixes and incubated at 55°C for 30 minutes. Tubes were agitated every 10 minutes to prevent settling or clumping of the beads. After 30 minutes the beads were pelleted and the supernatant removed and stored. Beads were then washed for 10 minutes in 180$\mu$l of Wash Buffer 2 (MYcroarray) diluted to 0.2X in 0.1% SDS. Every three minutes the tubes were agitated, and the wash was repeated three more times, with fresh 0.2X Wash Buffer 2 being introduced for each wash. After the fourth wash the final supernatant was removed and the tubes spun down to ensure all liquid was removed. The final pellet was eluted in 18.8$\mu$l of EBT.

### 2.5.3 Amplification

In order to re–amplify the captured sequences, a LibQ Master Mix was prepared. This contained 20$\mu$l of KAPA SYBR® FAST qPCR Master Mix (2X), 0.60$\mu$l Forward Primer 1469 (150nM) and 0.60$\mu$l Reverse Primer 1470 (150nM) per reaction. The LibQ Mix was added to the 18.8$\mu$l of captured template and amplified on a CFX. Amplification cycling protocols were as follows: 95°C for 5 minutes; cycle 12 times through 95°C for 30 seconds, 60°C for 45 seconds; finally hold at 60°C for 3 minutes. Following this the supernatant was removed and saved, yielding the captured library. This was purified using a Minelute PCR Purification Kit (Qiagen) using their standard protocol, yielding a final enriched and purified library suspended in 15$\mu$l of EB.

## 2.6 Quantification and Sequencing

All libraries to be sequenced were pooled together at varying concentrations with the aim of creating a single solution containing approximately 250 pM of DNA post size selection. In general, each library was calculated to ideally produce one million reads for sequencing. Libraries then underwent size selection to decrease the amount of non–target DNA and increase sequencing efficiency. Size selection was carried out on a 2% gel (50 ml agarose/1X TAE with2 $\mu$l EtBr). Loading dye equivalent to 1/5 of the library volume was added and samples were then run through the gel for 30 minutes at 100V. A 50 bp ladder was used for determining band position and size, and the bands from approximately 50 to 150 bp were excised.

The excised gel was then purified using a MinElute Gel Extraction Kit (Qiagen) following their standard protocol. Purification took place over one column and the size selected library was eluted into 60 $\mu$l of EB buffer. Final pool concentrations prior to sequencing were verified using a 2100 Bioanalyzer (Agilent). Sequencing of the first enrichment set was performed on a single lane on an Illumina HiSeq 1500, while the second set took a single lane on an Illumina MiSeq. Illumina Bustard software (MiSeq) or FreeIbis27 (HiSeq) were used to call bases (Renaud *et al.*, 2013).

## 2.7 Data Processing

Sequences not matching perfectly with one of the expected index combinations were discarded. Index and adapter sequences were removed from the raw reads and paired reads were merged using leeHom with standard aDNA settings (Renaud *et al.*, 2014). Reads shorter than 24 bp were filtered from the analysis with Samtools (Li *et al.*, 2009), with the remaining reads being mapped to the reference exon sequences using BWA (Li & Durbin, 2009). Duplicates with overlapping start and end coordinates were then removed using the Samtools rmdup function, resulting in a single aligned bam file for each sample. The bam files were then imported into Geneious version 9 (Kearse *et al.*, 2012) for assessment by eye of enrichment success. A gene was considered to have been enriched successfully if the mean base coverage was three or more, with no gaps in the consensus. Consensus sequences were called with a 50% threshold. GATK (McKenna *et al.*, 2010; DePristo *et al.*, 2011) was used to call SNP variants in accordance with

the pipeline described in Appendix D.1.

## 2.8 Alignment and Tree Estimation

### 2.8.1 Nuclear genes

The consensus sequences of the most successfully enriched genes across the greatest number of species (four) were aligned using MUSCLE with standard parameters (Edgar, 2004). Equivalent genes in the African savannah elephant (*Loxodonta africana*; NC_000934) were used as an outgroup. Ambiguously aligned sites were then removed with Gblocks (Castresana, 2000) using default relaxed settings. Tree estimations were performed both with individual genes and with genes concatenated with 10 N spacers. jModelTest 2 was used to pick an appropriate substitution model using 5 gamma–rate categories (Darriba *et al.*, 2012). For concatenated genes, the model deemed best according to a corrected Akaike Information Criterion (AIC) (Hurvich & Tsai, 1989) was GTR+I+G4 with gamma shape parameter (-a 0.809) and proportion of invariant sites (-i 0.270).

A maximum likelihood (ML) tree was generated using PhyML (Guindon & Gascuel, 2003; Guindon *et al.*, 2010) using the parameters obtained from jModelTest. One hundred bootstrap iterations were performed to assess statistical reliability (Pattengale *et al.*, 2010). Bayesian support for these trees was estimated using BEAST 2 (Bouckaert *et al.*, 2014), with XML files prepared in Beauti. Tip dates for each sample were incorporated and a relaxed molecular clock (Drummond *et al.*, 2006), as suggested in previous studies involving concatenated nuclear loci (Douady & Douzery, 2003; Springer *et al.*, 2003; Delsuc *et al.*, 2004), was used for a constant coalescent population model. Clock values were typical of nuclear DNA (1.0E-12). Data partitioning was automatically performed using the inbuilt RBS package (Bouckaert *et al.*, 2014) across three partitions, which is more appropriate than other methods for use in Bayesian inference. No clades were pre–established.

Under these parameters, the model was run for 10 million generations, sampling every thousand generations. After a 10% burn–in, Tracer (Rambaut *et al.*, 2014) was employed to assess convergence, with all parameters having an effective sample size

(ESS) of over 200. Treeannotator (Drummond *et al.*, 2012) was used to annotate the tree with greatest clade probability, showing mean node heights. The resulting trees are discussed in Chapter 3.2.

### 2.8.2 Mitochondrial genomes

Methods for aligning mitochondrial genomes and generating accurate an phylogenetic framework followed a similar methodology as with the nuclear data. The 33 new mitochondrial genomes from the 2015 Gibb et al paper representing all extant Xenarthra were added to those of the dugong (*Dugong dugon*; NC_003314), the African savannah elephant (*Loxodonta africana*; NC_000934), and the aardvark (*Orycteropus afer*; NC_002078), which were used as outgroups. Further, the recently published *Doedicurus clavicaudatus* mitochondrial genome (Delsuc *et al.*, 2016) and seven unpublished mitogenomes for other Xenarthra were also incorporated. These included three Antillean sloth (*Acratocnus*, *Parocnus*, *Megalonyx*), *Nothrotheriops*, *Mylodon listaii*, and two mitogenomes of *Mylodon darwinii*.

The mitogenome sequences were aligned using MUSCLE with standard parameters (Edgar, 2004). Ambiguously aligned sites were then removed by Gblocks (Castresana, 2000) with default relaxed settings, yielding sequences with 14995 sites across 44 taxa. An appropriate set of parameters for phylogenetic reconstruction were investigated with jModelTest. This resulted in using a GTR+I+G4 model of nucleotide substitution, with gamma (-a 0.381) and invariant proportion (-i 0.658). PhyML and BEAST were used for tree generation as in the previous section, and analysed in much the same fashion. One core difference was in the molecular clock rate, which was adjusted to a more appropriate value for mitochondrial DNA (1.0E-9).

# Chapter 3

# Results and Discussion

## 3.1 Enrichment

### 3.1.1 First Enrichment Set

Analyses with preseq (Daley & Smith, 2013) indicated that many of the libraries were of low complexity, with an average of 27.1% of the reads being unique. Furthermore this appeared to account the majority of unique reads with the libraries, with an average estimated exhaustion of 86.5%. Each sample produced on average just 7327 reads that uniquely mapped to any of the reference baits, with a range of values between 0 and 67966, indicating great disparity of enrichment success. Rather than the complete extant phylogeny we had sought, only twenty species generated enough reads to be reliably represented. There did appear to be some distinction in enrichment success based on which enrichment subset they samples were in, as described in Section 2.5. Ancient non–paleofeces specimens met their enrichment targets, as did many of the second modern subset. However, the first and third modern subsets performed much more poorly. A number of the libraries in these subsets are slightly older than those that were more successful.

Some explanations for the wide variation in enrichment success are discussed in Section 3.1.3. However, there are two additional complications that apply only to this first enrichment set. Specifically, the first modern enrichment was performed with ten times the intended concentration of blocker (salmon sperm DNA). This may have

inhibited the ability of baits to anneal to target sequences. Additionally, due to a math error all enrichments in this subset were performed with a lower concentration of baits that recommended by protocols which, intuitively, would inhibit targeted enrichment. In light of these errors and the need for more sequence data, a second enrichment was performed.

### 3.1.2 Second Enrichment Set

Barring differences based on the volumes of library used, the second enrichment generated more useable data than the first. For analyses, the sequence data from the two enrichments were combined together. The full results of the combined enrichment can be found in Appendix E. Twenty eight extant species generated enough reads to be used in phylogenetic analyses, as well as two mylodon. The other ancient species, while slightly improved, still underperformed substantially. Furthermore, only 38 baits generated any level of coverage.

Going forwards, it was decided to focus on optimising the number of species included in analyses over the number of loci used. As such, four genes – ADORA3, BCHE, BRCA1, and TTN – were selected for further examination. These four represented a range of different functions that optimised the balance between having sufficient read coverage for sequence consensus calling and the total number of species accounted for. While there were multiple other genes that could have been incorporated, doing so would have quickly decreased the total number of species that could be investigated as the coverage depth was too shallow for many of them. Mean coverage for each sequences ranged between 249X (TTN, *Cyclopes didactylus*) to 3x (ADORA3, *Chaetophractus vellerosus*).

### 3.1.3 Variable Success

The wide variation in sequencing success across our genes and samples is curious. Given that all libraries were enriched identically, mostly in concert with one another, and that they had been successfully enriched for mtDNA previously, one would expect similar levels of enrichment success here. Instead we see some baits failing to generate any reads whatsoever, and of the baits that work there are some samples that have vastly different numbers of reads generated. There exist a number of hypotheses that

might explain this variation.

One consideration is that the GC content of certain genes may have inhibited their ability to be enriched and to be sequenced, as has been suggested in the literature (Dohm *et al.*, 2008; Clark *et al.*, 2011; Wang *et al.*, 2011). As such we investigated the GC content of each gene and the mean base coverage achieved after sequencing. Mean base coverage was normalised by taking the mean coverage across all samples and dividing all means through by the value of the largest mean. Both GC content and normalised mean base coverage were normally distributed. A Spearman's Ranked Rho (-0.77) derived from these two variables indicates there may be a strong negative monotonic relationship between them; that is, generally we observe that as GC content increases, our ability to successfully enrich reads decreases. This conflicts with previous reports that GC content and hybridisation success are positively correlated in genes up to a GC content of around 45%. However, our sample is biased in this regard, as of the 75 genes investigated only 8 of them have a GC content less than 40. The majority have a GC content of higher than 50.

In contrast, the number of reads generated for the mitochondrial genomes in the Gibb etl al (2015) paper was a comparatively poor predictor for nuclear enrichment success. One might imagine that should there be some positive correlation between the successful sequencing of mitochondrial DNA and the sequencing of nuclear DNA, barring well known trends that suggest mtDNA has far greater survivability than nuclear DNA. This did not however hold true. While a few samples in Gibb et al's paper that sequenced comparatively poorly, here meaning less than one million reads generated, corresponded to a low number of reads generated in nuclear genes, there were equally as many that generated huge numbers of mitochondrial reads and essentially no nuclear data.

There is also something to be said for the function of particular genes and our success in enriching them. Genes involved in the development of dentine and enamel, such as AMBN, AMELX, and DMP1, were consistently among the highest performing baits. Likewise signalling genes (ADORA3, ADRB2, GJA10) and those relating to cone and rod cells (CNGB1, PDE6C) generated high coverage across many samples. Meanwhile genes associated with chitin production (CHIA, CHID1, CHIT1) experienced some

of the worst enrichment, generating only very few reads. This difference may be due to the evolutionary history of each of these gene families. If several genes are more closely related to one another and bear more similar sequences, then there might be "competition" between different enrichment baits for particular sequences leading to overall lower levels of enrichment for each gene or a biased enrichment for one gene over the over. If instead genes in the same family should be more diverged, or share a function as a result of convergent evolution, then this competitive element will disappear. BLAST results of poorly performing genes indeed show some such similarity when restricted to Xenarthra, but further analysis is required.

