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Phylogenetic Relationships and Divergence Times in Rodents Based on Both Genes and Fossils

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PHYLOGENETIC RELATIONSHIPS AND DIVERGENCE TIMES IN RODENTS BASED ON BOTH GENES AND FOSSILS

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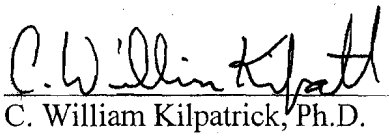

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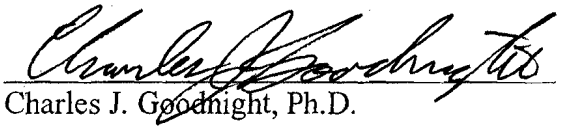

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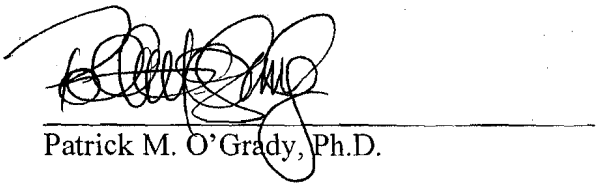

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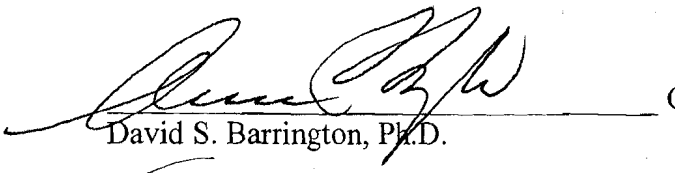

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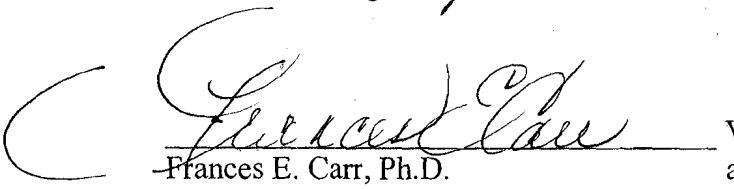

Frances E. Carr, Ph.D. — Vice President for Research and Dean of Graduate Studies

Date: October 24, 2008

Abstract

Molecular and paleontological approaches have produced extremely different estimates for divergence times among orders of placental mammals and within rodents with molecular studies suggesting a much older date than fossils. We evaluated the conflict between the fossil record and molecular data and find a significant correlation between dates estimated by fossils and relative branch lengths, suggesting that molecular data agree with the fossil record regarding divergence times in rodents. Our approach includes a correction for tree hierarchy involving simulating the random appearance of fossils. We also present a ghost lineage approach that attempts to incorporate the potential for the discovery of older fossils into a Bayesian analysis of divergence dates. Applying this approach to a set of Eocene rodent fossils, we estimated the earliest divergence in rodents appears to have occurred at approximately the K/T boundary, but interordinal splits were estimated to have taken place late in the Cretaceous. We propose that some molecular clock studies may overestimate divergence times due to periods of accelerated molecular evolution across multiple lineages or due to saturation of data that is not adequately corrected by the evolutionary model.

We have sequenced the complete mitochondrial genomes of three rodent species, *Anomalurus beecrofti*, *Castor canadensis*, and *Dipodomys ordii*, and attempt to resolve phylogenetic relationships within rodents using the mitochondrial genome, a nuclear dataset of comparable size, and a combined analysis containing 26 kbp of sequence data. The combined analysis recovered a Sciuromorpha – Hystricomorpha clade with strong support. Our data suggest that increased character sampling improves resolution at these early nodes while better taxon sampling of mitochondrial genomes has led to better supported clades that converge on conclusions obtained from nuclear datasets.

Several molecular studies have concluded that the zokors, genus *Myospalax*, evolved from within the rodent subfamily Cricetinae. We tested this conclusion using mitochondrial data and determined that *Myospalax* is sister to a clade containing the subfamilies Spalacinae and Rhizomyinae, and all three of these lineages appear to be basal to the superfamily Muroidea. Based on the position of these three lineages, we suggested that they be placed in a distinct family, the Spalacidae.

The murine genera *Mus* and *Rattus* are thought to have diverged about 12 million years ago (Ma) based on a series of fossils from the Siwaliks of Pakistan, but assumptions of murid relationships that led to this conclusion have been shown to be false by molecular data. Equally parsimonious hypotheses can be proposed which place the 12 million year old *Progonomys* fossil at the base of the family Muridae, basal to the subfamily Murinae, or at the *Mus* - *Rattus* divergence. We here test the dates of evolutionary divergences in murids. Our results indicate that the family Muridae probably diverged earlier than the Siwalik fossils, but *Mus* and *Rattus* diverged at the same time or prior to the 12 Ma fossil date. We also cannot reject the hypothesis that the 12 Ma date represents the oldest split in the Murinae instead of the more derived *Mus – Rattus* date. We also recovered phylogenetic results suggesting that *Taterillus* is related to the tribe Gerbillini and not to other genera that are treated as Taterillini and that *Gerbillurus* evolved from within *Gerbilliscus*.

Citation

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Chapter 1

Evolution and dating in rodents: A review of the literature

DIVERGENCE TIMES IN PLACENTAL MAMMALS

Placental mammals appear to have diverged from marsupials in the Early Cretaceous (Wible et al., 2005). The oldest record of the Eutheria (a clade that unites placentals with their fossil relatives) is *Eomaia* from Chinese deposits dated at about 125 million years ago (Ma; Ji et al., 2002). The oldest metatherian (a clade uniting marsupials with their fossil relatives), *Sinodelphys*, has also been found in China from the same time period (Luo et al., 2003). No fossils exist which can be unambiguously assigned to modern placental orders until the Cenozoic (McKenna and Bell, 1997; Wible et al., 2005; 2007). The traditional view has been that, although some basal forms coexisted with nonavian dinosaurs, modern placental mammals arose suddenly after the mass extinction event at the end of the Cretaceous. According to this view, both interordinal (between order) and intraordinal (within order) diversification of placental mammals took place after the Cretaceous-Tertiary (K/T) extinction event. The extinction of dinosaurs provided for an ecological release that allowed for the explosive radiation of placental mammals into open niches. This traditional hypothesis is currently regarded as The Explosive Model (Archibald and Deutschman, 2001; Springer et al., 2003; 2005) and still receives wide support among paleontologists (Foote et al., 1999; Wible et al., 2005; 2007).

The development of molecular approaches to phylogeny reconstruction was followed by a hypothesis that mutations accumulate at a constant rate over time termed the "molecular clock" (Zuckerkandl and Pauling, 1962). Although the assumption of rate constancy has been largely discarded over time, statistical approaches applying molecular clocks to molecular data have become powerful and frequently used tools (Bromham and Penny, 2003). The application of these molecular dating approaches to the question of placental mammal diversification yielded results that suggested that both interordinal and even many intraordinal evolutionary splits in placental mammals took place in the Cretaceous (Kumar and Hedges, 1998; Bininda-Emonds et al., 2007). These results essentially suggest that placental mammals evolved at the feet of dinosaurs. Characters associated with specific ecologies such as flight in bats, gnawing teeth in rodents, and carnassial teeth in carnivorans would have evolved in spite of competition with dinosaurs, pterosaurs, and other Cretaceous tetrapods. This hypothesis is based entirely on molecular data, has no support in the fossil record, and is termed the Short Fuse Model (Archibald and Deutschman, 2001; Springer et al., 2003; 2005).

The Long Fuse Model represents a compromise between the Explosive and Short Fuse models. It states that interordinal diversification took place in the Cretaceous, but intraordinal diversification took place after the K/T event. Presumably, primitive placentals diversified in the Cretaceous, but extreme shifts in morphology associated with ecological specialization, such as flight in bats and carnassials in carnivorans, took place after these niches became available due to the mass extinction event. Paleontologists have considered some Cretaceous eutherians to be allied to modern orders. Archibald

(1996; 2003) and Archibald et al. (2001) have suggested that the Cretaceous zalambdelestids and zhelestids are related to Glires (rodents and lagomorphs) and ungulates respectively. Their findings have been refuted by other paleontologists (Meng and Wyss, 2001; Meng et al., 2003; Wible et al., 2005; 2007). Molecular evidence for this hypothesis has also been limited. Unlike many other studies where nearly all orders dated to the Cretaceous (Kumar and Hedges, 1998; Bininda-Emonds et al., 2007), Springer et al. (2003) recovered Cenozoic divergence dates for all mammal orders except for Rodentia, Primates, Xenarthra, and Eulipotyphla. Nevertheless, the paleontological and molecular communities do not appear to be converging on the Long Fuse Model, and the conflict still appears intractable.

DIVERGENCE TIMES IN RODENTS

Although some dissent exists (D'Erchia et al., 1996; Grauer et al., 1991; 1996; Misawa and Janke, 2003), the closest relative to the order Rodentia is widely recognized to be the rabbits and pikas (order Lagomorpha) by both morphologists (Luckett and Hartenberger, 1993; Landry, 1999; Meng and Wyss, 2001; 2005) and molecular biologists (Murphy et al., 2001; Huchon et al., 2002; Douzery and Huchon, 2004; Bininda-Emonds et al., 2007; Huchon et al., 2007). Following a terminology employed by paleontologists (Wyss and Meng, 1996; Meng and Wyss, 2001; 2005), the Glires are defined as the most recent common ancestor of Rodentia and Lagomorpha and all its descendents. Rodentia are defined as the most recent common ancestor of all extant rodents (including *Anomalurus*, *Castor*, *Cavia*, *Mus*, and *Sciurus*) and all its descendants.

Simplicidentata is defined as all mammals sharing a more recent common ancestor with Rodentia than Lagomorpha. Rodentia is the more exclusive definition whereas Simplicidentata includes early fossil taxa that are basal to the clade that unites all extant rodents.

Simplicidenta are characterized by possessing a single pair of ever-growing incisors on both the upper and lower toothrow, an enlarged diastema with the upper diastema longer than the lower, and a lack of P^2 (Meng and Wyss, 2005). The earliest known simplicidentate is the Asian genus *Heomys* from the early Paleocene, dated about 64.6 million years ago (Ma; Li, 1977; Marivaux et al., 2004). The earliest known relative of modern lagomorphs, *Mimotona*, is known from the same formation and is dated to about the same time (Li, 1977; Marivaux et al., 2004).

The first morphologically modern rodents appear about 57 Ma in the Late Paleocene (Clarkforkian) of North America (Meng and Wyss, 2005; The Paleobiology Database [PBDB] http://paleodb.org). According to the fossil record, rodents underwent an explosive diversification through the Eocene (Fig. 1) and all modern suborders (as defined by Carleton and Musser, 2005) are present by its end (McKenna and Bell, 1997). A total of 76 genera of rodents have been described from the Paleogene (Marivaux et al., 2004). In terms of diversity, rodents are the most successful group of mammals. Modern rodents comprise nearly half of all described mammal species (33 families, 481 genera, 2,277 species). McKenna and Bell (1997) list an additional 743 extinct genera of rodents.

The discrepancy between molecular and fossil estimates for divergence dates in rodents is among the most extreme in mammals. Molecular analyses that employ a molecular clock have only recovered a few orders of placentals (Afrosoricida, Eulipotyphla, and Primates) that are comparable in their early age of intraordinal divergences (Kumar and Hedges, 1998; Springer et al., 2003; Bininda-Emonds et al., 2007). Molecular clock analyses using non-rodent calibration points consistently place early rodent splits in the Cretaceous period (Kumar and Hedges, 1998; Cao et al., 2000) even when using techniques that account for rate heterogeneity (Adkins et al., 2001; Huchon and Douzery, 2001; Mouchaty et al., 2001; Adkins et al., 2003; Douzery et al., 2003; Springer et al., 2003; Delsuc et al., 2004; Springer et al., 2005; Poux et al., 2006; Bininda-Emonds et al., 2007; Huchon et al., 2007). The only molecular clock studies that date the earliest split in Rodentia are those that apply calibration points within the rodents, usually with strong upper bounds on those dates (Huchon et al., 2002; Montgelard et al., 2002; Douzery et al., 2003).

In chapter 2, I evaluate the conflict between molecular and paleontological estimates for divergence times in rodents. I assess the paleontological literature to estimate a date of divergence for rodent splits that took place in the Eocene and compare them to relative age estimates obtained from molecular results. I evaluate the observed correlation between fossil and molecular estimates against a distribution of randomly appearing fossils to determine whether the molecular and fossil results actually disagree. I also develop a novel approach that incorporates the uncertainty inherent in the assumption that a given fossil represents the true date of divergence between lineages.

Finally, I generate estimates for divergences in rodents and between rodents and their closest relatives and evaluate the Explosive, Long Fuse, and Short Fuse Models of placental mammal evolution.

RELATIONSHIPS AMONG RODENTS

Multiple major proposals have been advanced attempting to divide rodents into subordinal ranks (Brandt, 1855; Tullberg, 1899; Ellerman, 1940; Simpson, 1945; Wood, 1955; 1959; 1965; Chaline and Mein, 1979; Hartenberger, 1985; Wilson and Reeder, 1993; Landry, 1999; Carleton and Musser, 2005), but the majority of these have centered around two principal characters, the morphology of the zygomasseteric system and the shape of the mandible. Brandt (1855), and other 19th century researchers developed a taxonomy based on Waterhouse's (1839) description of characters of the zygomasseteric system, the relationship of the masseter muscles to the zygomatic arch and infraorbital canal. Tullberg (1899) suggested that rodents be divided into two groups, those with a hystricognathous jaw and those with a sciurognathous jaw. Subsequent morphology-based taxonomies have largely been modifications of these two early proposals. Numerous well-sampled molecular studies have greatly clarified the relationships among rodents (Nedbal et al., 1994; 1996; Huchon et al., 1999; Adkins et al., 2001; DeBry and Sagel, 2001; Huchon and Douzery, 2001; Huchon et al., 2002; Montgelard et al., 2002; Adkins et al., 2003; DeBry, 2003; Huchon et al., 2007).

Although they applied many of the terms of Brandt (1855), Carleton and Musser (2005) proposed an updated taxonomy of extant Rodentia that incorporates molecular

results. They recognize five suborders: Sciuromorpha, Castorimorpha, Myomorpha, Anomaluromorpha, and Hystricomorpha. I apply the taxonomy of Carleton and Musser (2005) throughout the dissertation with the exception of chapter 4, which was published in 2004 (Norris et al., 2004) or except where specifically noted.

The Sciuromorpha unites the dormice (family Gliridae) with the mountain beaver (Aplodontiidae) and squirrel family (Sciuridae). The Sciuridae and Aplodontiidae have been found to be sister taxa in a number of well-supported studies (Huchon et al., 1999; Adkins et al., 2001; DeBry and Sagel, 2001; Huchon et al., 2002; Adkins et al., 2003; DeBry, 2003; Huchon et al., 2007). I follow the trend among many molecular studies (Huchon et al., 1999; Michaux and Catzeflis, 2000; Montgelard et al., 2002; DeBry, 2003; Douzery et al., 2003; Horner et al., 2007) in using the term Sciuroidea to refer to the Aplodontiidae + Sciuridae, but do so with reservation because paleontologists tend to refer to Sciuroidea and Aplodontioidea (or Aplodontoidea) to refer to clades uniting certain fossil families with the extant sciurids and aplodontiids (Wood, 1955; Meng, 1990; McKenna and Bell, 1997; Marivaux et al., 2004). The relationship between glirids and sciuroids has been recovered with good support, but in fewer studies (Adkins et al., 2003; Reyes et al., 2004; Horner et al., 2007; Huchon et al., 2007).

The Castorimorpha unites the beavers (Castoridae), pocket gophers (Geomyidae), and kangaroo rats (Heteromyidae). The sister relationship between the geomyids and heteromyids has been widely recognized by both molecular biologists (DeBry and Sagel, 2001; Huchon et al., 2002; Adkins et al., 2003; DeBry, 2003; Huchon et al., 2007) and morphologists (Wood, 1955; Hartenberger, 1989; Landry, 1999; Marivaux et al., 2004).

The position of the Castoridae as sister to the Geomyoidea is more preliminary and Carleton and Musser (2005) emphasized that further study was required to verify their hypothesis. Huchon et al. (2007) have since supported monophyly of Castorimorpha with reasonably high support.

The Myomorpha is an extremely successful group that includes the birch mice, jumping mice, and jerboas (Dipodidae), and the wildly successful superfamily Muroidea, a group that contains almost one quarter of all mammal species including mice, rats, gerbils, voles, hamsters, and their relatives. The Myomorpha represents another relatively uncontroversial grouping that has been supported in many studies (DeBry and Sagel, 2001; Adkins et al., 2003; DeBry, 2003; Reyes et al., 2004; Huchon et al., 2007).

The Anomaluromorpha contains the scaly-tailed flying squirrels (Anomaluridae) and the springhare (Pedetidae). This suborder combines two families that have a hystricomorphous zygomasseteric system, a hystricognathous mandible, and are currently restricted to sub-Saharan Africa. Most published molecular phylogenies have included one of these two families, but not both. Montgelard et al. (2002) recovered a well-supported Anomaluromorpha clade, but their study was restricted to the application of weighted parsimony to two mitochondrial genes. Carleton and Musser (2005) united the two families in a single suborder largely due to a lack of alternative hypotheses. Huchon et al. (2007) have since supported monophyly of Anomaluromorpha with good support.

The Hystricomorpha is a clade that includes the recently described *Laonastes*, the gundis, and the diverse Hystricognathi. This clade has been subjected to considerable study and many opposing hypotheses have been proposed, but both morphological

(Luckett and Hartenberger, 1985; Flynn et al., 1986; Landry, 1999; Marivaux et al., 2002; 2004; Dawson et al., 2006) and molecular (Huchon et al., 2000; Adkins et al., 2001; Huchon et al., 2002; Adkins et al., 2003; Huchon et al., 2007) studies have converged on its current composition. Although *Laonastes* was described in 2005 (Jenkins et al., 2005), its inclusion in this suborder receives unanimous support among those who have analyzed it (Jenkins et al., 2005; Dawson et al., 2006; Huchon et al., 2007). The terms Entodacrya (Landry, 1999) and Ctenohystrica (Huchon et al., 2000) have been coined to refer to this suborder, but I agree with Carleton and Musser (2005) that, although suprafamilial ranks are not covered by the International Code of Zoological Nomenclature, there is no compelling reason to create a new term whenever the composition of a taxonomic group changes due to new information. The core of Brandt's (1855) definition of Hystricomorpha is retained in modern classifications and new terms are not required.

In addition to evaluating divergence times in chapter 2, I also test monophyly of the suborders of Carleton and Musser (2005) using a dataset containing over 8,000 bp from seven genes. I attempt to determine the relationships among suborders in both chapter 2 and chapter 3. Chapter 3 employs sequencing the full mitochondrial genomes of three rodents, *Anomalurus beecrofti*, *Castor canadensis*, and *Dipodomys ordii*, combining these data with published mitochondrial genomes, and comparing these results with a nuclear dataset of comparable size (16 genes, >13,000 bp) in an attempt to determine how rodent suborders are related.

THE POSITION OF MYOSPALACINAE WITHIN THE MUROIDEA

The superfamily Muroidea represents the largest radiation of mammals with 1,518 extant species in 310 genera (Musser and Carleton, 2005). This represents nearly ¼ of described mammal species (Wilson and Reeder, 2005). The evolutionary relationships among these rodents are extremely complex leading Musser and Carleton (1993) to treat all members as a single family in the absence of viable alternative hypotheses. The application of molecular data to the question of muroid relationships (Furano et al., 1994; Usden et al., 1995; Robinson et al., 1997; Huchon et al., 1999; Michaux and Catzeflis, 2000; Chevret et al., 2001; DeBry and Sagel, 2001; Michaux et al., 2001) began to greatly improve understanding of relationships among muroids and certain patterns began to emerge. One of the most prominent of these patterns was the existence of a fossorial clade containing the blind mole rats (subfamily Spalacinae), the bamboo rats (subfamily Rhizomyinae), and the African mole rats in the genus *Tachyoryctes* (Robinson et al., 1997; Huchon et al., 1999; Michaux and Catzeflis, 2000; DeBry and Sagel, 2001; Michaux et al., 2001) basal to a monophyletic group containing the remaining sampled muroid subfamilies. As a result, Michaux et al. (2001) suggested that these taxa be placed in the family Spalacidae, while applying the family name Muridae to all remaining subfamilies.

The position of the zokors (subfamily Myospalacinae) was more storied. Genetic information from a single individual, identified as *Myospalax* sp. from an "unknown locality, Russia" was the only representative of the subfamily Myospalacinae applied to

several phylogenetic studies of muroid relationships (Furano et al., 1994; Usden et al., 1995; Michaux and Catzeflis, 2000; Chevret et al., 2001). The results of these studies indicated a phylogenetic position of the Myospalacinae nested within the subfamily Cricetinae (Michaux and Catzeflis, 2000; Chevret et al., 2001; Michaux et al., 2001). Specifically, *Myospalax* appeared to be sister to the hamster genus *Phodopus*.

In prior studies based on morphology, the Myospalacinae had been allied to several different muroid subfamilies including Rhizomyinae and Spalacinae (Tullberg, 1899), Spalacinae (Miller and Gidley, 1918; Chaline et al., 1977), Arvicolinae (Kretzoi, 1955), and Cricetinae (Gromov and Polyakov, 1977). Carleton and Musser (1984) considered the myospalacines to be primitive cricetids, whereas Lawrence (1991) concluded that they were derived from a fossorially adapted lineage basal relative to all muroids. Although the myospalacines had been considered related to the cricetines, their placement as sister to *Phodopus* within the Cricetinae represented a novel idea unique to these molecular studies (Michaux and Catzeflis, 2000; Chevret et al., 2001; Michaux et al., 2001).

We tested the position of *Myospalax* as a derived hamster in chapter 4, which was published in 2004 (Norris et al., 2004). We concluded that the tissue from the individual used in previous studies to advocate a position of zokors as derived hamsters was probably from an actual hamster and had been mislabeled. We determined that zokors are a part of the same basal fossorial radiation that includes the Spalacinae, Rhizomyinae, and *Tachyoryctes*, a conclusion supported by Jansa and Weksler (2004) who published a similar conclusion at about the same time. We recommended that Myospalacinae be

treated as a subfamily within Spalacidae, a position adopted by Musser and Carleton (2005).

Musser and Carleton (2005) further divided the Muroidea into six families in total. These are the spiny and pygmy dormice or tree mice (Platacanthomyidae), the blind mole rats, zokors, bamboo rats, and African mole rats (Spalacidae), the mouse-like hamsters, referred to by Norris et al. (2008) as brush-tailed mice (Calomyscidae), a clade of African and Malagasy endemics (Nesomyidae), the hamsters, voles, and New World rats and mice (Cricetidae), and the gerbils and Old World rats and mice (Muridae). The position of two subfamilies in Musser and Carleton's (2005) taxonomy is particularly preliminary. They place the Togo Mouse, *Leimacomys buetnerri*, in a new subfamily in the Muridae primarily due to a cladistic study of dental characters by Denys et al. (1995), who recovered a relationship of *Leimacomys* with the Gerbillinae, but with essentially no support. Although treatment of *Leimacomys* as a murid is probably as valid as any other hypothesis, the genus is essentially *incertae sedis*. Musser and Carleton (2005) also treat the maned rat, *Lophiomys imhausii*, as a member of a monotypic subfamily, but in the Cricetidae. In the only molecular study to include it, Jansa and Weksler (2004) determined, with strong support, that *Lophiomys* is a member of the Muridae clade.

RELATIONSHIPS AND DIVERGENCE TIMES AMONG THE MURIDAE: THE *MUS* – *RATTUS* DIVERGENCE

The classic view of the origin of *Mus* and *Rattus* is that they are part of two separate radiations that arose from the earliest split of the subfamily Murinae. A series of

paleontological studies (Jacobs, 1978; Jaeger et al., 1986; Flynn et al., 1990; Jacobs and Downs, 1994; Jacobs and Flynn, 2005) have estimated the *Mus* - *Rattus* divergence date as having occurred 10-14 million years ago (Ma) based on the temporally well-defined Siwalik fossil series from Pakistan. Jacobs and Downs (1994) describe the transition of molar characters from the plesiomorphic condition found in *Potwarmus* 14.4 Ma through transitionary intermediates to the first appearance of *Antemus*, the presumed ancestor of all murines, 14.0 Ma (Flynn et al., 1990; Jacobs and Flynn, 2005). The earliest species in the genus *Progonomys*, the first fully modern murine, appeared by 12.3 Ma (Jacobs and Flynn, 2005). Later species of *Progonomys*, thought to be on the line leading to *Mus*, appeared at 10.4 Ma, and *Karnimata*, the presumed ancestor of *Rattus*, appeared by 11.1 Ma (Jacobs and Flynn, 2005). Benton and Donaghue (2007) define the hard minimum value of this divergence time to be represented by the first appearance of *Karnimata* 11.1 Ma, and the soft maximum to be at the first appearance of modern murines, early forms of *Progonomys*, at 12.3 Ma. Because of the quality of this fossil series and the importance of these species, the 12 Ma *Mus* - *Rattus* divergence date has become one of the most widely used calibration points for molecular clocks and studies of molecular evolution (Catzeflis et al., 1987; Li et al., 1987; Furano et al., 1994; Nedbal et al., 1994; Adkins et al., 1996; Agulnik and Silver, 1996; Dubois et al., 1996; Edwards et al., 1997; Robinson et al., 1997; Ducroz et al., 1998; Huchon et al., 2000; Martin et al., 2000; Michaux and Catzeflis, 2000; Suzuki et al., 2000; Barome et al., 2001a, 2001b; Chevret et al., 2001; Ducroz et al., 2001; Fadda et al., 2001; Michaux et al., 2001; Weinreich, 2001; Huchon et al., 2002; Michaux et al., 2002; Smith and Eyre-Walker, 2002).

Some studies have used other calibration points to estimate the time of divergence between *Mus* and *Rattus*. I evaluated 75 estimates of this date from 18 molecular studies published before 2004 (O'hUigin and Li, 1992; Janke et al., 1994; Frye and Hedges, 1995; Kumar and Hedges, 1998; Messer et al., 1998; Cao et al., 2000; Huchon et al., 2000; Michaux and Catzeflis, 2000; Yoder and Yang, 2000; Adkins et al., 2001; Ducroz et al., 2001; Michaux et al., 2001; Nei et al., 2001; Nikaido et al., 2001; Michaux et al., 2002; Montelgard et al., 2002; Nei and Glazko, 2002; Adkins et al., 2003). Estimates range from 11.5 Ma to 86.9 Ma with a mean of 35.8 Ma. With only six exceptions (Michaux and Catzeflis, 2000 [11.5]; Yoder and Yang, 2000 [12.9, 13.7]; Ducroz et al., 2001 [12.5]; Michaux et al., 2001[12]; 2002 [11.9]), molecular estimates were consistently earlier than the 12-14 Ma as estimated by fossils.

Yoder and Yang (2000) used multiple primate calibration points separately and employed a variety of global and local clock estimates. Their estimates of the *Mus – Rattus* split range from 12.9 Ma to 56.9 Ma with a mean of 40.0 Ma. The presence of two values at the low end of such a broad range can clearly not be viewed as confirmation of the paleontological date estimate. The remaining four studies that produced a *Mus – Rattus* estimate <15 Ma are the only studies among these that employed calibration points from within the Muroidea. Two of these calibration points are problematic. Michaux et al. (2001) cite Tong (1989) as the source for a *Gerbillus – Tatera* calibration point of 8-10 Ma. Tong (1989) actually presents evidence that the calibration point between *Tatera* and *Gerbillus* is 6 Ma based on the paleontologic record. He notes that DNA-DNA hybridization studies (e. g. Brownell, 1983) produced a

Gerbillus – Tatera estimate of 8-12 Ma when calibrated with *Mus* - *Rattus*. Michaux et al.'s (2001) use of the *Gerbillus – Tatera* calibration to determine the *Mus – Rattus* split is invalid because it derives from a prior *Mus – Rattus* calibration. Ducroz et al. (2001) employ a Gerbillinae – Murinae calibration point, but this interpretation of fossils is potentially subject to the same problems of uncertainty as are described below for the *Mus – Rattus* date. Consequently, only two studies (Michaux and Catzeflis, 2000; Michaux et al., 2002) using a Spalacidae - Muridae (20 Ma) and an *Apodemus mystacinus – A. sylvaticus* (7 Ma) calibration have tested the *Mus – Rattus* divergence date using muroid calibrations. Neither study employs multiple calibration points or a method of estimation that accounts for rate heterogeneity. Estimates that do not use a muroid calibration point consistently yield *Mus – Rattus* dates that are unreasonably high (mean = 37.2 Ma) when compared to the paleontological evidence. This emphasizes the need to employ calibration points from sister taxa in this superfamily or at least calibration points within the Rodentia.

Molecular systematic studies of muroids have shed additional doubt on the current interpretation of the fossil record. A series of DNA-DNA hybridization studies (Chevret et al., 1993; Denys et al., 1995), DNA sequencing studies (Agulnik and Silver, 1996; Chevret et al., 2001; Michaux et al., 2001; Jansa and Weksler, 2004; Steppan et al., 2004), and other molecular studies (Furano et al., 1994; Usdin et al., 1995) have demonstrated that the spiny mouse, *Acomys*, is more closely related to the gerbils than to the Murinae. This led researchers (Michaux et al., 2001; Steppan et al., 2004; Musser

and Carleton, 2005) to recognize a new subfamily, Deomyinae, which contains *Acomys* and related genera.

The molar morphology of *Acomys* is extremely similar to *Mus*. Jacobs (1978) considered the genus *Acomys* to be sister to *Mus* and suggested that both were derived from *Progonomys debruijni*. Under that interpretation, the divergence time between *Acomys* and *Mus* should be about 8.5 Ma while *Acomys* and *Rattus* would have diverged when *Mus* and *Rattus* diverged 11.1-12.3 Ma. Subsequent morphological studies have also supported the affinity of *Acomys* with the murines (Denys et al., 1992; 1995; Xu et al., 1996) and none have suggested a reinterpretation of the Siwalik fossil series.

If only extant taxa are considered, two equally parsimonious explanations exist for the extreme similarity seen between *Acomys* and *Mus*. The *Mus*-like molar could be the plesiomorphic state for the family Muridae and could have evolved into the derived tooth morphology seen in gerbils. Under this scenario, a *Progonomys* – like ancestor would have given rise to all taxa in this clade. Alternatively, the *Mus*-like molar may have evolved independently in both the murines and deomyines. *Antemus* and early *Progonomys* could be the ancestors to the Murinae. Even under this scenario, the use of *Karnimata* and later species of *Progonomys* to represent the ancestors of *Rattus* and *Mus* respectively may be problematic, as recent molecular results have suggested that the split between *Mus* and *Rattus* does not represent the earliest divergence among the Murinae. Instead a clade of Philippine endemic rodents including *Phloeomys* and *Batomys* represents the most basal lineage of murines (Jansa and Weksler, 2004; Steppan et al., 2004; Steppan et al., 2005; Jansa et al., 2006; Rowe et al., 2008). Steppan et al. (2004)

and Jansa et al. (2006) chose to use the Siwalik fossil series as a calibration point to represent the split between this Philippine clade and the remaining murines. The 11.1-12.3 Ma date may therefore apply to a Deomyinae – Murinae split, a *Phloeomys – Rattus* split, or a *Mus – Rattus* split.

In chapter 5, I estimate the divergence date between *Mus* and *Rattus* using the same dataset that was used in chapter 2 with the addition of *Rattus*. This dataset involves a large amount of sequence data (>8,000 bp) and includes eight well-corroborated fossil calibrations. I also test among the three potential positions for the 12.3 Ma *Progonomys* date using a mitochondrial dataset that involves a 1,336 bp segment of the mitochondrial genome containing all or part of the protein coding genes COX1, COX2, and ATPase 8 as well as three transfer RNAs: tRNA-Ser, tRNA-Asp, and tRNA-Lys. In addition to testing divergence times, the mitochondrial dataset in chapter 5 is used to evaluate phylogenetic relationships within the Gerbillinae and involving additional samples of African Murinae obtained from Guinea and Sierra Leone over the course of my dissertation research (Norris, 2006; Decher et al., 2007; 2008).

The family Muridae is perhaps the single most important family of animals in laboratory science. The genera *Mus* and *Rattus* specifically are of vital importance to numerous fields of biological sciences. Both have been the subjects of genome projects (Bouchie, 1999; Chinwalla et al., 2002) and the information gained from study of these two taxa has led to advancement in a vast array of biology related fields. Much of this research has had broader application to mammals as a whole (Bradley, 2002). Few

advances in medicine and human biology have been made that did not involve preliminary or parallel study in a mouse or rat system.

In a series of papers that are not published in this dissertation, we applied systematic techniques to evaluate the molecular evolution of the genes involved in the endocannabinoid system across organisms whose complete genomes are available (McPartland et al., 2007a; 2007b; 2007c). Among the assumptions that were required to conduct these investigations were those made concerning phylogenetic relationships of model organisms and dates that evolutionary splits took place. By applying a *Mus – Rattus* date of about 12 Ma, we compared how the dates applied to a *Mus – Rattus* split in a prior study (Dorus et al., 2004) would influence results (McPartland et al., 2007c). Dorus et al. (2004) assumed that *Rattus* and *Mus* split at about the same time as *Homo – Macaca*. According to paleontological estimates the divergence time between *Mus* and *Rattus* is about half that of *Homo – Macaca*. Based upon the estimate of Dorus et al. (2004) we would conclude that the endocannabinoid system in rodents evolves about 2.7 times faster than in primates. Using the dates derived from fossils we concluded that it is actually evolving at 5.4 times the rate (McPartland et al., 2007c). In order to understand how differing results in rodent models have implications in primates it is vital to understand the evolutionary history of both groups. Estimating relationships and divergence times in rodents has the potential to affect conclusions in biomedicine and comparative genomics as well as systematics, evolution, paleoecology, and morphology.

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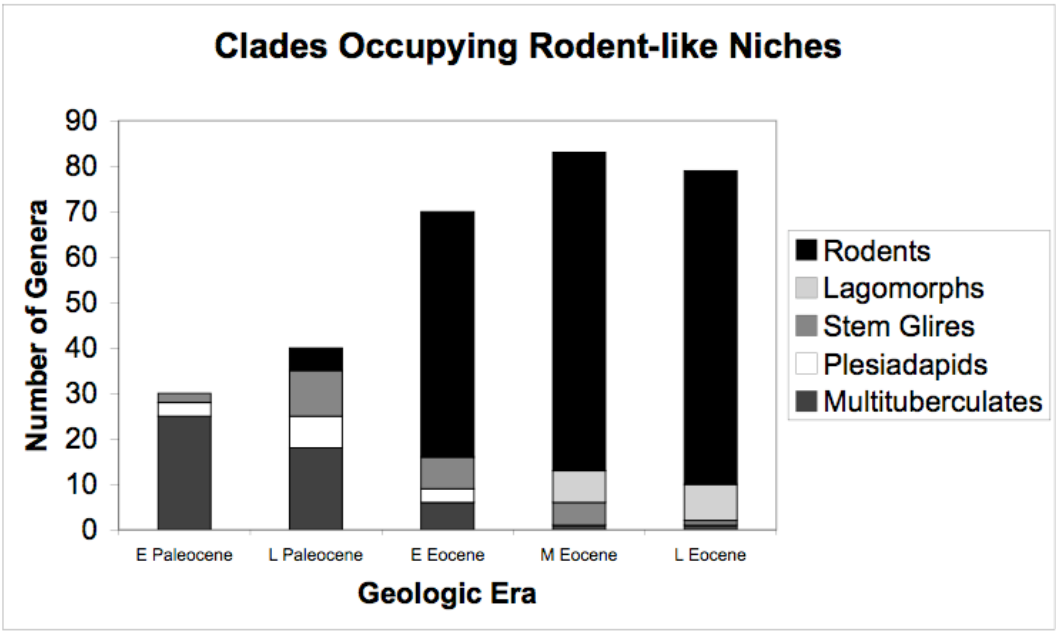
FIGURE LEGENDS

FIGURE 1. Mammals occupying ecological niches broadly similar to modern Rodentia in the Paleocene and Eocene. Data taken from McKenna and Bell (1997).

FIGURE 1.


Clades Occupying Rodent-like Niches
Number of Genera
90
80
70
60
50
40
30
20
10
0
E Paleocene
L Paleocene
E Eocene
M Eocene
L Eocene
Geologic Era
Rodents
Lagomorphs
Stem Glires
Plesiadapids
Multituberculates

Chapter 2

Phylogeny and divergence times of major rodent clades: Agreement between molecular clock and fossils

Abstract-

Molecular and paleontological approaches have produced extremely different estimates for divergence times among orders of placental mammals and within rodents. Molecular studies have suggested a Cretaceous origin for the Rodentia and other orders, but the fossil record shows no indication of any member that can be assigned to a modern order until the Paleocene. Here we evaluate the conflict between the fossil record and molecular data and find a significant correlation between dates estimated by fossils and relative branch lengths, suggesting that molecular data agree with the fossil record regarding divergence times in rodents. Our approach includes a correction for tree hierarchy involving simulating the random appearance of fossils and holds true across different molecular clock techniques. We also present a ghost lineage approach that attempts to incorporate the potential for the discovery of older fossils into a Bayesian analysis of divergence dates. We apply this approach to a set of Eocene rodent fossils and estimate divergence times within rodents and among the Euarchontoglires orders. The earliest divergence in rodents appears to have occurred at approximately the K/T boundary, but interordinal splits in the Euarchontoglires are estimated to have taken place late in the Cretaceous. We propose that some molecular clock studies may overestimate divergence times due to a period of accelerated molecular evolution across multiple

lineages or due to saturation of data that is not adequately corrected by the evolutionary model.

INTRODUCTION

The introduction of molecular data has greatly expanded the scientific community's understanding of the interordinal (between order) relationships in placental mammals (Murphy et al., 2001; Springer et al., 2004). Although certain specific clades are only recently being resolved, such as the relationships among the three Euarchontan orders (Janečka et al., 2007), the broader story has been acquiring wide acceptance over the past two decades. In contrast, assigning dates to both interordinal and intraordinal splits in placental mammals has proven highly controversial.