It is worth noting that across the two sets of enrichments there is little variability in which samples performed well. Any variation could possibly be explained by experimental errors, and indeed some known errors have been described previously. Where there are large differences between the number of reads generated by each enrichment, it appears to be explained by the lower volumes used in the second enrichment. The overall mixed enrichment results might be a result of long term sample storage and the inevitable damage to sequencing libraries. Libraries that had been re–indexed performed no better or worse than those that were not. Many of the samples had been in cold storage between two and six years. Additionally they had experienced thawing and refreezing over that time due to being moved, amongst other things, which might result in further damage.

## 3.2 Phylogenetic analysis of nuclear loci

The resulting well supported phylogenetic tree from the nuclear data can be found in Figure 3.1. The overall topology of the tree is in strong agreement with Gibb et al's 2015 mitogenomic framework, here on referred to as the Gibb framework, as well as the topology of other genetic studies (Delsuc *et al.*, 2001; Delsuc *et al.*, 2002; Delsuc *et al.*, 2003; Möller-Krull *et al.*, 2007; Delsuc *et al.*, 2012; Delsuc *et al.*, 2016). There are a number of Dasypodes that have slightly different placement. These differences may be due to fluctuations in the mammalian mitochondrial clock, which would lead to inconsistencies depending on the data used to generate any phylogeny (Nabholz *et al.*, 2009). The Dasypodes also show a node of lower statistical support between *D. hybridus* and *D. septemcinctus*. While all members of the genus appear to be

closely related, it should also be noted that there is almost no distinction between *D. sabanicola* and *D. yepesi*, which may indicate either that their distinct taxonomic classification is in error, or that they may be only recently separated from one another, given their distinct locales (Abba & Superina, 2010), a point echoed by Gibb et al (2015).

The positioning of Mylodon, the only successfully enriched ancient species in these experiments, has had a number of hypotheses tested. Our results agree with the positioning of Mylodon as a sister taxon to two–toed sloths (*Choloepus*), a positioning that is well established (Gaudin, 1995; Hoss *et al.*, 1996; Slater *et al.*, 2016).The absence of nuclear loci for other ancient species in these analysis makes it difficult to corroborate our framework with morphological studies that place the Mylodontidae as sister to all other Megatherids (Gaudin, 2004; Farina *et al.*, 2013), but this is addressed somewhat more thoroughly with the incorporation of additional ancient mitogenomes.

Branch lengths are quite heterogeneous across clades and show a wide range of evolutionary rates. Dasypodes in particular show this high variation. Three–toed and two–toed sloths show a clear divergence from one another, and the categorising of Cyclopodidae as it's own single species clade is supported by the deep divergence between them and the Myrmecophages. In three toed sloths we see that the maned sloth *Bradypus torquatus* forms a sister group to the three other Bradypodes, a distinction that has some literature support (Barros *et al.*, 2003). There have been suggestions that it be placed in a genus of its own (*Scaeopus)* to represent its distinctiveness (Barros *et al.*, 2008; Gibb *et al.*, 2016), which makes implications for conservation given its scarcity in the wild (Superina *et al.*, 2010). The same holds for the also endangered *Bradypus pygmaeus*, so named for its realtively small body size.

In short, we see strong evidence to support Mylodontidae as a sister taxon to two–toed sloths. This new nuclear framework representing all genera within Xenarthra is highly congruent with the Gibb framework, and makes similar suggestions in terms of rates of evolution across all clades. The unfortunate lack of success in enriching naked tailed armadillos (*Cabassous*) prevents further resolution of this genera, which is a long standing problem (Gaudin & Wible, 2011; Delsuc *et al.*, 2002), and there are some disagreements amongst the Dasypodes that are likely explained by their rapid

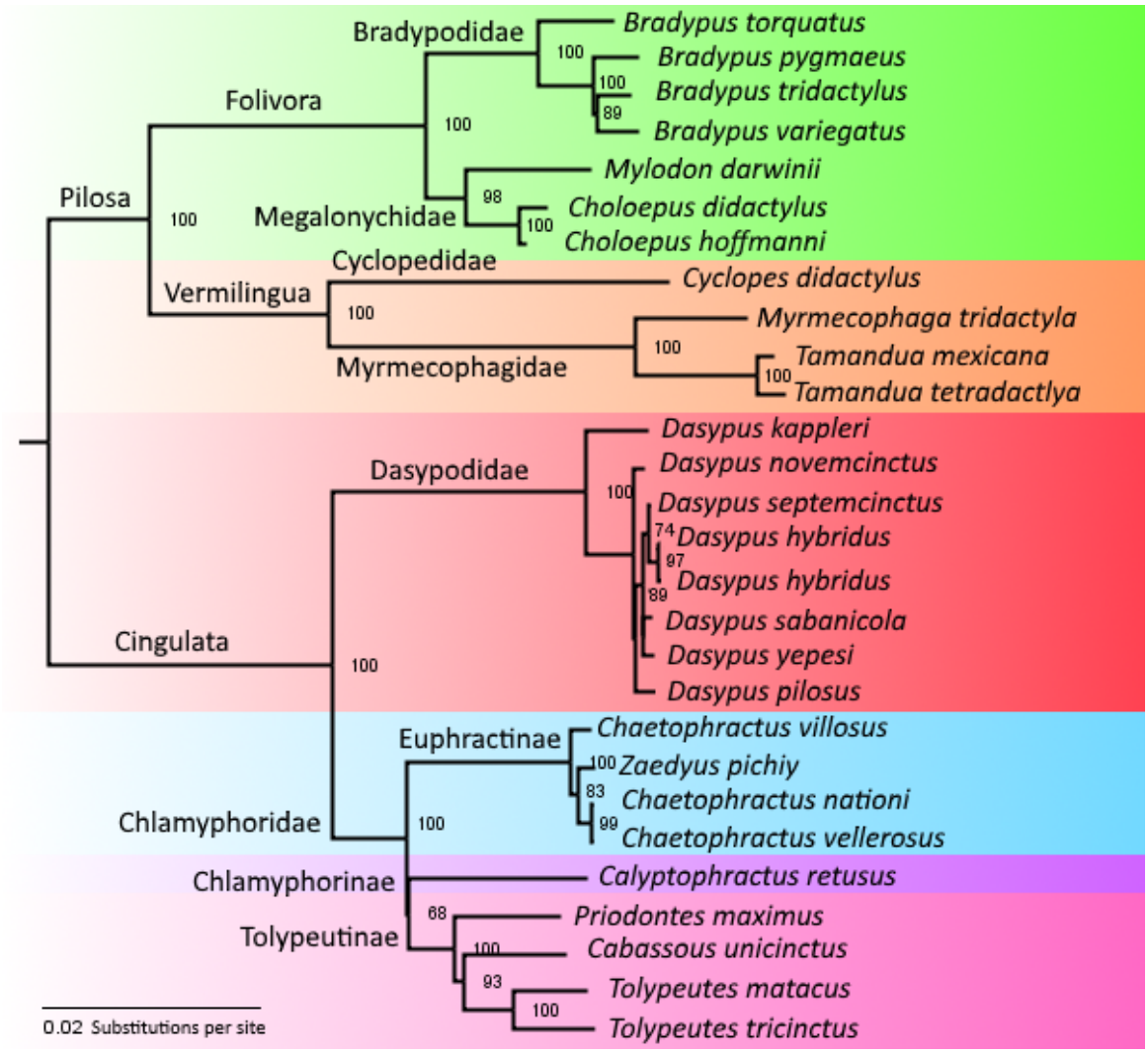


Figure 3.1: Phylogenetic relationships of 27 extant xenarthran species and 1 ancient species. Consensus tree obtained using PhyML and BEAST2. Values at nodes indicate maximum-likelihood bootstrap percentages (BP) obtained under the optimal model. Nodes with a score of 100 all had a Bayesian posterior probability of 1 as well. The outgroup, *Loxodonta africana*, is not shown.

evolution. We also note that nuclear data confirms the paraphyly of *Chaetophractus* suggested in the Gibb framework. In this same genus, there is little distinction between *C. nationi* and *C. vellerosus*; collapsing these as one genus might reasonably resolve this paraphyly.

## 3.3 Phylogenetic analysis of mitochondrial genomes

The ultrametric tree (Figure 3.2) gives approximate divergences in agreements with Gibb et al 2015 and Delsuc et al 2016, which is to be expected. When scaled to fit a chronogram as in Figure 3.3, we see divergence times matching that of the Gibb framework within the margin of error (Gibb *et al.*, 2016). The overall topology of the tree is uncontroversial, and many modern species tell much the same story as the nuclear data. *B. torquatus* appears long diverged from other Bradypodes; *C. didactylus* is likewise anciently diverged from other anteaters. In the modern armadillo species we observe an early divergence between *D. kappleri* and other Dasypodes, before the other members of that group rapidly evolve. The Euphractinae also show periods of rapid evolution, resulting in the paraphyly of the *Chaetophractus* genus. In all, these observations are well corroborated with the Gibb framework.

When investigating the placement of ancient species we see more controversial results. Firstly the ancient glytpdodon *Doedicurus* is placed more in line with (Gaudin & Wible, 2011) and (Billet *et al.*, 2011) as a sister taxon to the Euphractinae, which are based on morphological data. This stands in contrast to the Gibb framework, which places *Doedicurus* as most closely related to the Chlamyphorinae rather than the Euphractinae. It does however reconfirm that glyptodonts formed a group within armadillos and were not a sister taxon distinct from modern armadillos. Placing them within armadillo crown group implies their giant size is a derived feature.

The two nodes following the split in Chlamyphoridae have comparatively low posterior support, at 0.61 and 0.65. This may be indicate that the placement of *Doedicurus* next to Euphractinae is not necessarily the correct placement. Delsuc et al

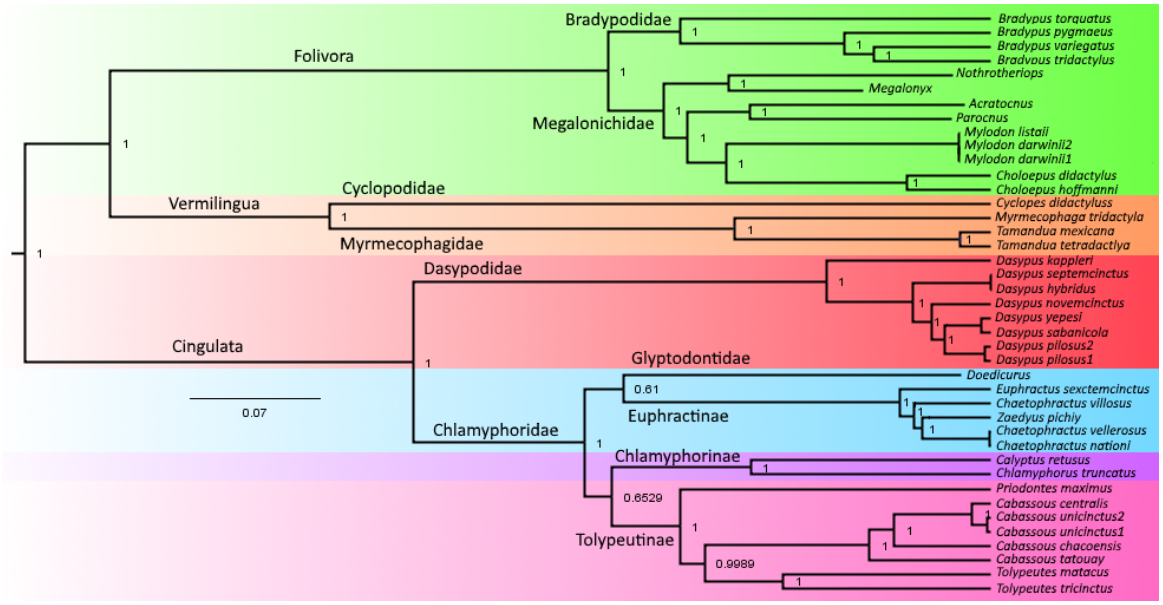


Figure 3.2: Phylogenetic relationships of all extant xenarthran species and 8 extinct species. Ultrametric consensus tree obtained using PhyML and BEAST2. Values at nodes indicate posterior value obtained under the optimal model. High posterior values (<0.97) are rounded up to 1. The outgroup, *Loxodonta africana*, is not shown.