Archibald and Deutschman (2001) and Springer et al. (2003; 2005) provide a simple overview of the state of dating controversy by outlining three basic competing hypotheses: the Explosive, Long Fuse, and Short Fuse models. These hypotheses vary based on the timing of interordinal and intraordinal diversification of placentals relative to the mass extinction event at the Cretaceous-Tertiary (K/T) boundary. The Explosive Model places both inter- and intra- ordinal diversification after the K/T boundary. It is widely supported by paleontologists (Foote et al., 1999; Wible et al., 2005a; 2007) and was the traditional hypothesis prior to the introduction of molecular data (Gingerich, 1977). The Long Fuse Model places interordinal splits in the Cretaceous, while placing intraordinal diversification in the Cenozoic. This compromise approach is supported at least in part by some paleontologists (Archibald et al., 2001; Archibald, 2003) as well as

for certain orders in some molecular analyses (Springer et al., 2004; 2005). The third hypothesis, the Short Fuse Model, places both interordinal and intraordinal diversification in the Cretaceous. The Short Fuse Model has no support from the fossil record, yet is supported for many orders by the majority of molecular analyses (Kumar and Hedges, 1998; Bininda-Emonds et al., 2007).

Perhaps no group of placental mammals shows a stronger conflict between the fossil record and the results of molecular clock analyses than the order Rodentia. For the sake of consistency we apply the terminology of Meng and Wyss (2005): Rodentia refers to the most recent common ancestor of *Mus*, *Sciurus*, and *Hystrix*. Simplicidentata is a stem-based term referring to all taxa more related to modern Rodentia than any other living taxa. Glires is defined as the most recent common ancestor of rodents and lagomorphs and all its decendents.

The earliest fossils that may be attributable to Rodentia are known from the Clarkforkian (55.4-56.8 million years ago [Ma]) of North America (Meng and Wyss, 2005). The earliest fossils attributable to Glires may date back to the early or middle Paleocene of Asia (less than 65.5 Ma; McKenna and Bell, 1997; Marivaux et al., 2004; Asher et al., 2005; Meng and Wyss, 2005; Li et al., 2007). No fossils attributable to either the order Rodentia or the superorder Glires that date prior to the K/T boundary have been discovered (Meng and Wyss, 2005; Wible et al., 2007).

Nevertheless, molecular clock analyses using non-rodent calibration points consistently place early rodent splits in the Cretaceous period (Kumar and Hedges, 1998; Cao et al., 2000) even when using techniques that account for rate heterogeneity (Adkins

et al., 2001; Huchon and Douzery, 2001; Mouchaty et al., 2001; Adkins et al., 2003; Douzery et al., 2003; Springer et al., 2003; Delsuc et al., 2004; Springer et al., 2005; Poux et al., 2006; Bininda-Emonds et al., 2007; Huchon et al., 2007). The only molecular clock studies that date the earliest split in Rodentia are those that apply calibration points within the rodents, usually with strong upper bounds on those dates (Huchon et al., 2002; Montgelard et al., 2002; Douzery et al., 2003).

The majority of these results based on molecular clocks tend to differ from the fossil record by considerable values. In one of the most extensive analyses to date, Bininda-Emonds et al. (2007) inferred a date for the earliest rodent split at about 85 Ma, about 30 million years before the first animals with rodent characters appear in the fossil record. They also suggest that about 8 lineages of rodents survived the K/T extinction event and have given rise to modern descendents. Other studies produce similar results (Kumar and Hedges, 1998; Cao et al., 2000; Springer et al., 2003; 2005; Huchon et al., 2007).

The implications of an early diversification of placental mammal orders require a reevaluation of many aspects of both macroevolutionary processes and paleoecology. Penny and Philips (2007) note that molecular results, such as those of Bininda-Emonds et al. (2007), suggest that pulses of rapid diversification in placental groups no longer align with mass extinction events. Both the Explosive Model and the Long Fuse Model would suggest that the rapid ecological diversification of placental mammals, suggested by the origin of modern orders, took place after the extinction of nonavian dinosaurs. Under the Short Fuse Model placental mammals would have diversified into broad ecological

niches alongside the nonavian dinosaurs. The carnassial pair in Carnivora, the unguligrade posture in Artiodactyla, flight in bats, and the ever-growing incisors of rodents would all be present prior to K/T event. Recent discoveries suggest that gliding (Meng et al., 2006), myrmecophagous and fossorial (Luo and Wible, 2005), and semi-aquatic (Ji et al., 2006) mammals and mammaliaformes did exist in Mesozoic, but none are attributable to modern placental lineages and no placental fossils have been discovered that support the idea of Cretaceous ecological diversification (Wible et al., 2007).

Several other implications of early placental diversification exist. McKenna (2007) summarized the literature on the implications of the asteroid impact that marks the K/T boundary and emphasized that a only limited number of individuals from a limited number of species with specific ecological requirements should have been capable of surviving the event. These conclusions based on molecular clock results increase both the number of hypothetical survivors and their ecological diversity. Foote et al. (1999) demonstrate that these ancient divergence times conflict with standard birth-death models for higher taxa and fossil preservation rates. Finally, a 30+ million year gap in the fossil record invalidates the use of many intraordinal fossil calibration points in analyses that apply molecular clocks to more recent events.

Objectives

We seek here to evaluate whether the fossil record and molecular clock results are truly in conflict statistically. To do this we include data from five nuclear (ADRA2B, BRCA1, GHR, IRBP, and VWF) and two mitochondrial (12S rRNA and CYTB) genes. In addition to outgroup taxa, we include representatives from 14 lineages of rodents. According to the fossil record, this includes all lineages of rodents present at 33.1 Ma (shortly after the end of the Eocene at 33.9 Ma) with the possible exception of certain Hystricomorpha (dependent on whether *Gaudeamus* and *Protataromys* are stem or crown taxa within their respective lineages) and potentially ancient families (such as the Dipodidae, Gliridae, and Sciuridae). We also present a novel approach to estimate upper confidence intervals on fossil calibration points, evaluate the Explosive, Long Fuse, and Short Fuse hypotheses in rodents, and discuss reasons why molecular analyses may yield such disparate results compared to the fossil record.

MATERIALS AND METHODS

Gene and Taxon Sampling

We included genetic data from representatives of 14 clades of rodents that correspond to those lineages present at 33.1 Ma according to the fossil record (Table 1). Outgroup taxa included were the two families in the order Lagomorpha, Ochotonidae (pikas) and Leporidae (rabbits and hares), and representatives from two Euarchontan orders, Scandentia (tree shrews) and Primates. Lagomorpha is widely recognized as the sister taxon to Rodentia based on both molecular (Murphy et al., 2001; Huchon et al.,

2002; Douzery and Huchon, 2004; Bininda-Emonds et al., 2007; Huchon et al., 2007) and morphological data (Luckett and Hartenberger, 1993; Landry, 1999; Meng and Wyss, 2001; 2005); the two orders comprise the clade Glires. The Euarchonta and Glires together form the clade Euarchontoglires. In several cases, data from multiple species were combined to construct a concatenated sequence (Table 1).

Genes were selected to take maximum advantage of the available data in GenBank resulting from the numerous studies conducted on rodents to date (Nedbal et al., 1994; 1996; Huchon et al., 1999; 2000; Adkins et al., 2001; DeBry and Sagel, 2001; Huchon and Douzery, 2001; Huchon et al., 2002; Montgelard et al., 2002; Adkins et al., 2003; DeBry, 2003; Huchon et al., 2007). Approximately 8.3 kbp of sequence data were compiled using data from five nuclear genes: alpha 2B andrenergic receptor (ADRA2B), breast cancer gene 1 (BRCA1), growth hormone receptor (GHR), interphotoreceptor retinoid binding protein (IRBP), and the von Willebrand factor (vWF), as well as two mitochondrial genes: small subunit RNA (12S rRNA) and cytochrome *b* (cytb). Data from GenBank were supplemented through sequencing of the GHR and BRCA1 genes in *Anomalurus beecrofti*. All genes were included for all 14 ingroup and four outgroup taxa. GenBank accession information is shown in Table 1.

DNA Sequencing

Data from two genes, GHR and BRCA1, were gathered from ethanol preserved liver from an individual *Anomalurus beecrofti* collected on 19 November 1999 from Agumatsa Wildlife Sanctuary, Volta Region, Ghana, and catalogued in the collection of

tissues in the Biology Department, University of Vermont (reference #1516). DNA was extracted using the DNeasy QIAGEN kit. PCR conditions are the same as described by Adkins et al. (2001), but our use of primers varied slightly for the BRCA1 gene due to difficulty amplifying. For the BRCA1 gene, the reverse primers BRCA1-2R and BRCA1-3R (Adkins et al., 2001) were used, but the following additional primers were also developed: BRCA1-CF: GARCRTCCCCTCACAAAYAAA (modified from Jugessur et al., 2000), BRCA1-DF: ATRRCACTCAGRACAGTRTNT (modified from Jugessur et al., 2000), BRCA1-N0F: CCAGCTYATTACAGCNTGRGA, BRCA1-N2F: TAAAGANGCNARYTCAGGCAGT, and BRCA1-N02R: AYGTYTCTYNCTTAYNTNYTCANYTGGC. PCR was performed using Illustra puReTaq Ready-To Go PCR Beads. Double stranded PCR products were purified using PEG precipitation (Maniatis et al., 1982). Sequencing was performed on an ABI 3130x1 Genetic Analyzer using dye terminator (ABI PRISM) cycle sequencing. The following primers were used to obtain BRCA1 sequence: CF, DF, N2F, 2R, and 3R. Sequences were assembled and edited using CodonCode Aligner (CodonCode Corporation).

Phylogenetic analyses

Nucleotides from protein coding regions were aligned in MacClade (Maddison and Maddison, 1998) while referencing corresponding alignments of amino acid sequences in Clustal X (Thompson et al., 1997). Sequences for 12S rRNA were initially aligned in MacClade according to secondary structure as indicated by Springer et al.

(1995). Individual stem and loop regions were aligned using ClustalX and edited by eye. Ambiguously aligned regions were excluded from the final analyses.

Tree reconstruction was conducted under both maximum parsimony (MP) and maximum likelihood (ML) frameworks in PAUP* (version 4.0b8, Swofford, 2002). Nodal support was evaluated using bootstrapping in PAUP* for both MP (1,000 replicates) and ML (300 replicates). Bayesian posterior probability values were obtained using MrBayes (version 3.1.1; 1,000,000 generations, sampled every 1,000 generations, burnin=250, 4 chains, 2 runs; Ronquist and Huelsenbeck, 2005). Modeltest 3.04 (Posada and Crandall, 1998) was used to determine the appropriate likelihood model for this combined data set, and a GTR + I + Γ model was used in the maximum likelihood and Bayesian analyses. Because our goal was to have an accurate representation of branch lengths for molecular clock analyses, we excluded all sites containing gaps or missing data in the ML and Bayesian analyses. The MP analysis included 8,356 bp and the ML and Bayesian analyses included 6,454 bp. Gaps were coded as missing data in the parsimony analysis.

Determining fossil dates at nodes

An extensive review of the fossil literature for rodents was conducted, including use of the Paleobiology Database (PBDB; http://paleodb.org) and the Neogene Mammal Database (NOW; http://www.helsinki.fi/science/now/). First appearance dates were determined for the clade uniting the relevant extant taxon with all related fossil taxa to the exclusion of all other extant taxa in order to evaluate the minimum constraint on the

divergence date at nodes as defined by Benton and Donaghue (2007). For example, members of the extinct family Eutypomyidae are widely considered to form a clade with the beavers (Castoridae) to the exclusion of all other extant taxa. The first appearance date of the Castoridae lineage is based on the first appearance date of the eutypomyid genus *Mattimys*, which is older than all other eutypomyids, castorids, or other members of this clade (Table 2). The date is not based on the first appearance of the Castoridae, but on the first appearance of a member of the most inclusive clade that includes the Castoridae while excluding all other extant taxa.

Numerous cladistic analyses of morphological data that include fossil taxa have been performed (Marivaux et al., 2002; Lopez Antoñanzas et al., 2004, Marivaux et al., 2004; Wible et al., 2005b). Many of these analyses produce results that are quite consistent with molecular-based phylogenies (Marivaux et al., 2002; 2004). Nevertheless, cladistic analyses that contain the breadth of sampling required for our purposes are lacking, due in no small part to the sheer size of the order Rodentia and the limited amount of characters that can be gleaned from limited remains (often only teeth). For the purposes of this study we include a taxon if there appears to be broad agreement among paleontologists as to its phylogenetic position. Care was also taken to avoid being misled by Linnean ranks that are known to be paraphyletic.

Fossil beds are usually dated with a range of values. Because we were interested in minimum divergence time, first appearance dates were evaluated using the minimum value in a range of values. Thus a fossil dated to a more precise 34.1-34.2 Ma using

radiometry or Appearance Event Ordination (AEO; Alroy, 1994) was selected over a fossil dated as "Late Eocene" (33.9-37.2 Ma).

Carleton and Musser (2005) divide the order Rodentia into five extant suborders, an approach we apply here. Monophyly of these suborders is moderately well to well supported in molecular (Nedbal et al., 1994; 1996; Huchon et al., 1999; Adkins et al., 2001; DeBry and Sagel, 2001; Huchon and Douzery, 2001; Huchon et al., 2002; Montgelard et al., 2002; Adkins et al., 2003; DeBry, 2003; Huchon et al., 2007) and, to a lesser degree, morphological (Luckett and Hartenberger, 1985; Meng, 1990; Landry, 1999; Emry, 2007) studies. Higher-level relationships are more poorly understood and are based almost entirely on molecular data. Early simplicidentates appear in the early to middle Paleocene and potential crown-rodents trace back to the end of the Paleocene (Meng and Wyss, 2005). Among this rich record there are no doubt representatives that are related to modern suborders, but the paleontological community has not reached a consensus as to the nature of these relationships due to the limitations of morphological characters at this level. Therefore we have excluded all nodes higher than suborder in our fossil-based dating. First appearance dates were determined for the remaining 18 lineages of rodents. They are displayed in Table 2 and are explained in more detail in the Appendix.

Dates were assigned at each node consistent with the hard minimum date defined by Benton and Donoghue (2007). The fossil-based date at a given node was defined as the older of the first appearance dates of the two daughter lineages that split from that node. These fossil-based dates at nodes are shown in Table 3

Relative molecular dating

In order to ensure robustness across techniques, we conducted molecular clock analyses using both a relatively simple technique that assumes autocorrelation of ancestral and descendent evolutionary lineages and a more complex model-based technique that makes no *a priori* assumption of autocorrelation. We estimated relative divergence times using the nonparametric rate smoothing method (NPRS; Sanderson, 1997) in the program r8s (Sanderson, 2003). This technique seeks to minimize the change in the rate of evolution along lineages over time. Relative divergences times were also estimated using a Bayesian approach as implemented in the program BEAST (version 1.4; Drummond and Rambaut, 2007), an approach that makes no *a priori* assumption of autocorrelation. In both instances, the root of the tree was set as either 1.0 or 100 and no fossil calibration points were applied. This allows for an estimate of relative divergence times of evolutionary events on the tree instead of absolute date estimates.

Both the topology and branch lengths of the tree with the best maximum likelihood score were used as input for the NPRS analysis in r8s. The root of the tree was set at 1.0 to allow for relative dates to be determined. Our NPRS approach represents the simpler analysis as it applies a basic algorithm that minimizes the change in the rate of evolution across the tree, and incorporates branch lengths from the ML tree, which used an evolutionary model that was not partitioned by gene. The program requires a rooted tree. The Euarchontan outgroups (Primates and Scandentia) were used to root the tree, but were not included in the actual analysis.

The Bayesian approach was conducted using the program BEAST with the same raw dataset as the ML analysis. To facilitate comparisons, tree topology was constrained to match the results of the ML analysis (Fig. 1). GTR + I + Γ was again used as the model of evolution, but data were partitioned by gene and the program optimized the model parameters by gene. The uncorrelated lognormal relaxed molecular clock model was used and the mean substitution rate was not fixed. The age of the root was set with a prior of a normal distribution where mean = 100.0 and standard deviation = 0.01 in order to yield results that round to 100.0 within two decimal places. The program was run for 10,000,000 generations, sampled every 1,000 generations with a burnin of 1,000. All taxa were included in the BEAST analysis. Although the topological constraints clearly defined that the root of the tree was along the branch connecting Glires and Euarchonta, its specific placement was determined in the analysis (Fig. 2).

Assessing conflict

Absolute date estimates based on fossil results were compared to relative ages estimated through molecular analyses using linear regression (Conroy and van Tuinen, 2003) in the statistical package JMP (version 5.0.1.2, SAS Institute Inc.). Because phylogenetic trees are hierarchical in nature, a "significant" correlation can be obtained through a linear regression in the absence of any relationship. By definition an ancestral node is older than its descendent in molecular analyses in any tree where branch lengths are greater than zero. Likewise, the first appearance date of an ancestral lineage will always be estimated as older than or equivalent to any descendent lineages.

Two approaches were used to deal with the problem of performing linear regression in the presence of tree hierarchy. In the first approach, we performed a linear regression comparing the lengths of internal branches obtained from the molecular analysis to the duration of time that passed between the fossil-based dates at the two nodes. Only four internal branches had dates at both ancestral and descendent nodes and these four data points were subjected to a linear regression analysis.

Because of the limited sample size of the approach restricted to internal branches, we also evaluated the correlation between molecular results and the observed fossils and compared the same molecular data against a simulation of fossils appearing in a random fashion that is consistent with our tree shape. In order to simulate first appearance dates, we assigned all tip lineages with a random age between 32.1 and 55.5 Ma. This interval represents the observed range of fossil dates at nodes plus one million years. Like the approach we applied to the observed fossils, we defined the divergence dates at all nodes in the simulation as the older of the two daughter lineages descending from that node. Simulated first appearance dates for internal lineages were assigned a random age between the simulated age of the descendent node as defined previously and 55.1 Ma. Dates at internal nodes were then also defined as the older of the two daughter lineages descending from that node. The age estimates at nodes in the simulated fossil data set were then compared to the relative molecular-based ages from both the NPRS and BEAST analyses using linear regression. The R^2 value was recorded. This was repeated for 500 simulated fossil datasets and the observed R^2 value was compared to the simulated distribution.

Date estimates

Absolute divergence dates were also estimated in a second analysis using BEAST. Parameters in BEAST were set as explained previously except that no date was imposed on the root of the tree and fossil calibration estimates were included for only 8 of the 9 dated nodes (Fig. 3). The split between the Hystricidae and the Phiomorpha + Caviomorpha clade was not dated because the fossil used (*Gaudeamus*) is the same as that used to date the split between Caviomorpha and Phiomorpha (Fig. 3, Table 3).

To date, most molecular studies have treated fossil calibrations as precise points. This is mathematically equivalent to arguing for 100% certainty in the assumption that the fossil in question represents the precise time when two taxa split (Graur and Martin, 2003). In reality the only certain information about an evolutionary divergence date that can be provided by fossils is that the split is not younger than the first appearance date of the older of the two daughter lineages. Ranges are sometimes used in other studies, but the ranges employed to date have generally been somewhat arbitrary. They are often set as the observed fossil date plus a few million years, often five or ten.

Paleontologists have developed several approaches to determine variance around the endpoints of a given taxon in the fossil record. Marshall (1998) provided an overview of several approaches to estimating the actual point of origination or extinction of a taxon based on the frequency at which it is observed across its known stratigraphic range. These approaches focus on the number of layers containing a record of this taxon and the duration of gaps that separate these records. Likewise, Tavare et al. (2002) generated a

speciation rate model in primates and compared this with their fossil record to estimate that primates may have arisen over 25 million years prior to the earliest known primate fossil. Presumably, Meehl's (1983) discovery asymptote could also be modified to estimate actual chronological range of a taxonomic group.

Applying one of these techniques to our molecular dataset would require an almost comprehensive knowledge of all rodent fossils discovered. Although tools such as the Paleobiology Database show promise that such information may one day be more accessible, this requirement of comprehensive knowledge currently prohibits wide scale application of these potential techniques.

Our approach is based upon similar logic to the stratigraphic consistency index (Huelsenbeck, 1994) and cladistic gap analysis (Paul, 1988). After establishing first appearance dates for rodent lineages, we compared the difference in first appearance dates for the two daughter lineages descending from each dated node. Because both daughter lineages should date to the same age, the difference between them represents the minimum length of a ghost range for the younger lineage. This is a quantifiable gap in the fossil record. We used this gap size to estimate an overall distribution of gap sizes between sister clades for early Tertiary rodent diversification (mean = 8.2 myr, standard deviation = 5.3 myr). This distribution was then assumed to represent a rough estimate of how much older the actual dates at nodes may be compared to the observed dates of the nodes. In this case, the upper 95% confidence interval of 12.3 million years suggests that observed dates may be as much as 12.3 million years younger than the actual evolutionary split. All 9 dated nodes were used to calculate this value.

This confidence interval was implemented in BEAST by using an exponential prior at the 8 nodes used as fossil calibrations. The prior was set such that the "zero offset" parameter was equal to the minimum age estimate at the node and the "exponential mean" parameter was set so that the upper 95% C.I. of the resulting distribution was 12.3 million years. For fossils dated with a range of values, the "zero offset" was set to the minimum value in the range and the 95% C.I. was set to be equal to the length of the range + 12.3 million years. BEAUti (version 1.4.7, part of the BEAST package) was used to visualize the exponential distribution.

Universal priors were applied to two nodes (origin of Lagomorpha and the Euarchonta) within outgroup taxa. These dates were set as a range between the estimated fossil minimum value and a maximum value equal to the molecular results of Bininda-Emonds et al. (2007). The minimum value for the lagomorph split was set as 42.4 Ma based on the first appearance of *Desmatolagus* (Ochotonidae) from Swift Current Creek fauna, Saskatchewan, Canada (PBDB reference number 16626; Storer, 1984). The minimum value for the euarchontan split was set as 61.7 Ma based on the first appearance of *Paromomys* (Primates) from Hanna Formation, Wyoming (PBDB 14858; Secord, 1998). Both *Purgatorius* and the plesiadapiforms are older than *Paromomys*, but they are often treated as basal euarchontans instead of true primates (Benton and Donoghue, 2007). Maximum values were set as 66.8 Ma for Lagomorpha and 94.3 Ma for Euarchonta based on Bininda-Emonds et al. (2007). The result of this BEAST analysis was used to evaluate among the Explosive, Long Fuse, and Short Fuse hypotheses as they pertain to rodents and their nearest outgroups.

RESULTS

Relationships

The results of the phylogenetic analyses are shown in Figure 1. Maximum support (bootstrap percentages = 100%, Bayesian PP = 1.0) is present for the orders Rodentia and Lagomorpha, the suborders Anomaluromorpha, Myomorpha, Hystricomorpha, and Sciuromorpha, and for the Hystricognathi, Sciuroidea, and Geomyoidea. Strong support (boostraps > 90%, PP = 1.0) is also present for Glires, the suborder Castorimorpha, a clade uniting the Phiomorpha and Caviomorpha, and a clade uniting the Anomaluromorpha, Myomorpha, and Castorimorpha (referred to as the "mouse-related clade" by Huchon et al., 2002). Relationships among the three suborders in the "mouse-related clade" and relationships among the "mouse-related clade" the Sciuromorpha, and the Hystricomorpha remain largely unresolved.

Correlation between fossils and molecular clock

An example ultrametric chronogram showing relative time from the Bayesian analysis is shown in Figure 2. The branches on this tree represent molecular-based time estimates, but only in a relative sense. Figure 3 shows the fossils from Table 2 applied to the tree topology with branch lengths proportional to actual dates. A significant correlation was recovered between molecular and fossil results using both the NPRS (R^2 = 0.847, p = 0.0004) and Bayesian (R^2 = 0.847, p = 0.0004; Fig. 4a) approaches. In spite of a sample size of only four, internal branches also yield a significant relationship for both NPRS (R^2 = 0.977, p = 0.012) and Bayesian (R^2 = 0.974, p = 0.013; Fig. 4b).

Simulated data produced correlations that ranged from $R^2 = 0.000$ to $R^2 = 0.840$ (mean = 0.154) for NPRS and ranging from $R^2 = 0.000$ to $R^2 = 0.840$ (mean = 0.187) for the Bayesian analysis. Simulated fossils yielded a "significant" result ($p < 0.05$) for 51 (10.2%) runs when compared to the NPRS data and for 57 (11.4%) runs compared to the Bayesian data. Nevertheless, the R^2 values from observed fossil data were higher than all 500 (100%) simulated fossil datasets for both molecular approaches.

Molecular date estimates

The results of the second BEAST analysis are shown in Table 4 and Figure 5. The root of the tree, the Euarchonta – Glires split, was estimated as 76.3 Ma (95% C.I. = 68.9-79.9 Ma) rejecting a Cenozoic (<65.5 Ma) origin. A Paleocene origin for the Glires is also rejected (best tree = 72.7 Ma, 95% C.I. = 67.4-77.5 Ma). Although a Paleocene date for the divergence of the two orders in the Euarchonta cannot be rejected (best tree = 75.3 Ma, 95% C.I. = 63.9-78.1 Ma), the Explosive Model hypothesis is rejected according to this analysis because the other interordinal splits occurred in the Cretaceous.

The origin of Rodentia is estimated to be at the Cretaceous/Tertiary boundary (best tree = 65.7 Ma, 95% C.I. = 62.3-70.8 Ma). Neither the Long Fuse nor the Short Fuse hypothesis can be rejected, because the earliest intraordinal splits could have taken place on either side of the K/T boundary (65.5 Ma). A Cretaceous origin is rejected for all rodent suborders and for the order Lagomorpha.

DISCUSSION

Relationships among rodents

All nodes recovered in our phylogenetic analysis are consistent with the results of Huchon et al. (2007), from whence some of our sequences originate, with the exception of how the "mouse-related clade," the Sciuromorpha, and the Hystricomorpha resolve. Huchon et al. (2007) recovered a clade uniting the "mouse-related" suborders with the Sciuromorpha whereas our results unite the "mouse-related" suborders with the Hystricomorpha (Fig. 1). Neither analysis is well supported at these nodes. A clade uniting the "mouse-related" suborders with the Hystricomorpha is consistent with some other molecular analyses (Springer et al., 2003; Poux et al., 2006), but a clade uniting the "mouse-related" suborders with the Sciuromorpha has been advocated by paleontologists, termed Ischyromyiformes by Marivaux et al., (2004), and bears a closer resemblance to the composition of the traditional Sciurognathi (Tullberg, 1899). Further research is clearly needed to resolve this relationship. Its accurate recovery has important implications on determining character polarity and resolving the early fossil history of rodents (Marivaux et al., 2004). Genome sequencing decisions are also being made based on a potentially incorrect understanding of relationships of rodents at this level. According to its summary page at NCBI, part of the rationale for sequencing the complete genome of *Spermophilus tridecemlineatus* (family Sciuridae) is that it "will expand rodent sequence diversity to another family within the suborder sciurognathi [sic]" (http://www.ncbi.nlm.nih.gov/sites/entrez?Db=genomeprj&cmd=ShowDetailView

&TermToSearch=13936). Much of the objective behind expanding full genome sequencing to additional rodents is to apply the knowledge gained from model organisms such as *Mus* and *Rattus* to more evolutionarily distant taxa such as primates. A vital first step is to understand how rodents are related to these model organisms.

Molecular analyses, including ours, have also failed to resolve the relationships among the suborders within the "mouse-related clade". With the exception of these two unresolved portions of the tree, we feel comfortable with imposing this tree topology onto our other analyses. These two unresolved regions are separated by short internal branches, suggesting that the effect on time estimates will be limited. No well-supported conflicts exist between the results of our analysis and the myriad of rodent phylogenetic analyses that have been conducted to date (Huchon et al., 1999; DeBry and Sagel, 2001; Huchon et al., 2002; Adkins et al., 2003; Poux et al., 2006; Huchon et al., 2007).

Fossils and molecules agree

Although it should be viewed as merely a heuristic technique to crudely estimate the completeness of a fossil record, our recovery of a mean gap size of only 8.2 million years in the Eocene rodent fossil record is probably indicative of a more complete record than would be expected if rodent diversification had taken place in the Cretaceous. If the sudden appearance of rodents in the Paleogene fossil record was merely a chance occurrence due to an increase in absolute numbers of individuals or a migration event from a region with a poor fossil record, the estimated size of this gap might be larger. Even this relatively small gap size estimate is inflated by the existence of lineages where

relationships between extant taxa and fossil taxa are poorly understood. The Pedetidae and Hystricidae, for example, both appear suddenly in the Miocene and no consensus exists as to their connections with earlier fossil taxa. They are the only families listed as *incertae sedis* by Hartenberger (1998). Until recently, paleontologists have tended to treat the Pedetidae as close relatives to the Diatomyidae (McKenna and Bell, 1997; Marivaux et al., 2004). Dawson et al. (2006) have only recently shown that the diatomyids are related to *Laonastes*, which is itself a relative of the Ctenodactylidae (Huchon et al., 2007). A new consensus is yet to emerge around an alternative hypothesis regarding the relationship of pedetids to fossil rodents.

A much stronger case for the relative completeness of the Eocene rodent fossil record can be made based on the results of our regression analyses. The significant correlation between dates obtained by fossils compared to molecules is not consistent with a random appearance of those fossils following a substantial gap in the fossil record. This nonrandom association holds true whether tree hierarchy problems are ignored ($R^2 = 0.847$, $p = 0.0004$), only internal branches are evaluated ($R^2 > 0.97$, $p < 0.02$), or the random appearance of fossils is simulated (observed values are better than 100% of simulated datasets) for both Bayesian and NPRS approaches. This result is strong evidence that both molecular techniques and fossils are tracking the same evolutionary event from the same timeframe and against the suggestion made by some researchers that the fossils are erroneous and that rodents diversified deep in the Cretaceous.

Why do fossils and molecules appear to conflict?

This correlation between molecular and paleontological data raises a further question. Why do molecular clock analyses so frequently overestimate the divergence times of rodents? The tendency for error is clearly not bidirectional, but is instead heavily biased towards overestimates as opposed to younger estimates.

Ho et al. (2005) demonstrated that using an older calibration point to date recent (<2 million years) events systematically leads to an overestimation of divergence times. This phenomenon has since been observed using ancient DNA from bison (Ho et al., 2007), across multiple bird taxa, in balaenid whales, and in brown bears (Ho et al., 2008). They attribute this phenomenon to a discrepancy between the mutation rate and substitution rate (Ho et al., 2005; Ho and Larson, 2006; Ho et al., 2007; 2008). Recent evolutionary events are characterized by the presence of an elevated short-term mutation rate whereas older events are characterized by a slower long-term substitution rate. Essentially most mutations are likely to be eliminated by selection or drift unless the mutation is genuinely neutral or positive leading to the reduced substitution rate relative to the mutation rate (Ho and Larson, 2006).

All of the evolutionary divergences evaluated in our study pertain to events that took place more than 33 million years ago. At that level of divergence, the recent elevated mutation rate will have long since been displaced by the long-term slower substitution rate. Although a similar scenario where older calibration points overestimate more recent evolutionary events appears to be at work, the reason is likely to be different from that suggested by Ho and Larson (2006).

McKenna (2007) argued that analyses based on molecular clocks have been misled by an assumption of constancy of rates of evolution over time. Rates of molecular evolution may be elevated at times of crisis, such as the K/T extinction event and lead to incorrect date estimates. Figure 6a shows the hypothetical effect of a period of rapid evolution that affects all lineages simultaneously. The dotted line represents the mean substitution rate calculated if the analysis was calibrated at the root of the tree. Under this scenario, the molecular clock will overestimate evolutionary events that took place prior to or in the early stages of the period of rapid evolution. It will underestimate evolutionary events at the later stages and after the period of rapid evolution.

We evaluated how our date estimates would change if such a scenario as suggested by McKenna (2007) took place. If the overall rate of molecular evolution in Euarchontoglires in general and rodents in particular was consistently higher during the Cretaceous through the Eocene than it has been since the Eocene, several patterns should emerge. The slope of the regression line comparing fossils and relative branch length should be relatively steep and the intercept should be negative (Fig. 6a). The regression of our observed fossil dates against the BEAST analysis employing relative ages at nodes (Fig. 4a) produced a best fit line with the equation "Branch Length = 1.79 x (Date) – 14.8". Both a steep slope and a negative intercept are present. To determine how correcting for this effect might change the results, divergence date estimates for all nodes were calculated using this equation (Table 4). No dates estimated through this approach fall in the Cretaceous. The tree root, the Euarchonta – Glires split, is estimated at 63.5 Ma. All suborders are estimated to have diverged in the Eocene, and all evolutionary

splits higher than the level of suborder are estimated as having taken place in the Paleocene except for the Anomaluromorpha – Myomorpha split which is at the Paleocene/Eocene border. These values are consistent with the Explosive Model. Note that error in such an analysis is bidirectional; the first appearance of the Aplodontiidae lineage appeared at 42.2 Ma, but this approach estimates an aplodontiid – sciurid split at 36.5 Ma.

Saturation of data that is not adequately corrected by the model of evolution also has the potential to inflate age estimates when the calibration point is at the root of the tree (Fig. 6b). According to the data logged during our second BEAST analysis, the slowest evolving gene in our dataset was ADRA2B (mean of substitution rate per branch = 0.0018 substitutions/site/million years) and the fastest evolving gene was CYTB (mean of substitution rate per branch = 0.0200 substitutions/site/million years). We conducted two additional analyses in BEAST using the same parameters as the analysis described previously that calculated relative rates with a root set at 100.0. The sequence data were limited to only the ADRA2B data or the CYTB data. The CYTB dataset yielded a tree with significantly longer terminal branches than the ADRA2B tree ($P<0.001$). Jansa et al. (2006) recovered a similar result when calculating divergence times in rodents. The slower evolving nuclear IRBP gene yielded divergence estimates that were roughly consistent with the paleontological data, but CYTB yielded estimates considerably older. They chose to exclude the CYTB estimates. Our results can only serve to inform that inadequate models of evolution can yield older results relative to data that are less likely to be saturated. We cannot directly assess how much uncorrected saturation may be

present in our data. The possibility exists that inadequately corrected saturation of sequence data may play a role in a wider variety of studies that employ a molecular clock than is identified.

When did rodents diversify?

Although our results do not reject the idea that rodents arose in the Cretaceous, they clearly suggest that, if such diversification of rodents took place, it was limited to the earliest splits and did not involve origin of any suborders. At most, the lineages leading to the five modern suborders were present at the K/T boundary. Clearly any early rodents that may have existed in the Cretaceous would have been present only at the very end of the Cretaceous as the upper 95% confidence interval for our estimate of the first split in Rodentia is 70.8 Ma.

Our best estimate for the origin of rodents (67.7 Ma) is right at the K/T boundary (65.5 Ma), but the lower bound of the 95% C.I. (62.3 Ma) suggests that this may have taken place at almost any point in the Early Paleocene (61.7 – 65.5 Ma). Rodent suborders appear to have diversified in the Late Paleocene to Middle Eocene, and clades within suborders during and subsequent to the Eocene. These results are in much closer agreement with the fossil record than has been suggested in many prior studies. Dates derived from Eocene rodent fossils are well corroborated with one another and display significant agreement with molecular data, suggesting that the eight calibration points described here may prove useful in later studies.

We are not able to fully reject any of the three hypotheses (Explosive, Long Fuse, and Short Fuse), but our study represents a step in the direction of finding convergence between molecular and paleontological conclusions. We are, however, able to reject the most extreme versions of the Short Fuse hypothesis in rodents. If rodents diversified in the Cretaceous, it was only at the end of the Cretaceous. Our second BEAST analysis rejects the Explosive Model, but a hypothetical period of accelerated evolution across lineages from the K/T boundary through the Eocene has the potential to mislead the analysis. Correction for this may place all evolutionary splits after the Cretaceous for the placental mammals in our dataset.

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FIGURE LEGENDS

FIGURE 1. Maximum likelihood phylogram obtained for analysis of all genes combined. Boxes indicate nodal support and are divided into three sections arranged from left to right indicating Bayesian posterior probability, ML bootstrap, and MP bootstrap. Black indicates maximum support: Bayesian PP = 1.00, BP = 100%. Dark gray indicates 0.95 < PP < 1.00 or 90% < BP <100%. Light gray indicates 0.75 < PP < 0.95 or 50% < BP < 90%. White indicates PP < 0.75 or BP < 50%. The five suborders of rodents are indicated with black bars. Other clades referred to in the text are indicated with gray bars.

FIGURE 2. Chronogram resulting from the analysis in BEAST where no fossil calibration points were used. The complete length from root to tip represents 100.0. Branch lengths indicate relative time and are shown as a percentage of total rooted tree length. The branch lengths of this tree were compared with fossil dates to determine if these molecular derived results agree with dates obtained by fossils.

FIGURE 3. Chronogram showing the phylogenetic position of the 16 fossils used in the analyses. Dated nodes are indicated.

FIGURE 4. Regression analyses showing correlation between molecular results and observed fossils. (a.) Relationship among time estimates for all dated nodes. (b.) Relationship among internal branch lengths.

FIGURE 5. Tree resulting from Bayesian analysis including fossil calibrations. Paleogene geologic epochs are delineated with gray lines. Gray bars at nodes represent 95% confidence intervals on estimates. The five suborders of rodents are indicated with black bars next to taxon names. Other clades referred to in the text are indicated with gray bars next to taxon names.

FIGURE 6. Biases that may exist in analyses using a molecular clock that is dated with a single calibration point at the root of the tree. (a.) Effect of a period of accelerated molecular evolution across multiple lineages. The solid line indicates the average number of substitutions that accumulate across all lineages on the tree through time. The period of rapid evolution is circled. The dotted line indicates the assumed accumulation of substitutions as calculated in the analysis. The analysis will either overestimate or underestimate dates depending on the position on the graph. (b.) Effect of saturation that is not corrected by the model of evolution. The dashed line indicates the true average number of substitutions accumulated over time. The solid line indicates the accumulation of substitutions that are recovered using a model of evolution that does not adequately correct for saturation. The dotted line indicates the assumed accumulation of substitutions as calculated in the molecular clock analysis. The analysis always overestimates divergence dates.

FIGURE 1.

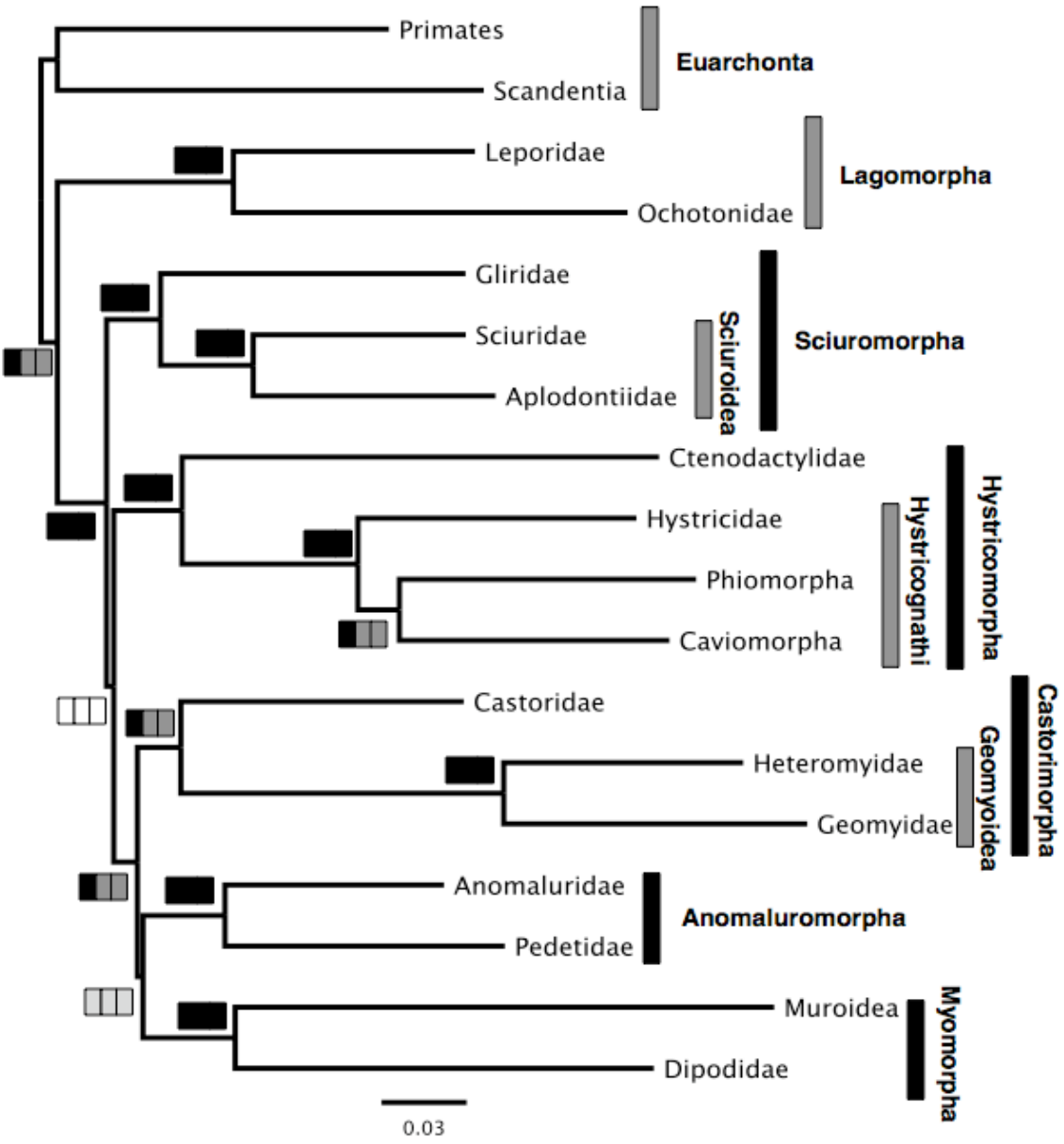

Primates
Scandentia
Euarchonta
Leporidae
Ochotonidae
Lagomorpha
Gliridae
Sciuridae
Aplodontiidae
Sciuroidea
Sciuromorpha
Ctenodactylidae
Hystricidae
Phiomorpha
Caviomorpha
Hystricognathi
Hystricomorpha
Castoridae
Heteromyidae
Geomyidae
Geomyoidea
Castorimorpha
Anomaluridae
Pedetidae
Anomaluromorpha
Muroidea
Dipodidae
Myomorpha
0.03

FIGURE 2.

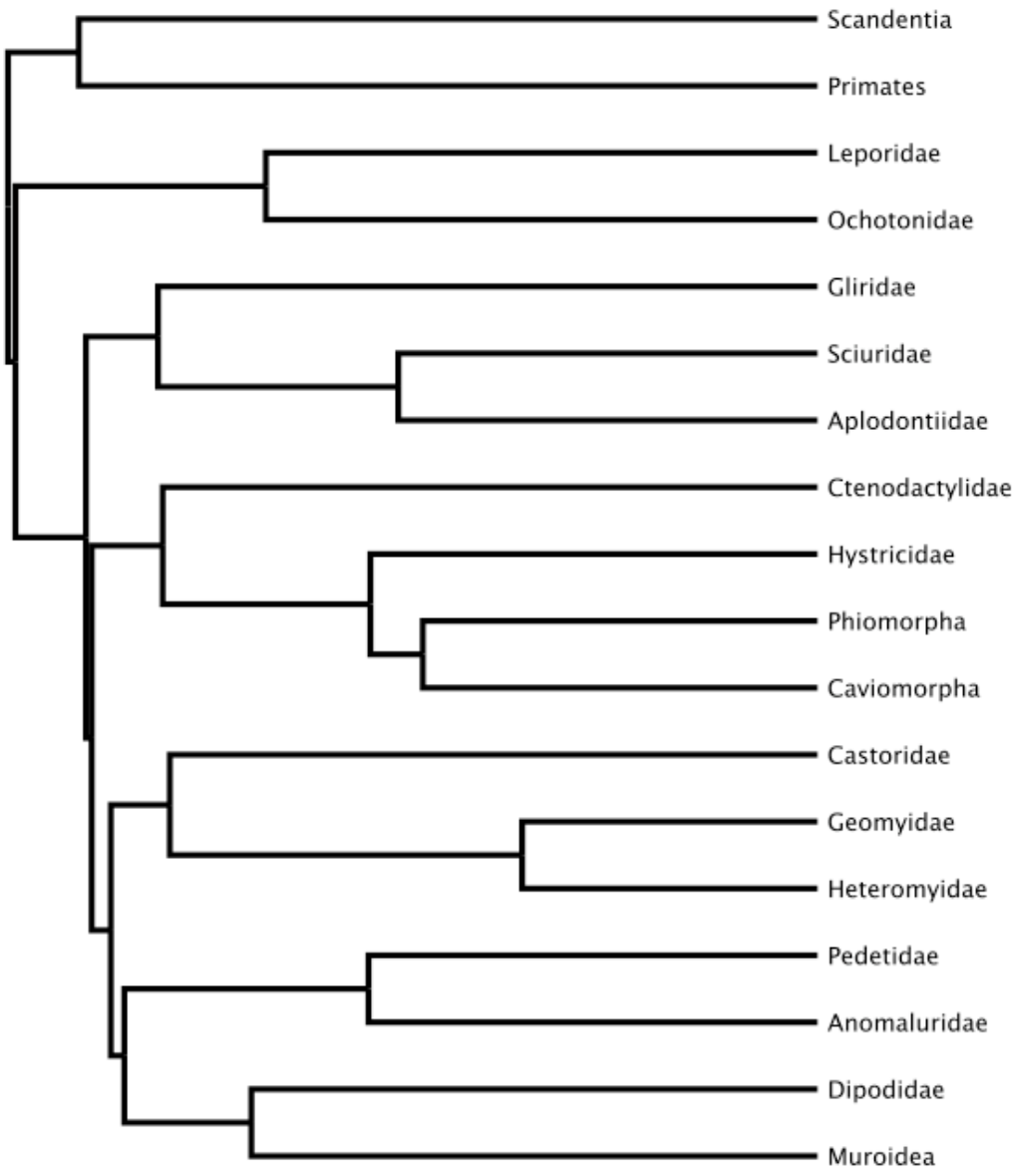

Scandentia
Primates
Leporidae
Ochotonidae
Gliridae
Sciuridae
Aplodontiidae
Ctenodactylidae
Hystricidae
Phiomorpha
Caviomorpha
Castoridae
Geomyidae
Heteromyidae
Pedetidae
Anomaluridae
Dipodidae
Muroidea

FIGURE 3.

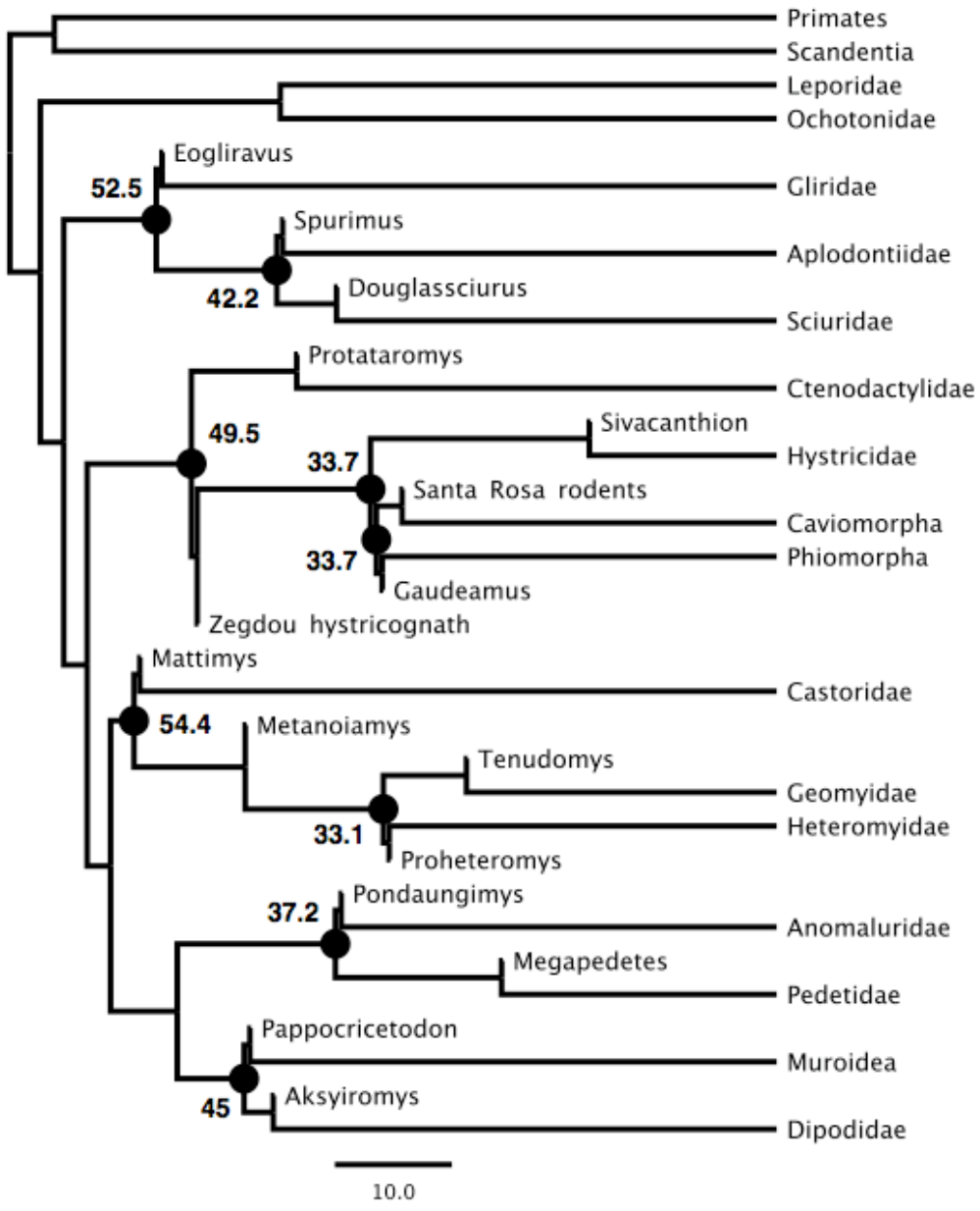
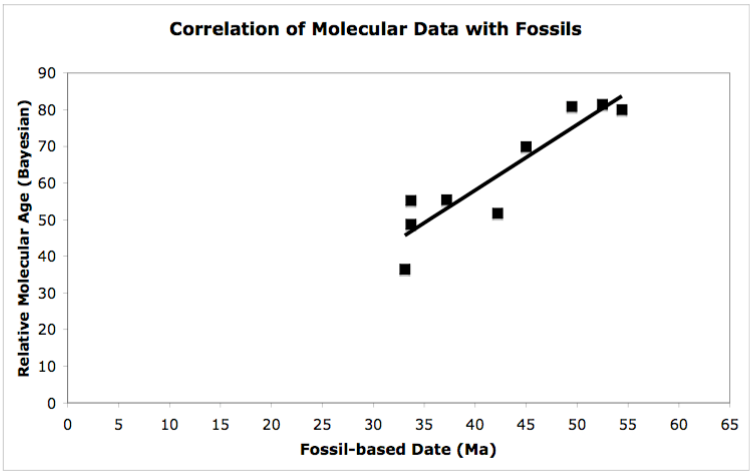

Primates
Scandentia
Leporidae
Ochotonidae
Eogliravus
52.5
Gliridae
Spurimus
Aplodontiidae
42.2
Douglassciurus
Sciuridae
Protataromys
Ctenodactylidae
49.5
Sivacanthion
Hystricidae
33.7
Santa Rosa rodents
Caviomorpha
33.7
Phiomorpha
Gaudeamus
Zegdou hystricognath
Mattimys
Castoridae
54.4
Metanoiamys
Tenudomys
Geomyidae
33.1
Heteromyidae
Proheteromys
Pondaungimys
37.2
Anomaluridae
Megapedetes
Pedetidae
Pappocricetodon
Muroidea
45
Aksyiromys
Dipodidae
10.0

FIGURE 4.

A.)


Correlation of Molecular Data with Fossils
Relative Molecular Age (Bayesian)
0
10
20
30
40
50
60
70
80
90
0
5
10
15
20
25
30
35
40
45
50
55
60
65
Fossil-based Date (Ma)

B.)

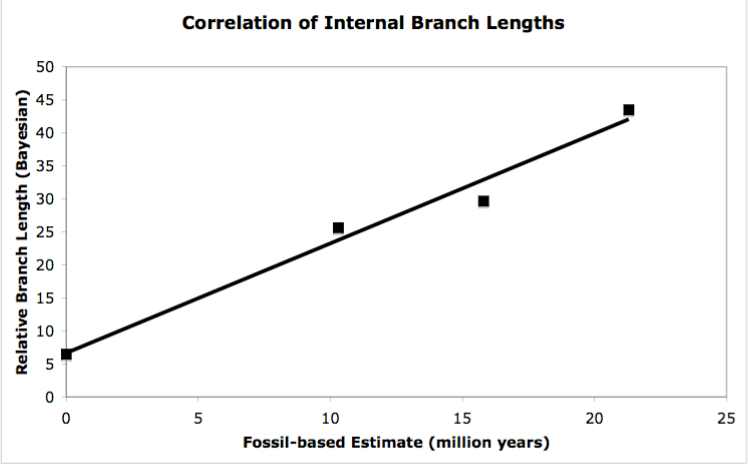

Correlation of Internal Branch Lengths
Relative Branch Length (Bayesian)
0
5
10
15
20
25
30
35
40
45
50
0
5
10
15
20
25
Fossil-based Estimate (million years)

FIGURE 5.

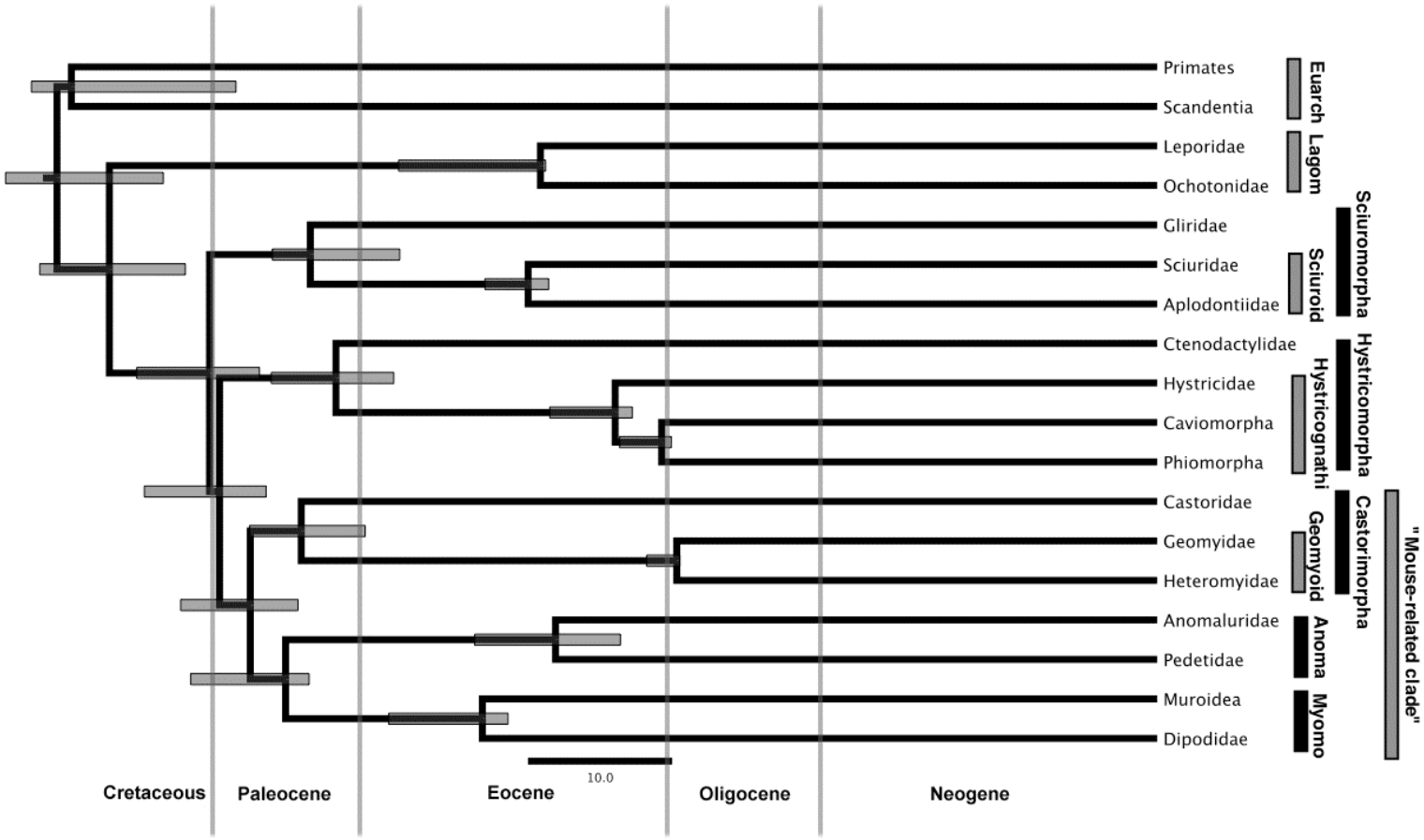

Primates
Scandentia
Leporidae
Ochotonidae
Gliridae
Sciuridae
Aplodontiidae
Ctenodactylidae
Hystricidae
Caviomorpha
Phiomorpha
Castoridae
Geomyidae
Heteromyidae
Anomaluridae
Pedetidae
Muroidea
Dipodidae
Euarch
Lagom
Sciuroid
Hystricognathi
Geomyoid
Anoma
Myomo
Sciuromorpha
Hystricomorpha
Castorimorpha
"Mouse-related clade"
10.0
Cretaceous
Paleocene
Eocene
Oligocene
Neogene

FIGURE 6.

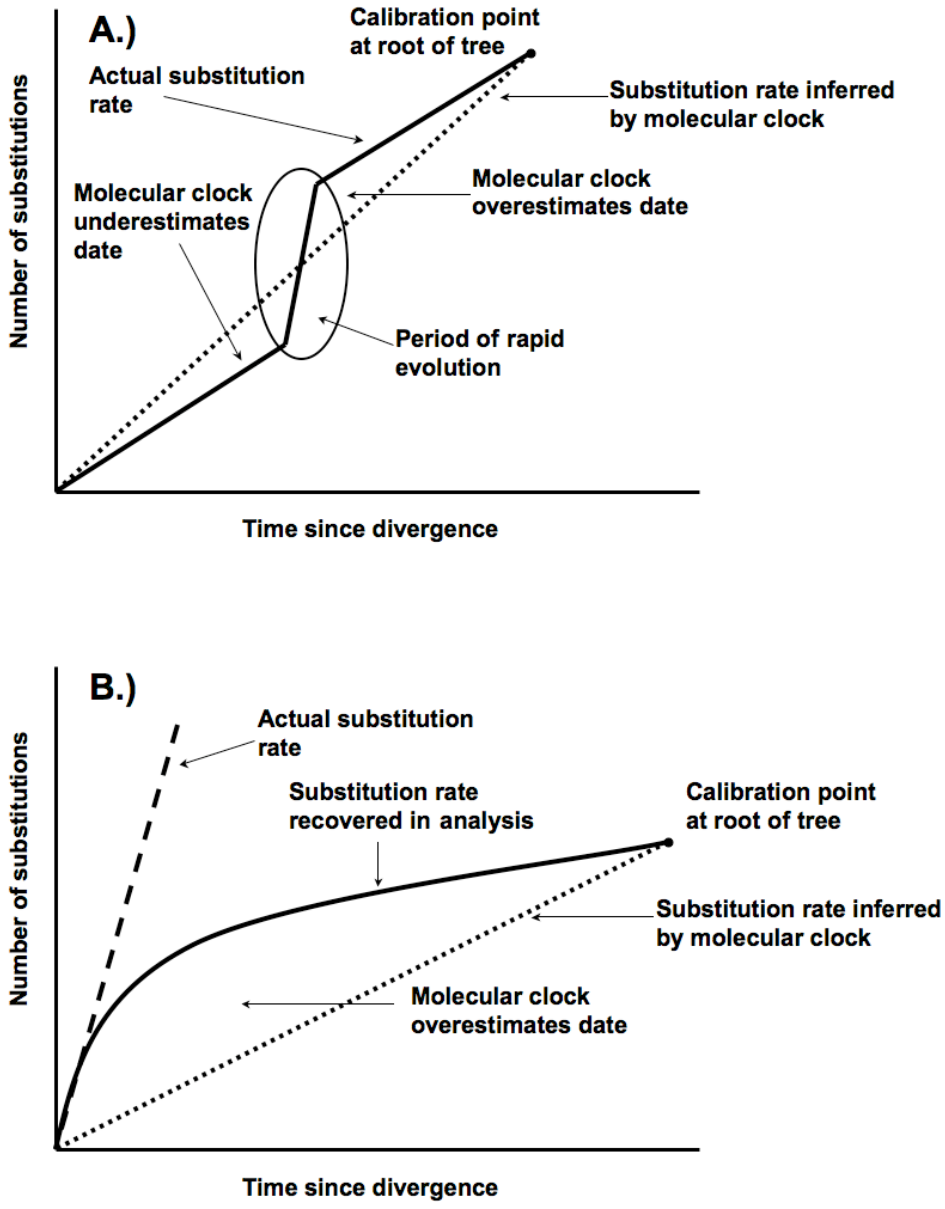

A.)
Calibration point
at root of tree
Actual substitution
rate
Substitution rate inferred
by molecular clock
Molecular clock
underestimates
date
Molecular clock
overestimates date
Period of rapid
evolution
Number of substitutions
Time since divergence
B.)
Actual substitution
rate
Substitution rate
recovered in analysis
Calibration point
at root of tree
Substitution rate inferred
by molecular clock
Molecular clock
overestimates date
Number of substitutions
Time since divergence

TABLE 1. GenBank accession numbers for taxa used in this study.

Lineage	ADRA2B	BRCA1	GHR	IRBP
Primates	*Homo sapiens* M34041	*Homo sapiens* NM007302	*Homo sapiens* NM000163	*Homo sapiens* NM002900
Scandentia	*Tupaia belangeri* AY150333	*Tupaia tana* AF284006	*Tupaia belangeri* AF332018	*Tupaia glis* Z11808
Leporidae	*Lepus crawshayi* AJ427254	*Lepus capensis* AF284005	*Lepus capensis* AF332016	*Lepus crawshayi* AJ427250
Ochotonidae	*Ochotona princeps* AJ427253	*Ochotona princeps* AY057827	*Ochotona princeps* AF332015	*Ochotona princeps* AY057832
Anomaluridae	*Anomalurus* sp. AJ427259	*Anomalurus beecrofti* this study	*Anomalurus beecrofti* this study	*Anomalurus* sp. AJ427240
Pedetidae	*Pedetes capensis* AM407920	*Pedetes capensis* AF332047	*Pedetes capensis* AF332025	*Pedetes capensis* AJ427241
Dipodidae	*Dipus sagitta* AJ427263	*Napaeozapus insignis* AF540634	*Allactaga sibirica* AY294897	*Allactaga sibirica* AY326076
Muroidea	*Mus musculus* M94583	*Mus musculus* U36475	*Mus musculus* AF120489	*Mus musculus* NM015745
Heteromyidae	*Dipodomys merriami* AJ427261	*Perognathus flavus* AF540638	*Perognathus flavus* *AF332029*	*Dipodomys merriami* AJ427233
Geomyidae	*Thomomys talpoides* AJ427262	*Geomys bursarius* AF540629	*Geomys bursarius* AF332028	*Thomomys talpoides* AJ427234

Castoridae	*Castor canadensis* AJ427260	*Castor canadensis* AF540622	*Castor canadensis* AF332026	*Castor canadensis* AJ427239
Ctenodactylidae	*Massoutiera mzabi* AJ427265	*Ctenodactylus gundi* AF540624	*Ctenodactylus gundi* AF332042	*Massoutiera mzabi* AJ427242
Hystricidae	*Trichys fasciculata* AJ427266	*Hystrix africaeaustralis* AF540631	*Hystrix africaeaustralis* AF332033	*Trichys fasciculata* AJ427245
Phiomorpha	*Heterocephalus glaber* AM407924	*Heterocephalus glaber* AF540630	*Heterocephalus glaber* AF332034	*Bathyergus suillus* AJ427251
Caviomorpha	*Erethizon dorsatum* AJ427270	*Erethizon dorsatum* AF540626	*Erethizon dorsatum* AF332037	*Erethizon dorsatum* AJ427249
Sciuridae	*Sciurus vulgaris* AJ315942	*Glaucomy volans* AF284003	*Sciurus niger* AF332032	*Glaucomy volans* AY227598
Aplodontiidae	*Aplodontia rufa* AJ427256	*Aplodontia rufa* AF332045	*Aplodontia rufa* AF332030	*Aplodontia rufa* AJ427238
Gliridae	*Glis glis* AJ427258	*Graphiurus murinus* AF332046	*Graphiurus murinus* AF332031	*Graphiurus murinus* AY303219

TABLE 1 CONTINUED

Lineage	**vWF**	**12S rRNA**	**CYTB**
Primates	*Homo sapiens* NM000552	*Homo sapiens* NC001807	*Homo sapiens* NC001807
Scandentia	*Tupaia glis* U31624	*Tupaia tana* AJ421453	*Tupaia belangeri* AJ421453

Leporidae	*Lepus crawshayi* AJ224669	*Lepus capensis* AY292706	*Lepus europaeus* NC004028
Ochotonidae	*Ochotona princeps* AJ224672	*Ochotona princeps* AJ537415	*Ochotona princeps* AJ537415
Anomaluridae	*Anomalurus* sp. AJ427229	*Anomalurus* sp. AJ389539	*Anomalurus* sp. AJ389526
Pedetidae	*Pedetes capensis* AJ238389	*Pedetes capensis* AY012113	*Pedetes capensis* AJ389527
Dipodidae	*Allactaga elater* AJ224661	*Allactaga elater* AJ389534	*Allactaga elater* AJ389534
Muroidea	*Mus musculus* NM011708	*Mus musculus* NC005089	*Mus musculus* NC005089
Heteromyidae	*Dipodomys merriami* AJ427226	*Perognathus flavus* U67298	*Dipodomys merriami* AY926383
Geomyidae	*Thomomys talpoides* AJ427227	*Geomys bursarius* AF084297	*Geomys bursarius* U65291
Castoridae	*Castor canadensis* AJ427228	*Castor canadensis* AY787823	*Castor fiber* AJ389529
Ctenodactylidae	*Massoutiera mzabi* AJ238388	*Massoutiera mzabi* AJ389544	*Massoutiera mzabi* AJ389533
Hystricidae	*Trichys fasciculata* AJ224675	*Hystrix africaeaustralis* U12448	*Hystrix africaeaustralis* X70674

Phiomorpha	*Heterocephalus glaber* AJ251134	*Heterocephalus glaber* AY425847	*Heterocephalus glaber* AF155870
Caviomorpha	*Erethizon dorsatum* AJ251135	*Erethizon dorsatum* AY012118	*Coendu bicolor* U34852
Sciuridae	*Glaucomy volans* AJ224667	*Sciurus vulgaris* NC_002369	*Sciurus vulgaris* NC_002369
Aplodontiidae	*Aplodontia rufa* AJ224662	*Aplodontia rufa* AJ389541	*Aplodontia rufa* AJ389528
Gliridae	*Glis glis* AJ224668	*Graphiurus murinus* AY303187	*Glis glis* NC_001892

TABLE 2. Summary of first appearance dates of lineages of rodents based on the fossil record.

Lineage	First Appearance	Date (Ma)	Primary Reference
Anomaluridae	*Pondaungimys*	37.2 +/- 1.3	Dawson et al., 2003
Pedetidae	*Megapedetes*	23.5-23.6	PBDB 27855: Pickford and Andrews, 1981
Muroidea	*Pappocricetodon*	45	Wang and Dawson, 1994
Dipodidae	*Aksyiromys*	43	Marivaux et al., 2004
Geomyoidea	*Metanoiamys*	45.4-45.5	PBDB 16752: Walsh, 1991
Geomyidae	*Tenudomys*	26.5-26.6	PBDB 17495: Swisher, 1982
Heteromyidae	*Proheteromys*	33.1	PBDB 17336: Simpson, 1985
Castoridae	*Mattimys*	54.4	PBDB 15660: McKenna, 1960
Ctenodactylidae	*Protataromys*	41	Marivaux et al., 2004
Hystricognathi	Zegdou phiomyid	49.5	Hartenberger, 1998
Hystricidae	*"Hystrix"* or *Sivacanthion*	~15.97	McKenna and Bell, 1997; Hartenberger, 1998
Phiomorpha + Caviomorpha clade	*Gaudeamus*	33.7-34.8	PBDB 60127: Gagnon, 1987
Caviomorpha	Santa Rosa rodent fauna	32-35	Frailey and Campbell, 2004
Gliridae	*Eogliravus*	52.5	Marivaux et al., 2004
Sciuroidea	*Spurimus*	42.2	PBDB 16514: Krishtalka and Black, 1975
Sciuridae	*Douglassciurus*	37.6	PBDB 16961: Emry, 1979
Aplodontiidae	*Spurimus*	42.2	PBDB 16514: Krishtalka and Black, 1975

TABLE 3. Calibration points used in analyses. "Gap" column indicates the minimum gap size present in the fossil record based on the difference in first appearance dates between daughter lineages at node.

Node	Fossil Calibration	Date (Ma)	Gap (million years)
Anomaluromorpha	*Pondaungimys*	37.2 +/- 1.3	13.7
Myomorpha	*Pappocricetodon*	45	2.0
Castorimorpha	*Mattimys*	54.4	9.0
Geomyoidea	*Proheteromys*	33.1	6.6
Hystricomorpha	Zegdou phiomyid	49.5	8.5
Hystricognathi	*Gaudeamus*	33.7-34.8	17.73
Phiomorpha + Caviomorpha clade	*Gaudeamus*	33.7-34.8	1.7
Sciuromorpha	*Eogliravus*	52.5	10.3
Sciuroidea	*Spurimus*	42.2	4.6

TABLE 4. Divergence times as estimated by fossils, standard BEAST analysis using the fossil calibrations shown, and based on a linear regression of uncalibrated ultrametric Bayesian tree against fossil dates. All values are represented in millions of years ago.

Node	Fossil Estimate	BEAST Estimate	95% C.I. for BEAST Estimate	Regression Estimate
Euarchontoglires	---	76.3	68.9-79.9	63.5
Euarchonta	61.7	75.3	63.9-78.1	58.6
Glires	---	72.7	67.4-77.5	63.0
Lagomorpha	42.4	42.8	42.4-52.6	45.7
Rodentia	---	65.7	62.3-70.8	58.1
Hystricomorpha + "Mouse-related" clade	---	65.0	61.8-70.2	57.7
"Mouse-related clade"	---	62.9	59.6-67.7	56.4
Anomaluromorpha + Myomorpha	---	60.4	58.8-67.0	55.5
Anomaluromorpha	37.2 +/- 1.3	41.7	37.2-47.3	38.6
Myomorpha	45	46.8	45.0-53.3	46.7
Castorimorpha	54.4	59.4	54.9-62.9	52.3
Geomyoidea	33.1	33.3	33.1-35.4	30.0
Hystricomorpha	49.5	56.6	52.9-61.4	52.8
Hystricognathi	(excluded)	37.6	36.4-42.1	38.4
Phiomorpha + Caviomorpha	33.7-34.8	34.4	33.7-37.3	34.8
Sciuromorpha	52.5	58.7	52.5-61.4	53.1
Sciuroidea	42.2	43.6	42.2-46.6	36.5

APPENDIX – Justification for calibration points used in this study

The oldest representative of the Anomaluridae lineage appears to be *Pondaungimys* from the Pondaung Formation, Myanmar (Dawson et al., 2003). This fossil dates to 37.2 +/- 1.3 Ma (Tsubamoto et al., 2002; Dawson et al., 2003). The Late Eocene *Nementchamys* has been classically considered the earliest anomalurid (Hartenberger, 1998; Bininda-Emonds, 2007), but *Pondaungimys* is clearly older. Many authorities consider the fossil family Zegdoumyidae of the Early/Middle Eocene to have affinities with the Anomaluridae (Hartenberger, 1998; McKenna and Bell, 1997), but the zegdoumyids have also been treated as relatives of glirids (Vianey-Liaud and Jaeger, 1996; Dawson et al., 2003), basal to the suborder Anomaluromorpha (Montgelard et al., 2002), or as members of a more basal stock of rodents (Marivaux et al., 2004).

Megapedetes is the oldest representative of the Pedetidae (McKenna and Bell, 1997; Hartenberger, 1998). *Megapedetes* from the Muhoroni Agglomerate, Kenya, is dated at 23.5-23.6 Ma (PBDB reference number 27855; Pickford and Andrews, 1981).

The oldest representative of the Muroidea is widely recognized to be *Pappocricetodon* (Dawson and Tong, 1998; de Bruijn et al., 2003). *Pappocricetodon* first appears in Jiangsu, China, (Wang and Dawson, 1994) 45 Ma (PBDB reference number 37493; Beard et al., 1994).

Assessing the first appearance of the Dipodidae lineage is more difficult due to the potential that the term Dipodoidea is frequently used in the paleontological literature to refer to a paraphyletic group that includes the basal stock from whence two extant

lineages, Dipodidae and Muroidea, arose. Two characters traditionally used to define the Dipodoidea, hystricomorphy and the presence of P^4, are present in the earliest muroids and are probably primitive characters for the Myomorpha (Wang and Dawson, 1994; de Bruijn et al., 2003). *Armintomys*, dated 49-50 Ma (Dawson et al., 1990), has been treated as an early representative of the Dipodidae lineage (Dawson et al., 1990; McKenna and Bell, 1997; Hartenberger, 1998), but is now usually considered to be basal to the suborder Myomorpha (Wang and Dawson, 1994; Holden and Musser, 2005). *Elymys* is another ancient genus attributed to the Dipodoidea (Hartenberger, 1998), dated to 49.7-49.8 Ma (PBDB 16218; Emry and Korth, 1989). Emry (2007) recently argued that in light of new material *Elymys* is likely to be a basal myomorph and not related to extant dipodids to the exclusion of Muroidea. He suggests instead that early myomorphs arose in North America, migrated to Asia, and diverged there into muroids and dipodids, noting the appearance of *Pappocricetodon* and *Aksyiromys* in the same fossil beds (see also Emry et al., 1998). We use the oldest dipodoid from Asia, *Aksyiromys*, as the first appearance date of the Dipodidae lineage due to the controversy surrounding *Elymys*. *Aksyiromys* from the Kolpak Formation, Shinzhaly, Kazakhstan, date at 43 Ma (Marivaux et al., 2004).

The oldest representative of the Geomyoidea (Heteromyidae + Geomyidae) may be either *Zaisaneomys*, or *Metanoiamys*. *Zaisaneomys* was described as an eomyid (superfamily Geomyoidea) by Shevyreva (1993) who considered it to be early Eocene (48.6-55.8 Ma) in date, a position adopted by McKenna and Bell (1997). Lucas (1998) argued that the material is no older than Irdinmanhan (37.2-48.6 Ma), and Emry et al.

(1997) questioned whether *Zaisaineomys* is even a geomyoid. Instead, we use the middle Eocene *Metanoiamys* as the first appearance of the Geomyoidea lineage. *Metanoiamys* is known from numerous Uintan deposits in California (PBDB), the earliest dates to 45.4-45.5 Ma from San Diego County (PBDB 16752; Walsh, 1991; 1997; Alroy, 2002). The first appearance for the Geomyidae lineage is *Tenudomys* from the Gering Formation, Nebraska dated 26.5-26.6 Ma (PBDB 17495; Swisher, 1982). *Proheteromys* of southwestern South Dakota, dated 33.1 Ma (PBDB 17336; Simpson, 1985), represents the first appearance for the Heteromyidae lineage.