(2016) received stronger support for their placement of *Doedicurus* as a sister to the Chlamyphorinae, though both indicate similar divergence times and this placement is more congruent with recent morphological investigations (Billet *et al.*, 2011). This distinction may be a result of differing partition schemes (Kainer & Lanfear, 2015); Delsuc et al utilised PartionFinder (Lanfear *et al.*, 2012), which is not always optimal for Bayesian analyses on small data sets.

Perhaps most interesting are the positioning of the ancient sloth species. Mylodontidae is again places as a sister taxon alongside two–toed sloths, as before (Hoss *et al.*, 1996; Slater *et al.*, 2016). Furthermore there is very little to distinguish *M. listaii* from *M. darwinii*. The two Antillean sloths, *Acratocnus* and *Parocnus* are grouped as sister taxa, closely related to the other Megalonychid, *Megalonyx*. The placing of *Nothrotheriops* as a sister species to *Megalonyx* is however more curious. This positioning is in agreement with morphological data (Gaudin, 2004; Farina *et al.*, 2013) that places three–toed sloth as a sister group to all other species of sloth. This would also support the placement of *Nothrotheriops* closer to *Megalonyx* than the other

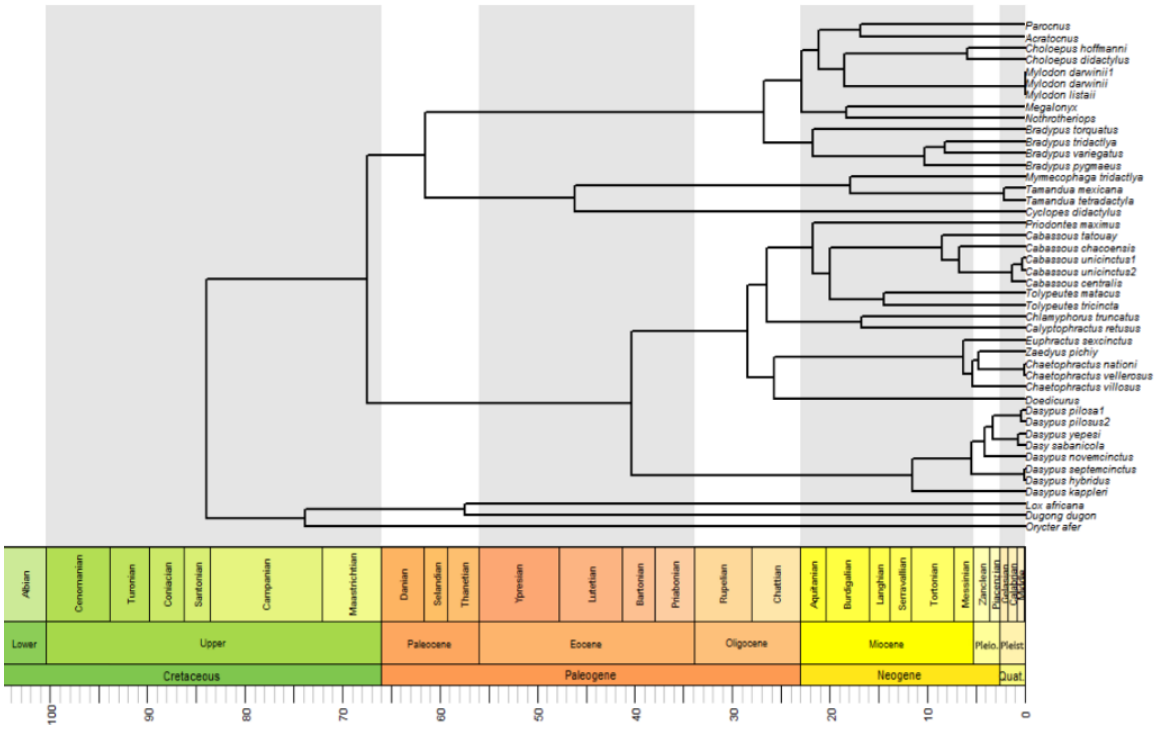


Figure 3.3: Chronogram from all extant Xenarthra and 8 extinct species. Created using the ape, strap, phyloch, and OutbreakTools in R. Scaled from tree in Figure 3.2. Major divergences in the overall phylogeny are very similar to those in the Gibb et al (2015) mitogenome paper.

Megalonychids. It does however conflict with recent limited retrovirus data (Slater *et al.*, 2016) that argues Bradypodidae and Nothrotheriidae are in fact sister taxa. That said, the retroviral analysis is based on a much smaller number of Xenarthran species (5), and may represent a divergence of host/virus phylogeny, evidenced by the fact the authors suggest numerous repeated infections to explain their tree.

The trees presented here could perhaps be improved by better calibrating the tree–building methods used. For example, the split between the Afrotheria outgroup and Xenarthra occurs much approximately 10 million years more recently than has been suggested in elsewhere (Hallström *et al.*, 2007). As such the mitochondrial trees may likely require further analysis and refinement.

# Chapter 4

# Concluding Remarks

Here we present an increase in nuclear DNA across the majority of extant Xenarthra, as well as a number of mitogenomes for extinct species. With this new data we have examined the evolutionary history and relationships of Xenarthra. Our analyses provide strong support for the phylogenetic framework proposed by Gibb et al (2015), showing a strong corroboration between both nuclear and mitochondrial genetic phylogenies.

With regard to the newly incorporated ancient species, certain parts of our proposed phylogeny have mixed evidence from the literature, but receive strong posterior support nonetheless. In particular, the positioning of Nothrotheriops has repeatedly experienced poorer resolution compared to other groups (Greenwood *et al.*, 2001; Slater *et al.*, 2016) Some inconsistencies across trees could be resolved by focusing only on non–neutral loci and by using both mitochondrial and nuclear loci simultaneously in analyses, though this was hampered by the mixed enrichment success of our DNA libraries. We attribute this variable success to enrichment inhibition caused by high GC content of some baits, as well as exhaustion and damage long term storage to some libraries.

This increase in genetic data marks a step forwards for resolution of the Xenarthran phylogeny. There is room for analyses of more nuclear loci that experienced lower enrichment success in these experiments. Further enrichment attempts of ancient Xenarthran species will allow for better calibration of clades that have proven difficult to consistently resolve. Future studies focusing on phylogeography of Xenarthrans

will benefit from this work. Perhaps most importantly, better understanding of these difficult to study organisms and their relation to each other will enable informed conservation decisions to be made, as both the genetic and morphological diversity of the clade becomes more evident.

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# Appendix A

# Abbreviations

- AIC: Akaike Information Criteria
- DNA: Deoxyribonucleic Acid
- dNTP: Deoxynucleotide Triphosphate
- dsDNA: Double Stranded DNA
- DTT: Dithiothreitol
- EDTA: Ethylenediaminetetraacetic Acid
- ESS: Effective sample size
- GATK: Genome Analysis ToolKit
- ML: Maximum Likelihood
- PCR: Polymerase Chain Reaction
- PTB: N–phenacyl thiazolium bromide
- PVP: Polyvinylpyrrolidone
- ssDNA: Single Stranded DNA
- TE: Tris–EDTA

# Appendix B

# True Enrichment Volumes

As discussed in the methods section, there were limited quantities of library available for the second enrichment set. As such, it was not possible to use the desired $5\mu$l of library for every library. Here are the true volumes used in each enrichment. Values of 0.01 indicate that the volume was so low as to essentially be zero.

| Samples | uL used | Samples | uL used |
|---|---|---|---|
| AnX18 | 3.45 | Mod21 | 5 |
| AnX25 | 4.72 | Mod22 | 5 |
| AnX32 | 3.46 | Mod23 | 2.34 |
| AnBlkX1 | 4.72 | Mod24 | 5 |
| Mod02 | 1.61 | Mod25 | 5 |
| Mod03 | 5 | Mod26 | 2.87 |
| Mod04 | 3.72 | Mod27 | 5 |
| Mod06 | 4.68 | Mod28 | 0.01 |
| Mod07 | 4.34 | Mod29 | 0.9 |
| Mod08 | 5 | Mod30 | 5 |
| Mod09 | 5 | MoL32 | 3.28 |
| Mod10 | 5 | MoL33 | 1.45 |
| Mod11 | 1.4 | MoL34 | 5 |
| Mod12 | 2.63 | MoL35 | 5 |
| Mod13 | 1.36 | MoL36 | 5 |
| Mod14 | 5 | MoL37 | 5 |
| Mod15 | 5 | MoL38 | 2.96 |
| Mod16 | 5 | MoL40 | 2.48 |
| Mod17 | 5 | MoL41 | 1.85 |
| Mod18 | 5 | MoL42 | 3.3 |
| Mod19 | 0.01 | MBlk1 | 5 |
| Mod20 | 5 | | |

# Appendix C

# Primer Combinations

Detailed here are the indexing primer combinations used in the sequencing of each sample. The library name can be cross referenced to the species as given in tables 2.1 and 2.2. As described in the Methods chapeter, all indexes are sourced from (Meyer & Kircher, 2010).