The family Eutypomyidae is widely recognized as the sister group to the Castoridae (Wahlert, 1977; McKenna and Bell, 1997; Hartenberger, 1998; Korth, 2001). As the earliest eutypomyid, *Mattimys* also represents the first appearance of the Castoridae lineage. *Mattimys* dates to 54.4 Ma (PBDB 15660; McKenna, 1960; Korth, 1984) from the Wasatch Formation, Colorado.

The suborder Hystricomorpha has been among the most widely studied groups of rodents (Flynn et al., 1986; Marivaux et al., 2002; 2004; Wible et al., 2005; Dawson et al., 2006), but a consensus opinion as to the phylogenetic position of many of the early forms has yet to emerge. McKenna and Bell (1997) introduced the concept of a suborder Sciuravida, which united a wide range of rodents into one group. These included the Ctenodactylidae and the fossil families Ivantoniidae, Sciuravidae, Cylindrodontidae, and a broadly defined Chapattimyidae that included baluchimyines, yuomyids, cocomyids, tamquammyids, *Protophiomys*, and *Fallomus*. Their concept of Sciuravida has been widely refuted by subsequent authorities as a polyphyletic assemblage of taxa whose

members are compiled from all corners of the rodent tree (Hartenberger, 1998; Marivaux et al., 2002; 2004; Wible et al., 2005; Dawson et al., 2006). The recent discovery of *Laonastes* (Jenkins et al., 2005) and studies supporting a sister relationship between the Diatomyidae, to which *Laonastes* belongs, and Ctenodactylidae through both morphological (Dawson et al., 2006) and molecular (Huchon et al., 2007) evidence further complicate assigning a first appearance date to this lineage. Potential early representatives of the Ctenodactylidae lineage (including the distinct family Diatomyidae) include *Tamquammys* and *Tsilingomys* (Marivaux et al., 2002; 2004), but more recent analyses suggest a more basal position for these taxa (Wible et al., 2005; Dawson et al., 2006). We use *Protataromys* to represent the first appearance of the Ctenodactylidae lineage (Marivaux et al., 2002; de Bruijn et al., 2003; Marivaux et al., 2004). *Protataromys* dates to 41 Ma from the Hedi Formation, Henan, China (Marivaux et al., 2004). If *Protataromys* forms a clade with extant Ctenodactylidae to the exclusion of Diatomyidae, then the split between these two families represents an additional Eocene divergence that is not represented in our analysis.

Marivaux et al. (2002; 2004) recovered a clade uniting the baluchimyines with the Hystricognathi. This hypothesis is one of several suggested by Flynn et al. (1986) upon their description of the subfamily. The oldest baluchimyine, and the oldest representative of the Hystricognathi lineage, is *Protophiomys*. *Protophiomys* has been dated to at least 36 Ma from Nementchas, Bir el Ater, Algeria (Marivaux et al., 2004). The Hystricognathi lineage clearly dates to 36 Ma or earlier. Hartenberger (1998) claims that material from Glib Zegdou, Algeria, was misidentified as a zegdoumyid (*Glibia*) in a prior study on

which he was an author (Vianey-Liaud et al., 1994) and actually represents the earliest stem Hystricognathi. This material is dated to 49.5 Ma (Marivaux et al., 2004). We have adopted Hartenberger's (1998) suggestion that this material represents the earliest member of the Hystricognathi lineage, but suggest that further study of this material and verification of its phylogenetic position would be useful.

The fossil record for the family Hystricidae does not appear to extend any earlier than the Miocene. McKenna and Bell (1997) list Oligocene with a question mark in their record for *Hystrix*. The only other references that we can find that includes an Oligocene date for hystricids are early versions of Vaughan's (1972; 1978) mammalogy texbook. Subsequent editions (Vaughan, 1986; Vaughan et al. 2000) state that hystricids appear in the Miocene, a position supported by other authors (Flynn et al., 1986; Hartenberger, 1998). The appropriate first appearance date for the Hystricidae lineage is either "*Hystrix*" from the early Miocene (15.97-23.03 Ma; McKenna and Bell, 1997) or *Sivacanthion* of the early-middle Miocene boundary (15.97 Ma; Hartenberger, 1998; Flynn et al., 1986). We apply a minimum date estimate of 15.97 Ma for this lineage.

The basal position of the family Hystricidae relative to the rest of the Hystricognathi is supported primarily through molecular analyses (Adkins et al., 2001; Huchon and Douzery, 2001; Huchon et al., 2002; Adkins et al., 2003; Poux et al., 2006) as opposed to morphological characters. As such, early representatives of the Phiomorpha + Caviomorpha clade are essentially indistinguishable from basal hysricognaths. By necessity, we use the older of the first appearance dates between the two lineages in this clade to also represent the first appearance of the Phiomorpha +

Caviomorpha clade. Most recent studies have used the Tinguiririca caviomorph discovered by Wyss et al. (1993) as the first appearance of Caviomorpha (Hartenberger, 1998; Huchon et al., 1999; Vucetich et al., 1999; Huchon and Douzery, 2001; Huchon et al., 2002; Marivaux et al., 2002; Adkins et al., 2003; Marivaux et al., 2004; Poux et al., 2006). Frailey and Campbell (2004) have recently described a number of new genera of rodents from Santa Rosa, Peru, that appears to predate the Tinguiririca fauna. A wide diversity of forms of caviomorphs are already present suggesting that this material may represent the divergence date among superfamilies within the Caviomorpha and that caviomorphs were present prior to this time frame (Frailey and Campbell, 2004; Martin, 2004; Martin, 2005). Frailey and Campbell (2004) also argue that the age of Tinguiririca fauna has been overestimated. The Santa Rosa rodent fauna dates to 32-35 Ma (Frailey and Campbell, 2004).

Gaudeamus (family Thryonomyidae) represents the first appearance of the Phiomorpha in the fossil record (Hartenberger, 1998). Frequently usage of the term Phiomorpha and even Thryonomyidae in the literature refers to paraphyletic groups (Hartenberger, 1998). Nevertheless, Lopez Antoñanzas et al. (2004) and Lopez Antoñanzas and Sen (2005) yielded a close relationship between modern *Thryonomys* and the fossil genus *Gaudeamus* in cladistic analyses including a variety of thryonomyid genera suggesting that they are unlikely to hold a basal position among hystricognaths. *Gaudeamus* may even form a clade with extant Thryonomyidae to the exclusion of Bathyergidae and indicate that the bathyergid-thryonomyid split extends into the Eocene. *Gaudeamus* is known from L-41 Quarry, Fayum, Egypt (PBDB 60127; Gagnon, 1987),

which dates to 33.7-34.8 Ma (Seiffert, 2006). As the oldest representative of either the Phiomorpha or Caviomorpha lineage, *Gaudeamus* at 33.7-34.8 Ma also represents the first appearance of the combined Phiomorpha + Caviomorpha clade in our analysis.

The first member of the Gliridae lineage is widely recognized as being *Eogliravus* from Europe (Hartenberger, 1998; Reyes et al., 1998; Montgelard et al., 2003; Marivaux et al., 2004). *Eogliravus* from Prémontré, France are dated to 52.5 Ma (Marivaux et al., 2004).

Although a number of ischyromyoid rodents bear a close resemblance to the Sciuroidea (Sciuridae + Aplodontiidae), a consensus has not emerged that defines stem taxa of this clade to the exclusion of glirids. We use the older of the first appearance dates between these two lineages to also represent the first appearance of the Sciuroidea. The first member of the Sciuridae lineage is *Douglassciurus* (= *Protosciurus*; Thorington and Hoffman, 2005), which is known from the White River Formation, Wyoming, and dated at 37.6 Ma (PBDB 16961; Emry, 1979; Alroy, 2002). The earliest member of the Aplodontiidae lineage is the allomyid genus *Spurimus* from the middle Eocene of North America (McKenna and Bell, 1997; Hartenberger, 1998). *Spurimus* appears 42.2 Ma from the Wagon Bed Formation, Wyoming (Krishtalka and Black, 1975; Black, 1971). As the oldest representative of either the Sciuridae or Aplodontiidae lineage, *Spurimus* at 42.2 Ma also represents the first appearance of the Sciuroidea lineage in our analysis.

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Chapter 3

New mitochondrial genomes from scaly-tailed flying squirrel, beaver, and kangaroo rat: Assessing rodent relationships with large amounts of mitochondrial and nuclear data

Abstract-

Although the subject of much study, there remains a great deal of uncertainty concerning certain basal level relationships in the Rodentia. We have sequenced the complete mitochondrial genomes of three rodent species, *Anomalurus beecrofti*, *Castor canadensis*, and *Dipodomys ordii*, and attempt to resolve phylogenetic relationships within rodents using the mitochondrial genome, a comparable sized nuclear dataset, and a combined analysis containing over 26,000 bp of sequence data. We determine that although the nuclear and mitochondrial datasets conflict, the combined analysis recovers a Sciuromorpha – Hystricomorpha clade with strong support. Our data suggest that increased character sampling improves resolution at these early nodes while improved taxon sampling of mitochondrial genomes has led to better support in mitochondrial studies and a convergence towards the conclusions obtained from nuclear datasets.

INTRODUCTION

In spite of being the subject of numerous studies, the evolutionary relationships of rodents remain controversial. Multiple major proposals have been advanced attempting to divide rodents into subordinal ranks (Brandt, 1855; Tullberg, 1899; Ellerman, 1940;

Simpson, 1945; Wood, 1955; 1959; 1965; Chaline and Mein, 1979; Hartenberger, 1998; Wilson and Reeder, 1993; Landry, 1999; Carleton and Musser, 2005), but the majority of these have centered around two principal characters, the morphology of the zygomasseteric system and the shape of the mandible. Brandt (1855), and other 19th century researchers developed a taxonomy based on Waterhouse's (1839) description of characters of the zygomasseteric system, the relationship of the masseter muscles to the zygomatic arch and infraorbital canal. Tullberg (1899) suggested that rodents be divided into two groups, those with a hystricognathous mandible and those with a sciurognathous mandible. Subsequent morphology-based taxonomies have largely been modifications of these two early proposals. Numerous well-sampled molecular studies have greatly clarified the relationships among rodents (Nedbal et al., 1994; 1996; Huchon et al., 1999; Adkins et al., 2001; DeBry and Sagel, 2001; Huchon and Douzery, 2001; Huchon et al., 2002; Montgelard et al., 2002; Adkins et al., 2003; DeBry, 2003; Huchon et al., 2007). A summary of the relationships among rodents that have been recovered with good support is shown in Figure 1. We apply the taxonomy of Carleton and Musser (2005), which is in general agreement with the results of these molecular studies, except where indicated. They recognized five suborders of rodents: Sciuromorpha, Castorimorpha, Myomorpha, Anomaluromorpha, and Hystricomorpha.

The Sciuromorpha unites the dormice (family Gliridae) with the mountain beaver (Aplodontiidae) and squirrel family (Sciuridae). The Sciuridae and Aplodontiidae have been found to be sister taxa in a number of well-supported studies (Huchon et al., 1999; Adkins et al., 2001; DeBry and Sagel, 2001; Huchon et al., 2002; Adkins et al., 2003;

DeBry, 2003; Huchon et al., 2007; Norris et al., chapter 2) and have been termed Sciuroidea in many molecular studies (Huchon et al., 1999; Michaux and Catzeflis, 2000; Montgelard et al., 2002; DeBry, 2003; Douzery et al., 2003; Horner et al., 2007). The relationship between glirids and sciuroids has also been recovered with good support, but in fewer studies (Adkins et al., 2003; Reyes et al., 2004; Horner et al., 2007; Huchon et al., 2007; Norris et al., chapter 2).

The Castorimorpha unites the beavers (Castoridae), pocket gophers (Geomyidae), and kangaroo rats (Heteromyidae). The sister relationship between the geomyids and heteromyids has been widely recognized by both molecular biologists (DeBry and Sagel, 2001; Huchon et al., 2002; Adkins et al., 2003; DeBry, 2003; Huchon et al., 2007; Norris et al., chapter 2) and morphologists (Wood, 1955; Hartenberger, 1998; Landry, 1999; Marivaux et al., 2004). The position of the Castoridae as sister to the Geomyoidea is more preliminary and Carleton and Musser (2005) emphasized that further study was required to verify their hypothesis. Huchon et al. (2007) and Norris et al. (chapter 2) have since supported monophyly of Castorimorpha with reasonably high support.

The Myomorpha is an extremely successful group that includes the birch mice, jumping mice, and jerboas (Dipodidae), and the superfamily Muroidea, which includes mice, rats, gerbils, voles, hamsters, and their relatives. The Myomorpha represents a relatively uncontroversial grouping that has been supported in many studies (DeBry and Sagel, 2001; Adkins et al., 2003; DeBry, 2003; Reyes et al., 2004; Huchon et al., 2007; Norris et al., chapter 2).

The Anomaluromorpha contains the scaly-tailed flying squirrels (Anomaluridae) and the springhare (Pedetidae). Most published molecular phylogenies have included one of these two families, but not both. Montgelard et al. (2002) recovered a well-supported Anomaluromorpha clade, but their study was restricted to the use of a weighted parsimony analysis applied to a dataset containing two mitochondrial genes. Carleton and Musser (2005) united the two families in a single suborder largely due to a lack of alternative hypotheses. Huchon et al. (2007) and Norris et al. (chapter 2) have since supported monophyly of Anomaluromorpha with good support.

The Hystricomorpha is a clade that includes the recently described *Laonastes*, the gundis, and the diverse Hystricognathi. This clade has been subjected to considerable study and many opposing hypotheses have been proposed, but both morphological (Luckett and Hartenberger, 1985; Flynn et al., 1986; Landry, 1999; Marivaux et al., 2002; 2004; Dawson et al., 2006) and molecular (Huchon et al., 2000; Adkins et al., 2001; Huchon et al., 2002; Adkins et al., 2003; Huchon et al., 2007; Norris et al., chapter 2) studies have converged on its current composition. Although *Laonastes* was described too recently (Jenkins et al., 2005) to be included in Carleton and Musser's (2005) taxonomy, its inclusion in this suborder receives unanimous support among those who have analyzed material (Jenkins et al., 2005; Dawson et al., 2006; Huchon et al., 2007).

Two recent molecular studies (Huchon et al., 2007; Norris et al., chapter 2) have applied datasets that combine multiple genes (~5.5 kbp from 6 genes + SINES and ~8.4 kbp from 7 genes respectively) to achieve improved resolution among major clades of rodents. Two important nodes of the rodent phylogenetic tree remain essentially

unresolved in spite of these studies (Fig. 1). The first evolutionary splits among rodents produce three clades: the suborder Hystricomorpha, the suborder Sciuromorpha, and a clade called the "mouse-related clade" by Huchon et al. (2002) that unites the suborders Anomaluromorpha, Myomorpha, and Castorimorpha. Achieving adequate resolution among these three clades strikes to the core of 150 years of debate concerning rodent relationships. A basal position for the Hystricomorpha would retain the core of Tullberg's (1899) Sciurognathi vs. Hystricognathi dichotomy intact and would be consistent with the Ichyromyiformes hypothesis developed by Marivaux et al. (2004) based on paleontological evidence. Recently, the Broad Institute has justified its sequencing of a squirrel (Sciuridae) genome by citing its supposed relationship to the mouse and rat (Myomorpha) (http://www.ncbi.nlm.nih.gov/sites/entrez?Db=genomeprj&cmd=ShowDetailView&TermToSearch=13936), a claim that is neither supported nor refuted by molecular phylogenetic analyses.

Likewise, the relationships among the three suborders in the "mouse-related clade" have not been resolved in molecular analyses (Fig. 1). Resolving the relationships among the Anomaluromorpha, Castorimorpha, and Myomorpha has implications for assessing character polarity, Paleogene biogeography, and comparative genomics. Clarification will aid in determining the number of independent origins of the different morphologies of the zygomasseteric system, the primary character of Brandt's (1855) taxonomy. The Anomaluromorpha and Myomorpha are both derived from hystricomorphous stock, whereas the Castorimorpha are sciuromorphous. Determining the relationships among modern members of these suborders and their fossil counterparts

will also contribute to a better understanding of the complex biogeographic connections among North America, Europe, Asia, and even potentially Africa (in the Anomaluromorpha) in the Paleogene. Finally, one of the primary goals of comparative genomics is to improve the applicability of results found in model organisms to a wider array of taxa. Understanding the relationships between model organisms such as mice and rats (suborder Myomorpha) and other rodents is essential to understanding how genetic findings in these animals apply to other taxa. Phylogenetic trees, particularly those involving model organisms, are an important part of the process of selecting which animals deserve full genome sequencing.

In the first study to employ full mitochondrial genomes to evaluate relationships among rodents and between rodents and other mammals, D'Erchia et al. (1996) titled their paper: "The guinea-pig is a not a rodent", emphasizing their inability to recover rodent monophyly. The addition of the mitochondrial genomes from a dormouse (Reyes et al., 1998), a squirrel (Reyes et al., 2000a; 2000b), and a cane rat (Mouchaty et al., 2001) still resulted in phylogenetic analyses that failed to recover even rodent monophyly. Nevertheless, as mitochondrial genomes became available for more species of rodents, the phylogenetic trees produced have begun to converge on the results of studies employing multiple nuclear genes (Lin et al., 2002; Reyes et al., 2004; Horner et al., 2007). With the continued addition of more taxa, phylogenies generated using full mitochondrial genomes may theoretically converge on the results obtained using nuclear data.

Our goals in this study were to attempt to obtain further clarity regarding the relationships among major rodent groups by adding taxa relative to previous full mitochondrial genome studies, and by analyzing far more characters than had been previously analyzed in rodent phylogenetic studies. In all, 16 nuclear genes were analyzed comprising about 13.5 kbp of data. Complete mitochondrial genomes were sequenced for three species of rodent: *Anomalurus beecrofti*, *Castor canadensis*, and *Dipodomys ordii*. Although Horner et al. (2007) published a mitochondrial genome for an unidentified species of *Anomalurus* midway through our project, we had selected this animal because no mitochondrial genome was available for a member of the suborder Anomaluromorpha. No mitochondrial genome was available for the suborder Castorimorpha, and *Dipodomys* and *Castor* represent two highly divergent members of this clade. Norris et al. (chapter 2) suggest that they split about 59.4 million years ago (Ma) and the fossil record suggests a divergence time of 54.4 Ma (PBDB – the Paleobiology Database – reference #15660: McKenna, 1960). Nuclear and mitochondrial data were analyzed both separately and combined, and conflict between the two was evaluated.

MATERIALS AND METHODS

Gene and Taxon Sampling

Genetic data were obtained for 16 nuclear genes (~13,500 bp) from 7 clades of rodents. We obtained the maximum amount of nuclear data available from GenBank that allowed for sampling of representatives of all of the suborders Anomaluromorpha,

Myomorpha, and Hystricomorpha, as well as the families Gliridae and Sciuridae in Sciuromorpha, and the Heteromyidae and Castoridae in Castorimorpha. Each of these clades has been shown to be monophyletic based on a number of previous studies (Huchon et al., 1999; Adkins et al., 2001; DeBry and Sagel, 2001; Huchon et al., 2002; Adkins et al., 2003; DeBry, 2003; Reyes et al., 2004; Huchon et al., 2007; Norris et al., chapter 2). A complete list of Genbank accession numbers and species used is shown in Table 1. Sequence data were also obtained for euarchontan and lagomorph outgroups. Full mitochondrial genomes were obtained from Genbank for 13 species of rodents as well as 4 lagomorphs, a primate, and a tree shrew (Table 2). Lagomorphs are widely recognized as the sister taxon to the Rodentia (Murphy et al., 2001; Huchon et al., 2002; Douzery and Huchon, 2004; Springer et al., 2004; 2005; Bininda-Emonds et al., 2007; Huchon et al., 2007); the two orders together form the clade Glires. The Euarchonta (primates + dermopterans + tree shrews) and Glires comprise a clade referred to as Euarchontoglires (Murphy et al., 2001; Springer et al., 2004; 2005).

DNA Sequencing

Full mitochondrial genomes were sequenced from three individual rodents housed in the collection of tissues in the Biology Department, University of Vermont. Ethanol preserved tissue was used to obtain sequence data from an individual Beecroft's scaly-tailed flying squirrel, *Anomalurus beecrofti* collected on 19 November 1999 from Agumatsa Wildlife Sanctuary, Volta Region, Ghana, (catalog #1516). Frozen tissue was used to obtain sequence data from an individual American beaver, *Castor canadensis*,

obtained from a local trapper and collected at an unknown locality in central Vermont, USA (catalog# RWN 223). Ethanol preserved tissue was used to obtain sequence data from an individual Ord's kangaroo rat, *Dipodomys ordii*, from an unknown locality in Texas (catalog #60; CWK 1815). DNA was extracted using the DNeasy QIAGEN kit. Ethanol preserved material was soaked overnight in lysis buffer prior to extraction.

The entire mitochondrial genome was amplified in segments ranging in length from 500 to 3,000 bp, with most reactions ~900 bp in length. The deterioration of tissue over time, particularly for those stored in ethanol, seemed to preclude the possibility of amplifying fragments over 3,000 bp in length. Primers were designed so that sequences obtained from different PCR reactions overlapped considerably (by at least 100 bp) to increase the probability that pseudogenes would be detected if amplified. In the few instances where overlapping regions appeared to conflict, nested PCR was performed where the initial outer primer pair amplified at least 3,000 bp of DNA. All final protein coding regions were in an open reading frame that matched known vertebrate mitochondrial structure, leading us to believe that pseudogenes were not incorporated.

PCR was performed using the following parameters: 35 cycles of 94^{o}C (1 min) denaturing, 50^{o}C annealing (1-3 min), and 72^{o}C (1 min, 10 sec) extension. Amplification reactions were performed using Illustra puReTaq Ready-To Go PCR Beads. Double stranded PCR products were purified using PEG precipitation (Maniatis et al., 1982). Sequencing was performed on an ABI 3130x1 Genetic Analyzer using dye terminator (ABI PRISM) cycle sequencing. Primers used for PCR and sequencing are shown in Table 3. Initially primers were designed to encompass the complete mitochondrial

genome based upon published primers (Irwin et al., 1991; Simon et al., 1994; Riddle 1995; Sullivan et al., 1997; Sorenson et al., 1999; Tieman-Boese et al., 2000; Quérouil et al., 2001; Steppan et al., 2005). These published primers were modified to match a consensus sequence of *Mus*, *Sciurus*, *Cavia*, and *Ochotona*. New primers were generated based on successful sequences in order to amplify regions where PCR was initially unsuccessful.

Phylogenetic analyses

Three datasets were analyzed: a nuclear dataset, a mitochondrial dataset, and a combined dataset. The mitochondrial dataset consisted of protein coding regions, 12S rRNA and 16S rRNA; tRNAs, D-Loop and intergenic regions were excluded. Although some genes on the mitochondrial genome overlap, such regions were assigned to one of the two genes involved to prevent duplicating them in the analysis. Alignments were performed in MacClade (Maddison and Maddison, 1989) while referencing corresponding alignments of amino acid sequences in Clustal X (Thompson et al., 1997) for both nuclear and mitochondrial protein coding regions and while referencing secondary structure for ribosomal RNAs (Springer et al., 1995). Ambiguously aligned regions were excluded from the final analyses. The nuclear dataset included 16 genes comprising 13,465 bp (including gaps) whereas the mitochondrial dataset included 15 genes (12,638 bp). The combined analysis involved 31 genes and 26,103 bp.

Tree reconstruction was conducted under both maximum parsimony (MP) and maximum likelihood (ML) frameworks in PAUP* (version 4.0b8, Swofford, 2002).

Nodal support was evaluated using MP bootstrapping in PAUP* (1,000 replicates) and Bayesian posterior probability values using MrBayes (version 3.1.1; 500,000 generations, sampled every 1,000 generations, burnin=250, 4 chains, 2 runs; Ronquist and Huelsenbeck, 2005). Modeltest 3.04 (Posada and Crandall, 1998) determined the appropriate likelihood model for these datasets. GTR + I + Γ, TVM + I + Γ, and GTR + I + Γ were used in the maximum likelihood analyses for the nuclear, mitochondrial, and combined datasets respectively. Because MrBayes does not distinguish between TVM and GTR, GTR + I + Γ was used in all Bayesian analyses.

RESULTS

Characterization of mitochondrial genomes

The mitochondrial genomes of *Anomalurus beecrofti*, *Castor canadensis*, and *Dipodomys ordii* exhibit the typical vertebrate organization. The mitochondrial genome of *Anomalurus beecrofti* is 16,925 bp in length. This is the longest mitochondrial genome yet reported in rodents. The previously published genome of an unidentified species of *Anomalurus* is 16,923 bp (Horner et al., 2007). The Tamura and Nei (1993) genetic distance between these two *Anomalurus* individuals is 0.158, a value comparable to the genetic distance between *Microtus levis* and *M. kikuchii* (0.157) and between *Mus musculus* and *M. terricolor* (0.134). Although congeners, it is unlikely that these two individuals are members of the same species of *Anomalurus*. L-strand base composition of the *Anomalurus beecrofti* mitochondrion is A: 33.3%, T: 27.4%, C: 26.4%, and G: 12.9%. The mitochondrial genome in *Castor canadensis* is 16,733 bp long. L-strand

base composition is A: 33.7%, C: 28.0%, T: 24.7%, and G: 13.5%. The complete mitochondrion of *Dipodomys ordii* is 16,260 bp in length. This is the shortest mitochondrial genome yet reported in rodents, but falls within the range reported in mammals. L-strand base composition is A: 33.2%, T: 29.5%, C: 24.7%, and G: 12.7%.

Phylogenetic analyses

The results of the ML analysis for the nuclear dataset are shown in Figure 2. A single most parsimonious tree was recovered and is not shown, but is discussed later. Monophyly of the order Rodentia (MP BP = 99%, Bayesian PP = 1.00) and the suborders Castorimorpha (Castoridae + Heteromyidae; MP BP = 59%, Bayesian PP = 1.00) and Sciuromorpha (Gliridae + Sciuridae; MP BP = 97%, Bayesian PP = 1.00) were recovered in MP, ML, and Bayesian analyses. All analyses recover a basal position for the Sciuromorpha, but with poor support (MP BP = 47%, Bayesian PP = 0.87). The "mouse-related clade" was recovered in the ML and Bayesian (PP = 1.00) analyses and by a plurality of replicates in the MP bootstrap (43%), but not in the single most parsimonious tree. The MP tree produced an Anomalomorpha + Castorimorpha clade and a Myomorpha + Hystricomorpha clade. The Anomaluromorpha+ Castorimorpha clade received essentially no support in the MP bootstrap analysis (<50%).

Figure 3 shows the results of the ML analysis of the mitochondrial data. Monophyly of the suborders Sciuromorpha (MP BP = 87%, Bayesian PP = 1.00) and Hystricomorpha (MP BP = 97%, Bayesian PP = 1.00) were recovered in all analyses. Myomorpha monophyly (Bayesian PP = 1.00) and monophyly of the "mouse-related

clade" (Bayesian PP = 1.00) was recovered only in the ML and Bayesian analyses, but with strong support. Monophyly of Glires was recovered only in the MP tree, but with essentially no support (<50%). Castorimorpha is not monophyletic in either analysis. The MP tree recovers a Dipodidae + Heteromyidae clade that is supported by a 93% bootstrap value. No other nodes with a bootstrap >50% were recovered in the MP analysis that differ from the tree shown in Figure 3.

The results of the combined analysis are shown in Figure 4. The ML and Bayesian analyses recover monophyly of the Rodentia (MP BP = 100%, Bayesian PP = 1.00) Castorimorpha (Bayesian PP = 1.00), Sciuromorpha (Bayesian PP = 1.00), and the "mouse-related clade" (Bayesian PP = 1.00). A sister relationship is suggested between the Sciuromorpha and the Hystricomorpha (MP BP = 65%, Bayesian PP = 1.00) and between the Anomaluromorpha and Myomorpha (Bayesian PP = 0.87). The clades supported by bootstrap values > 50% in the MP analyses are a Myomorpha + Heteromyidae clade (MP BP = 87%) and Gliridae + Hystricognathi clade (MP BP = 66%). In both instances, the longest two branches within a clade are drawn together in the parsimony analysis, but are part of separate clades in model-based analyses.

Conflict among datasets

A partition homogeneity test was conducted in PAUP* under a parsimony framework to evaluate the nuclear vs. mitochondrial datasets. The two were significantly different (P = 0.001). In order to test how differing signals affected tree topology, a series of Shimodaira Hasegawa (1999) tests were conducted in PAUP under a likelihood

framework (Table 4). Each dataset was constrained to fit each of the tree topologies shown in Figures 2-4 and the likelihood scores for these trees were compared. Tests involving the mitochondrial dataset were conducted with a set of taxa pruned to match the 9 taxa used in the nuclear and combined analyses. The Shimodaira Hasegawa tests showed that the mitochondrial topology (Fig. 3) was significantly worse than either the nuclear topology (Fig. 2) or the combined topology (Fig. 4) when evaluated using the nuclear dataset ($P = 0.001$ for both).

DISCUSSION

Relationships among rodents

This study is the first to show good support for resolution among the Sciuromorpha, Hystricomorpha, and "mouse-related clade" at the base of the Rodentia. This support is predominantly derived from analyses that incorporate a model of evolution (Bayesian PP = 1.00), but limited support is also present in the parsimony analysis (BP = 65%). In order to ensure that this is not an artifact of where the Rodentia is rooting, we performed an ML analysis excluding *Homo* and another excluding *Ochotona*. Both recovered the Sciuromorpha + Hystricomorpha clade shown in Figure 4. Both trees differed from Figure 4 in recovering a Castorimorpha + Anomaluromorpha clade instead of the Anomaluromorpha + Myomorpha clade recovered when both outgroups are present. Although this clade was not recovered in the majority of trees sampled in the Bayesian analysis performed on the nuclear dataset (Bayesian PP = 0.87 for "mouse-related clade" + Hystricomorpha clade), it represented the next most

commonly sampled clade in that analysis (Bayesian PP = 0.11). This may be a situation where hidden support from a seemingly conflicting dataset emerges when the two are combined (Sullivan et al., 1995).

Even those studies that employ multiple genes and dense taxon sampling have failed to resolve the relationships among the three clades at the base of the Rodentia. Huchon et al. (2007) sampled 5,500 bp of sequence data from 25 families of rodents and recovered the Hystricomorpha as the most basal clade, but with ML BP < 50% and Bayesian PP < 0.75. Norris et al. (chapter 2) sampled 8,300 bp from 14 families of rodents and recovered a basal position of the Sciuromorpha, but with MP and ML BP <50% and Bayesian PP <0.75.

The conclusion that the Sciuromorpha and Hystricomorpha form a clade is unusual from the perspective of morphology. Uniting the Hystricomorpha with the "mouse-related clade" unites a group with a hystricomorphous ancestral condition (Hystricomorpha) with a clade that contains the only other hystricomorphous rodents (suborders Anomaluromorpha and Myomorpha) excluding the dormouse *Graphiurus* which probably derived from the pseudomyomorphy seen in other glirids. Unifying the Sciuromorpha with the "mouse-related clade" retains the core of Tullberg's (1899) Sciurognathi, a suborder defined by their retention of the primitive rodent jaw shape, and is consistent with the Ischyromyiformes hypothesis which Marivaux et al. (2004) constructed based on a suite of dental and cranial characters in early fossil members of modern groups. Nevertheless, Norris et al. (chapter 2), using a different assumption of tree topology than is shown in Figure 4, suggested that about 400,000 or 700,000 years

separated the first and second evolutionary splits in rodents. Such a rapid succession of evolutionary events about 60 million years ago may prove very difficult to track in the fossil record and in genetic analyses. Our results should be confirmed with denser taxon sampling, with particular emphasis on greater diversity within the Hystricomorpha. Specifically, full mitochondrial genome sequencing combined with more extensive nuclear sampling for either gundis (family Ctenodactylidae) or the recently described *Laonastes*, would improve this analysis.

In contrast to the improved resolution at the base of Rodentia, our data show no improvement in resolution among the three suborders of the "mouse-related clade" when compared to prior studies. Both Huchon et al. (2007) and Norris et al. (chapter 2) recovered comparable support at this node as shown in Figure 4. Prior studies showed essentially no resolution among these three subfamilies. The conclusions shown in Figure 4 can be called into further question because the topology changed when individual outgroup taxa were excluded. Increasing taxon sampling to include both anomaluromorph families, in particular a *Pedetes* mitochondrial genome, and increased nuclear sampling for Dipodidae may improve this analysis.

A common dilemma facing molecular phylogeneticists and a source of much discussion is whether it is better to add taxa or characters to resolve difficult nodes (Graybeal, 1998; Mitchell et al., 2000; Wortley et al., 2005). Our results would seem to provide arguments for both. Extremely large numbers of characters have yielded strong support for a node (Hystricomorpha + Sciuromorpha) that had proven unrecoverable in analyses with fewer characters. We await confirmation as to whether other studies will

also find this clade to be well-supported. In contrast, reducing taxa may have prevented any improvement in nodal support for an Anomaluromorpha + Myomorpha relationship. Finally, the importance of denser taxon sampling for inherently noisy data can be confirmed by the way that mitochondrial genome studies in rodents have yielded a slow, but steady improvement of nodal support and a convergence toward nuclear-derived topologies.

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FIGURE LEGENDS

FIGURE 1. Relationships among the Rodentia based on a summary of well-supported clades in prior molecular studies. Still unresolved are the relationships among the Sciuromorpha, Hystricomorpha, and the "mouse-related clade" and among the Anomaluromorpha, Myomorpha, and Castorimorpha.

FIGURE 2. Phylogenetic relationships among rodents based on nuclear data. The maximum likelihood tree is shown. Values at nodes are Bayesian posterior probabilities followed by maximum parsimony bootstrap percentages.

FIGURE 3. Phylogenetic relationships among rodents based on mitochondrial data. The maximum likelihood tree is shown. Values at nodes are Bayesian posterior probabilities followed by maximum parsimony bootstrap percentages.

FIGURE 4. Phylogenetic relationships among rodents based on the combined nuclear and mitochondrial data. The maximum likelihood tree is shown. Values at nodes are Bayesian posterior probabilities followed by maximum parsimony bootstrap percentages.

FIGURE 1.

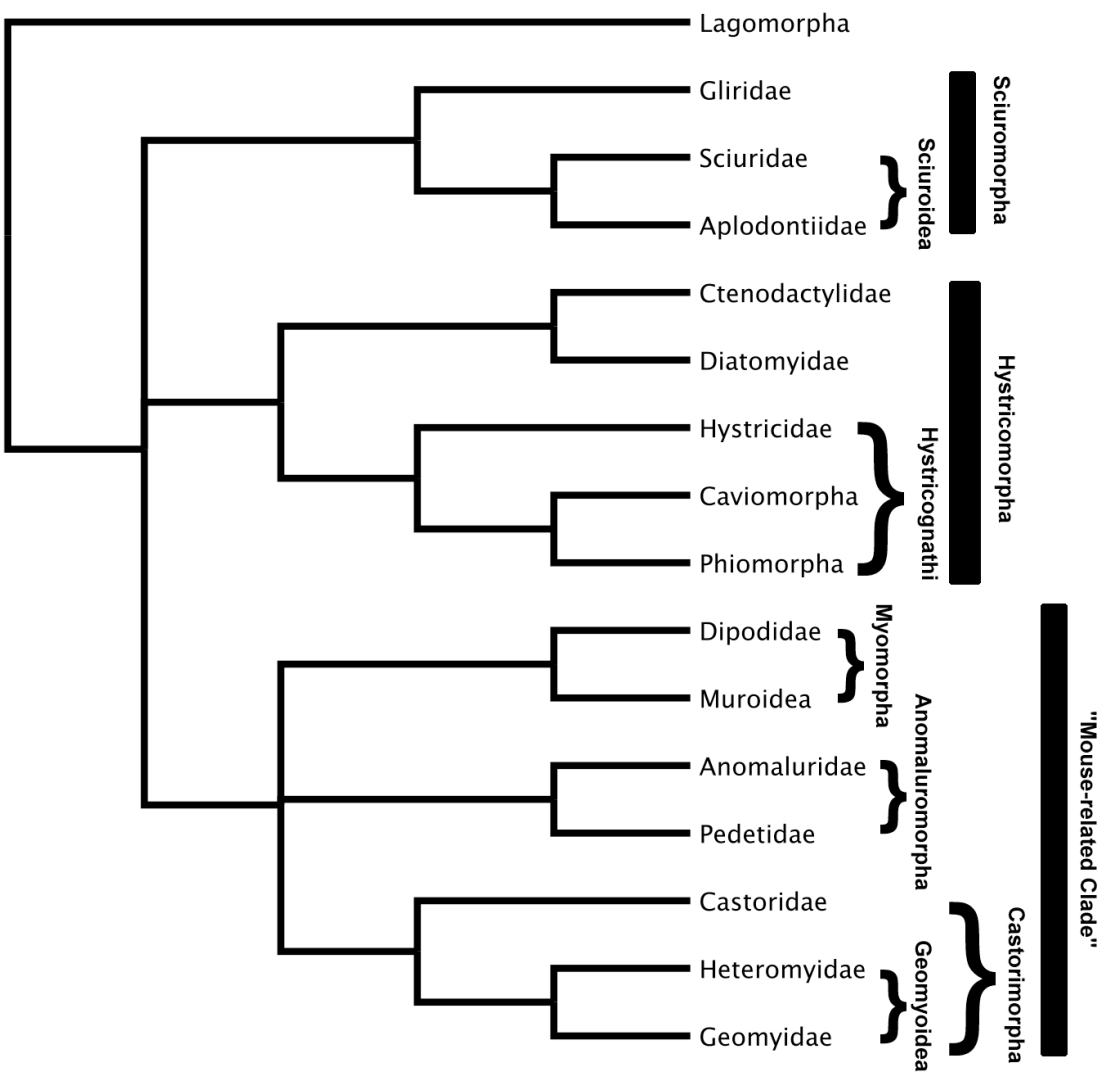
Lagomorpha
Gliridae
Sciuridae
Aplodontiidae
Sciuroidea
Sciuromorpha
Ctenodactylidae
Diatomyidae
Hystricidae
Caviomorpha
Phiomorpha
Hystricognathi
Hystricomorpha
Dipodidae
Muroidea
Myomorpha
Anomaluridae
Pedetidae
Anomaluromorpha
Castoridae
Heteromyidae
Geomyidae
Geomyoidea
Castorimorpha
"Mouse-related Clade"

FIGURE 2.

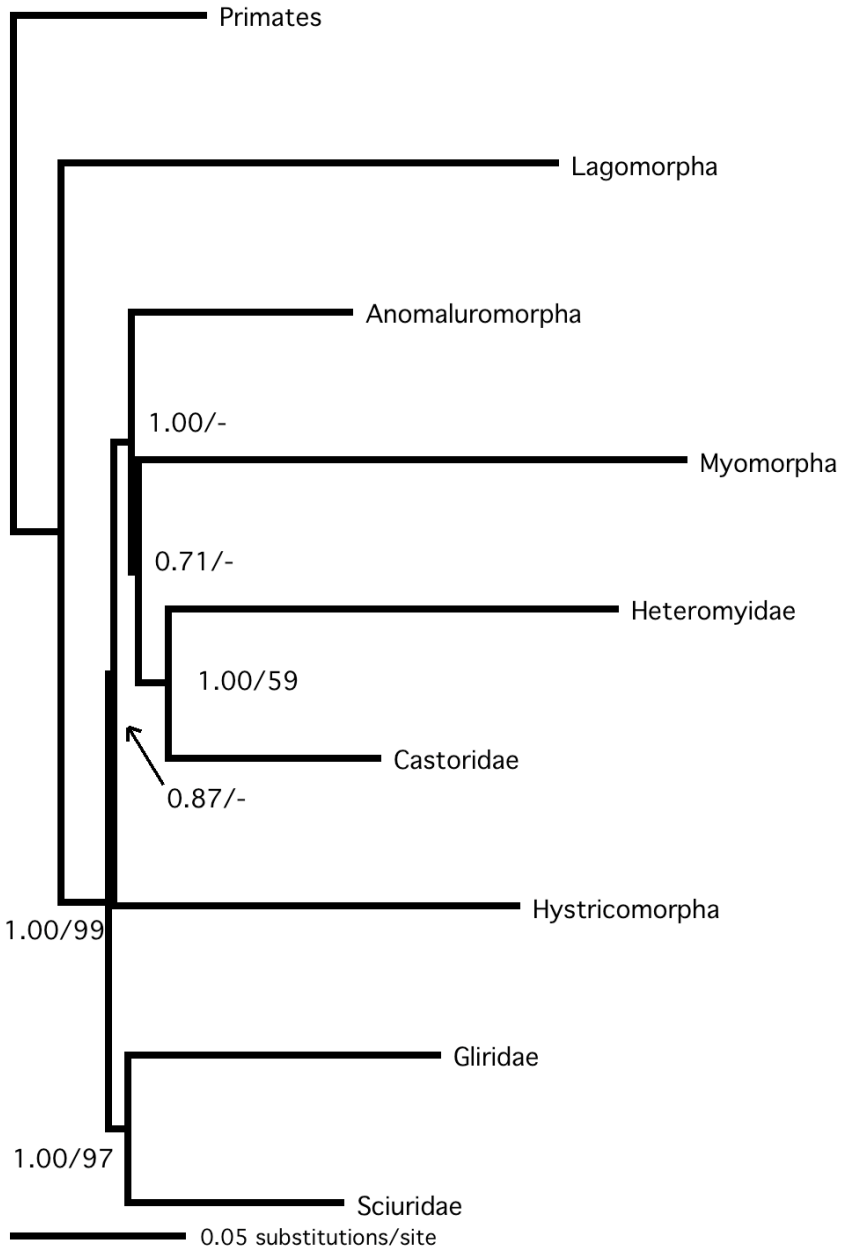
Primates
Lagomorpha
Anomaluromorpha
1.00/-
Myomorpha
0.71/-
Heteromyidae
1.00/59
Castoridae
0.87/-
Hystricomorpha
1.00/99
Gliridae
1.00/97
Sciuridae
0.05 substitutions/site

FIGURE 3.

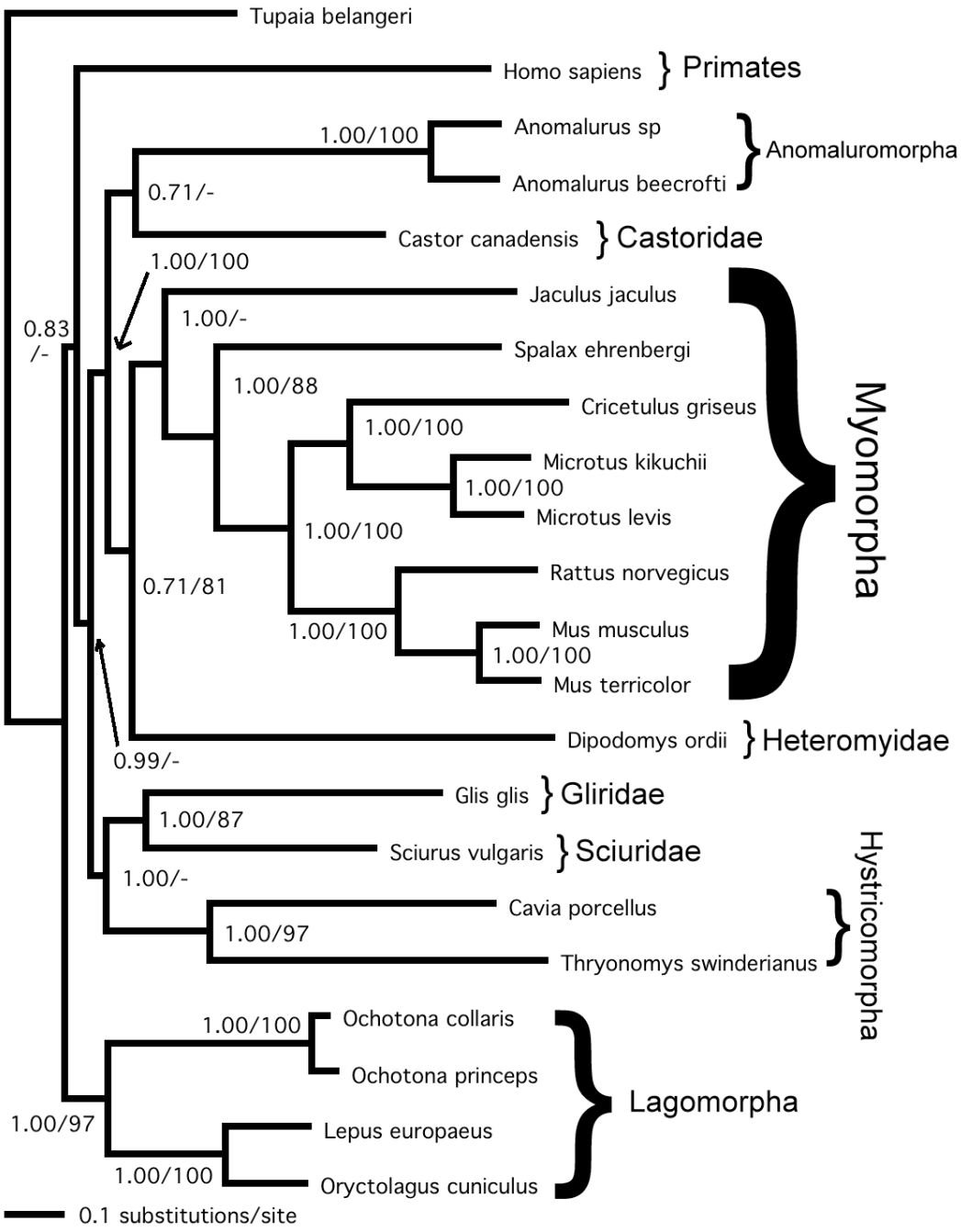
Tupaia belangeri
Homo sapiens
Primates
1.00/100
Anomalurus sp
Anomalurus beecrofti
Anomaluromorpha
0.71/-
Castor canadensis
Castoridae
1.00/100
Jaculus jaculus
1.00/-
0.83
/-
Spalax ehrenbergi
1.00/88
Cricetulus griseus
1.00/100
Microtus kikuchii
1.00/100
Microtus levis
1.00/100
Rattus norvegicus
0.71/81
1.00/100
Mus musculus
1.00/100
Mus terricolor
Myomorpha
Dipodomys ordii
Heteromyidae
0.99/-
Glis glis
Gliridae
1.00/87
Sciurus vulgaris
Sciuridae
1.00/-
Cavia porcellus
1.00/97
Thryonomys swinderianus
Hystricomorpha
1.00/100
Ochotona collaris
Ochotona princeps
1.00/97
Lepus europaeus
1.00/100
Oryctolagus cuniculus
Lagomorpha
0.1 substitutions/site

FIGURE 4.

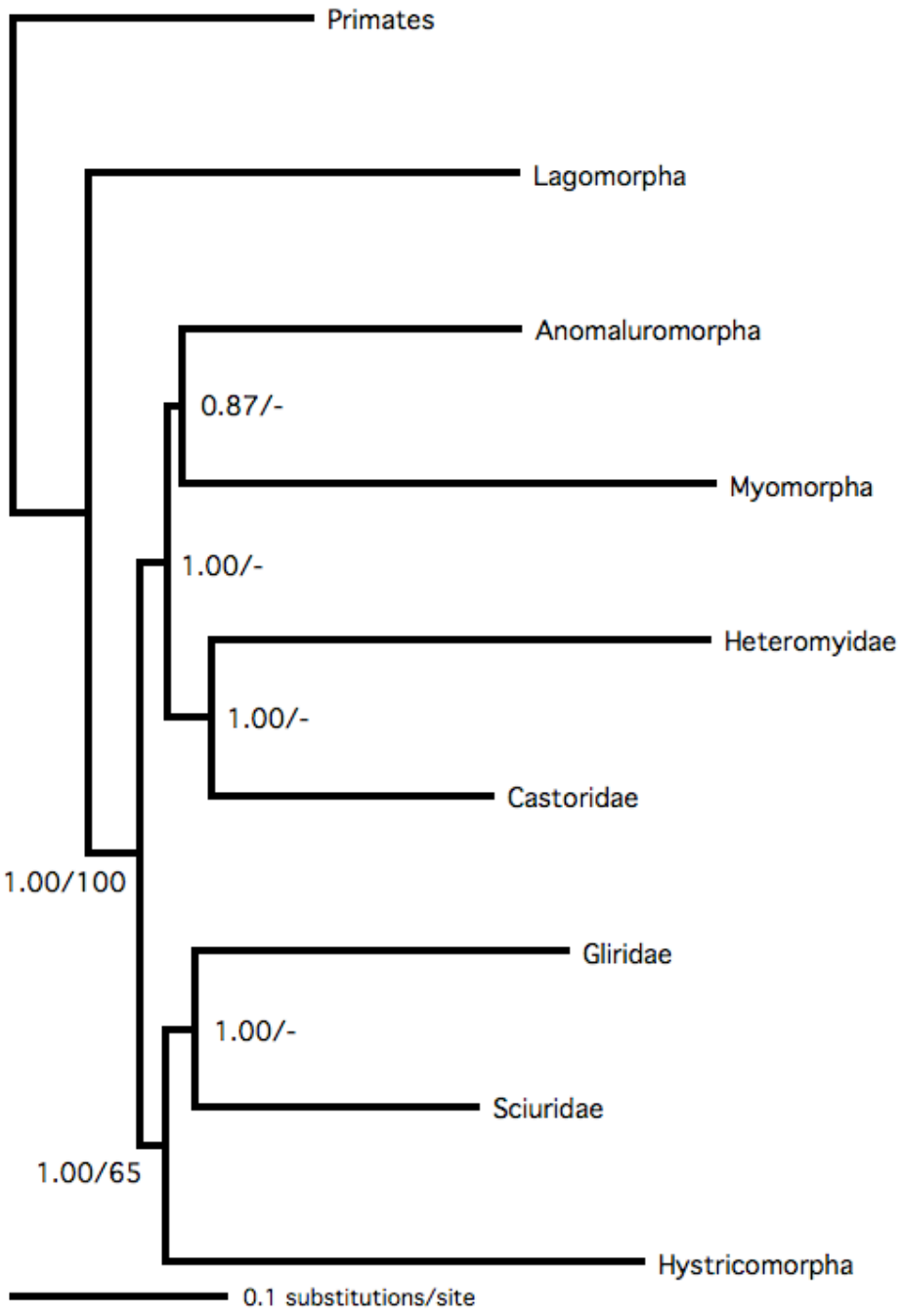
Primates
Lagomorpha
Anomaluromorpha
0.87/-
Myomorpha
1.00/-
Heteromyidae
1.00/-
Castoridae
1.00/100
Gliridae
1.00/-
Sciuridae
1.00/65
Hystricomorpha
0.1 substitutions/site

TABLE 1. GenBank accession numbers for nuclear genes used in this study.

Lineage	ADRA2B	BRCA1	GHR	IRBP
Primates	*Homo sapiens* M34041	*Homo sapiens* NM007302	*Homo sapiens* NM000163	*Homo sapiens* NM002900
Ochotonidae	*Ochotona princeps* AJ427253	*Ochotona princeps* AY057827	*Ochotona princeps* AF332015	*Ochotona princeps* AY057832
Anomaluromorpha	*Anomalurus sp.* AJ427259	*Anomalurus beecrofti* Norris et al., chapter 2	*Anomalurus beecrofti* Norris et al., chapter 2	*Anomalurus sp.* AJ427240
Heteromyidae	*Dipodomys merriami* AJ427261	*Perognath flavus* AF540638	*Perognath flavus* *AF332029*	*Dipodomys merriami* AJ427233
Castoridae	*Castor canadensis* AJ427260	*Castor canadensis* AF540622	*Castor canadensis* AF332026	*Castor canadensis* AJ427239
Caviomorpha	*Erethizon dorsatum* AJ427270	*Erethizon dorsatum* AF540626	*Erethizon dorsatum* AF332037	*Erethizon dorsatum* AJ427249
Sciuridae	*Sciurus vulgaris* AJ315942	*Glaucomy volans* AF284003	*Sciurus niger* AF332032	*Glaucomy volans* AY227598
Gliridae	*Glis glis* AJ427258	*Graphiurus murinus* AF332046	*Graphiurus murinus* AF332031	*Graphiurus murinus* AY303219
Myomorpha	*Mus musculus* M94583	*Mus musculus* U36475	*Mus musculus* AF120489	*Mus musculus* NM015745

TABLE 1 CONTINUED

Lineage	vWF	CNR1	RAG1	RAG2 (section 1)
Primates	*Homo sapiens* NM000552	*Homo sapiens* BC074812	*Homo sapiens* NM000448	*Homo sapiens* BC022397
Ochotonidae	*Ochotona princeps* AJ224672	*Ochotona princeps* AY303188	*Ochotona hyperborea* AY011896	*Ochotona hyperborea* AY011953
Anomaluromorpha	*Anomalurus sp.* AJ427229	*Pedetes capensis* AY011578	*Pedetes capensis* AY011882	*Pedetes capensis* AY011939
Heteromyidae	*Dipodomys merriami* AJ427226	*Dipodomys heermani* AY011584	*Dipodomys heermani* AY011888	*Dipodomys heermani* AY011945
Castoridae	*Castor canadensis* AJ427228	*Castor canadensis* AY303180	*Castor canadensis* AY011880	*Castor canadensis* *AY011937*
Caviomorpha	*Erethizon dorsatum* AJ251135	*Erethizon dorsatum* AY011583	*Erethizon dorsatum* AY011887	*Erethizon dorsatum* AY011944
Sciuridae	*Glaucomys volans* AJ224667	*Tamias striatus* AY011575	*Tamias striatus* AY011879	*Tamias striatus* AY011936
Gliridae	*Glis glis* AJ224668	*Graphiurus murinus* AY303187	*Graphiurus murinus* AY294934	*Muscardinus avellanarius* AY011938
Myomorpha	*Mus musculus* NM011708	*Mus musculus* NM007726	*Mus musculus* M29475	*Mus musculus* NM009020

TABLE 1 CONTINUED

Lineage	**RAG2 (section 2)**	**HSPD3**	**EDG1**	**PNOC**
Primates	*Homo sapiens* BC022397	*Homo sapiens* NM006308	*Homo sapiens* AK312493	*Homo sapiens* NM006228
Ochotonidae	*Ochotona Princeps* AY303207	*Ochotona Princeps* AJ550791	*Ochotona hyperborea* AY011717	*Ochotona hyperborea* AY011836
Anomaluromorpha	*Pedetes capensis* AY303208	*Anomalurus sp.* AJ550794	*Pedetes capensis* AY011705	*Pedetes capensis* AY011824
Heteromyidae	*Dipodomys heermani* AY303202	*Dipodomys merriami* AJ550793	*Dipodomys heermani* AY011710	*Dipodomys heermani* AY011829
Castoridae	*Castor canadensis* AY303199	*Castor canadensis* AJ550795	*Castor canadensis* AY011703	*Castor canadensis* AY011822
Caviomorpha	*Erethizon dorsatum* AY303205	*Erethizon dorsatum* AJ550797	*Erethizon dorsatum* AY011709	*Erethizon dorsatum* AY011828
Sciuridae	*Tamiasciurus hudsonius* AY303214	*Sciurus vulgaris* AJ550800	*Tamias striatus* AY011702	*Tamias striatus* AY011821
Gliridae	*Graphiurus murinus* AY303206	*Glis glis* AJ550799	*Muscardinus avellanarius* AY011704	*Muscardinus avellanarius* AY011823
Myomorpha	*Mus musculus* NM009020	*Mus musculus* NM019960	*Mus musculus* NM007901	*Mus musculus* NM010932

TABLE 1 CONTINUED

Lineage	PLCB4	CREM	ATP7A	APP
Primates	*Homo sapiens* NM000933	*Homo sapiens* AY011664	*Homo sapiens* AY011418	*Homo sapiens* AY011354
Ochotonidae	*Ochotona hyperborea* AY011779	*Ochotona hyperborea* AY011655	*Ochotona hyperborea* AY011409	*Ochotona hyperborea* AY011346
Anomaluromorpha	*Pedetes capensis* AY011765	*Pedetes capensis* AY011642	*Pedetes capensis* AY011396	*Pedetes capensis* AY011333
Heteromyidae	*Dipodomys heermani* AY011771	*Dipodomys heermani* AY011648	*Dipodomys heermani* AY011402	*Dipodomys heermani* AY011339
Castoridae	*Castor canadensis* AY011763	*Castor canadensis* AY011640	*Castor canadensis* AY011394	*Castor canadensis* AY011331
Caviomorpha	*Erethizon dorsatum* AY011770	*Erethizon dorsatum* AY011647	*Erethizon dorsatum* AY011401	*Erethizon dorsatum* AY011338
Sciuridae	*Tamias striatus* AY011762	*Tamias striatus* AY011639	*Tamias striatus* AY011393	*Tamias striatus* AY011330
Gliridae	*Muscardinus avellanarius* AY011764	*Muscardinus avellanarius* AY011641	*Muscardinus avellanarius* AY011395	*Muscardinus avellanarius* AY011332
Myomorpha	*Mus musculus* AY011766	*Mus musculus* AY011643	*Mus musculus* AY011397	*Mus musculus* AY011334

TABLE 1 CONTINUED

Lineage	BDNF
Primates	*Homo sapiens* AY011481
Ochotonidae	*Ochotona hyperborea* AY011473
Anomaluromorpha	*Pedetes capensis* AY011460
Heteromyidae	*Dipodomys heermani* AY011466
Castoridae	*Castor canadensis* AY011458
Caviomorpha	*Erethizon dorsatum* AY011465
Sciuridae	*Tamias striatus* AY011457
Gliridae	*Muscardinus avellanarius* AY011459
Myomorpha	*Mus musculus* AY011461

TABLE 2. GenBank accession numbers for mitochondrial genomes used in this study.

Suborder/Order	Species	Accession
Primates	*Homo sapiens*	NC_001807
Scandentia	*Tupaia belangeri*	NC_002521
Lagomorpha	*Ochotona collaris*	NC_003033
Lagomorpha	*Ochotona princeps*	NC_005358
Lagomorpha	*Lepus europaeus*	NC_004028
Lagomorpha	*Oryctolagus cuniculus*	NC_001913
Sciuromorpha	*Glis glis*	NC_001892
Sciuromorpha	*Sciurus vulgaris*	NC_002369
Hystricomorpha	*Thryonomys swinderianus*	NC_002658
Hystricomorpha	*Cavia porcellus*	NC_000884
Anomaluromorpha	*Anomalurus sp.*	NC_009056
Myomorpha	*Jaculus jaculus*	NC_005314
Myomorpha	*Spalax ehrenbergi*	NC_005315
Myomorpha	*Cricetulus griseus*	NC_007936
Myomorpha	*Microtus kikuchii*	NC_003041
Myomorpha	*Microtus levis*	NC_008064
Myomorpha	*Mus musculus*	NC_005089
Myomorpha	*Mus terricolor*	NC_010650
Myomorpha	*Rattus norvegicus*	NC_001665

TABLE 3. Primers used to sequence mitochondrial genomes. Primers were modified from the original source based on a consensus sequence of *Cavia*, *Sciurus*, and *Mus*. "Position" refers to position on *Mus* genome (H-strand). If primer was used for *Anomalurus* (A), *Castor* (C), or *Dipodomys* (D), it is indicated with an X in that column.

Primer	Sequence	Citation	F/R	Position	A	C	D
12S2GW	TGGGAAGAAATGGGCTACATT		F	772	X		X
12SC	GGTAAATTTCGTGCCAGCCAC		F	293	X	X	X
16SARN	TTACCAAAAACATCACCTCTA	Quérouil et al., 2001	F	1935	X	X	X
16SF1	ANCGAGCYTGGTGATAGCTG	Sorenson et al., 1999	F	1427	X	X	X
6520F	GCWGGMTTYGTNCACTGATTCCC	Steppan et al., 2005	F	6498	X	X	
7101F	CAYGAYCAYACNYTWATAAT	Steppan et al., 2005	F	7082	X		
7481R	CARGARTGNARNACRTCTTC	Steppan et al., 2005	R	7481	X	X	
7927R	GAGGMRAAWARATTTTCGTTCAT	Steppan et al., 2005	R	7927	X		
AF1	GACCAATCGGTCCTAAGGACACTCA	this study	F		X		
AF2	CCATCGCTACCACCATTATTACACTA	this study	F		X		
AF3	TTATCCCCACAATTATACTTATTCCAT	this study	F		X		
AF4	CACATCTGCACCCACGCATTTTT	this study	F		X		
AF5	ACCATGAGGTGTATTCTTTCCATGC	this study	F		X		
AF6	AATGAAGCACGTACACACCGCCC	this study	F		X		
AF7	TTCTCACATCCTCAACCCTATCTA	this study	F		X		
AF8	ATTCACCGATCTCAACCAGAAATCAACC	this study	F		X		
AF9	AACGAAAATCTATTCACCTCCTTCATC	this study	F		X		
AR1	TATCTTTATTGATGGCTGCTTTCGG	this study	R		X		
AR10	ATTGTTGCATATTTTACTAACCATA	this study	R		X		
AR12	AGCCTGAGGCTATTAATAGGGCGGA	this study	R		X		
AR2	CATAGATGAGGAGGTTAATTATTGC	this study	R		X		
AR3	GGCTCCTGTTAGGGGTCAGGGGCTA	this study	R		X		
AR4	CCATAAGAGGGAGTATGGTTTGAAG	this study	R		X		

AR5	GGACGTATCCTATAAAAGCAGTAGC	this study	R		X		
AR6	TTGAAATTCGTTGAGTTTACGGCTAA	this study	R		X		
AR8	ATATGTGGGGTGTTTATGGTGGTGG	this study	R		X		
AR9	GCATGGAAAGAATACACCTCATGG	this study	R		X		
ATP6F2	GCNGTNGCNNTAATYCAAGCNTACGT	Sorenson et al., 1999	F	8539	X	X	X
ATP6R1	TGTCCNGCNGTAATRTTRGCNGTNA	Sorenson et al., 1999	R	8405	X	X	
ATP8F1	AYYTATTTGCCTCNTTCATTACNCC	Sorenson et al., 1999	F	7937		X	X
MTF8	YTNCAACCNNTYGCNGAYGC	Simon et al., 1994	F	2886	X		X
CADR2	GAARATAAARCCTARNGCTCANA	this study	R	6343	X	X	
CAF1	GARTACCAGAAGTNACYCAAGGA	this study	F		X		
CAR1	TAANAGGATTGNNGGTTTNTTGTT	this study	R	8361		X	
CAR2	TAGRTGGATATAAAGCACCGCCAAGT	this study	R	590		X	
CF1	AGTATACTATGCCTATTCACCCTAA	this study	F			X	
CF10	CATTAGCGCATTAAAGTCATAAACAA	this study	F			X	
CF11	CAATTGAACTGAGCAATGAAGCAC	this study	F			X	
CF12	CAACACATGAACAAAGAGCCAGTAG	this study	F			X	
CF4	ACAACCCGTTGAACCCCCATTCATT	this study	F			X	
CF5	CCCTAGTAGCACTAACTATAAAA	this study	F			X	
CF6	GAGTAAAAGTCTTCAGCTGACTGGC	this study	F			X	
CF7	TTAATTCTAGTCACAGCAAATAACC	this study	F			X	
CF8	CCAACCCTACCTGTCATTCCTCCA	this study	F			X	
CF9	CCCACGAACCCCAACACAAACATA	this study	F			X	
CO3F1	ATYACNTGANCNCAYCAYAGCYTNATAGA	Sorenson et al., 1999	F	9036	X	X	X
CR1	ATTGAAGGTTGTATCCGTATCT	this study	R			X	
CR2	AGTGGGGGTAAGAGGATTGAGGGT	this study	R			X	
CR4	TGGGTGGAGTCCGAATTGGGCTGAT	this study	R			X	
CR5	AATGTGGCTATTTGAAGTGCTTTG	this study	R			X	

CR6	TTTGCTTTTTAGTGCTTTGAGTTAAT	this study	R			X	
CR7	GGGTTAGGAGTATGGTTCGGCTGTG	this study	R			X	
CR8	GCGTACTCACTGGGGCACGGATATTT	this study	R			X	
CYTBA	GATATGAAAAACCATCGTTG	Sullivan et al., 1997	F		X	X	X
CYTBAM	ACATGAAAAATCATCGTTG	Sullivan et al., 1997	F			X	
CYTBAR	TACAACRRTGRTTTTTCAT	Sullivan et al., 1997	R		X	X	X
CYTBD	CTTCATGAGGACAAATATC	Sullivan et al., 1997	F	14542			X
CYTBE	ACTCCTGTTTATAGTAAGAC	Sullivan et al., 1997	R				X
CYTBECAS	CAAAAGGATATTTGCCCTCA	this study	R			X	
CYTBEND2	TAAGAATNTCAGCTTTGGGTGCTG	this study	R	15384	X	X	X
CYTBG	ATAGACAAAATCCCATTCCA	Irwin et al., 1991	F		X	X	
CYTBJ	CTGCAGTCATCTCCGGTTTACAAGAC	Irwin et al., 1991	F	15315			X
CYTB752R	GCAGGAGTGTAATTATCGGGGTCTC	Tieman-Boese et al., 2000	R	14896	X		X
DF3	TAGCCCCATTCCACCTCTGAGTCC	this study	F				X
DF4	ACCCCTGTTCGTTTGATCCGTACTT	this study	F				X
DF5	AGACGTACTACATTCATGAGCAGT	this study	F				X
DF6	TTATCGCATTCTCAACTTCTAGTCA	this study	F				X
DF7	GGACTTGGCGGTGCTTTATATCCA	this study	F				X
DF8	ATCCACGATTTCAACCTATATCCCC	this study	F				X
DF9	GACAAATCGCATCAGTCCTCTACTTC	this study	F				X
DF10	AGACCTTACATTCACTAAAACACCCAA	this study	F				X
DF11	AACTTGATTTATCCAATTTTACGACT	this study	F				X
DF12	AATCCACACCCTACCCCACCACTAAT	this study	F				X
DF13	TGATACTGACATTTCGTAGACGTAGT	this study	F				X
DF15	CCATAGCCCTCGCTGTACGACTAACT	this study	F				X
DF16	TTATAGCAACAGGTTTCCACGGACTT	this study	F				X

DF17	CTAGCTGCATGTGAAGCAGCTGTTGGC	this study	F				X
DPD6	TCCTTGTCCATATGACTATC	this study	F				X
DR2	AAGAGCTGTACCTCTTTAGGTTAGC	this study	R				X
DR3	TGGTGTAGTAGAGATGGATAAAGAAT	this study	R				X
DR4	TTTGGAGTGGATAAGCCATAAAGAT	this study	R				X
DR5	TGGTGTTGGCTAAGCAAAATAGGGC	this study	R				X
DR6	GGGTGTCAGGCGTATAATGTATTGCT	this study	R				X
DR7	AGTGGGGTATTTAATCCCAGTTTAG	this study	R				X
DR9	AGGGCTATAAGAGGAAGTATTGTTTG	this study	R				X
DR10	TAAAGCATTCATAAAATGTCCAAGCG	this study	R				X
DR11	AATGAAAGTTGAGATACGTATTATT	this study	R				X
DR13	ATAGCGGATGAGTCATCCGTAATTC	this study	R				X
DR14	AGGCTTACTAGAAGGGTGAATACGTA	this study	R				X
DR16	GGGATTCAAAAGGCAATTAAGATTAG	this study	R				X
DR15	ATGCCTGATGTAAGTAAGAGGGCTGA	this study	R				X
DR17	TTTTCATGTAATTGGTTCTTTTGCTAT	this study	R				X
HISF1	AAAACAYTAGAYTGTGAATCTRRYAAYA	Sorenson et al., 1999	F	11562	X	X	X
LEUR1	TTTTTGGYTCCTAAGACCAAYGGAT	Sorenson et al., 1999	R	11691	X	X	X
MTF12	GCNTCNTAYCCACGNTTCCGNTACGAYC	Simon et al., 1994	F	3573	X	X	X
MTF14	TGRGGRGGNCTNAACCAAAC	Simon et al., 1994	F	4412	X	X	X
MTF15	TNGGAGGNCTYCCNCCAYTNACNGG	Simon et al., 1994	F	4665	X	X	X
MTF16	TTTACAGYCTAAYGCYTACTCRGCC	Simon et al., 1994	F	5294	X	X	X
MTF20	GGRACNGGNTGRACNGTYTACCCNCC	Simon et al., 1994	F	5694	X	X	X
MTF23	CCYACNGGNGTNAAAGTNTTYAGCTGRYTNGC	Simon et al., 1994	F	6270	X	X	X
MTF26	AACYYCCNNNRRYTRGTTTCAAGCC	Simon et al., 1994	F	6888	X	X	X
MTF32	AGAYGTNCTNCAYTCNTGA	Simon et al., 1994	F	12099	X	X	
MTF40	GARTGRAYNCARAAAGGNYTN	Simon et al., 1994	F	9771	X		

MTF42	CNTAYTCNTCNRTYAGYCA	Simon et al., 1994	F	11025	X	X	X
MTF8	YTNCAACCNNTYGCNGAYGC	Simon et al., 1994	F	2886	X	X	X
MTFB1	GACCGGAGNAATCCAGGTCGG	Simon et al., 1994	F	2517	X	X	X
MTR13	CTATCAAAGTAAYTCTTTTRTCAGACA	Simon et al., 1994	R	3713	X	X	X
MTR14	GTTTGGTTNAGNCCYCCYCA	Simon et al., 1994	R	4412	X	X	X
MTR16	GGCYGAGTARGCRTTAGRCTGTAAA	Simon et al., 1994	R	5294	X	X	X
MTR17	NGTACCYACTATNCCNGCYCA	Simon et al., 1994	R	5400	X	X	X
MTR21	CCAAARAATCARAAYARRTGYTG	Simon et al., 1994	R	6021	X	X	X
MTR24	GTRNARCCNGNRAAYARNGG	Simon et al., 1994	R	6458		X	
MTR28	TCYTCTATRATNGGRGANGNRGC	Simon et al., 1994	R	7046	X	X	
MTR33	CCACARATYTCNGARCATTGNCCR	Simon et al., 1994	R	7591	X	X	X
MTR38	YTRTTYATYRTNTCNGAAGT	Simon et al., 1994	R	8859		X	
MTR39	TGRTACTGACAYTTYGTNGA	Simon et al., 1994	R	9324	X	X	
MTR4	GCTCCATAGGGTCTTCTCGT	Simon et al., 1994	R	2154	X	X	X
MTR40	NARNCCTTTYTGNRTYCAYTC	Simon et al., 1994	R	9771	X		
MTR7	GGNCCTTTNCGNAGTTGTATRTANCCN	Simon et al., 1994	R	2834	X	X	X
MTR9	GGNCCTTTNCGNAGTTGTATRTANCCN	Simon et al., 1994	R	3105	X	X	X
MTRB1	CCGACCTGGATTNCTCCGGTC	Simon et al., 1994	R	2517	X		
ND3F1	YCNTATGARTGYGGNTTYGAYCC	Sorenson et al., 1999	F	9564	X	X	
ND4R1	GGNGNRGATARNGRRTCNGANRAGAA	Sorenson et al., 1999	R	10261	X	X	X
ND4R2	GTNGGRATYAARGTNGYYTCAAA	Sorenson et al., 1999	R	10530	X	X	
ND4R3	GTNCGGCTGTGRATNCGTTC	Sorenson et al., 1999	R	11169	X		X
ND4F1	TTCTYNTCNGAYYCNYTATCYNCNCC	Sorenson et al., 1999	F	12686			X
ND5F1	AAAYACNGCAGCNCTNCAAGC	Sorenson et al., 1999	F	12233	X	X	X
ND5F3	CACATYTGYACYCACGCNTTCTT	Sorenson et al., 1999	F	12723	X	X	
ND5R2	AGNCCAAATTGNGCNGATTTTCC	Sorenson et al., 1999	R	12405	X	X	X
ND5R4	TCCTATYTTTCGRATGTCYTGTTC	Sorenson et al., 1999	R	12798	X	X	X

ND5R6	TTNGGNRYNRYTTTTTCTANYCARRT	Sorenson et al., 1999	R	13407	X		
ND6F1	CCAAANACNACCANCATNCCNCC	Sorenson et al., 1999	F	13868		X	
R95F2	CATGATAACACATAATGACCCACCAA	Riddle, 1995	F	8593			X
R95R2	ACTAAGAGAGTAGGATCCTCATCAATA	Riddle, 1995	R	9375			X
VALR1	ATCTYCNGGGTGTARGCCRGRTGC	Sorenson et al., 1999	R	1047	X	X	X

TABLE 4. Results of Shimodaira-Hasegawa tests comparing topologies obtained from ML analysis of three datasets. Each topology was compared with the other two topologies for each individual dataset. Asterisks indicate significant results after correcting for multiple tests.

Dataset	Nuc. topology vs. mt. topology	Nuc. topology vs. comb. topology	Mt. topology vs. comb. topology
Nuclear	Nuc. P = 0.001*	Nuc. P = 0.504	Comb. P = 0.001*
Mitochondrial	Mt. P = 0.027	Comb. P = 0.054	Mt. P = 0.185
Combined	Nuc. P = 0.457	Comb. P = 0.122	Mt. P = 0.110

Chapter 4

The phylogenetic position of the zokors (Myospalacinae) and comments on the families of muroids (Rodentia)

Abstract

Recent molecular studies have concluded that the genus *Myospalax* evolved from within the rodent subfamily Cricetinae. This conclusion was tested using the complete sequences from the mitochondrial 12S rRNA and cytochrome b genes. Based on our analyses, *Myospalax* appears to be sister to a clade containing the subfamilies Spalacinae and Rhizomyinae, and all three of these lineages appear to be basal to the superfamily Muroidea. Based on the position of these three lineages, we suggest that they be placed in a distinct family, the Spalacidae, rather than subsumed as subfamilies in the family Muridae. Finally, our analyses suggest that the earlier placement of *Myospalax* as a member of the Cricetinae is the result of a single misidentified specimen, which was not a *Myospalax*.

Introduction

Genetic information from a single individual (Tissue # T-394), identified as *Myospalax* sp. from an "unknown locality, Russia" (Michaux and Catzeflis, 2000), was used to represent the subfamily Myospalacinae in several phylogenetic studies of muroid relationships (Furano et al., 1994; Usdin et al., 1995; Michaux and Catzeflis, 2000; Chevret et al., 2001). The results of these studies indicate a phylogenetic position of the

Myospalacinae nested within the subfamily Cricetinae (Michaux and Catzeflis, 2000; Chevret et al., 2001; Michaux et al., 2001). Specifically, *Myospalax* appears to be sister to the hamster genus *Phodopus*.

Fossils of myospalacines extend to the Late Miocene, and Lawrence (1991) considered all fossil and recent species similar enough to be placed in a single genus. The estimated time of divergence between *Myospalax* and *Phodopus*, based on the molecular data, ranges from 4.5 to 6.7 Myr (Michaux and Catzeflis, 2000; Michaux et al., 2001). If dates obtained in these molecular analyses (Michaux and Catzeflis, 2000; Michaux et al., 2001) are close to being accurate, then a massive amount of morphological evolution has occurred over a short period of time in the myospalacine lineage. This case requires the origin of a fossorial lifestyle, complete with numerous morphological specializations (long claws, small eyes and ears, large keratinized nose, strong zygomatic arch, distinct occipitum, fused cervical vertebrae, enlarged olecranon process; Tullberg, 1899; Carleton and Musser, 1984; Lawrence, 1991) arising from a hamster-like phenotype. In addition to these specializations, *Myospalax* differs from typical hamsters in other characters including increased diploid chromosome number, hypsodont molars with prismatic cusps, a triangular braincase, oval shaped infraorbital canals, small incisive foramina, the lack of internal cheek pouches, and the absence of sebaceous flank glands (Carleton and Musser, 1984; Lawrence, 1991).