| Library # | R index (i7) | | | F index (i5) | | for Illumina |
|---|---|---|---|---|---|---|
| | Index # | Sequence (5' to 3') | Reverse complement | Index # | Sequence (5' to 3') | input file |
| An016 | 9 | AGTTGGT | ACCAACT | 4 | TTGATCC | ACCAACT-TTGATCC |
| An028 | 10 | GTACCGG | CCGGTAC | 5 | ATCTTGC | CCGGTAC-ATCTTGC |
| An054 | 22 | CCAATGC | GCATTGG | 17 | GGTACCT | GCATTGG-GGTACCT |
| An058 | 24 | CATATTG | CAATATG | 19 | GAGATTC | CAATATG-GAGATTC |
| An067 | 30 | GCGGCAT | ATGCCGC | 25 | GACGTCA | ATGCCGC-GACGTCA |
| An069 | 32 | TACTATT | AATAGTA | 27 | GTAATTG | AATAGTA-GTAATTG |
| AnX18 | 40 | TTGCGAA | TTCGCAA | 35 | CGGTTCT | TTCGCAA-CGGTTCT |
| AnX25 | 45 | ACCTGCT | AGCAGGT | 40 | TTGCGAA | AGCAGGT-TTGCGAA |
| AnX32 | 46 | CCGGTAC | GTACCGG | 41 | TTGAATT | GTACCGG-TTGAATT |
| AnBX1 | 9 | AGTTGGT | ACCAACT | 50 | TCTAGTT | ACCAACT-TCTAGTT |
| Mod01 | 2 | CCTGCGA | TCGCAGG | P5 | TCTTTCC | TCGCAGG-TCTTTCC |
| Mod02 | 3 | ACCTAGG | CCTAGGT | P5 | TCTTTCC | CCTAGGT-TCTTTCC |
| Mod03 | 4 | TTGATCC | GGATCAA | P5 | TCTTTCC | CCTAGGT-TCTTTCC |
| Mod04 | 5 | ATCTTGC | GCAAGAT | P5 | TCTTTCC | GGATCAA-TCTTTCC |
| Mod05 | 6 | TCTCCAT | ATGGAGA | P5 | TCTTTCC | GCAAGAT-TCTTTCC |
| Mod06 | 7 | CATCGAG | CTCGATG | P5 | TCTTTCC | ATGGAGA-TCTTTCC |
| Mod07 | 8 | TTCGAGC | GCTCGAA | P5 | TCTTTCC | CTCGATG-TCTTTCC |
| Mod08 | 9 | AGTTGGT | ACCAACT | P5 | TCTTTCC | GCTCGAA-TCTTTCC |
| Mod09 | 10 | GTACCGG | CCGGTAC | P5 | TCTTTCC | ACCAACT-TCTTTCC |
| Mod10 | 11 | CGGAGTT | AACTCCG | P5 | TCTTTCC | CCGGTAC-TCTTTCC |
| Mod11 | 12 | ACTTCAA | TTGAAGT | P5 | TCTTTCC | TTGAAGT-TCTTTCC |
| Mod12 | 13 | TGATAGT | ACTATCA | P5 | TCTTTCC | ACTATCA-TCTTTCC |
| Mod13 | 14 | GATCCAA | TTGGATC | P5 | TCTTTCC | TTGGATC-TCTTTCC |
| Mod14 | 15 | CAGGTCG | CGACCTG | P5 | TCTTTCC | CGACCTG-TCTTTCC |
| Mod15 | 16 | CGCATTA | TAATGCG | P5 | TCTTTCC | TAATGCG-TCTTTCC |
| Mod16 | 17 | GGTACCT | AGGTACC | P5 | TCTTTCC | AGGTACC-TCTTTCC |
| Mod17 | 18 | GGACGCA | TGCGTCC | P5 | TCTTTCC | TGCGTCC-TCTTTCC |
| Mod18 | 19 | GAGATTC | GAATCTC | P5 | TCTTTCC | GAATCTC-TCTTTCC |
| Mod19 | 20 | GAGCATG | CATGCTC | P5 | TCTTTCC | CATGCTC-TCTTTCC |
| Mod20 | 21 | GTTGCGT | ACGCAAC | P5 | TCTTTCC | ACGCAAC-TCTTTCC |
| Mod21 | 22 | CCAATGC | GCATTGG | P5 | TCTTTCC | GCATTGG-TCTTTCC |
| Mod22 | 23 | CGAGATC | GATCTCG | P5 | TCTTTCC | GATCTCG-TCTTTCC |
| Mod23 | 24 | CATATTG | CAATATG | P5 | TCTTTCC | CAATATG-TCTTTCC |
| Mod24 | 25 | GACGTCA | TGACGTC | P5 | TCTTTCC | TGACGTC-TCTTTCC |
| Mod25 | 26 | TGGCATC | GATGCCA | P5 | TCTTTCC | GATGCCA-TCTTTCC |
| Mod26 | 27 | GTAATTG | CAATTAC | P5 | TCTTTCC | CAATTAC-TCTTTCC |
| MoL27 | 6 | TCTCCAT | ATGGAGA | 2 | TGCAGAG | ATGGAGA-TGCAGAG |
| MoL28 | 7 | CATCGAG | CTCGATG | 3 | ACCTAGG | CTCGATG-ACCTAGG |
| MoL29 | 9 | AGTTGGT | ACCAACT | 5 | ATCTTGC | ACCAACT-ATCTTGC |
| MoL30 | 10 | GTACCGG | CCGGTAC | 6 | TCTCCAT | CCGGTAC-TCTCCAT |
| MoL31 | 12 | ACTTCAA | TTGAAGT | 8 | TTCGAGC | TTGAAGT-TTCGAGC |
| MoL32 | 13 | TGATAGT | ACTATCA | 9 | AGTTGGT | ACTATCA-AGTTGGT |
| MoL33 | 14 | GATCCAA | TTGGATC | 10 | GTACCGG | TTGGATC-GTACCGG |
| MoL34 | 15 | CAGGTCG | CGACCTG | 11 | CGGAGTT | CGACCTG-CGGAGTT |
| MoL35 | 16 | CGCATTA | TAATGCG | 12 | ACTTCAA | TAATGCG-ACTTCAA |
| MoL36 | 17 | GGTACCT | AGGTACC | 13 | TGATAGT | AGGTACC-TGATAGT |
| MoL37 | 18 | GGACGCA | TGCGTCC | 14 | GATCCAA | TGCGTCC-GATCCAA |
| MoL38 | 19 | GAGATTC | GAATCTC | 15 | CAGGTCG | GAATCTC-CAGGTCG |
| MoL39 | 20 | GAGCATG | CATGCTC | 16 | CGCATTA | CATGCTC-CGCATTA |
| MoL40 | 21 | GTTGCGT | ACGCAAC | 17 | GGTACCT | ACGCAAC-GGTACCT |
| MoL41 | 23 | CGAGATC | GATCTCG | 19 | GAGATTC | GATCTCG-GAGATTC |
| MoL42 | 24 | CATATTG | CAATATG | 20 | GAGCATG | CAATATG-GAGCATG |
| ModB1 | 8 | TTCGAGC | GCTCGAA | 4 | TTGATCC | GCTCGAA-TTGATCC |

# Appendix D

# Scripts and code

## D.1 GATK

The Genome Analysis ToolKit (GATK) was used for SNP variant calling in the aligned nuclear sequences. This workflow begins with a fasta reference and the bam files generated by the data processing pipeline described in chapter 2.7. Here the reference is named reference.fasta, which was represented by the complete bait set, and the initial bam file is named sorted.bam.

Create sequence dictionary: > java -jar /bin/picard-tools-1.8.5/CreateSequenceDictionary.jar REFERENCE=reference.fasta OUTPUT=reference.dict

Create sequence index: > samtools faidx reference.fasta

Mark duplicates: > java -jar /bin/picard-tools-version/MarkDuplicates.jar I=sorted.bam O=dedup.bam METRICS_FILE=metrics.txt

Sort bam file: > java -jar /bin/picard-tools-version/BuildBamIndex.jar INPUT=dedup.bam

Create realignment targets: > java -jar /bin/GATK3.3/GenomeAnalysisTK.jar -T RealignerTargetCreator -R reference.fasta -I dedup.bam -o targetintervals.list

Indel realignment: > java -jar /bin/GATK3.3/GenomeAnalysisTK.jar -T IndelRe-

aligner -R PA01.fasta -I dedup.bam -targetIntervals targetintervals.list -o realigned.bam

Call variants (HaplotypeCaller): > java -jar /bin/GATK3.3/GenomeAnalysisTK.jar -T HaplotypeCaller -R reference.fasta -I realigned.bam -ploidy 1 -stand_call_conf 30 -stand_emit_conf 10 -o raw.vcf

# Appendix E

# Enrichment Results

The following table shows the mean base coverage for every sample across every loci of interest. Only the combined results are shown; that is, those from both the first and second enrichment sets, rather than each individually. Values represent the mean base coverage achieved from sequencing a given locus. Blanks are not included, as very few sequences mapped and those that did had a very poor mapping quality, resulting in no reads that could be further utilised.

| Library | Species | ADORA3 | ADRA2B | ADRB2 | AMBN | AMELX | AMTN |
|---|---|---|---|---|---|---|---|
| An016 | Mylodon | 4.7 | 0.1 | 0.5 | 3.1 | 4.1 | 0.4 |
| An028 | Glyptodon doedicurus | 0 | 0.1 | 0.1 | 0.1 | 0.1 | 0 |
| An054 | Parocnus | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| An058 | Acratocnus | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| An067 | Mylodon | 1.6 | 0.1 | 0.5 | 0.9 | 0.6 | 0 |
| An069 | Megalonyx | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| AnX18 | Pilosa | 0 | 0.1 | 0 | 0 | 0 | 0 |
| AnX25 | Pilosa | 0 | 0.1 | 0 | 0 | 0.1 | 0 |
| AnX32 | Megatheriidae | 0 | 0.1 | 0.1 | 0 | 0.1 | 0 |
| Mod01 | Dasypus novemcinctus | 5.1 | 0 | 0.6 | 0.8 | 2 | 0.3 |
| Mod02 | Dasypus kappleri | 12.7 | 0.5 | 25.8 | 3.9 | 5.8 | 1.1 |
| Mod03 | Dasypus pilosus | 0 | 0 | 0 | 0 | 0 | 0 |
| Mod04 | Dasypus septemcinctus | 9.9 | 0.5 | 9.9 | 3.3 | 12.8 | 1 |
| Mod05 | Chlamyphorus truncatus | 0 | 0 | 0 | 0 | 0 | 0 |
| Mod06 | Chaetophractus villosus | 1.3 | 0.7 | 4.5 | 6.7 | 2.7 | 0.1 |
| Mod07 | Chaetophractus vellerosus | 3 | 0.1 | 6 | 1.9 | 4.2 | 0.1 |
| Mod08 | Euphractus sexcinctus | 0 | 0 | 0 | 0 | 0 | 0 |
| Mod09 | Zaedyus pichiy | 10 | 0.2 | 44.5 | 30.9 | 31.3 | 0.1 |
| Mod10 | Cabassous unicinctus | 13.5 | 10.4 | 45.1 | 27.5 | 73.4 | 0.1 |
| Mod11 | Cabassous unicinctus | 0 | 1.4 | 0 | 0 | 0 | 0 |
| Mod12 | Cabassous chacoensis | 0.7 | 1.3 | 1.4 | 1 | 2.5 | 0 |
| Mod13 | Priodontes maximus | 3.5 | 1.2 | 14.1 | 5.2 | 36.7 | 0 |
| Mod14 | Tolypeutes matacus | 3.4 | 1.7 | 10.7 | 1 | 0.7 | 0 |
| Mod15 | Cyclopes didactylus | 177.1 | 6.5 | 73.4 | 0.1 | 43.7 | 0 |
| Mod16 | Myrmecophaga tridactyla | 0 | 0 | 0.1 | 0 | 0 | 0 |
| Mod17 | Tamandua mexicana | 66.5 | 0.1 | 19.3 | 0.1 | 53.4 | 0 |
| Mod18 | Bradypus tridactylus | 52.4 | 4.8 | 72.3 | 13.5 | 34.5 | 0 |
| Mod19 | Bradypus variegatus | 6.6 | 0.3 | 2.2 | 0.2 | 1.1 | 0 |
| Mod20 | Choloepus hoffmanni | 48.3 | 1.85 | 16.2 | 13.5 | 21.5 | 4.4 |
| Mod21 | Choloepus didactylus | 141.1 | 1.2 | 33.9 | 29.6 | 33 | 2.2 |
| Mod22 | Myrmecophaga tridactyla | 26.3 | 1.9 | 18.3 | 0.1 | 10.1 | 0 |
| Mod23 | Chaetophractus nationi | 1.4 | 0.1 | 4.3 | 3.5 | 10.1 | 0 |
| Mod24 | Dasypus yepesi | 9.9 | 1 | 4 | 1.5 | 3.5 | 0.1 |
| Mod25 | Calyptophractus retusus | 8.19 | 1 | 13.7 | 4.9 | 7.2 | 0.1 |
| Mod26 | Dasypus pilosus | 0.1 | 0.1 | 1.2 | 13.5 | 1.1 | 0.1 |
| MoL27 | Dasypus hybridus | 2.9 | 0.4 | 1.8 | 1.4 | 2.2 | 0.5 |
| MoL28 | Cabassous centralis | 0.4 | 0.1 | 0.3 | 0.2 | 3.7 | 0 |
| MoL29 | Dasypus pilosus | 20.9 | 0.5 | 14 | 12 | 12.5 | 1.4 |
| MoL30 | Dasypus pilosus | 0.5 | 0.2 | 0.4 | 0.2 | 0.5 | 0.1 |
| MoL31 | Dasypus pilosus | 0 | 0 | 0 | 0 | 0 | 0 |
| MoL32 | Bradypus pygmaeus | 57.3 | 1.2 | 42 | 17 | 48 | 0 |
| MoL33 | Dasypus sabanicola | 125 | 1.6 | 42 | 29 | 18 | 2.9 |
| MoL34 | Bradypus torquatus | 0 | 0 | 0 | 0 | 0 | 0 |
| MoL35 | Dasypus hybridus | 15 | 0.5 | 3.9 | 5.7 | 13 | 1.3 |
| MoL36 | Dasypus hybridus | 11.8 | 0.2 | 4.8 | 4.4 | 6.5 | 1.2 |
| MoL37 | Dasypus hybridus | 69 | 0.9 | 19 | 21 | 16 | 1.9 |
| MoL38 | Dasypus hybridus | 43 | 0.4 | 13 | 15 | 5 | 2 |
| MoL39 | Cabassous tatouay | 0.2 | 0 | 0.1 | 0.1 | 0.1 | 0 |
| MoL40 | Tamandua tetradactyla | 51 | 0.2 | 17 | 0 | 29 | 0 |
| MoL41 | Bradypus torquatus | 147 | 1.7 | 32 | 8.6 | 28 | 0 |
| MoL42 | Tolypeutes tricinctus | 5.4 | 0.4 | 7.5 | 0.8 | 0 | 0 |