The phylogenetic position of the Myospalacinae within the superfamily Muroidea has been controversial in that this subfamily has been allied to several different muroid subfamilies including Rhizomyinae and Spalacinae (Tullberg, 1899), Spalacinae (Miller

and Gidley, 1918; Chaline et al., 1977), Arvicolinae (Kretzoi, 1955), and Cricetinae (Gromov and Polyakov, 1977). Carleton and Musser (1984) considered the myospalacines to be primitive cricetids, whereas Lawrence (1991) concluded that they were derived from a fossorially adapted lineage basal relative to all muroids. Although the myospalacines have been considered related to the cricetines, their placement as sister to *Phodopus* within the Cricetinae is a novel idea found only in several related studies (Michaux and Catzeflis, 2000; Chevret et al., 2001; Michaux et al., 2001). Given the amount of difference between *Myospalax* and cricetine rodents in general, further research is certainly warranted before the acceptance of *Myospalax* as being sister to *Phodopus*, a lineage well within the Cricetinae.

The subfamilies Spalacinae and Rhizomyinae are subfamilies of muroid rodents that also possess a number of morphological and physiological specializations for a fossorial or semi-fossorial lifestyle. Molecular phylogenies constructed using the LCAT (Robinson et al., 1997; Michaux and Catzeflis, 2000), vWF (Huchon et al., 1999; Michaux et al., 2001), IRBP (DeBry and Sagel, 2001), and 12S rRNA, LCAT and vWF combined (Michaux et al., 2001) all show strong support for a separate clade containing the subfamilies Spalacinae and Rhizomyinae that resides basal to a monophyletic group containing the remaining muroid subfamilies. As a result, Michaux et al. (2001) suggested that the subfamilies Rhizomyinae and Spalacinae be placed in the family Spalacidae, while applying the family name Muridae to all remaining subfamilies. The subfamilies Lophiomyinae, Petromyscinae, and Platacanthomyinae were not included in

their analysis and no comment was made as to their position. We follow the distinction of two family names, Spalacidae and Muridae, in this paper.

In previous molecular studies (Michaux and Catzeflis, 2000; Chevret et al., 2001; Michaux et al., 2001), individual T-394, identified as *Myospalax* sp., was used, and this specimen can be clearly assigned to the Muridae clade. Nevertheless, given the unusual placement of this individual, additional samples of myospalacines should be examined. Nucleotide sequence data from the mitochondrial 12S rRNA and cytochrome b genes are available for over 20 individuals of seven species of *Myospalax* (obtained by KYZ, CQZ, and GY; GenBank accession numbers AF326235-AF326252, AF326255-AF326272, AF387076-AF387084). In this paper, we incorporate this new information with existing data from T-394 and other subfamilies of Muridae to investigate the placement of the Myospalacinae.

Materials and Methods

Representative GenBank sequences of the complete 12S rRNA gene and complete cytochrome b gene were obtained for 36 and 30 species, respectively, and these data represent information from 15 subfamilies of muroid rodents (Table 1). Sequence data was available for both genes for only 26 species in 13 subfamilies and these taxa were used in the combined analysis. *Glis glis*, *Pedetes capensis*, and *Jaculus jaculus* were included as outgroups for the 12S data set. *Glis glis* and *Zapus trinotatus* were used as outgroups in the cytochrome b analysis. *Jaculus* and *Zapus* are members of the family Dipodidae, the presumed sister-group to the Muridae (Michaux and Catzeflis, 2000;

Adkins et al., 2001; DeBry and Sagel, 2001). In order to use this family as an outgroup in combined analyses, these two taxa were used to construct a concatenated sequence. Concatenated sequences of ingroup taxa included *Steatomys* sp. with *S. parvus* to represent the genus *Steatomys*, and *Macrotarsomys ingens* with *M. bastardi* to represent the genus *Macrotarsomys*. Sequence data for individual T-394 is available in GenBank for 12S rRNA, but not for cytochrome b. Therefore this individual was included in the 12S rRNA analyses, but not in the cytochrome b and the combined analyses.

Sequences for 12S rRNA were initially aligned by eye according to secondary structure as indicated by Springer et al. (1995). Individual stem and loop regions were aligned using ClustalX (Thompson et al., 1997) and edited by eye. Ambiguously aligned regions were not included in the final analysis.

Gap handling is an important part of phylogenetic analyses. Under a parsimony framework, PAUP* (version 4.0b8, Swofford, 1999) allows for gaps to be treated as either missing data or as a fifth character state. Under both of those frameworks, the important phylogenetic information of presence or absence of the indel is ignored. A numerical character state matrix was generated to indicate the presence or absence of insertion / deletion events (Nedbal, et al., 1994). Totally removing positions with gaps results in a loss of potentially valuable phylogenetic information resulting from substitution events among taxa without the deletion. Treating gaps as missing data or as a fifth character state when combined with the character state matrix will result in higher weight of the indels when compared with all sites without an insertion / deletion event. A weighting scheme of 1/2 for each column in the character state matrix and 1/N, where N

= the number of bases involved in the particular indel, for the positions with gaps can incorporate both the presence / absence of the indel and substitution information within the insertion while keeping the overall weight of the insertion / deletion event proportional to the weight of other positions.

Data for 12S was analyzed using both maximum parsimony and maximum likelihood in PAUP*. A total of 888 sequence characters and 36 numerical characters were analyzed under a parsimony framework. Separate analyses were conducted with gaps treated as missing and as a fifth character state both with and without the character state matrix. Positions in the character state matrix and all positions with gaps were treated with a weight of one and downweighted as described in the above paragraph. Nodal support using bootstrap (1,000 replicates; Felsenstein, 1985) and final results are presented using gaps as fifth character states and with the weighting scheme listed above. All sites with gaps and the numerical character state matrix were excluded for the maximum likelihood analysis leaving a total of 844 characters. Modeltest 3.04 (Posada and Crandall, 1998) was used to determine the appropriate likelihood model for this data set, and a GTR + I + gamma model was used in the maximum likelihood analysis. Bootstrap values (100 replicates) for the likelihood analysis were determined using NNI branch swapping to conserve computer time.

Cytochrome b sequences were aligned by eye. Maximum likelihood using a TVM + I + gamma model as determined by Modeltest 3.04 (Posada and Crandall, 1998) and maximum parsimony analyses were performed on the cytochrome b data set alone and bootstrap values (1,000 replicates with TBR branch swapping and 100 replicates with

NNI branch swapping respectively) were determined. These data were combined with the 12S rRNA data sets for a total evidence analysis. The partition homogeneity test of PAUP* was performed on both the maximum parsimony and maximum likelihood data sets and the two genes were not found to be providing conflicting data (1,000 replicates; P = 0.434 and P = 0.531 respectively). A total of 2031 sequence characters and 36 numerical characters were analyzed under a parsimony framework as described above for the 12S rRNA data. A total of 1987 sequence characters were analyzed under a maximum likelihood framework. For the combined data, GTR + I + gamma was chosen as the appropriate model using Modeltest 3.04 (Posada and Crandall, 1998) and bootstrap values (100 replicates, NNI branch swapping) were calculated to determine nodal support.

Results

Eleven most parsimonious trees were obtained for the analysis of the 12S rRNA data set. The strict consensus of these trees is shown in figure 1. Tree topology differed depending on how gaps were treated. Those nodes which were not present in all MP trees under all gap handling methods are indicated with an asterisk (Fig. 1). A total of 12 nodes shown in figure 1 were not present under all gap handling methods. This emphasizes that treatment of gaps can have a considerable effect on tree topology, even involving nodes supported by bootstrap values ranging from 56% to 62%. Nodal support for the maximum likelihood analysis of the 12S gene is also shown in figure 1. One most parsimonious tree was found for the analysis of cytochrome b alone (Fig. 2). Three trees

(not shown) were obtained from a maximum likelihood search with equal log likelihood scores. Bootstrap values for both the parsimony and likelihood analyses of cytochrome b are shown in figure 2. The maximum likelihood tree for the combined data set is shown in figure 3. A single most parsimonious tree was recovered for the data set combining cytochrome b and 12S (tree not shown; tree length = 5188.25, CI = 0.3291, RI = 0.4264). Four nodes were not present in all MP trees under all gap handling methods. Nodal support for the combined analyses under both parsimony and likelihood frameworks is indicated in figure 3.

In the 12S analyses (Fig. 1), individual T-394, identified as *Myospalax* sp., was sister to the subfamily Cricetinae. This placement is roughly consistent with the results from previous studies (Michaux and Catzeflis, 2000; Michaux and Catzeflis, 2001; and Michaux et al., 2001). In all analyses (Figs. 1-3), the subfamily Cricetinae was grouped within the Muridae clade (bootstrap support from <50% to 99% for the Muridae clade). All seven other representatives of the subfamily Myospalacinae formed a monophyletic group (bootstrap 79% to 100%; Figs. 1-3) within the Spalacidae clade (bootstrap <50% to 100%) along with the subfamilies Spalacinae and Rhizomyinae (Figs. 1 and 3).

The parsimony analysis of the cytochrome b gene is the only analysis that does not show nodal support for the separation of the families Muridae and Spalacidae. Bootstrap values for the other analyses range from 65% to 99% and from 86% to 100% for family Muridae and family Spalacidae respectively. *Rhizomys* is found on a long branch relative to *Nannospalax* and *Myospalax* and is sister to *Petromyscus* in the cytochrome b maximum parsimony tree. The parsimony analysis for 12S and

cytochrome b combined does not show diminished bootstrap support for the two families (both families are 99%; Fig. 3) compared to the support for the two families when 12S is analyzed alone (both families are 99%; Fig. 1). Additionally, a Rhizominae / Spalacinae clade is more strongly supported in the combined analysis (63%; Fig. 3) than it is in the 12S analysis (<50%; Fig. 1). These results suggest that although the two genes appear to conflict, there is hidden support for the topology indicated in Fig. 3 in the cytochrome b data (Sullivan, 1996).

Although other conflicts arise among the different analyses, few are supported with a bootstrap >50%. The Sigmodontinae is supported as a monophyletic group in the 12S analyses (58% and 68%; Fig. 1) and the combined maximum likelihood analysis (60%; Fig. 3). In the cytochrome b maximum likelihood analysis and the combined parsimony analysis, however, *Akodon* is basal to the rest of the Arvicolinae / Cricetinae / Sigmodontinae clade (bootstrap 59% and 51% respectively). Conflict also exists concerning the relationships among the myospalacines. Although *Myospalax aspalax* and *M. psilurus* form a clade consistently basal to the remaining species, there is conflict among the relationships of *M. baileyi*, *M. cansus*, *M. fontanieri*, *M. rothschildi*, and *M. rufescens*. The 12S maximum likelihood analysis supports *M. rufescens* as basal to the clade (bootstrap 68%; Fig. 1) while the cytochrome b and combined analyses support the topology shown in Figs. 2 and 3.

Discussion

Individual T-394 is clearly not a myospalacine and appears to represent a misidentified specimen. Using the likelihood model parameters, sequence divergence between this individual and members of the subfamily Cricetinae ranged from 0.0938 and 0.1227. This is comparable to the distance between *Mesocricetus* and the two species of *Cricetulus* (0.0883 and 0.0991). In contrast, the sequence divergences between this specimen and members of the genus *Myospalax* range from 0.2941 and 0.3377. Michaux and Catzeflis (2000) and Michaux et al. (2001) estimated the divergence time between T-394 and *Phodopus roborowskii* at 4.5-6.5 Myr (Michaux and Catzeflis, 2000; Michaux et al., 2001). These results suggest that T-394 is either a different species of *Phodopus* or belongs to a genus of hamster not included in our analysis. Musser and Carleton (1993) report two species of *Phodopus* in Russia, *P. campbelli* and *P. sungorus*, as well as species in the genera *Allocricetulus*, *Cricetus*, and *Tscherskia*. Without more data, it is not possible to assign T-394 to one of these genera or species.

Previous phylogenetic conclusions that associate *Myospalax* with hamsters have been based on a single specimen. Our data suggest that this particular specimen, T-394, has been misidentified, indicating the importance of museum vouchers and geographic information that can be used to verify assignment of specimens used in a molecular phylogenetic study. In addition, this finding substantiates the need for the use of multiple representatives of a major clade, especially in phylogenetic studies that infer relationships among higher taxonomic categories. Often obtaining multiple individuals is very difficult for phylogenetically important taxa (such as myospalacines) and we do not

suggest that this should preclude their inclusion in phylogenies. It is important, however, that caution be advised in conclusions that are not supported by multiple individuals. Based solely on the misidentified individual T-394, myospalacines would appear to belong to an entirely different part of the muroid radiation.

As is often the case with many attempts to reconstruct relationships among muroid rodents (Jansa et al., 1999; Michaux and Catzeflis, 2000), our analyses reveal a lack of resolution at several nodes, as well as contradictions in the separate and combined analyses and when different gap handling approaches are employed. Despite the observed incongruence, several conclusions can be drawn. First, two subfamilies (Spalacinae and Rhizomyinae) in the family Spalacidae appear to be sister to the subfamily Myospalacinae (Figs. 1 and 3). Second, within the family Muridae, monophyly of the subfamilies Arvicolinae, Cricetinae (including T-394), Gerbillinae, Murinae, and Nesomyinae is supported in all analyses. The monophyly of the subfamilies Acomyinae, Cricetomyinae, Dendromurinae, and Sigmodontinae is poorly supported, with the placement of taxa differing among analyses. Fourth, an arvicoline / cricetine / sigmodontine clade is present in all analyses. This relationship is consistent with several previous studies (Robinson et al., 1997; Michaux et al., 2001). Finally, save for the taxonomic position of Myospalacinae, no well-supported conflicts exist between this study and Michaux et al. (2001).

The recognition of the family Spalacidae containing the genera *Myospalax*, *Rhizomys*, *Tachyoryctes*, and *Spalax* was first proposed by Tullberg (1899). *Cannomys* (a rhyzomyine similar to *Rhizomys*) and *Nannospalax* (a spalacine similar to *Spalax*) are

also clearly a part of this family. The family Spalacidae contains a diverse group of muroids adapted to a fossorial way of life. Myospalacines dig using their forelimbs, while the forelimbs of spalacines are much reduced with animals using their protruding upper incisors for excavation. Rhizomyines use both their forelimbs and upper incisors. Spalacines have an olecranon process that is greatly enlarged (Carleton and Musser, 1984), suggesting that scratch digging is the plesiomorphic trait. The protrusion of the incisors outside of the mouth when closed and the use of the incisors in digging in both rhizomyines and spalacines is additional support for a basal position of the myospalacines among the Spalacidae. Tullberg (1899) clearly showed the similarities between the *Myospalax* molar pattern and that of a juvenile *Spalax* suggesting that the two are derived from a common ancestor. The dental morphology of the myospalacines is clearly not derived from a cricetine or arvicoline cusp pattern as has been suggested (Gromov and Polyakov, 1977). To the contrary, it is probably derived from a primitive muroid condition.

The family Spalacidae can be identified by the presence of several characteristics including a reduction or absence of external eyes, reduced pinnae, stocky body, short tail (< 50% head and body length), broad rostrum, triangular-shaped braincase, infraorbital canal ovoid shape and does not extend ventrally to the roof of the palate, zygomatic plate absent or much reduced, nasolacrimal canal inside infraorbital canal, incisive foramina small to medium-sized, extensive neck musculature and prominent points of attachment on the occipitum, minimal reduction in M^3 relative to M^1 and M^2, an equal number of

cusps on M^2 as compared to M^3, and a distinct orientation of the manubrium of the malleus bone (Tullberg, 1899; Carleton and Musser, 1984).

In contrast, the family Muridae clade has few diagnostic characters. In general, members of this family display two characteristics, infraorbital canal V-shaped and extends to the roof of the mouth and the incisive foramina medium to large sized. Although lacking in morphological synapomorphies, a monophyletic Muridae has been supported by numerous molecular studies (Robinson et al., 1997; Huchon et al., 1999; Michaux and Catzeflis, 2000; DeBry and Sagel, 2001; Michaux et al., 2001). Although our data are congruent with a monophyletic Muridae, we did not include two murid subfamilies (Lophiomyinae and Platacanthomyinae) recognized by Musser and Carleton (1993). The single species found in the Lophiomyinae also has a V-shaped infraorbital canal that extends to the palate, large incisive foramina that extend to the first molar, a zygomatic plate, and a molar cusp pattern that closely resembles the mystromyines or cricetines suggesting that *Lophiomys* is probably a member of the Muridae clade as well.

The subfamily Platacanthomyinae, however, has small incisive foramina, a distinct infraorbital canal, a cusp pattern unlike any other muroid, and a fossil record that extends to the Early Miocene (Carleton and Musser, 1984). Carleton and Musser (1984) considered the platacanthomyines to be muroids and not glirids, but the basis for their suggestion emphasizes a lack of glirid apomorphies instead of characters uniting this group with any muroid subfamilies. The absence of characters uniting this subfamily with any other representatives in either the Spalacidae or Muridae suggest that it may be

either basal to the family Muridae or basal to the superfamily Muroidea and it is probably not closely related to any extant muroid lineage.

The recognition of two separate families of muroids is in order. The family Spalacidae includes the subfamilies Myospalacinae, Rhizomyinae, and Spalacinae whereas the family Muridae contains the subfamilies Acomyinae, Arvicolinae, Calomyscinae, Cricetinae, Cricetomyinae, Dendromurinae, Gerbillinae, Lophiomyinae (based on simple morphological observations), Murinae, Mystromyinae, Nesomyinae, Petromyscinae, and Sigmodontinae. Additional study is needed to determine the appropriate position of the Platacanthomyinae.

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Figure Captions

Figure 1. Strict consensus of eleven most parsimonious trees for 12S rRNA (tree length = 1782, CI = 0. 3386, RI = 0.5528). Values above the lines represent bootstrap values >50% (1,000 replicates, TBR branch swapping). Nodes not present in MP analyses under all gap handling schemes (see text) are indicated by an asterisk. Values below the lines represent bootstrap values >50% (100 replicates, NNI branch swapping) for the maximum likelihood analysis under a GTR + I + gamma model of evolution. Maximum likelihood bootstrap values >50% not indicated on the tree are as follows: *Myospalax baileyi* / *M. cansus* / *M. fontanieri* / *M. rothschildi* = 68% and *Acomys* / *Deomys* / *Lophuromys* = 62%.

Figure 2. Maximum parsimony tree for cytochrome b (tree length = 4252, CI = 0.263, RI = 0.360). Values above the lines represent bootstrap values >50% (1,000 replicates, TBR branch swapping). Values below the lines represent bootstrap values >50% (100 replicates, NNI branch swapping) for the maximum likelihood analysis under a TVM + I + gamma model of evolution. Maximum likelihood bootstrap values >50% not indicated on the tree are as follows: Myospalacinae / Rhizomyinae / Spalacinae (family Spalacidae) = 86%; Acomyinae / Arvicolinae / Calomyscinae / Cricetinae / Cricetomyinae / Dendromurinae / Gerbillinae / Murinae / Mystromyinae / Nesomyinae / Petromyscinae / Sigmodontinae (family Muridae) = 65%; *Acomys* / *Lophuromys* (Acomyinae) = 86%; *Peromyscus* / Arvicolinae / Cricetinae = 59%; *Macrotarsomys* / *Nesomys* (Nesomyinae) = 58%; and *Mystromys* / *Petromyscus* = 51%. Subfamilies are indicated in Table 1.

Figure 3. Maximum likelihood tree for combined 12S rRNA and cytochrome b obtained under a GTR + I + gamma model of evolution. Values above the lines represent bootstrap values >50% (100 replicates, NNI branch swapping). Values below the line represent bootstrap values >50% (1,000 replicates, TBR branch swapping) under a parsimony framework. The maximum parsimony analysis yielded a bootstrap value of 51% for a clade consisting of *Peromyscus* / *Clethrionomys* / *Volemys* / *Mesocricetus* / *Cricetulus griseus* / *C. migratorius* that is not indicated on the tree.

Figure 1

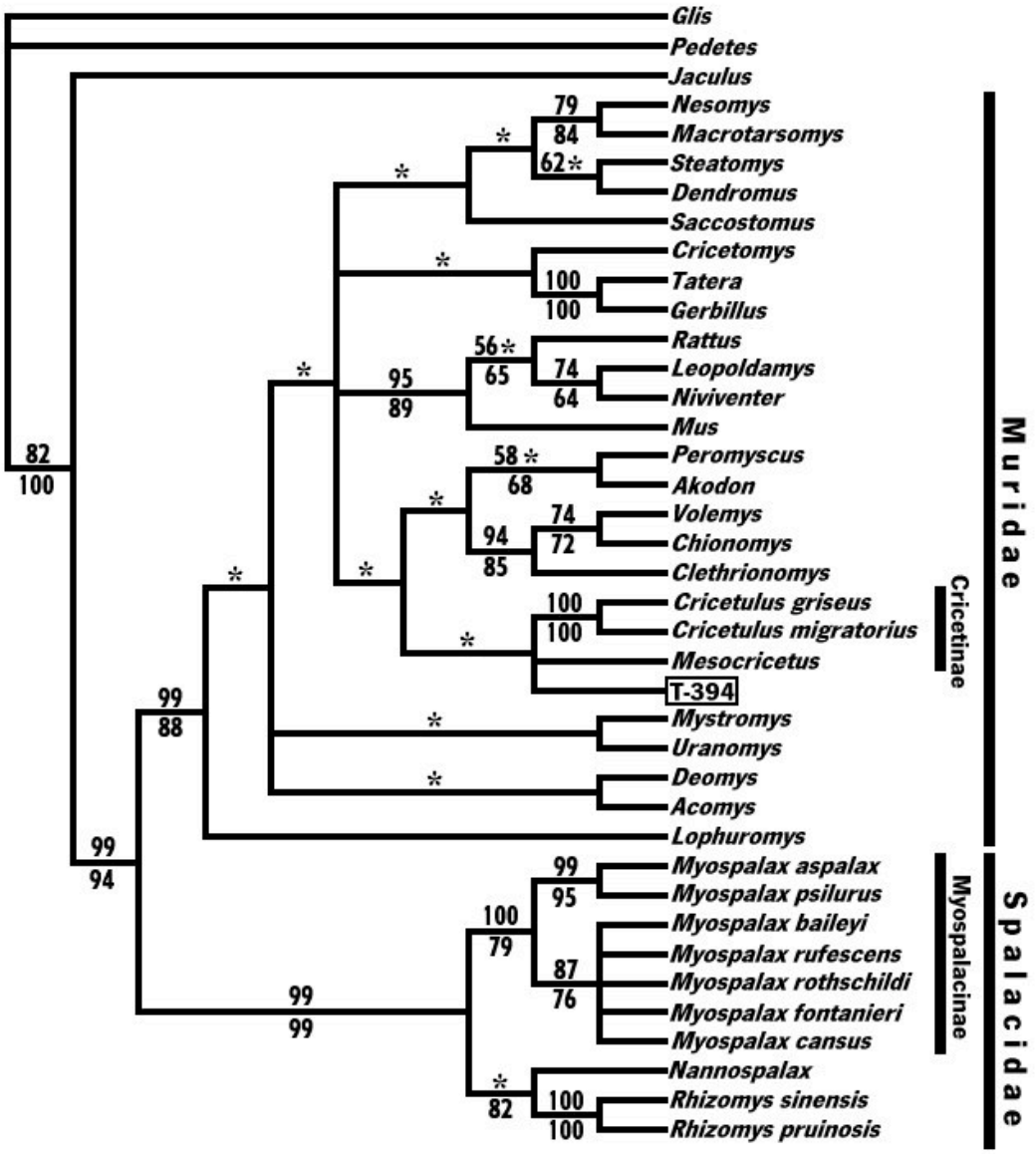
Glis
Pedetes
Jaculus
Nesomys
Macrotarsomys
Steatomys
Dendromus
Saccostomus
Cricetomys
Tatera
Gerbillus
Rattus
Leopoldamys
Niviventer
Mus
Peromyscus
Akodon
Volemys
Chionomys
Clethrionomys
Cricetulus griseus
Cricetulus migratorius
Mesocricetus
T-394
Mystromys
Uranomys
Deomys
Acomys
Lophuromys
Myospalax aspalax
Myospalax psilurus
Myospalax baileyi
Myospalax rufescens
Myospalax rothschildi
Myospalax fontanieri
Myospalax cansus
Nannospalax
Rhizomys sinensis
Rhizomys pruinosis
Cricetinae
Myospalacinae
Muridae
Spalacidae

Figure 2

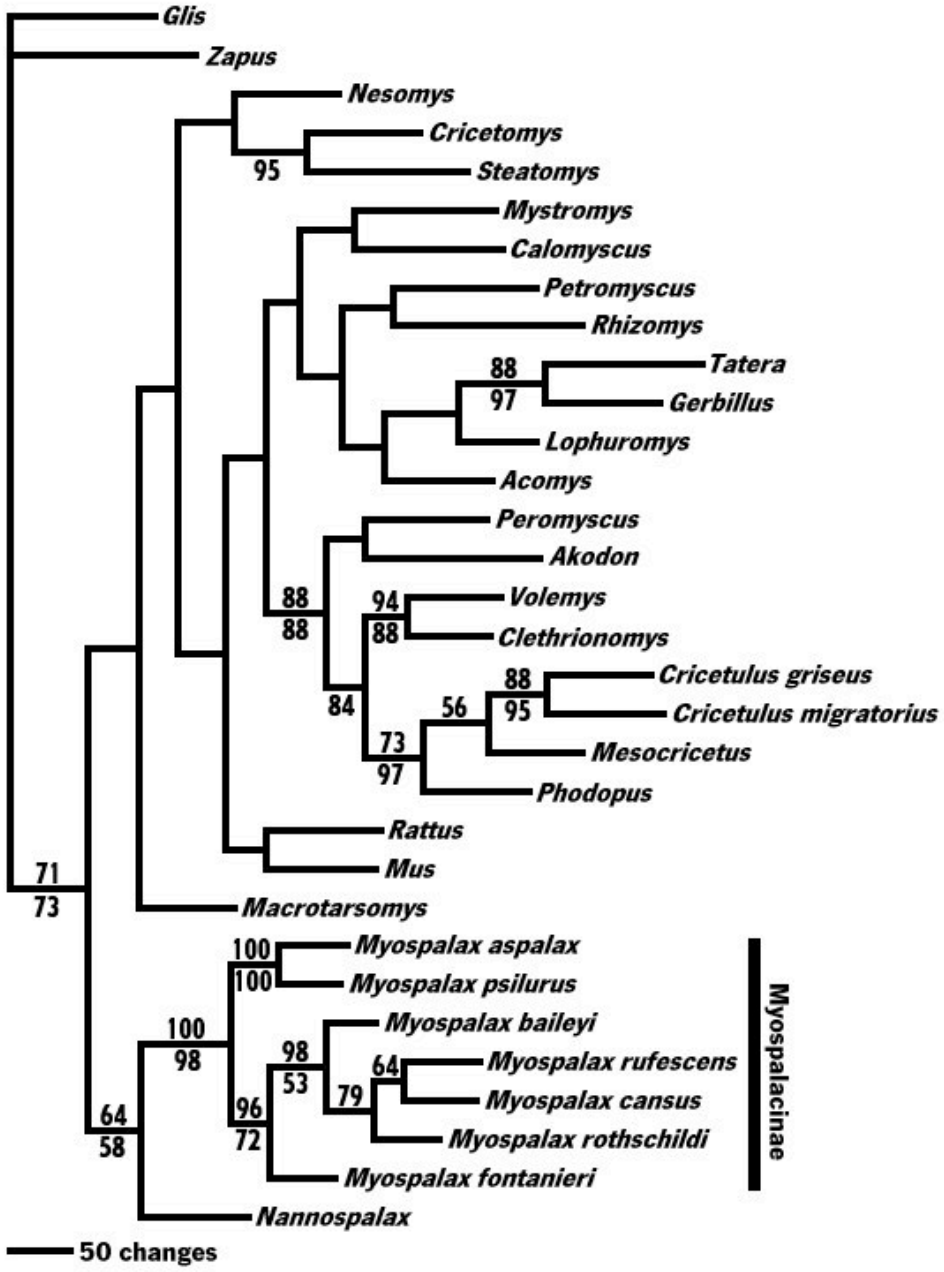
Glis
Zapus
Nesomys
Cricetomys
95
Steatomys
Mystromys
Calomyscus
Petromyscus
Rhizomys
88
97
Tatera
Gerbillus
Lophuromys
Acomys
Peromyscus
Akodon
88
88
94
88
Volemys
Clethrionomys
84
88
95
56
Cricetulus griseus
Cricetulus migratorius
73
97
Mesocricetus
Phodopus
Rattus
Mus
71
73
Macrotarsomys
100
100
Myospalax aspalax
Myospalax psilurus
100
98
Myospalax baileyi
98
53
64
Myospalax rufescens
79
Myospalax cansus
96
72
Myospalax rothschildi
64
58
Myospalax fontanieri
Myospalacinae
Nannospalax
50 changes

Figure 3

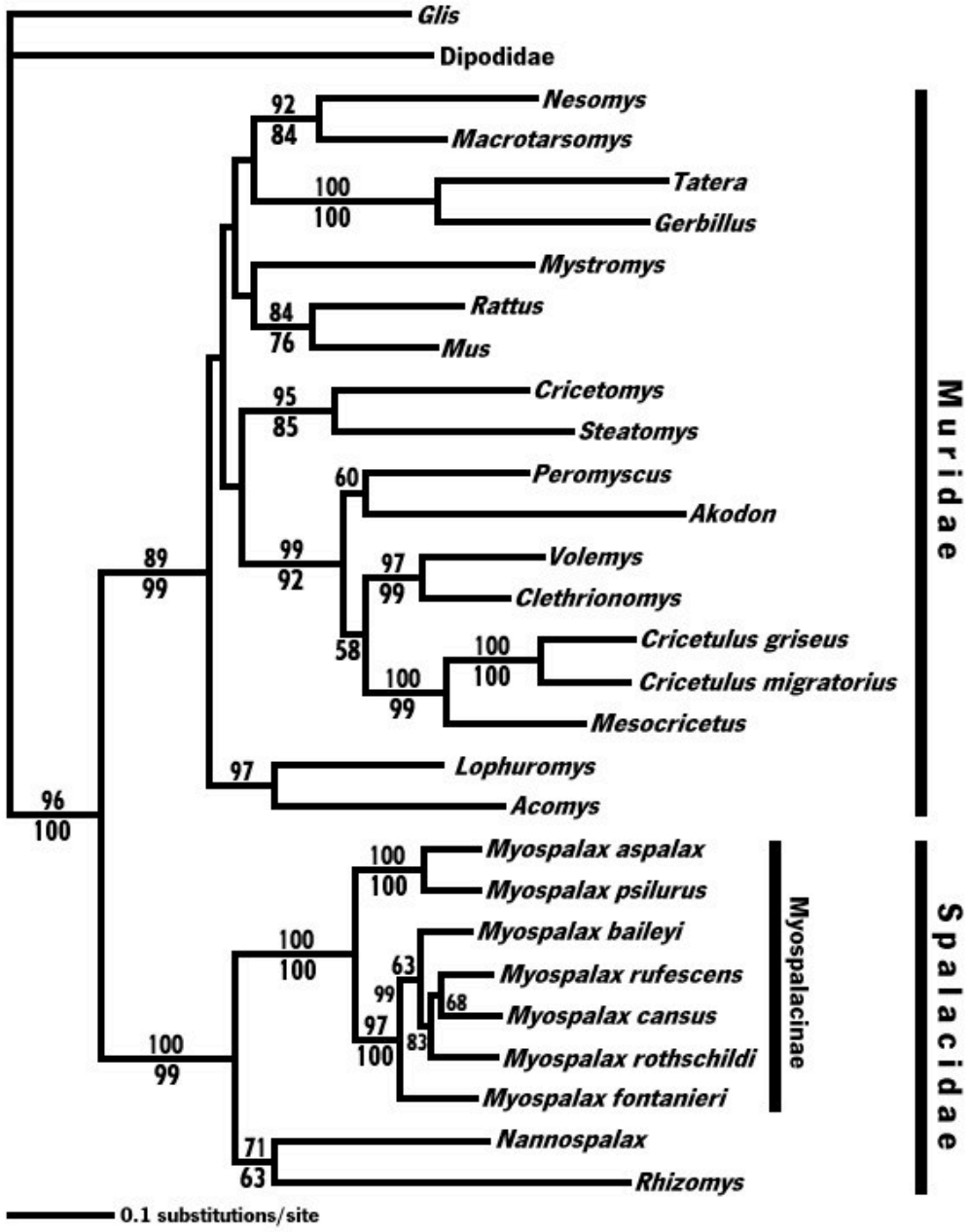
Glis
Dipodidae
Nesomys
Macrotarsomys
Tatera
Gerbillus
Mystromys
Rattus
Mus
Cricetomys
Steatomys
Peromyscus
Akodon
Volemys
Clethrionomys
Cricetulus griseus
Cricetulus migratorius
Mesocricetus
Lophuromys
Acomys
Myospalax aspalax
Myospalax psilurus
Myospalax baileyi
Myospalax rufescens
Myospalax cansus
Myospalax rothschildi
Myospalax fontanieri
Nannospalax
Rhizomys
Muridae
Spalacidae
Myospalacinae
0.1 substitutions/site

Table 1. GenBank accession numbers for taxa used in this study.

Species	Subfamily	12S rRNA	Cytochrome b
Tissue T-394	?	AJ250355	-
Myospalax aspalax	Myospalacinae	AF326252	AF326272
Myospalax baileyi	Myospalacinae	AF387080	AF387084
Myospalax cansus	Myospalacinae	AF326243	AF326263
Myospalax fontanierii	Myospalacinae	AF326245	AF326266
Myospalax psilurus	Myospalacinae	AF326250	AF326271
Myospalax rothschild	Myospalacinae	AF326247	AF326268
Myospalax rufescens	Myospalacinae	AF326248	AF326269
Acomys cahirinus	Acomyinae	X84387	AJ233953
Deomys ferrugineus	Acomyinae	AJ250350	-
Lophuromys sikapusi	Acomyinae	AJ250349	AJ012023
Uranomys ruddi	Acomyinae	X84388	-
Chionomys nivalis	Arvicolinae	X99464	-
Clethrionomys glareolus	Arvicolinae	AJ250356	AF318585
Volemys kikuchii	Arvicolinae	AF348082	AF348082
Calomyscus baluchi	Calomyscinae	-	AY288509
Cricetulus griseus	Cricetinae	AY012116	AB033693
Cricetulus migratorius	Cricetinae	X84389	AY288508
Mesocricetus auratus	Cricetinae	X84390	AF119265
Phodopus campbelli	Cricetinae	-	AF119278

Cricetomys gambianus	Cricetomyinae	X99461	AF160614
Saccostomus sp.	Cricetomyinae	AJ250353	-
Dendromus mystacalis	Dendromurinae	AJ250352	-
Steatomys parvus	Dendromurinae	-	AF160599
Steatomys sp.	Dendromurinae	AJ250351	-
Gerbillus nigeriae	Gerbillinae	X84381	AF141226
Tatera kempi	Gerbillinae	X84391	AJ012024
Leopoldamys edwarsi	Murinae	X84386	-
Mus musculus	Murinae	AB042432	AB042432
Niviventer cremoriventer	Murinae	AJ005779	-
Rattus norvegicus	Murinae	AY012115	AB033713
Mystromys albicaudatus	Mystromyinae	AJ250354	AF160607
Macrotarsomys ingens	Nesomyinae	X99460	-
Macrotarsomys bastardi	Nesomyinae	-	AF160579
Nesomys rufus	Nesomyinae	X99462	AF160592
Petromyscus collinus	Petromyscinae	-	AF160600
Rhizomys pruinosus	Rhizomyinae	AJ250358	-
Rhizomys sinensis	Rhizomyinae	AF326254	AF326274
Akodon jelskii	Sigmodontinae	AJ005782	M35714
Peromyscus leucopus	Sigmodontinae	X99463	AF131926
Nannospalax ehrenbergi	Spalacinae	AJ250357	AF155871
Jaculus jaculus	Dipodidae	U67296	-

Zapus trinotatus	Dipodidae	-	AF119262
Glis glis	Gliridae	NC_001892	NC_001892
Pedetes capensis	Pedetidae	AY012113	-

Chapter 5

Revisiting the *Mus* – *Rattus* divergence in light of advances in murid and basal rodent phylogenetics

Abstract -

The murine genera *Mus* and *Rattus* are thought to have diverged about 12 million years ago (Ma) based on the traditional interpretation of a series of fossils from the Siwaliks of Pakistan. The molecular-based discovery that the spiny mice, genus *Acomys*, and their relatives are more related to the gerbils than to the Murinae casts doubt on the use of the 12 Ma date as a *Mus* – *Rattus* divergence. *Acomys* possesses the same murine tooth morphology as the true Murinae (such as *Mus* and *Rattus*) and had been considered to be a close relative of *Mus*. Equally parsimonious hypotheses can be proposed which place *Progonomys* as basal to the family Muridae (including *Acomys*, gerbils, *Mus*, *Rattus*, and their relatives), basal to the subfamily Murinae (including *Mus*, *Rattus* and their relatives), or at the *Mus* - *Rattus* divergence to the exclusion of more basal Murinae. We here test among the potential positions using two datasets, one that employs a series of well-corroborated fossils that are only distantly related to the Muridae and another that involves a dense taxon sampling within the Muridae, but with a potentially less reliable set of fossils. Our results indicate that the family Muridae probably diverged earlier than the dates suggested by the Siwalik fossils. *Mus* and *Rattus*, however, appear to have diverged at about the same time or just prior to the 12 Ma date suggested by the appearance of *Progonomys*. We also cannot reject the hypothesis that the 12 Ma date

represents the oldest split in the Murinae instead of the more derived *Mus – Rattus* date. In addition to testing divergence dates, we recover interesting phylogenetic results suggesting that *Taterillus* is more closely related to members of the tribe Gerbillini than to other genera that have traditionally been treated as Taterillini. Additionally, our results suggest that the genus *Gerbilliscus* is paraphyletic as *Gerbillurus* is more related to *Gerbilliscus kempi* than either is to *G. robustus*.