| Library | APOB | ASIP | ATP7A | BCHE | BDNF | BRCA1 | BRCA2 | C4orf26 |
|---|---|---|---|---|---|---|---|---|
| An016 | 5.3 | 0 | 6.1 | 1.9 | 3.1 | 26 | 15 | 7.5 |
| An028 | 0.1 | 0 | 0.1 | 0.3 | 0.1 | 0.1 | 0.2 | 0.3 |
| An054 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| An058 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.2 |
| An067 | 2.9 | 0 | 1.6 | 6.7 | 1.2 | 13 | 5.2 | 3 |
| An069 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.8 | 0.1 | 0.1 |
| AnX18 | 0 | 0 | 0 | 0 | 0 | 0.1 | 0 | 0.1 |
| AnX25 | 0.1 | 0 | 0 | 0.1 | 0.1 | 0.1 | 0.1 | 0 |
| AnX32 | 0 | 0 | 0 | 0 | 0 | 0.1 | 0 | 0 |
| Mod01 | 8.1 | 0 | 4.3 | 12.5 | 1.3 | 13.6 | 11.8 | 4.2 |
| Mod02 | 17.7 | 0 | 3.5 | 21.2 | 6.8 | 27.7 | 11.8 | 13.1 |
| Mod03 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mod04 | 20.9 | 0.1 | 21.7 | 23.9 | 12.6 | 15.6 | 16.6 | 19 |
| Mod05 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mod06 | 4.5 | 0 | 3.2 | 11.2 | 7.2 | 15.2 | 1.3 | 2.2 |
| Mod07 | 4.7 | 0 | 2.6 | 25.9 | 16.3 | 18 | 2 | 0.4 |
| Mod08 | 0 | 0 | 0 | 0 | 0 | 0.9 | 0 | 0 |
| Mod09 | 13.3 | 0.1 | 7.5 | 47.9 | 63.6 | 37.9 | 3.8 | 20.9 |
| Mod10 | 15.5 | 0 | 31.2 | 121.8 | 15.5 | 95.9 | 6.8 | 17.5 |
| Mod11 | 1.6 | 0 | 0 | 0 | 0 | 12.9 | 0 | 0 |
| Mod12 | 1.4 | 0 | 1.1 | 5.1 | 2 | 4.4 | 0.7 | 1.3 |
| Mod13 | 18.5 | 0 | 24.9 | 59.1 | 13.2 | 94.3 | 5.6 | 8.3 |
| Mod14 | 5.8 | 0 | 5.7 | 27.2 | 6.8 | 23.9 | 3.9 | 3.2 |
| Mod15 | 129.4 | 0.1 | 144.8 | 247.8 | 63.3 | 167.5 | 131.6 | 0.1 |
| Mod16 | 0.1 | 0 | 0 | 0.1 | 0.1 | 0.1 | 0.1 | 0 |
| Mod17 | 105.7 | 0 | 88.8 | 11.5 | 38.7 | 147.5 | 111.3 | 0.6 |
| Mod18 | 56.9 | 2.4 | 53.4 | 56.5 | 43.8 | 33.9 | 44.4 | 41.8 |
| Mod19 | 7.3 | 0 | 1.5 | 13.9 | 2.7 | 13.8 | 12.4 | 5.8 |
| Mod20 | 42.2 | 2.8 | 25.86 | 51 | 22.4 | 45.5 | 44.2 | 39.9 |
| Mod21 | 97 | 4.3 | 59.6 | 67.9 | 35.2 | 42.8 | 105 | 0 |
| Mod22 | 46.6 | 0.4 | 14.5 | 31.8 | 25.4 | 24 | 27.6 | 0.1 |
| Mod23 | 2.2 | 0 | 3.6 | 8.8 | 5 | 10.6 | 2.2 | 1.6 |
| Mod24 | 6.5 | 0.7 | 2.5 | 6.9 | 4.8 | 7.2 | 5.5 | 4.7 |
| Mod25 | 17.3 | 0 | 23 | 48.9 | 10.1 | 16.5 | 44 | 6.9 |
| Mod26 | 0.1 | 0.1 | 0.1 | 3.3 | 3.9 | 2.2 | 0.1 | 0.8 |
| MoL27 | 4.5 | 0.1 | 4 | 4 | 3 | 4.9 | 4.7 | 5.5 |
| MoL28 | 1.3 | 0 | 1.7 | 6.6 | 0.9 | 10.3 | 1.6 | 2.1 |
| MoL29 | 51 | 0.1 | 54 | 79 | 18 | 71.7 | 74 | 93 |
| MoL30 | 0.3 | 0 | 0.8 | 0.4 | 0.1 | 0.5 | 0.4 | 0.6 |
| MoL31 | 0 | 0 | 0 | 0 | 0 | 0.1 | 0 | 0 |
| MoL32 | 155 | 0.5 | 36 | 138 | 44 | 87.2 | 138 | 88 |
| MoL33 | 142 | 1.1 | 75 | 197 | 46 | 113 | 141 | 53 |
| MoL34 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MoL35 | 16 | 0.9 | 16 | 31 | 0.3 | 38 | 27 | 14 |
| MoL36 | 13 | 2.1 | 6 | 22 | 5.6 | 22 | 21 | 11 |
| MoL37 | 78 | 0.7 | 87 | 134 | 22 | 143 | 130 | 39 |
| MoL38 | 48 | 0.7 | 57 | 75 | 14 | 53 | 72 | 41 |
| MoL39 | 0.1 | 0 | 0.1 | 1.5 | 0.1 | 2.4 | 0.3 | 0.1 |
| MoL40 | 67 | 0 | 55 | 68 | 18 | 87 | 53 | 11 |
| MoL41 | 155 | 0 | 111 | 185 | 30 | 160 | 119 | 103 |
| MoL42 | 7.2 | 0 | 6.2 | 41 | 11 | 21 | 2.1 | 1.7 |

| Library | CHIA | CHID1 | CHIT1 | CLOCK | CNGA1 | CNGA3 | CNGB1 | CNGB3 |
|---|---|---|---|---|---|---|---|---|
| An016 | 0.1 | 3.3 | 0.1 | 106 | 12 | 0.2 | 0.1 | 14 |
| An028 | 0 | 0 | 0 | 1.6 | 0.2 | 0 | 0 | 0.2 |
| An054 | 0.1 | 0.1 | 0.1 | 1.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| An058 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| An067 | 0.1 | 0.7 | 0 | 17 | 4 | 0 | 0.1 | 5.2 |
| An069 | 0.1 | 0.1 | 0.1 | 0.5 | 0.1 | 0.1 | 0.1 | 0.1 |
| AnX18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| AnX25 | 0 | 0.1 | 0 | 0.1 | 0 | 0 | 0 | 0 |
| AnX32 | 0 | 0 | 0 | 0 | 0 | 0.1 | 0 | 0.4 |
| Mod01 | 0.2 | 0 | 0 | 2.4 | 6.5 | 0 | 0 | 8.5 |
| Mod02 | 0.7 | 0.1 | 0.3 | 2.7 | 13.7 | 1 | 0 | 6.4 |
| Mod03 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mod04 | 2.7 | 0.6 | 0.4 | 5.7 | 16.3 | 0.6 | 0.9 | 16 |
| Mod05 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mod06 | 0.6 | 0.4 | 0 | 0.4 | 8 | 0.8 | 0.4 | 2.5 |
| Mod07 | 0.4 | 0 | 0.2 | 1.7 | 10 | 0.3 | 0.3 | 9.6 |
| Mod08 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mod09 | 9 | 0.1 | 1.2 | 4.7 | 22.1 | 1.7 | 4.5 | 7.7 |
| Mod10 | 6.6 | 4.8 | 1.4 | 17.3 | 82.1 | 1 | 2.5 | 22.4 |
| Mod11 | 0 | 0.1 | 0 | 0.8 | 9 | 0 | 0 | 3.7 |
| Mod12 | 1.2 | 0 | 0.2 | 0.7 | 6.6 | 0.4 | 0.2 | 0.5 |
| Mod13 | 1.4 | 0.1 | 0.5 | 13.8 | 32 | 1.7 | 1.6 | 13.2 |
| Mod14 | 1.7 | 0.1 | 0.4 | 6.3 | 14.2 | 0.9 | 0.6 | 7.1 |
| Mod15 | 1.4 | 0.1 | 0.1 | 54 | 89.9 | 0.1 | 0.4 | 96.7 |
| Mod16 | 0 | 0 | 0 | 0.1 | 0.1 | 0 | 0 | 0.1 |
| Mod17 | 0.1 | 0 | 0 | 12 | 35.7 | 0.1 | 0.9 | 8.5 |
| Mod18 | 1.1 | 0.3 | 2.6 | 8.1 | 45.8 | 42.1 | 0.5 | 45.6 |
| Mod19 | 0 | 0 | 0 | 1.86 | 5.2 | 3.2 | 0.1 | 6 |
| Mod20 | 4.5 | 8.4 | 7.6 | 33.2 | 35.78 | 8.1 | 0.4 | 47.2 |
| Mod21 | 3.2 | 6.3 | 11.8 | 42 | 125 | 11.9 | 0.5 | 155 |
| Mod22 | 0.1 | 0 | 0.1 | 8.2 | 13.2 | 0.4 | 0.1 | 8.5 |
| Mod23 | 0.1 | 0 | 0.3 | 0.7 | 6.4 | 0.1 | 0.1 | 4.5 |
| Mod24 | 1.2 | 0.8 | 0.2 | 2.1 | 5.2 | 0.5 | 0.6 | 9 |
| Mod25 | 0 | 2.5 | 0.3 | 27 | 26 | 0.9 | 1.3 | 21.7 |
| Mod26 | 0.1 | 0.1 | 0.1 | 2.9 | 1.4 | 0.1 | 0.1 | 155 |
| MoL27 | 0.9 | 0.1 | 0.1 | 2.8 | 3.1 | 0.1 | 0.1 | 6.4 |
| MoL28 | 0.3 | 0.1 | 0.1 | 6.4 | 8.1 | 0.2 | 0.1 | 2.2 |
| MoL29 | 2.7 | 0.4 | 0.5 | 94 | 47 | 1 | 0.88 | 53 |
| MoL30 | 0.3 | 0 | 0.1 | 0.6 | 0.6 | 0.1 | 0.1 | 3.6 |
| MoL31 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MoL32 | 0 | 0.1 | 0.2 | 26 | 89 | 19 | 0.1 | 88.9 |
| MoL33 | 6.8 | 1.1 | 0.8 | 41 | 125 | 1.8 | 2.7 | 166 |
| MoL34 | 0 | 0 | 0 | 0 | 0.1 | 0 | 0 | 0 |
| MoL35 | 1.3 | 1 | 0.2 | 29 | 17 | 0.6 | 1 | 24 |
| MoL36 | 1.8 | 1.4 | 0.1 | 17 | 14 | 1.1 | 1.1 | 15 |
| MoL37 | 3.7 | 2.2 | 0.2 | 44 | 76.5 | 0.5 | 1.7 | 54 |
| MoL38 | 2.8 | 1.3 | 0.3 | 27 | 48 | 0.1 | 1.2 | 26 |
| MoL39 | 0 | 0 | 0 | 1.9 | 0.9 | 0 | 0 | 0.2 |
| MoL40 | 0.1 | 0.1 | 0 | 11 | 21 | 0.6 | 0.1 | 14 |
| MoL41 | 0.2 | 0 | 9.1 | 20 | 131 | 7 | 0.1 | 127 |
| MoL42 | 0.5 | 0 | 0.3 | 4.3 | 18.8 | 0.2 | 0.1 | 11 |