INTRODUCTION

The family Muridae is perhaps the single most important family of mammals in laboratory science. Several genera of murids are used in experimental research such as *Acomys*, *Mastomys*, *Meriones*, *Mus*, *Psammomys*, and *Rattus* (Catzeflis et al., 1992; Walder et al., 2002). The genera *Mus* and *Rattus* specifically are of vital importance to numerous fields of biological sciences. Both have been the subjects of genome projects (Bouchie, 1999; Chinwalla et al., 2002) and the information gained from study of these two taxa has led to advancement in a vast array of biology related fields. Much of this research has had broader application to mammals as a whole (Bradley, 2002). Few advances in medicine and human biology have been made that did not involve preliminary or parallel study in a mouse or rat system.

The classic view of the origin of *Mus* and *Rattus* is that they are part of two separate radiations that arose from the earliest split of the subfamily Murinae. Jacobs (1978) and other paleontological studies (Jaeger et al., 1986; Flynn et al., 1990; Jacobs and Downs, 1994; Jacobs and Flynn, 2005) have estimated the *Mus* - *Rattus* divergence

date as having occurred 10-14 million years ago (Ma) based on the temporally well-defined Siwalik fossil series from Pakistan (Fig. 1a). Jacobs and Downs (1994) describe the transition of molar characters from the plesiomorphic condition found in *Potwarmus* 14.4 Ma through transitionary intermediates to the first appearance of *Antemus*, the presumed ancestor of all murines, 14.0 Ma (Flynn et al., 1990; Jacobs and Flynn, 2005). The earliest species in the genus *Progonomys*, the first fully modern murine, appeared by 12.3 Ma (Jacobs and Flynn, 2005). Later species of *Progonomys*, thought to be on the line leading to Mus, appeared at 10.4 Ma, and *Karnimata*, the presumed ancestor of *Rattus*, appeared by 11.1 Ma (Jacobs and Flynn, 2005). Benton and Donaghue (2007) define the hard minimum value of this divergence time to be represented by the first appearance of *Karnimata* 11.1 Ma, and the soft maximum to be at the first appearance of modern murines, early forms of *Progonomys*, at 12.3 Ma.

Because of the quality of this fossil series and the importance of these species, the 12 Ma *Mus* - *Rattus* divergence date has become one of the most widely used calibration points for molecular clocks and studies of molecular evolution (Catzeflis et al., 1987; Li et al., 1987; Furano et al., 1994; Nedbal et al., 1994; Adkins et al., 1996; Agulnik and Silver, 1996; Dubois et al., 1996; Edwards et al., 1997; Robinson et al., 1997; Huchon et al., 2000; Martin et al., 2000; Michaux and Catzeflis, 2000; Suzuki et al., 2000; Barome et al., 2001a, 2001b; Chevret et al., 2001; Ducroz et al., 2001; Fadda et al., 2001; Michaux et al., 2001; Weinreich, 2001; Huchon et al., 2002; Michaux et al., 2002; Smith and Eyre-Walker, 2002).

Numerous studies have used other calibration points to estimate the time of divergence between *Mus* and *Rattus* with estimates ranging from 11.5-86.9 Ma (O'hUigin and Li, 1992; Janke et al., 1994; Frye and Hedges, 1995; Kumar and Hedges, 1998; Messer et al., 1998; Cao et al., 2000; Huchon et al., 2000; Michaux and Catzeflis, 2000; Yoder and Yang, 2000; Adkins et al., 2001; Ducroz et al., 2001; Michaux et al., 2001; Nei et al., 2001; Nikaido et al., 2001; Michaux et al., 2002; Montelgard et al., 2002; Nei and Glazko, 2002; Adkins et al., 2003; Springer et al., 2003; Bininda-Emonds et al., 2007). The vast majority of these studies estimate this divergence to be well above 11 Ma.

Recent molecular systematic studies of muroids have shed additional doubt on the current interpretation of the fossil record. A series of DNA-DNA hybridization studies (Chevret et al., 1993; Denys et al., 1995), DNA sequencing studies (Agulnik and Silver, 1996; Chevret et al., 2001; Michaux et al., 2001; Jansa and Weksler, 2004; Steppan et al., 2004), and other molecular studies (Furano et al., 1994; Usdin et al., 1995) have demonstrated that the spiny mouse, *Acomys*, is more closely related to the gerbils than to the Murinae (Fig. 1b). This led researchers (Michaux et al., 2001; Steppan et al., 2004; Musser and Carleton, 2005) to recognize a new subfamily, Deomyinae, which contains *Acomys* and related genera. We use the taxonomy of Musser and Carleton (2005) here, including their informal use of divisions of genera, except where specifically noted.

The molar morphology of *Acomys* is extremely similar to *Mus*. Jacobs (1978) considered the genus *Acomys* to be sister to *Mus* and suggested that both were derived from *Progonomys debruijni* (Fig. 1a). Under that interpretation, the divergence time

between *Acomys* and *Mus* should be about 8.5 Ma while *Acomys* and *Rattus* would have diverged when *Mus* and *Rattus* diverged 11.1-12.3 Ma. Subsequent morphological studies have also supported the affinity of *Acomys* with the murines (Denys et al., 1992; Denys et al., 1995; Xu et al., 1996) and none have suggested a reinterpretation of the Siwalik fossil series.

If only extant taxa are considered, multiple equally parsimonious explanations exist for the extreme similarity seen between *Acomys* and *Mus*. The *Mus*-like molar could be the plesiomorphic state for the family Muridae and could have evolved into the derived tooth morphology seen in gerbils. Under this scenario, a *Progonomys* – like ancestor would have given rise to all taxa in this clade (Fig. 1b). Alternatively, the *Mus*-like molar may have evolved independently in both the murines and deomyines. *Antemus* and early *Progonomys* could be the ancestors to the Murinae. Even under this scenario, the use of *Karnimata* and later species of *Progonomys* to represent the ancestors of *Rattus* and *Mus* respectively may be problematic, as recent molecular results have suggested that the split between *Mus* and *Rattus* does not represent the earliest divergence among the Murinae. Instead a clade of Philippine endemic rodents including *Phloeomys* and *Batomys* represents the most basal lineage of murines (Jansa and Weksler, 2004; Steppan et al., 2004; Steppan et al., 2005; Jansa et al., 2006; Rowe et al., 2008). Steppan et al. (2004) and Jansa et al. (2006) chose to use the Siwalik fossil series as a calibration point to represent the split between this Philippine clade and the remaining murines. The 11.1-12.3 Ma date may therefore apply to a Deomyinae – Murinae split, a *Phloeomys* – *Rattus* split, or a *Mus* – *Rattus* split (Fig. 1b).

The ideal scenario for evaluating among these three hypotheses would be to use a series of well-established and highly corroborated fossil calibration points from within the Muridae that do not involve the controversial taxa and characters. The Muridae is the largest family of rodents with over 730 species in 150 genera (Carleton and Musser, 2005) found in three major subfamilies, with as many as three additional small subfamilies whose inclusion (Leimacomyinae and Lophiomyinae) or rank (Otomyinae) is controversial (Jansa and Weksler, 2004; Carleton and Musser, 2005). This family has received only limited attention in molecular studies, and many phylogenetic relationships remain uncertain. There are a limited number of well-dated murid fossils whose phylogenetic position is relatively uncontroversial. As a result, we have chosen to evaluate these dates using two datasets. The first employs a series of well-corroborated fossils representing divergences of rodents in the Eocene. These divergences are much older than the splits in question in the Muridae, but represent a high quality fossil record. The second dataset employs a more limited set of calibration points, but involves dense taxon sampling within the Muridae.

MATERIALS AND METHODS

Basal rodent dataset

We added previously published sequences for the brown rat, *Rattus norvegicus*, to the dataset of basal rodents used by Norris et al. (chapter 2). GenBank accession numbers are shown in Table 1. Norris et al. (chapter 2) were able to verify the reliability of 8 fossil calibration points representing evolutionary splits of rodents that took place in

the Eocene. Two analyses were performed in BEAST (version 1.4; Drummond and Rambaut, 2007) as described in Norris et al. (chapter 2) with the addition of sequences for *Rattus*. Tree topology was constrained to match that used by Norris et al. (Fig. 1 in chapter 2). GTR + I + Γ was used as the model of evolution as determined by Modeltest 3.04 (Posada and Crandall, 1998), and data were partitioned by gene and the program optimized the model parameters by gene. The uncorrelated lognormal relaxed molecular clock model was used and the mean substitution rate was not fixed. Exponential priors were used for 8 rodent calibrations such that the "zero offset" parameter was equal to the minimum divergence date estimate based on fossils. The upper 95% confidence interval was set to be equal to the size of the range + 12.3 million years, a value estimated by Norris et al. (chapter 2) to represent the upper 95% confidence interval on gap size of the rodent fossil record in the Eocene. Universal priors were set in outgroup taxa to range between fossil estimates and molecular estimates. Fossil calibration settings are shown in Table 2. With the exception of the *Rattus* sequences, these setting are identical to Norris et al. (chapter 2). The program was run for 5,000,000 generations, sampled every 1,000 generations with a burnin of 1,000.

Muridae dataset

Steppan et al. (2005) and Rowe et al. (2008) assessed relationships among members of the subfamily Murinae, particularly Asian and Australasian taxa, using several genes. We here expand their taxon sampling to: 1.) include a wider diversity within other murid subfamilies, Gerbillinae and Deomyinae, 2.) expand taxon sampling

of underrepresented clades of murines, such as African taxa, 3.) break up long branches, and 4.) allow more fossil calibration points within murids to be included. A 1336 bp segment of the mitochondrial genome was used to evaluate relationships and estimate divergence times among murids. This region contains all or part of the protein coding genes COX1, COX2, and ATPase 8 as well as three transfer RNAs: tRNA-Ser, tRNA-Asp, and tRNA-Lys. GenBank accession numbers for previously published samples are shown in Table 3.

This region of the mitochondrial genome was sequenced in whole or part for 34 individual murid rodents (Table 4 and Table 5). Tissues had been stored in either ethanol or lysis buffer and are cataloged at the University of Vermont or the Carnegie Museum of Natural History. Several were the product of recent collecting trips to Guinea and Sierra Leone (Norris, 2006; Decher et al., 2007; Decher et al., 2008.). DNA was extracted using the DNEasy QIAGEN kit. The primers and PCR protocols of Steppan et al. (2005) were used with the addition of multiple primers modified from their published primers. The following additional primers were used: 7101Fmod: AYAAAYTTYCAYGAYCAYACNCTNATAAT (modified from 7101F), 7481Rmod: GCTCATGAGTGNAGNACNTCTTC (modified from 7481R), 7927Rmod: GAGGNRAATARRTTTTCGTTCATTT (modified from 7927R). PCR was performed using Illustra puReTaq Ready-To Go PCR Beads. Double stranded PCR products were purified using PEG precipitation (Maniatis *et al*. 1982). Sequencing was performed on an ABI 3130x1 Genetic Analyzer using dye terminator (ABI PRISM) cycle sequencing. The same primers were used for cycle sequencing as are listed above for PCR

Sequences were aligned by eye in MacClade (Maddison and Maddison, 1989). Because of the potential for confounding mutation rate with substitution rate (Ho and Larson, 2006) and the separate settings required to correct for this problem in BEAST, intraspecific variation was eliminated by limiting the phylogenetic analyses to a single individual in each species. Excluded samples are listed in Table 5. Two exceptions were made. Two clades identified as *Gerbilliscus kempi* exhibited a level of sequence divergence comparable to other between species splits. Considering the potential that a less common West African *Gerbilliscus* species may have been mistaken for *G. kempi* we included both. The same degree of high sequence divergence was also true for two clades of *Gerbillus gerbillus*. Two individuals were included from each of these species. The sample identified in GenBank as *Gerbillurus vallianus* [sic] (accession # EU349708) exhibited an identical haplotype to one of the *Gerbillus gerbillus* samples and was highly divergent from other *Gerbillurus*, including *Gerbillurus vallinus*, and was also excluded from our analysis. The final dataset contained 86 taxa including 4 outgroup taxa (Table 3 and Table 4).

A maximum likelihood tree was constructed using GARLi (version 0.951; Zwickl, 2006). Nodal support was determined using 100 bootstrap replicates in GARLi. These results are shown in Figure 2. Nodes supported by >75% bootstrap percentage were constrained in all subsequent BEAST analyses. Nodal support was also evaluated using MP bootstrapping in PAUP* (version 4.0b8, Swofford, 2002). The following additional nodes were constrained to be monophyletic due to their consistently strong support in other analyses that involve multiple genetic markers and slower evolving

markers: Cricetidae (Michaux et al., 2001; Jansa and Weksler, 2004; Steppan et al., 2004), Deomyinae + Gerbillinae (Michaux et al., 2001; Jansa and Weksler, 2004; Steppan et al., 2004), Deomyinae (Michaux et al., 2001; Jansa and Weksler, 2004; Steppan et al., 2004), Murinae (Michaux et al., 2001; Jansa and Weksler, 2004; Steppan et al., 2004), and all murines except the Phloeomys Division (Jansa and Weksler, 2004; Steppan et al., 2004; 2005; Rowe et al., 2008). These basal relationships are well established and our results do not exhibit well-supported conflict with these earlier studies. Additionally, because we have added no additional taxa sampling to these groups and because of the use of multiple genes in prior studies, we imposed monophyly on the following well-supported clades of Phillippine and Sahul murines found in Steppan et al. (2005) and Rowe et al. (2008): a Sahul + Chrotomys Division clade, a Chrotomys Division clade (*Apomys* + *Rhynchomys*), a Sahul clade (Hydromys Division + Lorentzimys Division + Pogonomys Division + Pseudomys Division + Uromys Division + Xeromys Division), a Uromys Division clade (*Melomys* + *Paramelomys* + *Uromys*), a *Conilurus* + *Mesembriomys* + *Leporillus* clade, a *Leptomys* + *Parahydromys* clade, a *Lorentzimys* + *Anisomys* + *Chiruromys* + *Hyomys* + *Macrurumys* + *Pogonomys* clade, and an *Abeleomelomys* + *Mallomys* + *Mammelomys* clade. Our inclusion of additional taxa to other groups of murines prevented us from constraining any other nodes regardless of support in these prior studies. The Markov chain in the BEAST analyses was therefore permitted to sample trees that not only varied in substitution rate and model parameter, but in tree topology at these unconstrained nodes.

We initially performed a BEAST analysis in the absence of fossil constraints in order to generate an ultrametric tree where branch lengths represent relative time instead of absolute times. The age of the root was set with a prior of normal distribution where mean = 100.0 and standard deviation = 0.01 in order to yield results that round to 100.0 within two decimal places. GTR + I + Γ was used as the model of evolution and a Yule process of speciation (as recommended in the BEAST manual for interspecific taxa). The uncorrelated exponential relaxed molecular clock model was used and the mean substitution rate was not fixed. An exponential distribution of substitution rates is probably a more realistic shape for this dataset, because of the potential that many of the divergence times may have taken place as recently as one million years ago (Ho et al., 2007). A repeat of the analysis using a lognormal distribution yielded significantly worse likelihood scores. The program was run for 5,000,000 generations, sampled every 1,000 generations with a burnin of 1,000.

First appearance dates for many lineages were estimated based on survey of the literature and are listed in Table 6. The Siwalik series of fossils involving *Antemus*, *Progonomys*, and *Karnimata* was excluded because our goal was to evaluate their position. Tong and Jaeger (1993) suggest that an early myocricetontine dated at 16 Ma represents the date of divergence between the Gerbillinae and the other Muridae. If valid, this fossil provides strong evidence against the interpretation of the *Antemus* – *Progonomys* series at the base of the Muridae. We have excluded this potential calibration point from our analyses, because of its direct involvement in the hypothesis with the goal of evaluating it as well. Additionally, we treated the first appearance of

Rattus in the fossil record at 3 Ma (Zheng, 1993; Chaimanee et al., 1996; Benton and Donoghue, 2007) as the first appearance of the Rattus Division instead of the genus *s.s*. This group contains a number of specialized genera that have frequently been included in the genus *Rattus* and the current definition of the genus is still potentially p=araphyletic (Musser and Carleton, 2005).

Absolute dates of divergence times within the Muridae based on fossil results were compared to relative branch lengths obtained from the BEAST analysis using linear regression (Conroy and van Tuinen, 2003) in the statistical package JMP (version 5.0.1.2, SAS Institute Inc.). The regression was restricted to calibration points within the Muridae, because of concerns that substitution rates vary when recent divergences are included (Ho and Larson, 2006) and that saturation of molecular data or accelerated evolutionary rates during periods of rapid diversification (Norris et al. chapter 2) may influence the results across long time scales. Additionally, tree hierarchical problems (Norris et al., chapter 2) ensure that any regression involving old fossils and basal branches combined with very recent evolutionary events and tip branches are likely to generate a significant outcome regardless of the validity of fossils. This set of murid fossils involves ages less than 10 Ma and includes nesting of clades at only a single level.

For analyses involving multiple fossil calibrations, Near et al. (2005) suggested an iterative approach to removing successive inconsistent calibration points until the remaining calibrations were in agreement. Marshall (2008) argued that their approach was flawed because it did not distinguish between calibration points that are too old relative to the remaining fossils and those that are too young. Fossil calibrations

represent minimum estimates for divergence times and, unless an extinct taxon has been misplaced phylogenetically, these calibrations can only be too young. He modified the approach of Near et al. (2005) so that only fossils that are too young are removed in successive iterations. We would argue that there are two major problems with Marshall's (2008) approach. First, although his approach identifies and removes extremely old fossils that represent statistical outliers, it essentially calibrates the tree using the single fossil that produces the oldest estimate. Such an approach assumes that the accuracy of the molecular clock analysis is absolute and that all error derives from the fossils. The second problem stems from the potential for periods of explosive diversification, saturated data, or the appearance of accelerated evolution at the tip of the tree to influence the outcome. His approach is mathematically equivalent to forcing the regression analysis to pass through the point of origin. This restriction may be biologically unrealistic due to the problems noted above (see Norris et al., chapter 2 for further discussion).

We restricted the fossil calibration points to a more realistic dataset by removing inconsistent fossils that were too young using successive regression analyses. A simple regression was performed using JMP, and 95% C.I. around the resulting line was calculated. All fossil calibrations that fell outside of the 95% C.I. and were too young were removed. The regression was repeated and more fossils removed until all fossil calibration points fell within the 95% C.I. The six remaining murid fossil calibrations were the first appearance of *Desmodillus* at 3.5 Ma, *Apodemus* at 9.7 Ma, *Rhabdomys* at

3.5 Ma, *Leopoldamys* at 1.81 Ma, *Zelotomys* at 1.81 Ma, and *Arvicanthis* and *Lemniscomys* (sister taxa known from the same formation) at 2.95 Ma.

A final BEAST analysis was performed using absolute dates obtained from the fossils listed as "used" in Table 6. The root of the tree was fixed at 45 Ma based on the well-corroborated Dipodidae – Muroidea divergence (Norris et al., chapter 2). The six murid fossils identified by the successive regressions were also fixed (normal distribution with a standard deviation of 0.01). We excluded the Spalacidae – Eumuroida calibration point because it fell outside the 95% C.I. when a regression was performed that included the two basal calibrations and the 6 murid calibrations. All other input parameters are as described above for the prior BEAST analysis.

RESULTS

Basal rodent dataset

The results of the BEAST analysis for the basal rodent dataset are shown in Figure 2. Estimates for nodes are consistent with the results of Norris et al. (chapter 2). The best tree generated in the analysis yielded a divergence time for *Mus – Rattus* of 16.0 Ma (Table 7). The 95% confidence interval ranges from 13.8 Ma to 20.7 Ma. All trees sampled produced a *Mus – Rattus* divergence date between 12.2 and 23.8 Ma.

Murid dataset

The maximum likelihood tree is shown in Figure 3. Most of the phylogenetic implications involve taxa that derive from Steppan et al. (2005) and Rowe et al. (2008)

and are discussed there. Limited information can be obtained regarding those taxa that are novel to this study, as much of the tree is unresolved. Monophyly of the genera *Mus*, *Rattus*, *Malacomys*, *Lophuromys*, *Gerbillurus*, *Gerbillus*, and *Meriones* is supported with bootstrap values greater than 90%. The subgenera *Mus* (*M. musculus* and *M. terricolor*) and *Gerbillurus* (*G. setzeri* and *G. vallinus*) are also supported as monophyletic with bootstrap support >90%. *Taterillus* was found to belong to a clade including members of the tribe Merionini (bootstrap = 100%) as opposed to the clade that contains the remaining members of the Taterillini. Monophyly of *Gerbilliscus* was not recovered, as *Gerbillurus* was sister to *Gerbilliscus kempi*, albeit with more limited support (bootstrap = 83%). Monophyly of *Gerbillus gerbillus* was also refuted, but support was relatively poor (76%).

The results of the BEAST analysis that did not employ fossil calibrations are shown in Figure 4. Bayesian posterior probability support is shown. Nodes constrained in the analysis are indicated with a circled number 1. No significant correlation was found between branch lengths and fossil dates in murids (Figure 5a, $R^2 = 0.121$ P = 0.156), in spite of the presence of multiple situations where both an ancestral clade (such as the first appearance of *Apodemus*) and its descendent clade (such as a dated *Apodemus agrarius – A. semotus* clade) were included in the dataset. Six fossil calibrations remained after the successive removal of excessively young calibrations. Regression of the six fossils with branch lengths is shown in Figure 5b ($R^2 = 0.982$, P = 0.0001).

Final age estimates based on the second BEAST analysis are shown in Figure 6 and Table 7. Certain nodes were recovered in the best tree, but in less than 50% of

sampled trees. BEAST did not calculate a 95% confidence interval for these nodes and no error bar is shown. The timing of the first appearance of *Progonomys* at 12.3 Ma, the soft maximum for the *Mus – Rattus* date according to Benton and Donoghue (2007), relative to these BEAST results is also shown. The use of any of the three fossils - *Karnimata* at 11.1 Ma, *Progonomys* at 12.3 Ma, or *Antemus* at 14 Ma - cannot be rejected as the appropriate date for the *Mus – Rattus* split based on our results. *Progonomys* at 12.3 Ma and *Antemus* at 14 Ma cannot be rejected as appropriate dates for the earliest split in the Murinae (Phloeomys Division vs. all other murines). In contrast, our results reject the use of *Progonomys* at 12.3 Ma as a calibration point for the Murinae vs. Deomyinae + Gerbillinae divergence. The first appearance of *Antemus* at 14 Ma does, however, fall within this 95% confidence interval.

Tong and Jaeger (1993) suggested that an early myocricetontine dated at 16 Ma represents the date of divergence between the Gerbillinae and Deomyinae. We excluded this date as a calibration point because of its direct conflict with hypotheses shown in Figure 1. Our results are highly consistent with the concept of a 16 Ma date of divergence between Gerbillinae and Deomyinae as the estimate from the best tree obtained was 15.9 Ma (95% confidence interval ranges from 12.4-20.0).

DISCUSSION

Phylogenetic implications

Phylogeny was not explicitly tested using the basal rodent dataset as all nodes were constrained to be consistent with the results of Norris et al. (chapter 2). The murid

dataset yielded a tree that showed poor resolution across many nodes and was primarily constitent with the results of Steppan et al. (2005) and Rowe et al. (2008) from whence many of these sequences derive. Nevertheless, a few interesting results were recovered due to our expanded taxon sampling within the Gerbillinae. Two extensive morphological studies have been conducted attempting to resolve the relationships among genera in this subfamily (Tong, 1989; Pavlinov et al., 1990). Both studies proposed that gerbillines were comprised of three major groups, which Musser and Carleton (2005) defined as tribes. The Ammodillini is monotypic and was not sampled in this study. The composition of remaining two, Gerbillini and Taterillini, are roughly equivalent to clades proposed by both Tong (1989) and Pavlinov et al. (1990), but the two differ in the placement of *Desmodillus*. Tong (1989) proposed that the genus held a basal position within a clade that corresponds with Musser and Carleton's (2005) Gerbillini, whereas Pavlinov et al. (1990) suggested it was sister to *Gerbillurus*, a member of the Taterillini (which they treated as a subfamily). Our data suggest that *Desmodillus* belongs in a clade comprised of members of Musser and Carleton's (2005) tribe Taterillini (ML BP = 81%, MP BP <50%), but is not particularly related to *Gerbillurus* (a hypothesis rejected by ML BP = 83%, MP BP = 89%). A similar position for *Desmodilus* has been reported in other studies based on the mitochondrial 12S and cytochrome b genes (Chevret and Dobigny, 2005) and nuclear GHR and IRBP genes (Lecompte et al., 2008).

Our results strongly suggest (ML BP = 100%, MP BP = 80%) that *Taterillus* is a member of a clade containing the Gerbillini and is not allied with the remaining members of Taterillini (as defined by Musser and Carleton, 2005). Chevret and Dobigny (2005)

came to a similar conclusion with extremely strong nodal support. A position of *Gerbillurus* nested within the genus *Gerbilliscus* is also suggested by our data (ML BP = 83%, MP BP = 89%). Colangelo et al. (2007) recovered a similar pattern of *Gerbillurus* species sister to *Gerbilliscus kempi* and relatives to the exclusion of *Gerbilliscus robustus* and other *Gerbilliscus*, but also with limited support.

Two species, *Gerbilliscus kempi* and *Gerbillus gerbillus*, had individuals with highly divergent haplotypes. The molecular clock analysis suggested a divergence time of 4.3 Ma (95% C.I. = 1.1 - 4.0 Ma) and 4.1 Ma (95% C.I. = 2.0 - 5.2 Ma) for the *Gerbilliscus kempi* and *Gerbillus gerbillus* individuals respectively. These values are comparable to between species or between genera divergence times in other murids. Greater taxon sampling within the respective genera combined with reduced taxon sampling within the Gerbillinae relative to the Murinae may be biasing these results. Nevertheless, these results may suggest either an error in initial identification or the presence of additional undescribed species. More molecular work is clearly required on the evolutionary relationships Gerbillinae, which remain largely overlooked in molecular systematic studies in spite of their considerable diversity (103 species in 16 genera; Musser and Carleton, 2005). Only a single study has been published using sequence data to specifically investigate the relationships among genera in this subfamily (Chevret and Dobigny, 2005), although two have been published investigating the relationships in *Gerbilliscus* and *Gerbillurus* (Colangelo et al., 2005; 2007).

Dating murid evolutionary splits

The two approaches presented here produced distinct, but overlapping, estimates for the divergence time between *Mus* and *Rattus*. A strict application of the confidence intervals of the results obtained from the two datasets might suggest a divergence time of 13.8 - 14.4 Ma, a date highly consistent with the origin of *Antemus* at 14 Ma. Numerous authors have noted that, although *Antemus* appears to be related to the lineage leading to murines, it lacks the full three chevrons on M^1 that is characteristic of modern Murinae (Jacobs and Downs, 1994; Jacobs and Flynn, 2005). This character appears in the earliest *Progonomys* at 12.3 Ma, leading to the interpretation that *Progonomys* represents the oldest taxon that contains the full suite of characters found in extant murines. Benton and Donoghue (2007) termed the first appearance of *Progonomys* and not *Antemus* as the soft maximum for the paleontological estimate for the *Mus – Rattus* split for this reason.

Ultimately the very limited conflict between the results obtained from these two datasets may simply be an artifact of the treatment of calibration points. Norris et al. (chapter 2) emphasized the potential for the discovery of new fossils to change any existing fossil calibrations and attempted to incorporate that possibility into the analyses. This was achieved by using an exponential prior in BEAST with a 95% confidence interval that matches the 95% confidence interval on an estimate of the size of gaps in the Eocene fossil record of rodents. We repeated that approach for the basal rodent dataset in this study, but not for the murid dataset. No attempt was made to quantify calibration uncertainty in Muridae fossils because these relationships are so poorly understood and the ghost lineage approach of Norris et al. (chapter 2) requires a prior understanding of

tree topology and proper placement of fossil taxa within that known phylogeny. Graur and Martin (2003) describe these calibration points as a date +/- 0, and warn against the illusion of precision in such studies. Since only minimum dates for the intervals of these fossils were used and no potential for fossil uncertainty was incorporated, the murid dataset is probably best viewed as a rough minimum estimate. The calibration points in the basal rodent dataset probably represent a more reasonable estimate or slight overestimate.

Both analyses suffer from further problems. The analysis of the basal rodent dataset attempts to use dated evolutionary events from 33 to 55 Ma as calibrations to estimate an event that occurred about 10 to 15 Ma. Norris et al. (chapter 2) noted that periods of rapid evolution across multiple lineages and saturation of data that is inadequately corrected by the evolutionary model can lead to a directional bias in molecular clock estimates. This bias is presumably more pronounced as the time between the calibration point and the estimated event increases (see Fig. 6 in Norris et al., chapter 2). The murid dataset suffers from a severe problem with uncertainty in tree topology. This affects both the application of calibration points and the nodes estimated. For example the application of the 9.7 Ma first appearance date of *Apodemus* has the potential to have a different effect if *Apodemus* is considered to be basal to the *Mus* – *Rattus* split or if *Apodemus* is treated as more related to *Mus* than *Rattus*. *Apodemus* was treated as the latter, but neither our ML nor MP analyses provide any bootstrap support for either option.

Nevertheless, our results show a surprising convergence with dates suggested by paleontologists and appear to, in part, reject the alternate hypotheses proposed in Figure 1b. The first appearance of *Progonomys* at 12.3 Ma falls after the origin of the family Muridae at about 16.8 Ma (95% C.I. = 13.2 – 20.7 Ma). This would suggest that *Progonomys* is not on the direct line of ancestry for the deomyine genus *Acomys*, although the potential that it represents a sister taxon to the Muridae with a ghost lineage cannot be addressed by our data. The estimated date of 15.9 Ma (95% C.I. = 12.4 – 20.0 Ma) for the split between Deomyinae and Gerbillinae, along with a proposed first appearance date of the Gerbillinae lineage at 16 Ma (Tong and Jaeger, 1993) provide additional support rejecting the hypothesis that *Progonomys* is an ancestor of *Acomys*. Finally, in the only molecular study to include it, Jansa and Weksler (2004) recovered a sister relationship between the maned rat, *Lophiomys imhausi*, and the clade uniting the Deomyinae and Gerbillinae. Although they cited Jansa and Weksler (2004) elsewhere, Musser and Carleton (2005) did not make mention of their work in discussion of *Lophiomys* and treated *Lophiomys* as a member of the family Cricetidae. If, as strongly suggested by Jansa and Weksler (2004), *Lophiomys* holds a phylogenetic position nested within the Muridae, it provides further evidence against the presence of a murine-like tooth at the base of the Muridae. *Lophiomys* retains the primitive cricetid-like tooth morphology and its phylogenetic position led Jansa and Weksler (2004) to conclude that the murine-like tooth evolved independently in the Deomyinae and Murinae. The results of the analysis of the murid dataset also fail to reject the potential position of *Antemus* (14 Ma) at the base of the Muridae. It is unlikely, however, that a partial evolution of the

murine tooth occurred at the base of the family and completion of this trait occurred independently in the deomyines and murines, particularly if a reversal of the character took place in *Lophiomys*.

The results of our murid dataset cannot distinguish between a position of *Progonomys* (12.3 Ma) at the base of the Murinae (best estimate = 15.8 Ma, 95% C.I. = 11.7-17.8 Ma) and at the *Mus – Rattus* divergence (best estimate = 13.1, 95% C.I. = 10.7-14.4 Ma). That level of precision may be difficult to obtain in a molecular clock analysis, particularly one plagued with problems of poor resolution such as ours. We do see no reason to reject the idea of *Karnimata* (11.1 Ma) as an early member of the clade containing *Rattus* since we recovered a *Rattus – Maxomys* split at 9 Ma (95% C.I. = 6.5 – 11.8 Ma).

Although several recent studies have begun to treat the *Progonomys* calibration at 12 Ma as representing the earliest split in Murinae (Steppan et al., 2004; Jansa et al., 2006; Lecompte et al., 2008), we see no reason to reject the idea that it may hold a more nested position within the subfamily such as at the *Mus – Rattus* divergence. The earliest clade of murines, the Phloeomys Division, is restricted to the Philippines, a region with an essentially nonexistent small mammal fossil record (Heaney et al., 1998) and subject to considerable variation in exposed versus submerged land over time (Heaney, 1986). Other early Philippine endemics, as well as Sahul and eastern Indomalayan taxa also hold a relatively basal position among the murines (Steppan et al., 2005; Jansa et al., 2006; Rowe et al., 2008). The only murine groups with extensive species diversity west of the Himalayas are members of the African clades, *Apodemus*, *Mus*, *Millardia*, and the

monotypic genera *Golunda*, *Micromys*, and *Nesokia* (Corbet and Hill, 1992; Musser and Carleton, 2005). The bulk of murine diversity, particularly regarding early diverging clades is clearly east of the Himalayas. If only the distribution of extant taxa were considered relative to the phylogenetic tree, the origin of the Murinae might be considered to be farther east than Pakistan, perhaps even on Southeast Asian archipelagos. *Progonomys* may simply represent the recolonization of murines into South Central Asia from their point of origin farther east. The actual origin of the first true murine from *Antemus* or a relative of *Antemus* may have taken place earlier than 12.3 Ma in one of these areas with a poor fossil record or even on land now submerged. Under such a scenario, early *Progonomys* would represent a split near, but not at, the base of the Murinae.

Implications

The burgeoning field of comparative genomics makes frequent use of divergence time estimates to evaluate genetic information, but reliable divergence dates remain highly controversial. For example, McPartland et al. (2007) noted that Dorus et al. (2004), citing molecular estimates, relied on an assumption that the date of the *Homo – Macaca* divergence is roughly equivalent to that of *Mus – Rattus*, yet Benton and Donaghue (2007) suggest that the *Homo* - *Macaca* split is twice as old as the *Mus – Rattus* split. Whereas Dorus et al. (2004) calculated that murine rodents showed a rate of evolution across the endocannabinoid system that is 2.7 times that of primates, McPartland et al (2007) noted that a younger divergence date for *Mus – Rattus* would

indicate that murines actually evolve 5.4 times as fast in this system and discussed the implications. Such examples are rampant in the biomedical literature; resolution of important dates such as the *Mus – Rattus* divergence can improve the quality of research across several fields. The use of ages in excess of 20 Ma for a *Mus – Rattus* split is simply not supported by either paleontological or more robust molecular estimates.

We see no compelling reason to reject the hard minimum date of 11.1 Ma proposed by Benton and Donoghue (2007) for the divergence time between *Mus* and *Rattus*, but we cannot determine whether their "soft maximum" date of 12.3 Ma is better applied to the origin of the Murinae or to a more derived node such as the *Mus – Rattus* split. The use of a minimum date of 11.1 Ma for the origin of the clade leading to *Rattus* is probably the most conservative approach.

Our results also suggest that the first appearance of *Apodemus* at 9.7 Ma (Martin-Suàrez and Mein, 1998; Freudenthal and Martin-Suàrez, 1999), *Desmodillus* as 3.5 Ma (PBDB 59167: Muizon and Hendey, 1980), *Rhabdomys* at 3.5 Ma (PBDB 59167: Muizon and Hendey, 1980), *Leopoldamys* at 1.81 Ma (McKenna and Bell, 1997), *Zelotomys* at 1.81 Ma (Denys, 1999), and *Arvicanthis* and *Lemniscomys* at 2.95 Ma (PBDB 21546: Wesselman, 1984) may all prove to be useful calibration points in future studies on murid rodents. Our results were also highly consistent with the use of early myocricetodontines at 16 Ma (Tong and Jaeger, 1993) as a calibration point representing the Deomyinae – Gerbillinae split.

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FIGURE LEGENDS

FIGURE 1. Phylogenetic position of the Siwalik fossil series and evolution of the murine style molar. (a.) Traditional hypothesis based on fossils and morphology. *Antemus* first appears at 14.0 Ma, after the Murinae – Gerbillinae split. *Antemus* gives rise to the earliest modern murine rodent, *Progonomys*, 12.3 Ma. Early *Progonomys* gives rise to *Karnimata*, which eventually gives rise to *Rattus* and relatives. Early *Progonomys* also gives rise to later species of *Progonomys*, which are ancestors to both *Mus* and *Acomys*. (b.) Effect of molecular results on position of Siwalik series. Molecular results have demonstrated that *Acomys* is more closely related to the Gerbillinae than the Murinae. Molecular studies have also shown that the *Mus – Rattus* split is not the oldest divergence in the Murinae since the Phloeomys Division, a clade of Philippine endemics, holds a basal position. Three positions of *Progonomys* are possible: at the base of the family Muridae involving a reversion in tooth morphology in the Gerbillinae, at the base of the Murinae involving an independent evolution of the murine tooth in *Acomys*, and at its traditional position representing the *Mus – Rattus* split. Our study attempts to use a molecular clock to test where the 12.3 million year old early *Progonomys* fossils might fit.

FIGURE 2. Divergence times among major clades of rodents using a Bayesian approach to molecular clock in BEAST. Values at the nodes represent posterior divergence date estimates. Gray bars at nodes represent 95% confidence intervals for divergence date

estimates. The 12.3 Ma *Progonomys* fossil is younger than the 95% C.I. for the divergence of *Mus* and *Rattus*.

FIGURE 3. Maximum likelihood tree for the Muridae using mitochondrial data. Nodal support is indicated by color of circle at nodes. Black circles indicate bootstrap percentage >90% for both ML and MP. Dark gray indicates ML BP > 75% and MP BP > 50%. Light gray indicates ML BP > 75% and MP BP <50%. White circles indicate 50% < ML BP < 75% regardless of MP BP. All nodes supported by ML BP > 75% (black, dark gray, and light gray) were constrained in molecular clock analyses.