| Library | CNR1 | CRB1 | DMP1 | DSPP | ENAM | FGF5 | GHR | GJA10 |
|---|---|---|---|---|---|---|---|---|
| An016 | 0.2 | 2.96.4 | 37 | 51.9 | 8 | 20 | 1.9 | 2.6 |
| An028 | 0 | 0.1 | 0.6 | 0.5 | 0.1 | 0.1 | 0.1 | 0.1 |
| An054 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| An058 | 0.1 | 0.1 | 0.1 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 |
| An067 | 0.1 | 1.1 | 11.5 | 13.9 | 34.2 | 5.4 | 12.8 | 1 |
| An069 | 0.1 | 0.1 | 0.1 | 1.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| AnX18 | 0 | 0.1 | 0.1 | 0 | 0.1 | 0 | 0 | 0 |
| AnX25 | 0 | 0.1 | 0.1 | 0.1 | 0.1 | 0 | 0 | 0.1 |
| AnX32 | 0 | 0.1 | 0.1 | 0.1 | 0.2 | 0 | 0 | 0 |
| Mod01 | 0.6 | 2.7 | 12 | 23.8 | 5 | 0.9 | 5.2 | 0.1 |
| Mod02 | 5.3 | 9.7 | 14.4 | 17 | 16.9 | 5.2 | 12.8 | 1.6 |
| Mod03 | 0 | 0 | 0 | 0 | 0.1 | 0 | 0 | 0 |
| Mod04 | 7.2 | 12.8 | 15.4 | 20 | 13.2 | 6.4 | 13.8 | 1.2 |
| Mod05 | 0 | 0 | 0 | 0 | 0.1 | 0 | 0 | 0 |
| Mod06 | 4 | 3.8 | 15.4 | 14.9 | 15.1 | 3.8 | 4.7 | 0.1 |
| Mod07 | 5.3 | 5.4 | 18.7 | 11.3 | 9.3 | 6.2 | 5.1 | 0.1 |
| Mod08 | 0 | 0.3 | 0.5 | 1.7 | 0.5 | 0 | 0 | 0 |
| Mod09 | 34 | 14.1 | 79.2 | 37.9 | 59.5 | 15.1 | 20.3 | 0.1 |
| Mod10 | 12.7 | 14.6 | 94.5 | 70.7 | 42.3 | 14.2 | 18.1 | 0.4 |
| Mod11 | 0 | 0 | 15.4 | 16.5 | 42.3 | 0.8 | 1.6 | 0.2 |
| Mod12 | 1.5 | 1.1 | 4.1 | 3.8 | 1.6 | 1.7 | 2.5 | 0.1 |
| Mod13 | 8.8 | 7.3 | 88.8 | 135.6 | 49.9 | 12.1 | 19.3 | 0.6 |
| Mod14 | 7.4 | 4.1 | 19 | 26.1 | 16.2 | 8.9 | 5.1 | 0 |
| Mod15 | 41.7 | 14.9 | 208.3 | 5.6 | 84.5 | 70.5 | 72.7 | 0.1 |
| Mod16 | 0 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0 | 0.1 |
| Mod17 | 19.7 | 6 | 84.4 | 76 | 16.7 | 37.96 | 53.9 | 0 |
| Mod18 | 26.7 | 20.9 | 60 | 75.6 | 38.1 | 49.9 | 37.6 | 28.6 |
| Mod19 | 1.7 | 1.6 | 17.9 | 19.2 | 14.3 | 1.2 | 1.8 | 1.2 |
| Mod20 | 14.2 | 27.5 | 30.4 | 53.4 | 34.2 | 30.6 | 329.6 | 40 |
| Mod21 | 25.4 | 70 | 152 | 235 | 68.7 | 70 | 52 | 45 |
| Mod22 | 17.9 | 3.7 | 38.5 | 24.8 | 29.9 | 17.3 | 27.1 | 0.1 |
| Mod23 | 2.9 | 3.2 | 9.6 | 10.9 | 3.1 | 4.6 | 5.6 | 0 |
| Mod24 | 2.4 | 4 | 5.4 | 7.4 | 3.2 | 2.3 | 3.6 | 0.7 |
| Mod25 | 8.9 | 11.9 | 43 | 43.8 | 18.7 | 12 | 11.4 | 1.5 |
| Mod26 | 0.1 | 0.1 | 2.6 | 1.3 | 1.6 | 0.1 | 0.1 | 0.1 |
| MoL27 | 1.4 | 3.2 | 3.6 | 3.3 | 3.2 | 1.9 | 2.7 | 0.5 |
| MoL28 | 0.7 | 0.8 | 8.4 | 15.4 | 2 | 1.5 | 0.7 | 0.1 |
| MoL29 | 9.3 | 29 | 50 | 42 | 29 | 14 | 33 | 2 |
| MoL30 | 0.3 | 0.3 | 0.4 | 0.7 | 0.4 | 0.2 | 0.6 | 0.1 |
| MoL31 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MoL32 | 28 | 41 | 71 | 123 | 81 | 60 | 72 | 51 |
| MoL33 | 30 | 88 | 60 | 68 | 38 | 28 | 78 | 2.9 |
| MoL34 | 0.1 | 0 | 0 | 0 | 0.1 | 0 | 0 | 0 |
| MoL35 | 2.4 | 7 | 34 | 48 | 16 | 8.5 | 11 | 0.4 |
| MoL36 | 3.1 | 8.7 | 18 | 24 | 10 | 5.7 | 10 | 0.1 |
| MoL37 | 71 | 34 | 101 | 161 | 31 | 19.6 | 38 | 2 |
| MoL38 | 8 | 24 | 26 | 54 | 27 | 14 | 26 | 1.3 |
| MoL39 | 0.1 | 0.1 | 1.7 | 3.7 | 0.3 | 0.1 | 0.2 | 0 |
| MoL40 | 9.3 | 4 | 42 | 77 | 64.8 | 15 | 31 | 0 |
| MoL41 | 12 | 29.7 | 64 | 266 | 50 | 62 | 40 | 49 |
| MoL42 | 3.7 | 5.8 | 21 | 20 | 18 | 7.3 | 1.4 | 0 |

| Library | GNAT1 | GNAT2 | GNB1 | GNB3 | GNGT1 | GNGT2 | GRK1 | GRK7 |
|---|---|---|---|---|---|---|---|---|
| An016 | 0.8 | 9.2 | 0.1 | 0.1 | 1.1 | 0.1 | 0.1 | 4.3 |
| An028 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| An054 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| An058 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| An067 | 0.2 | 1.1 | 0 | 0 | 8.5 | 0.1 | 0.1 | 2.5 |
| An069 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| AnX18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| AnX25 | 0.7 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0.1 |
| AnX32 | 0 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mod01 | 0 | 0 | 0 | 0 | 2 | 0 | 1.2 | 8.1 |
| Mod02 | 0.4 | 5.2 | 0.6 | 2.5 | 1.5 | 0.7 | 2.7 | 12.2 |
| Mod03 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mod04 | 1.1 | 12 | 1.3 | 0 | 0 | 1.6 | 0 | 0 |
| Mod05 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mod06 | 0.5 | 0.5 | 0.1 | 1 | 1.3 | 0.3 | 1.4 | 0.3 |
| Mod07 | 0.1 | 2.4 | 0.1 | 0.3 | 3.4 | 0 | 1.6 | 0.2 |
| Mod08 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mod09 | 2.4 | 8.7 | 2.5 | 3.4 | 10.4 | 1.1 | 10 | 1.2 |
| Mod10 | 6.2 | 8.3 | 1.7 | 3.1 | 16.3 | 6.9 | 17.7 | 1.6 |
| Mod11 | 0 | 0 | 0 | 0 | 0 | 0 | 0.8 | 0 |
| Mod12 | 0.3 | 0.5 | 0.2 | 0.6 | 1 | 0 | 0.7 | 0.1 |
| Mod13 | 1.6 | 12.5 | 0.3 | 0.8 | 4.8 | 0.8 | 8.6 | 2.8 |
| Mod14 | 0.1 | 3.4 | 0.1 | 0.2 | 3.3 | 0 | 4.6 | 1.3 |
| Mod15 | 0.9 | 32.2 | 0.1 | 0.1 | 0.3 | 0 | 6.4 | 0.1 |
| Mod16 | 0 | 0.1 | 0 | 0 | 0 | 0 | 0.1 | 0 |
| Mod17 | 0.1 | 18.6 | 0 | 0.1 | 4.6 | 0 | 1 | 0.2 |
| Mod18 | 7 | 64.5 | 3 | 1.4 | 10.9 | 1.4 | 11.6 | 19 |
| Mod19 | 0.1 | 1.2 | 0.1 | 0 | 0.1 | 0.1 | 0.4 | 3.2 |
| Mod20 | 1.8 | 28.5 | 1.2 | 1.4 | 15.2 | 5.2 | 9.8 | 32.2 |
| Mod21 | 3.3 | 62 | 1 | 0.7 | 19.7 | 6.2 | 16.5 | 66 |
| Mod22 | 1 | 9.2 | 0.4 | 0.3 | 1.8 | 0 | 1.8 | 0.2 |
| Mod23 | 0 | 0.9 | 0.1 | 0 | 0 | 0 | 0.8 | 0.3 |
| Mod24 | 1.5 | 4.6 | 0.8 | 0.5 | 1.7 | 4.2 | 1.7 | 4.4 |
| Mod25 | 1.4 | 8.1 | 1.3 | 0.4 | 11 | 6 | 9.5 | 3.9 |
| Mod26 | 0.1 | 0.1 | 0.1 | 0.1 | 1 | 0.1 | 0.1 | 0.1 |
| MoL27 | 0.2 | 2.7 | 0.2 | 0.1 | 1.5 | 0.6 | 1.1 | 3.2 |
| MoL28 | 0.1 | 1.4 | 0.2 | 0 | 1.1 | 0 | 0.7 | 1 |
| MoL29 | 0.6 | 29 | 1.2 | 0.3 | 18 | 18 | 7 | 46 |
| MoL30 | 0.1 | 0.1 | 0.3 | 0.1 | 0.5 | 0.2 | 0.1 | 0.9 |
| MoL31 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MoL32 | 2.2 | 53 | 1 | 0.2 | 5.1 | 1.8 | 8.8 | 38 |
| MoL33 | 1.6 | 65 | 3.3 | 1.5 | 28 | 4.2 | 24 | 58 |
| MoL34 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MoL35 | 1 | 10 | 1 | 0.3 | 26 | 3 | 0.1 | 13 |
| MoL36 | 1.3 | 7.5 | 1.7 | 0.5 | 21 | 2.3 | 5.8 | 10.7 |
| MoL37 | 1.6 | 23 | 1.6 | 0.8 | 58 | 9 | 18 | 56 |
| MoL38 | 0.7 | 22 | 1.5 | 0.7 | 32 | 2.3 | 11 | 31 |
| MoL39 | 0 | 0.2 | 0.1 | 0 | 0.4 | 0 | 0.1 | 0.1 |
| MoL40 | 0.7 | 10 | 0.2 | 0.1 | 5.8 | 0 | 0.9 | 0 |
| MoL41 | 1.3 | 63 | 0.7 | 34 | 10.5 | 0.6 | 9 | 26 |
| MoL42 | 0.2 | 2.2 | 0 | 0.1 | 2.3 | 0 | 3.4 | 0.7 |

| Library | GUCA1A | GUCA1B | GUCY2D | GUCY2F | HR | IBSP | LWS | MC1R |
|---|---|---|---|---|---|---|---|---|
| An016 | 0.1 | 0.1 | 0.1 | 21.5 | 1 | 10 | 2 | 0.4 |
| An028 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| An054 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| An058 | 0.1 | 0.1 | 0.1 | 0.4 | 0.1 | 0.6 | 0.1 | 0.1 |
| An067 | 0.3 | 0 | 0.1 | 1.9 | 1.2 | 3.2 | 0.8 | 0.1 |
| An069 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| AnX18 | 0 | 0 | 0 | 0 | 0 | 0.1 | 0 | 0 |
| AnX25 | 0 | 0 | 0.1 | 0.2 | 0 | 0.9 | 0 | 0 |
| AnX32 | 0 | 0 | 0 | 0.4 | 0 | 0.3 | 0 | 0 |
| Mod01 | 0 | 0 | 0 | 4.2 | 0 | 3.3 | 0 | 0.4 |
| Mod02 | 0.4 | 0.7 | 0.2 | 5.8 | 0.3 | 5.6 | 2.1 | 1 |
| Mod03 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mod04 | 1.4 | 0 | 0 | 9 | 0 | 0 | 0 | 1.3 |
| Mod05 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mod06 | 1.2 | 0 | 0.1 | 0.2 | 0.1 | 0.6 | 0 | 0.1 |
| Mod07 | 0.1 | 2.2 | 0.1 | 0.2 | 0.2 | 0.4 | 0 | 0.2 |
| Mod08 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mod09 | 3.2 | 15.2 | 1.3 | 2.3 | 0.7 | 3.1 | 0.4 | 0.2 |
| Mod10 | 3.4 | 0.5 | 1.4 | 4.1 | 1 | 4.6 | 2.6 | 0 |
| Mod11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mod12 | 0.1 | 0 | 0.2 | 0.3 | 0.1 | 0.5 | 0 | 0.2 |
| Mod13 | 1.1 | 1.1 | 0.4 | 5.2 | 0.4 | 3.3 | 0.4 | 0.2 |
| Mod14 | 0.7 | 0.2 | 0.3 | 3.8 | 0.8 | 1 | 0.5 | 0 |
| Mod15 | 0.1 | 4.1 | 0.8 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Mod16 | 0 | 0 | 0 | 0.1 | 0.1 | 0 | 0 | 0 |
| Mod17 | 0 | 0 | 0.2 | 1.2 | 0.1 | 0 | 0.1 | 0 |
| Mod18 | 2.4 | 2.6 | 0.9 | 15.4 | 1.4 | 10.5 | 16.4 | 8.3 |
| Mod19 | 0 | 0.4 | 0.1 | 3.4 | 0.9 | 0.8 | 0.6 | 0.1 |
| Mod20 | 9.3 | 2.4 | 0.7 | 19 | 3.7 | 30 | 7.4 | 3.8 |
| Mod21 | 2 | 12.4 | 1 | 37 | 2.5 | 90 | 19.2 | 7.8 |
| Mod22 | 0 | 0 | 0.3 | 1.4 | 0.2 | 0.1 | 0.2 | 0.2 |
| Mod23 | 0.2 | 0 | 0.1 | 1 | 0.1 | 0.1 | 0 | 0 |
| Mod24 | 1.1 | 1.6 | 0.4 | 2.6 | 0.2 | 3.3 | 0.1 | 2 |
| Mod25 | 0.5 | 0.7 | 0.4 | 7.6 | 5 | 7.6 | 0.3 | 0.1 |
| Mod26 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| MoL27 | 0.1 | 0.1 | 0.1 | 3.7 | 0.1 | 2.9 | 0.4 | 0.1 |
| MoL28 | 0 | 0 | 0.1 | 0.6 | 0.3 | 0.8 | 0 | 0 |
| MoL29 | 0.7 | 3.2 | 0.1 | 21 | 0.1 | 21 | 0.5 | 2.1 |
| MoL30 | 0.3 | 0.2 | 0.1 | 0.9 | 0.1 | 0.1 | 0.2 | 0.2 |
| MoL31 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MoL32 | 1.4 | 1.3 | 0.2 | 59 | 4.4 | 19.7 | 11.9 | 6 |
| MoL33 | 2.3 | 6.1 | 0.6 | 68 | 0.5 | 50 | 0.8 | 20 |
| MoL34 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MoL35 | 0.2 | 0.8 | 0.1 | 22 | 0.2 | 14 | 0.2 | 1.2 |
| MoL36 | 0.6 | 0.1 | 0.2 | 7 | 0.1 | 12 | 0.2 | 0.9 |
| MoL37 | 1.1 | 1.8 | 0.3 | 31 | 0.1 | 43 | 0.4 | 3.4 |
| MoL38 | 0.7 | 1.5 | 0.3 | 22 | 0.1 | 23 | 0.2 | 1.4 |
| MoL39 | 0 | 0 | 0 | 0.1 | 0 | 0.1 | 0 | 0 |
| MoL40 | 0 | 0 | 0.1 | 2.3 | 1.7 | 0.8 | 0.6 | 0 |
| MoL41 | 0.4 | 2.2 | 0.3 | 27 | 1.6 | 15 | 6 | 4 |
| MoL42 | 0 | 0 | 0 | 0 | 0.1 | 1 | 0.1 | 0 |

| Library | MEPE | MLPH | MMP20 | ODAM | PDE6A | PDE6B | PDE6C | PDE6G |
|---|---|---|---|---|---|---|---|---|
| An016 | 15.5 | 2 | 1.5 | 0.3 | 3.7 | 0.9 | 21 | 0.1 |
| An028 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.4 | 0 |
| An054 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| An058 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| An067 | 5.5 | 0.5 | 0.3 | 0 | 0.8 | 0.3 | 6.4 | 0 |
| An069 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| AnX18 | 0 | 0 | 0 | 0 | 0 | 0 | 0.1 | 0 |
| AnX25 | 0.1 | 0 | 0 | 0.1 | 0.1 | 0.1 | 0.1 | 0 |
| AnX32 | 0 | 0 | 0 | 0.1 | 0.1 | 0 | 0 | 0 |
| Mod01 | 8.5 | 0.1 | 0.3 | 0.4 | 1 | 6 | 7.1 | 0 |
| Mod02 | 15.5 | 0.2 | 0.8 | 0.7 | 0.5 | 1.1 | 4.3 | 0 |
| Mod03 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mod04 | 17 | 0 | 1.4 | 0 | 0 | 0 | 0.2 | 0 |
| Mod05 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mod06 | 13.4 | 0 | 0 | 0 | 1.2 | 0.1 | 0.2 | 0 |
| Mod07 | 22.7 | 0 | 0.1 | 0 | 1.3 | 0.2 | 0.2 | 0 |
| Mod08 | 1.7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mod09 | 57.7 | 0.1 | 0.2 | 0 | 6.7 | 5.6 | 3.6 | 0 |
| Mod10 | 90.9 | 0.1 | 0.8 | 0 | 5.4 | 3 | 12.2 | 0.4 |
| Mod11 | 11.7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mod12 | 4.3 | 0.1 | 0.1 | 0 | 0.3 | 0.2 | 0.2 | 0 |
| Mod13 | 64.9 | 0.1 | 0.1 | 0.2 | 4.5 | 1.7 | 24.7 | 0 |
| Mod14 | 13.4 | 0 | 0.2 | 0.1 | 1.9 | 0.8 | 0.2 | 0 |
| Mod15 | 142.3 | 0 | 0.1 | 0 | 6.2 | 1 | 47.6 | 0 |
| Mod16 | 0.1 | 0 | 0 | 0 | 0.1 | 0 | 0.1 | 0 |
| Mod17 | 89.9 | 0.6 | 0.1 | 0 | 2.9 | 0.6 | 10.5 | 0 |
| Mod18 | 58.6 | 12.3 | 2.1 | 4.2 | 7.6 | 0 | 13.5 | 0 |
| Mod19 | 11.4 | 0 | 0 | 0.2 | 0.4 | 0.1 | 2.8 | 0 |
| Mod20 | 34.9 | 13 | 5.4 | 12 | 8.3 | 3.2 | 128.1 | 0 |
| Mod21 | 96.6 | 12 | 5.6 | 25 | 28 | 3.9 | 81 | 0 |
| Mod22 | 40.2 | 0.7 | 0 | 0.1 | 1.1 | 0.6 | 5 | 0 |
| Mod23 | 12.5 | 0 | 0.1 | 0.1 | 0.3 | 0.1 | 1.6 | 0 |
| Mod24 | 5.4 | 0.3 | 0.8 | 0.4 | 1.1 | 1.8 | 7 | 0 |
| Mod25 | 1.9 | 0.2 | 0.6 | 0.1 | 8.2 | 2.7 | 1.2 | 0.2 |
| Mod26 | 0.1 | 0.1 | 0.1 | 0 | 0.1 | 0.1 | 1.1 | 0.1 |
| MoL27 | 3.2 | 0.1 | 0.1 | 0.6 | 1.2 | 0.9 | 4.9 | 0 |
| MoL28 | 6.6 | 0 | 0.2 | 0.1 | 0.6 | 0.1 | 3.1 | 0 |
| MoL29 | 35.8 | 0.5 | 2.4 | 4.2 | 6.2 | 2.4 | 29 | 0 |
| MoL30 | 0.5 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| MoL31 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MoL32 | 85.1 | 5.8 | 1.6 | 0 | 4.9 | 1.7 | 138 | 0 |
| MoL33 | 57 | 0.7 | 5.4 | 10 | 13 | 9.1 | 87 | 0 |
| MoL34 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MoL35 | 27 | 0.4 | 5.6 | 3.5 | 5 | 4 | 20 | 0.1 |
| MoL36 | 19 | 0.5 | 3.5 | 4 | 4.2 | 3.3 | 11 | 0 |
| MoL37 | 46 | 0.9 | 12 | 10 | 15 | 10 | 55 | 0 |
| MoL38 | 43 | 0.7 | 5.9 | 8 | 9.5 | 6 | 44 | 0.1 |
| MoL39 | 1.3 | 0 | 0.1 | 0 | 0.1 | 0.1 | 0.8 | 0 |
| MoL40 | 60 | 1.1 | 0 | 0 | 1.4 | 0.5 | 16 | 0.2 |
| MoL41 | 80 | 6.4 | 1.6 | 6.6 | 7.5 | 2.4 | 76 | 0 |
| MoL42 | 12 | 0 | 0.2 | 0 | 1.6 | 0.3 | 5.1 | 0 |

| Library | PDE6H | PNOC | POMC | RAG2 | RBP3 | RH1 | S1PR1 | SAMHD1 |
|---|---|---|---|---|---|---|---|---|
| An016 | 0.6 | 1.6 | 0 | 3.7 | 0.3 | 0.1 | 1.2 | 9 |
| An028 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.1 |
| An054 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| An058 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| An067 | 0.2 | 0.1 | 0 | 2.2 | 0.1 | 0.1 | 0.1 | 14 |
| An069 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| AnX18 | 0 | 0 | 0 | 0 | 0 | 0 | 0.1 | 0.5 |
| AnX25 | 0 | 0 | 0 | 0 | 0 | 0.1 | 0 | 0.1 |
| AnX32 | 0 | 0.1 | 0 | 0.1 | 0.1 | 0 | 0 | 0 |
| Mod01 | 0 | 0 | 0 | 11.6 | 0 | 0.4 | 1.3 | 1.5 |
| Mod02 | 1.5 | 1.3 | 0 | 7.9 | 0 | 2.5 | 4.9 | 1.8 |
| Mod03 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mod04 | 1.4 | 0 | 0 | 20 | 0 | 6.2 | 10.9 | 4.1 |
| Mod05 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mod06 | 0 | 0.2 | 0 | 8.2 | 0 | 0.6 | 3.4 | 0.4 |
| Mod07 | 0 | 0 | 0 | 14.1 | 0 | 1.7 | 3.5 | 2.7 |
| Mod08 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mod09 | 0 | 0.2 | 0 | 35.5 | 0 | 10.7 | 21.8 | 3.9 |
| Mod10 | 0 | 0.2 | 0.1 | 94.9 | 0 | 11.9 | 37.1 | 14.2 |
| Mod11 | 0 | 0 | 0 | 3.1 | 0 | 0 | 1.3 | 0 |
| Mod12 | 0 | 0 | 0 | 4.3 | 0 | 0.5 | 1.5 | 0.6 |
| Mod13 | 0 | 0.6 | 0 | 45.4 | 0 | 7.7 | 11 | 4.1 |
| Mod14 | 0.1 | 0 | 0.2 | 17.3 | 0 | 4.7 | 8.4 | 4.8 |
| Mod15 | 0.1 | 26.5 | 0.1 | 131.9 | 18.7 | 20.8 | 66.9 | 20.3 |
| Mod16 | 0 | 0 | 0 | 0 | 0 | 0 | 0.1 | 0.1 |
| Mod17 | 0.2 | 19.6 | 0.1 | 76.38 | 4.9 | 44 | 18 | 9.5 |
| Mod18 | 6.7 | 12 | 0.4 | 87.3 | 11.9 | 14.4 | 31.2 | 4.9 |
| Mod19 | 0 | 0 | 0 | 3.8 | 0.8 | 0.9 | 1.4 | 0.8 |
| Mod20 | 15 | 8.3 | 0.1 | 26.3 | 7 | 9 | 14 | 9.5 |
| Mod21 | 18 | 15 | 0 | 88.6 | 15 | 13.8 | 27.3 | 40.4 |
| Mod22 | 0 | 13 | 0 | 28 | 5.5 | 4.3 | 16 | 5.5 |
| Mod23 | 0 | 0 | 0 | 6.1 | 0 | 1.2 | 2.4 | 0.1 |
| Mod24 | 15 | 0.1 | 0 | 3.4 | 0 | 3 | 5 | 2.1 |
| Mod25 | 1.1 | 0 | 0 | 29 | 0 | 5.7 | 6.6 | 17.2 |
| Mod26 | 0.1 | 0.1 | 0 | 1.4 | 0.1 | 0.1 | 0.1 | 0.1 |
| MoL27 | 0.3 | 0 | 0.1 | 1.6 | 0 | 1.4 | 1.7 | 2 |
| MoL28 | 0.1 | 0.1 | 0 | 1.5 | 0 | 0.3 | 1.1 | 5 |
| MoL29 | 6.3 | 0.3 | 0 | 10 | 0 | 4.7 | 21.6 | 12.8 |
| MoL30 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 2.9 |
| MoL31 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MoL32 | 7.5 | 7 | 0 | 95 | 25 | 8.7 | 36 | 10 |
| MoL33 | 8.7 | 0 | 0 | 128 | 0 | 17 | 73 | 33 |
| MoL34 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MoL35 | 1.5 | 0.4 | 0 | 15 | 0.1 | 1.5 | 5 | 17 |
| MoL36 | 1.7 | 0.1 | 0.1 | 12 | 0 | 1 | 5 | 13.2 |
| MoL37 | 5.4 | 0.2 | 0.1 | 63 | 0 | 3.9 | 15 | 40 |
| MoL38 | 3.1 | 0 | 0 | 47 | 0.1 | 2.1 | 12 | 29 |
| MoL39 | 0 | 0 | 0 | 1.2 | 0 | 0 | 0.2 | 0.6 |
| MoL40 | 0.3 | 7.4 | 0 | 42.8 | 2.6 | 1.7 | 14 | 9.5 |
| MoL41 | 4.7 | 4.2 | 0 | 106 | 5 | 6.3 | 32 | 24 |
| MoL42 | 0 | 0 | 0 | 18 | 0 | 1.2 | 4.5 | 2.7 |

| Library | SLC26A5 | SLN | SPARCL1 | SPP1 | SWS1 | TAS1R1 | TAS1R2 | TAS1R3 |
|---|---|---|---|---|---|---|---|---|
| An016 | 14 | 0 | 13 | 3.7 | 2.1 | 0.5 | 0.3 | 0.1 |
| An028 | 0.1 | 0 | 0.1 | 0 | 0 | 0 | 0 | 0 |
| An054 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| An058 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| An067 | 5 | 0 | 5.5 | 1.1 | 0.5 | 0.2 | 0.1 | 0 |
| An069 | 1.7 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| AnX18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| AnX25 | 0.1 | 0 | 0.1 | 0.1 | 0 | 0 | 0 | 0 |
| AnX32 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mod01 | 1.8 | 0 | 0.4 | 0.8 | 8 | 0.1 | 0.1 | 0.1 |
| Mod02 | 3.2 | 0.8 | 6.6 | 4.3 | 8.3 | 3.1 | 3 | 0 |
| Mod03 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mod04 | 5.2 | 0 | 0.6 | 0 | 24 | 0 | 0 | 0 |
| Mod05 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mod06 | 2 | 0 | 1.2 | 0.2 | 3.2 | 0.8 | 0.8 | 0 |
| Mod07 | 0.9 | 0 | 2.6 | 0.2 | 3 | 0.1 | 0.6 | 0 |
| Mod08 | 0.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mod09 | 21.5 | 0 | 6.8 | 0.2 | 29.6 | 6.1 | 4.8 | 0.2 |
| Mod10 | 15.7 | 0 | 0.7 | 3.2 | 23.1 | 13.7 | 4 | 0.1 |
| Mod11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mod12 | 0.7 | 0 | 0.8 | 0.3 | 1.3 | 0.8 | 0.3 | 0 |
| Mod13 | 8.5 | 0 | 5.1 | 0.6 | 17.5 | 2.1 | 2.9 | 0 |
| Mod14 | 12.7 | 0 | 4.7 | 1 | 10.3 | 0.2 | 0.1 | 0.1 |
| Mod15 | 65.5 | 0 | 16.5 | 4.7 | 0 | 0.1 | 0.1 | 0.1 |
| Mod16 | 0.1 | 0 | 0.1 | 0 | 0.1 | 0 | 0 | 0 |
| Mod17 | 38.5 | 0 | 4.6 | 0 | 0.6 | 0.1 | 0 | 0 |
| Mod18 | 22.3 | 3.7 | 10.8 | 32.8 | 34.5 | 6.7 | 1.4 | 0 |
| Mod19 | 2.4 | 0 | 1.3 | 1.3 | 3.7 | 0.3 | 0.1 | 0 |
| Mod20 | 15 | 2.2 | 21 | 28.5 | 32 | 6.6 | 3.2 | 0.2 |
| Mod21 | 30.5 | 3.9 | 28 | 49 | 85 | 11 | 6.4 | 0.1 |
| Mod22 | 11.7 | 0 | 1.8 | 0.2 | 0.8 | 0.5 | 0 | 0 |
| Mod23 | 2.6 | 0 | 0.6 | 0.3 | 1.3 | 0.8 | 0.3 | 0 |
| Mod24 | 2.4 | 0 | 3.6 | 2.1 | 8.6 | 2.4 | 1.2 | 0.2 |
| Mod25 | 12.1 | 0 | 12.8 | 2.9 | 6.7 | 5.2 | 1.4 | 0.1 |
| Mod26 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| MoL27 | 1.8 | 0 | 2.6 | 1.7 | 3.9 | 0.4 | 0.4 | 0.1 |
| MoL28 | 3.5 | 0 | 0.6 | 0.2 | 0.9 | 0 | 0 | 0 |
| MoL29 | 8.4 | 0 | 26 | 15 | 38 | 5.4 | 2.8 | 0.1 |
| MoL30 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| MoL31 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MoL32 | 24 | 3 | 31 | 36 | 47 | 13 | 1.3 | 0 |
| MoL33 | 33 | 0.9 | 52 | 35 | 186 | 18 | 11 | 0.4 |
| MoL34 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MoL35 | 15 | 0 | 16 | 8 | 19 | 1.6 | 0.1 | 0.1 |
| MoL36 | 11 | 0.5 | 13.9 | 6.1 | 11 | 1.4 | 1.3 | 0.1 |
| MoL37 | 13 | 0.7 | 58 | 26 | 83 | 6.5 | 5.6 | 0.1 |
| MoL38 | 11 | 0.2 | 30 | 17 | 51 | 4.6 | 2.7 | 0.1 |
| MoL39 | 0.4 | 0 | 0.1 | 0 | 1.8 | 0.1 | 0.1 | 0.1 |
| MoL40 | 23 | 0 | 7.4 | 0 | 0.8 | 0.1 | 0 | 0 |
| MoL41 | 27 | 3.8 | 36 | 18 | 42 | 15 | 0.8 | 0 |
| MoL42 | 11.7 | 0 | 2.5 | 0 | 7.2 | 0.9 | 0.1 | 0 |

| Library | TTN | TYR1 | UCP1 | VWF |
|---|---|---|---|---|
| An016 | 23.7 | 4.4 | 5.5 | 0.3 |
| An028 | 0.1 | 0 | 0 | 0 |
| An054 | 0.1 | 0.1 | 0.1 | 0.1 |
| An058 | 0.1 | 0.1 | 0.1 | 0.1 |
| An067 | 8.3 | 0.9 | 2.4 | 0.1 |
| An069 | 0.1 | 0.1 | 0.1 | 0.1 |
| AnX18 | 0.1 | 0 | 0 | 0 |
| AnX25 | 0.1 | 0 | 0 | 0 |
| AnX32 | 0.1 | 0.1 | 0 | 0 |
| Mod01 | 15.3 | 2.6 | 2.4 | 0.8 |
| Mod02 | 24.2 | 6.1 | 8 | 3.4 |
| Mod03 | 0.1 | 0 | 0 | 0 |
| Mod04 | 26.5 | 10.4 | 0.5 | 1 |
| Mod05 | 0.1 | 0 | 0 | 0 |
| Mod06 | 13.2 | 0.9 | 0 | 3.6 |
| Mod07 | 26.4 | 0.1 | 0 | 2.8 |
| Mod08 | 1.2 | 0 | 0 | 0 |
| Mod09 | 46.4 | 1.5 | 0.2 | 26.7 |
| Mod10 | 56.6 | 2.3 | 0.8 | 22 |
| Mod11 | 8.8 | 0 | 0 | 1.3 |
| Mod12 | 3.7 | 0.3 | 0 | 1.3 |
| Mod13 | 61.8 | 0.4 | 0.1 | 7.2 |
| Mod14 | 26.8 | 0.1 | 0.1 | 2.7 |
| Mod15 | 249.3 | 45.4 | 0.1 | 18.2 |
| Mod16 | 0.1 | 0 | 0 | 0 |
| Mod17 | 22.2 | 71 | 0.1 | 12.5 |
| Mod18 | 75.9 | 50.7 | 0.9 | 7.7 |
| Mod19 | 16 | 2 | 0 | 1 |
| Mod20 | 12 | 28.7 | 24 | 3.7 |
| Mod21 | 154.9 | 88 | 61 | 6.9 |
| Mod22 | 44 | 30.6 | 0 | 8.1 |
| Mod23 | 13 | 0 | 0.1 | 3.7 |
| Mod24 | 7 | 5.7 | 4.7 | 1.5 |
| Mod25 | 44.3 | 7.1 | 1.1 | 5.2 |
| Mod26 | 1.8 | 0.1 | 0.1 | 0.1 |
| MoL27 | 4.9 | 2.4 | 1.8 | 0.1 |
| MoL28 | 4.9 | 0.6 | 0.2 | 0.1 |
| MoL29 | 78.1 | 21.5 | 22 | 4 |
| MoL30 | 0.1 | 0.1 | 0.1 | 0.1 |
| MoL31 | 0 | 0 | 0 | 0 |
| MoL32 | 172.7 | 79 | 1 | 7 |
| MoL33 | 190 | 68 | 63 | 15 |
| MoL34 | 0 | 0 | 0 | 0 |
| MoL35 | 28.7 | 8 | 8 | 1.6 |
| MoL36 | 21 | 7 | 9.7 | 0.7 |
| MoL37 | 127 | 30 | 51 | 4.6 |
| MoL38 | 78.5 | 22 | 28 | 3.4 |
| MoL39 | 0.6 | 0.2 | 0 | 0.1 |
| MoL40 | 85 | 35 | 0 | 8.4 |
| MoL41 | 195 | 63 | 2.3 | 4.1 |
| MoL42 | 32.8 | 0 | 0.4 | 2.6 |