FIGURE 4. Ultrametric tree displaying relative ages as estimated in BEAST. Branch lengths indicate time relative to the root of the tree, but are not assigned absolute values because they were estimated in the absence of fossil calibration. Values at nodes indicate posterior probability value obtained from BEAST runs. Circled values correspond to those nodes where monophyly was enforced.

FIGURE 5. Regression analyses showing correlation between molecular results and murid fossils. (a.) Relationship between relative ultrametric branch length and all murid fossils (excluding dated node at the root of the tree). Molecular branch lengths are shown as a percentage of total rooted tree length. (b.) Relationship between relative ultrametric branch length and only those fossils used in the final analysis including the 45 Ma age at the root of the tree.

FIGURE 6. Ultrametric tree displaying absolute ages as estimated in BEAST when fossil calibrations are included. The branch leading to the outgroup has been cropped to improve visualization. Stars indicate nodes where a fossil calibration point was applied. Gray bars at nodes represent 95% C.I. of age estimate. A date of 12.3 Ma, corresponding with the earliest *Progonomys* fossils, is indicated with a gray vertical line. The three hypothesized phylogenetic positions of *Progonomys* from Figure 1 are shown with short gray bars.

FIGURE 1.

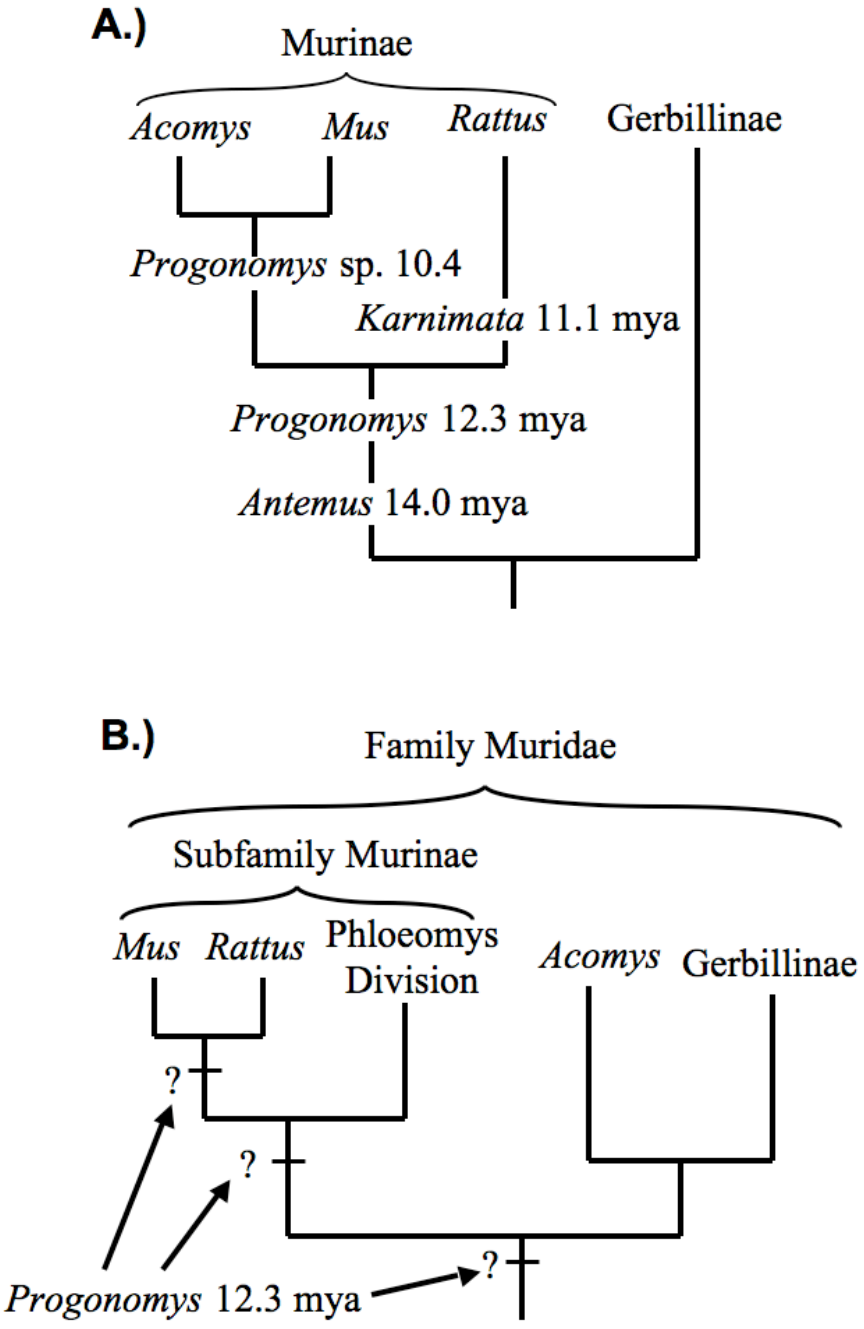

A.)
Murinae
Acomys
Mus
Rattus
Gerbillinae
Progonomys sp. 10.4
Karnimata 11.1 mya
Progonomys 12.3 mya
Antemus 14.0 mya
B.)
Family Muridae
Subfamily Murinae
Mus
Rattus
Phloeomys
Division
Acomys
Gerbillinae
?
?
?
Progonomys 12.3 mya

FIGURE 2.

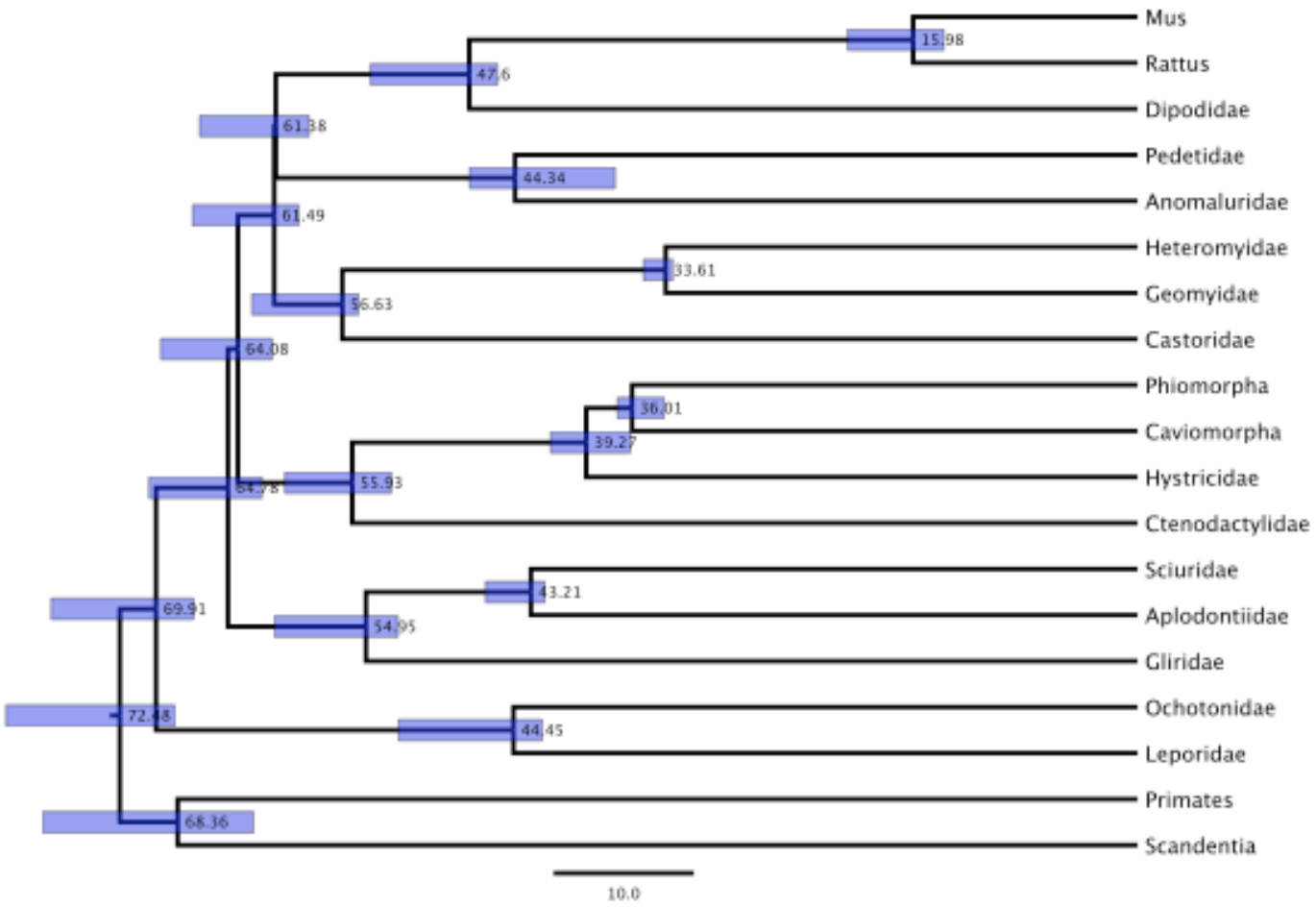

Mus
Rattus
Dipodidae
Pedetidae
Anomaluridae
Heteromyidae
Geomyidae
Castoridae
Phiomorpha
Caviomorpha
Hystricidae
Ctenodactylidae
Sciuridae
Aplodontiidae
Gliridae
Ochotonidae
Leporidae
Primates
Scandentia
15.98
47.6
61.38
44.34
61.49
33.61
56.63
64.08
36.01
39.27
55.93
43.21
69.91
54.95
72.48
44.45
68.36
10.0

FIGURE 3.

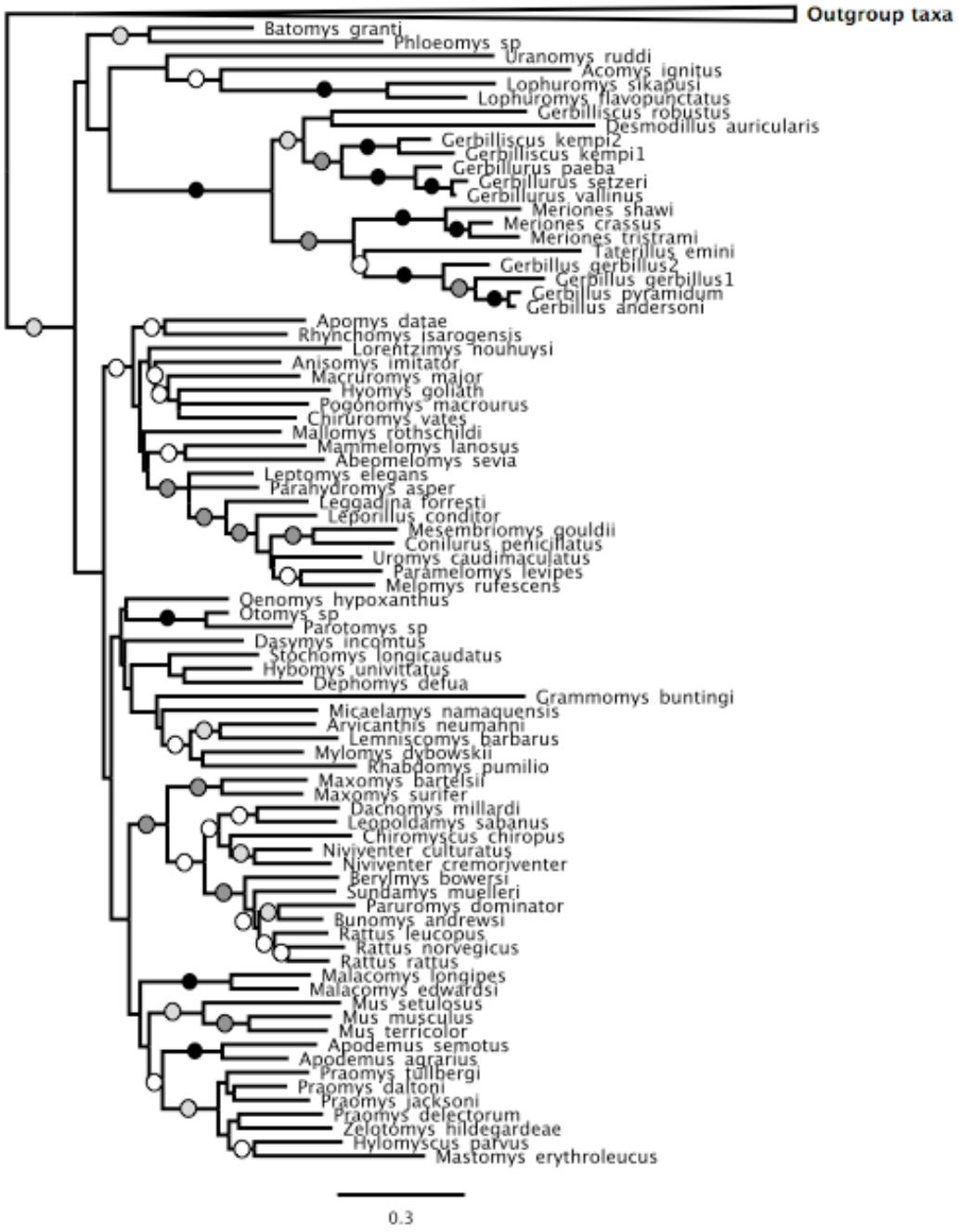
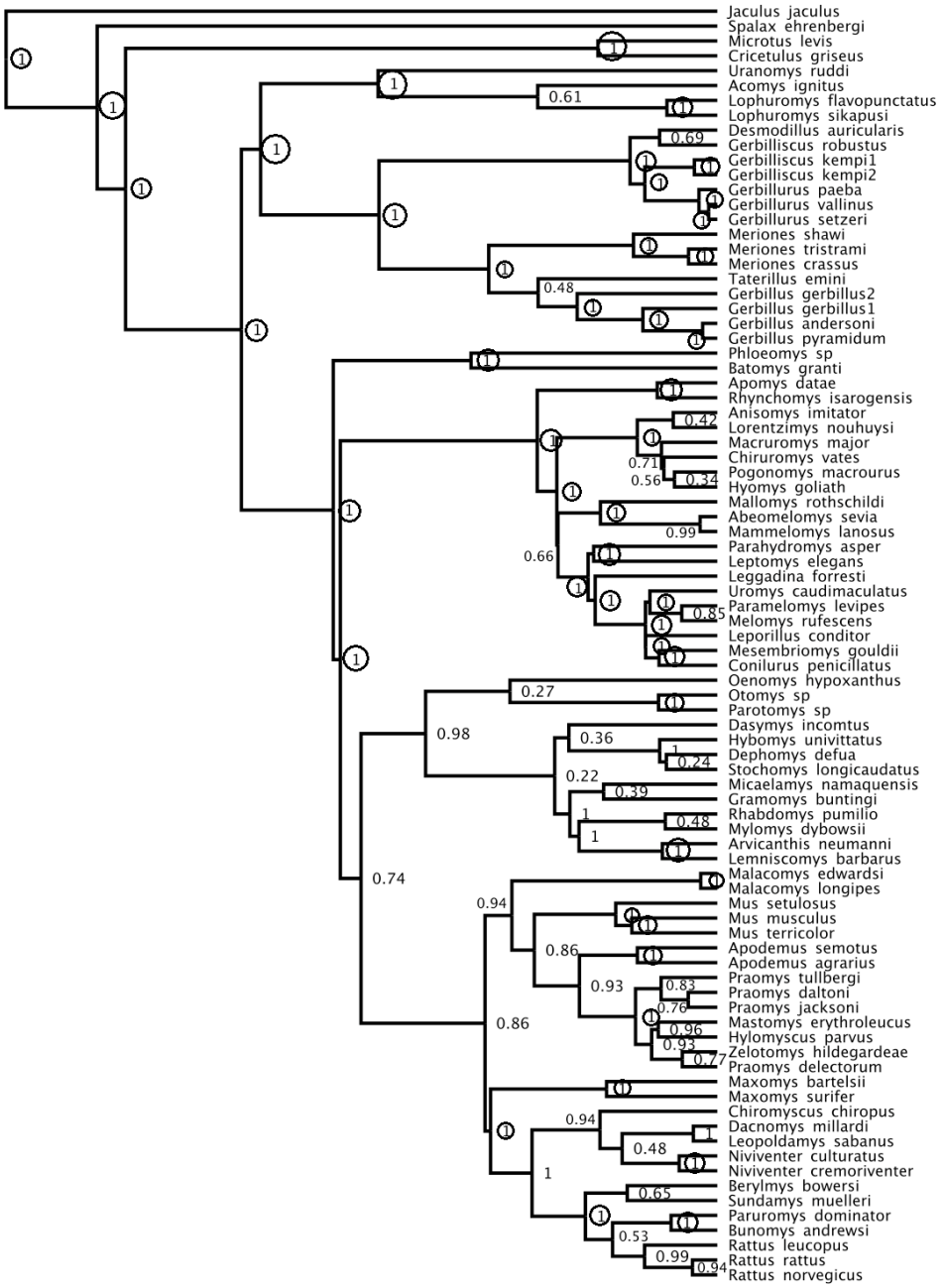

Outgroup taxa
Batomys granti
Phloeomys sp
Uranomys ruddi
Acomys ignitus
Lophuromys sikapusi
Lophuromys flavopunctatus
Gerbilliscus robustus
Desmodillus auricularis
Gerbilliscus kempi2
Gerbilliscus kempi1
Gerbillurus paeba
Gerbillurus setzeri
Gerbillurus vallinus
Meriones shawi
Meriones crassus
Meriones tristrami
Taterillus emini
Gerbillus gerbillus2
Gerbillus gerbillus1
Gerbillus pyramidum
Gerbillus andersoni
Apomys datae
Rhynchomys isarogensis
Lorentzimys nouhuysi
Anisomys imitator
Macruromys major
Hyomys goliath
Pogonomys macrourus
Chiruromys vates
Mallomys rothschildi
Mammelomys lanosus
Abeomelomys sevia
Leptomys elegans
Parahydromys asper
Leggadina forresti
Leporillus conditor
Mesembriomys gouldii
Conilurus penicillatus
Uromys caudimaculatus
Paramelomys levipes
Melomys rufescens
Oenomys hypoxanthus
Otomys sp
Parotomys sp
Dasymys incomtus
Stochomys longicaudatus
Hybomys univittatus
Dephomys defua
Grammomys buntingi
Micaelamys namaquensis
Aryicanthis neumanni
Lemniscomys barbarus
Mylomys dybowskii
Rhabdomys pumilio
Maxomys bartelsii
Maxomys surifer
Dacnomys millardi
Leopoldamys sabanus
Chiromyscus chiropus
Niviventer culturatus
Niviventer cremoriventer
Berylmys bowersi
Sundamys muelleri
Paruromys dominator
Bunomys andrewsi
Rattus leucopus
Rattus norvegicus
Rattus rattus
Malacomys longipes
Malacomys edwardsi
Mus setulosus
Mus musculus
Mus terricolor
Apodemus semotus
Apodemus agrarius
Praomys tullbergi
Praomys daltoni
Praomys jacksoni
Praomys delectorum
Zelotomys hildegardeae
Hylomyscus parvus
Mastomys erythroleucus
0.3

FIGURE 4.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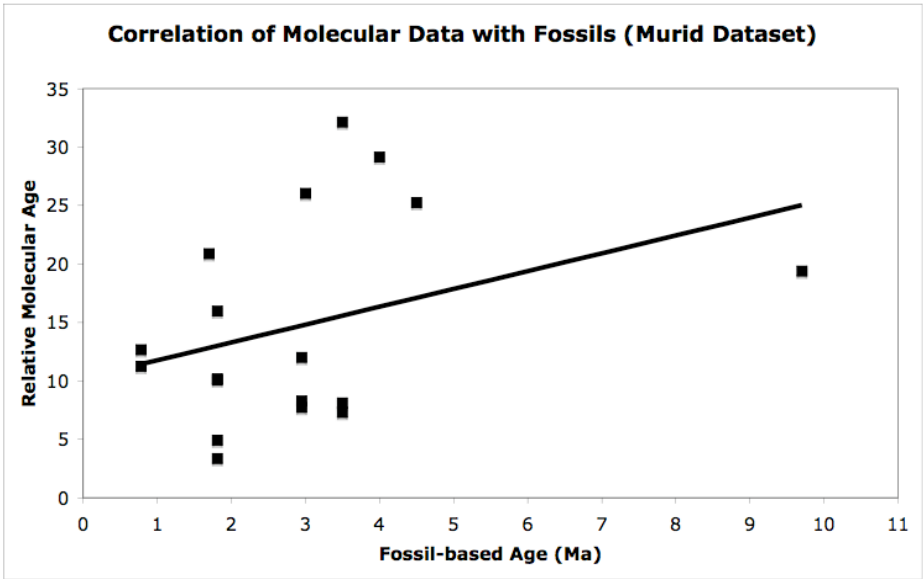
Spalax ehrenbergi
Microtus levis
Cricetulus griseus
Uranomys ruddi
Acomys ignitus
Lophuromys flavopunctatus
Lophuromys sikapusi
Desmodillus auricularis
Gerbilliscus robustus
Gerbilliscus kempi1
Gerbilliscus kempi2
Gerbillurus paeba
Gerbillurus vallinus
Gerbillurus setzeri
Meriones shawi
Meriones tristrami
Meriones crassus
Taterillus emini
Gerbillus gerbillus2
Gerbillus gerbillus1
Gerbillus andersoni
Gerbillus pyramidum
Phloeomys sp
Batomys granti
Apomys datae
Rhynchomys isarogensis
Anisomys imitator
Lorentzimys nouhuysi
Macruromys major
Chiruromys vates
Pogonomys macrourus
Hyomys goliath
Mallomys rothschildi
Abeomelomys sevia
Mammelomys lanosus
Parahydromys asper
Leptomys elegans
Leggadina forresti
Uromys caudimaculatus
Paramelomys levipes
Melomys rufescens
Leporillus conditor
Mesembriomys gouldii
Conilurus penicillatus
Oenomys hypoxanthus
Otomys sp
Parotomys sp
Dasymys incomtus
Hybomys univittatus
Dephomys defua
Stochomys longicaudatus
Micaelamys namaquensis
Gramomys buntingi
Rhabdomys pumilio
Mylomys dybowsii
Arvicanthis neumanni
Lemniscomys barbarus
Malacomys edwardsi
Malacomys longipes
Mus setulosus
Mus musculus
Mus terricolor
Apodemus semotus
Apodemus agrarius
Praomys tullbergi
Praomys daltoni
Praomys jacksoni
Mastomys erythroleucus
Hylomyscus parvus
Zelotomys hildegardeae
Praomys delectorum
Maxomys bartelsii
Maxomys surifer
Chiromyscus chiropus
Dacnomys millardi
Leopoldamys sabanus
Niviventer culturatus
Niviventer cremoriventer
Berylmys bowersi
Sundamys muelleri
Paruromys dominator
Bunomys andrewsi
Rattus leucopus
Rattus rattus
Rattus norvegicus

FIGURE 5.

A.)


Correlation of Molecular Data with Fossils (Murid Dataset)
Relative Molecular Age
Fossil-based Age (Ma)
0
5
10
15
20
25
30
35
0
1
2
3
4
5
6
7
8
9
10
11

B.)

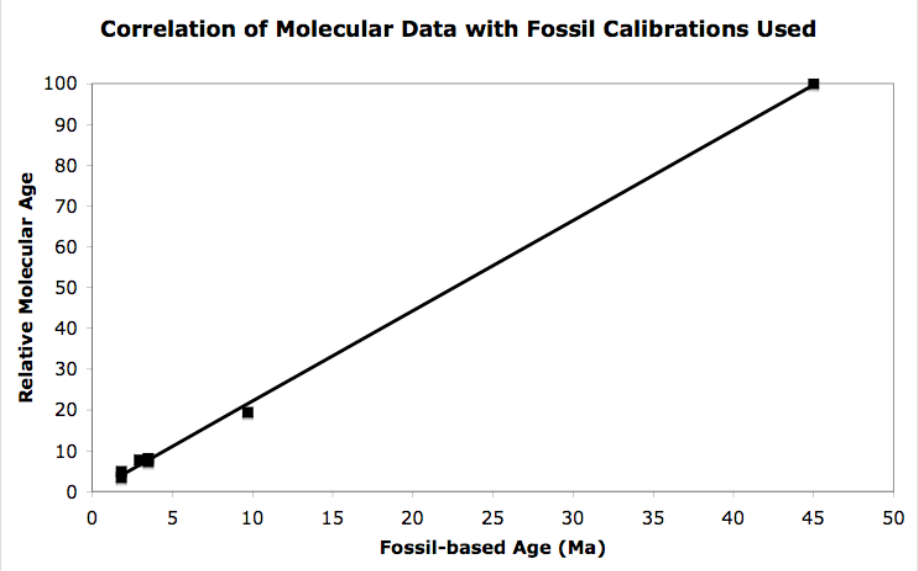

Correlation of Molecular Data with Fossil Calibrations Used
Relative Molecular Age
Fossil-based Age (Ma)
0
10
20
30
40
50
60
70
80
90
100
0
5
10
15
20
25
30
35
40
45
50

FIGURE 6.

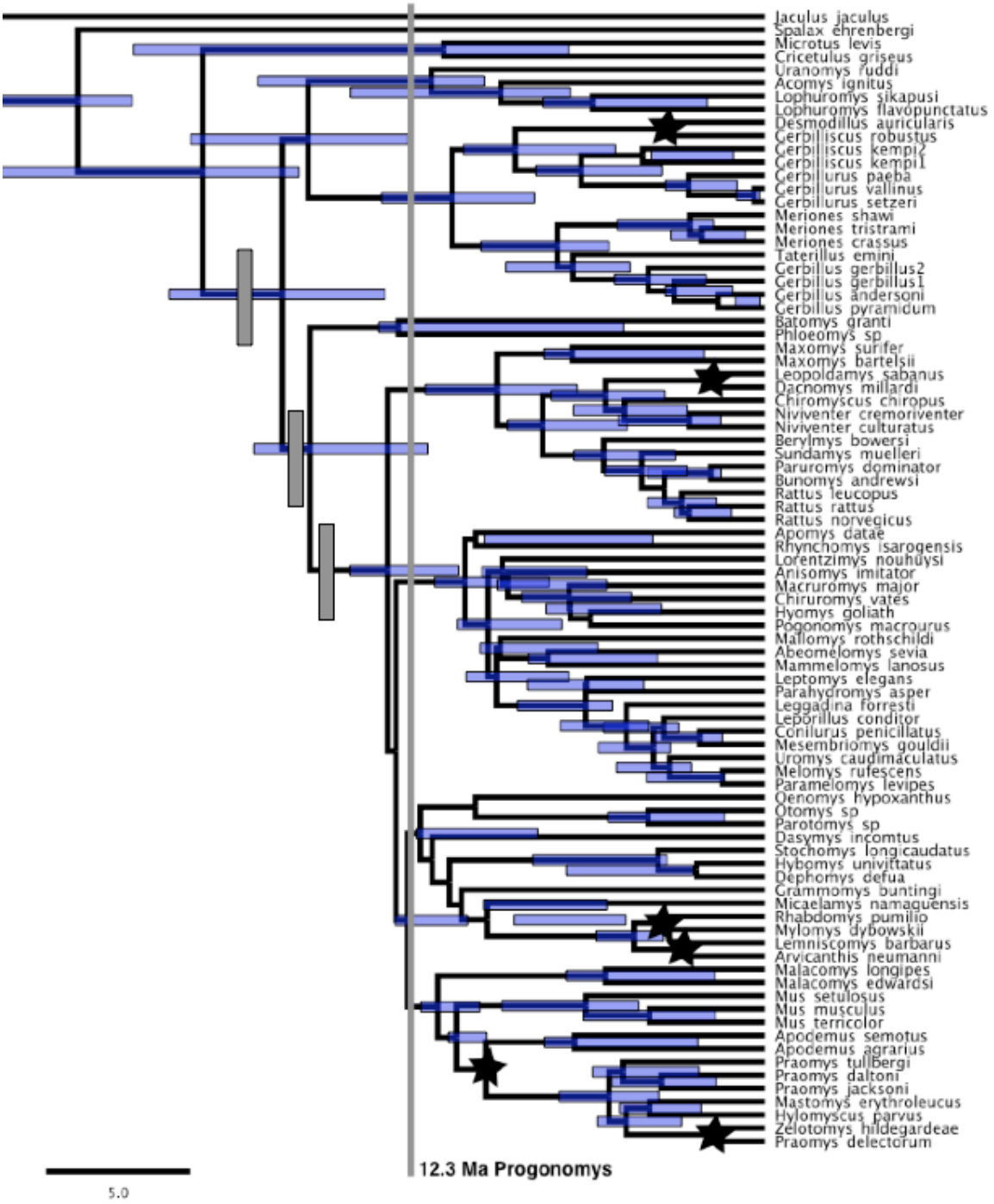

Jaculus jaculus
Spalax ehrenbergi
Microtus levis
Cricetulus griseus
Uranomys ruddi
Acomys ignitus
Lophuromys sikapusi
Lophuromys flavopunctatus
Desmodillus auricularis
Gerbilliscus robustus
Gerbilliscus kempi2
Gerbilliscus kempi1
Gerbillurus paeba
Gerbillurus vallinus
Gerbillurus setzeri
Meriones shawi
Meriones tristrami
Meriones crassus
Taterillus emini
Gerbillus gerbillus2
Gerbillus gerbillus1
Gerbillus andersoni
Gerbillus pyramidum
Batomys granti
Phloeomys sp
Maxomys surifer
Maxomys bartelsii
Leopoldamys sabanus
Dacnomys millardi
Chiromyscus chiropus
Niviventer cremoriventer
Niviventer culturatus
Berylmys bowersi
Sundamys muelleri
Paruromys dominator
Bunomys andrewsi
Rattus leucopus
Rattus rattus
Rattus norvegicus
Apomys datae
Rhynchomys isarogensis
Lorentzimys nouhuysi
Anisomys imitator
Macruromys major
Chiruromys vates
Hyomys goliath
Pogonomys macrourus
Mallomys rothschildi
Abeomelomys sevia
Mammelomys lanosus
Leptomys elegans
Parahydromys asper
Leggadina forresti
Leporillus conditor
Conilurus penicillatus
Mesembriomys gouldii
Uromys caudimaculatus
Melomys rufescens
Paramelomys levipes
Qenomys hypoxanthus
Otomys sp
Parotomys sp
Dasymys incomtus
Stochomys longicaudatus
Hybomys univittatus
Dephomys defua
Grammomys buntingi
Micaelamys namaquensis
Rhabdomys pumilio
Mylomys dybowskii
Lemniscomys barbarus
Arvicanthis neumanni
Malacomys longipes
Malacomys edwardsi
Mus setulosus
Mus musculus
Mus tericolor
Apodemus semotus
Apodemus agrarius
Praomys tullbergi
Praomys daltoni
Praomys jacksoni
Mastomys erythroleucus
Hylomyscus parvus
Zelotomys hildegardeae
Praomys delectorum
12.3 Ma Progonomys
5.0

TABLE 1. GenBank accession numbers for taxa used in this study.

Lineage	ADRA2B	BRCA1	GHR	IRBP
Primates	*Homo sapiens* M34041	*Homo sapiens* NM007302	*Homo sapiens* NM000163	*Homo sapiens* NM002900
Scandentia	*Tupaia belangeri* AY150333	*Tupaia tana* AF284006	*Tupaia belangeri* AF332018	*Tupaia glis* Z11808
Leporidae	*Lepus crawshayi* AJ427254	*Lepus capensis* AF284005	*Lepus capensis* AF332016	*Lepus crawshayi* AJ427250
Ochotonidae	*Ochotona princeps* AJ427253	*Ochotona princeps* AY057827	*Ochotona princeps* AF332015	*Ochotona princeps* AY057832
Anomaluridae	*Anomalurus* sp. AJ427259	*Anomalurus beecrofti* this study	*Anomalurus beecrofti* this study	*Anomalurus* sp. AJ427240
Pedetidae	*Pedetes capensis* AM407920	*Pedetes capensis* AF332047	*Pedetes capensis* AF332025	*Pedetes capensis* AJ427241
Dipodidae	*Dipus sagitta* AJ427263	*Napaeozapus insignis* AF540634	*Allactaga sibirica* AY294897	Allactaga *sibirica* AY326076
Heteromyidae	*Dipodomys merriami* AJ427261	*Perognathus flavus* AF540638	*Perognathus flavus* *AF332029*	*Dipodomys merriami* AJ427233
Geomyidae	*Thomomys talpoides* AJ427262	*Geomys bursarius* AF540629	*Geomys bursarius* AF332028	*Thomomys talpoides* AJ427234
Castoridae	*Castor canadensis* AJ427260	*Castor canadensis* AF540622	*Castor canadensis* AF332026	*Castor canadensis* AJ427239

Ctenodactylidae	*Massoutiera mzabi* AJ427265	*Ctenodactylus gundi* AF540624	*Ctenodactylus gundi* AF332042	*Massoutiera mzabi* AJ427242
Hystricidae	*Trichys fasciculata* AJ427266	*Hystrix africaeaustralis* AF540631	*Hystrix africaeaustralis* AF332033	*Trichys fasciculata* AJ427245
Phiomorpha	*Heterocephalus glaber* AM407924	*Heterocephalus glaber* AF540630	*Heterocephalus glaber* AF332034	*Bathyergus suillus* AJ427251
Caviomorpha	*Erethizon dorsatum* AJ427270	*Erethizon dorsatum* AF540626	*Erethizon dorsatum* AF332037	*Erethizon dorsatum* AJ427249
Sciuridae	*Sciurus vulgaris* AJ315942	*Glaucomy volans* AF284003	*Sciurus niger* AF332032	*Glaucomy volans* AY227598
Aplodontiidae	*Aplodontia rufa* AJ427256	*Aplodontia rufa* AF332045	*Aplodontia rufa* AF332030	*Aplodontia rufa* AJ427238
Gliridae	*Glis glis* AJ427258	*Graphiurus murinus* AF332046	*Graphiurus murinus* AF332031	*Graphiurus murinus* AY303219
Mus musculus	M94583	U36475	AF120489	NM015745
Rattus norvegicus	AF366899	NM012514	NM017094	AJ429134

TABLE 1 CONTINUED

Lineage	**vWF**	**12S rRNA**	**CYTB**
Primates	*Homo sapiens* NM000552	*Homo sapiens* NC001807	*Homo sapiens* NC001807
Scandentia	*Tupaia glis* U31624	*Tupaia tana* AJ421453	*Tupaia belangeri* AJ421453

Leporidae	*Lepus crawshayi* AJ224669	*Lepus capensis* AY292706	*Lepus europaeus* NC004028
Ochotonidae	*Ochotona princeps* AJ224672	*Ochotona princeps* AJ537415	*Ochotona princeps* AJ537415
Anomaluridae	*Anomalurus* sp. AJ427229	*Anomalurus* sp. AJ389539	*Anomalurus* sp. AJ389526
Pedetidae	*Pedetes capensis* AJ238389	*Pedetes capensis* AY012113	*Pedetes capensis* AJ389527
Dipodidae	Allactaga *elater* AJ224661	*Allactaga elater* AJ389534	*Allactaga elater* AJ389534
Heteromyidae	*Dipodomys merriami* AJ427226	*Perognathus flavus* U67298	*Dipodomys merriami* AY926383
Geomyidae	*Thomomys talpoides* AJ427227	*Geomys bursarius* AF084297	*Geomys bursarius* U65291
Castoridae	*Castor canadensis* AJ427228	*Castor canadensis* AY787823	*Castor fiber* AJ389529
Ctenodactylidae	*Massoutiera mzabi* AJ238388	*Massoutiera mzabi* AJ389544	*Massoutiera mzabi* AJ389533
Hystricidae	*Trichys fasciculata* AJ224675	*Hystrix africaeaustralis* U12448	*Hystrix africaeaustralis* X70674
Phiomorpha	*Heterocephalus glaber* AJ251134	*Heterocephalus glaber* AY425847	*Heterocephalus glaber* AF155870

Caviomorpha	*Erethizon dorsatum* AJ251135	*Erethizon dorsatum* AY012118	*Coendu bicolor* U34852
Sciuridae	*Glaucomy volans* AJ224667	*Sciurus vulgaris* NC_002369	*Sciurus vulgaris* NC_002369
Aplodontiidae	*Aplodontia rufa* AJ224662	*Aplodontia rufa* AJ389541	*Aplodontia rufa* AJ389528
Gliridae	*Glis glis* AJ224668	*Graphiurus murinus* AY303187	*Glis glis* NC_001892
Mus musculus	*Mus musculus* NM011708	*Mus musculus* NC005089	*Mus musculus* NC005089
Rattus norvegicus	XM001066203	NC001665	NC001665

TABLE 2. Calibration points used in analyses. "Gap" column indicates the minimum gap size present in the fossil record based on the difference in first appearance dates between daughter lineages at node.

Node	Fossil Calibration	Date (Ma)
Anomaluromorpha	*Pondaungimys*	37.2 +/- 1.3
Myomorpha	*Pappocricetodon*	45
Castorimorpha	*Mattimys*	54.4
Geomyoidea	*Proheteromys*	33.1
Hystricomorpha	Zegdou phiomyid	49.5
Phiomorpha + Caviomorpha clade	*Gaudeamus*	33.7-34.8
Sciuromorpha	*Eogliravus*	52.5
Sciuroidea	*Spurimus*	42.2

TABLE 3. GenBank accession numbers for mitochondrial sequence data used in murid dataset.

Subfamily	Species	Accession
Dipodidae	*Jaculus jaculus*	NC_005314
Spalacidae	*Spalax ehrenbergi*	NC_005315
Cricetidae	*Cricetulus griseus*	NC_007936
Cricetidae	*Microtus levis*	NC_008064
Deomyinae	*Acomys ignitus*	DQ019086
Deomyinae	*Lophuromys flavopunctatus*	DQ019087
Gerbillinae	*Gerbilliscus robustus*	DQ019084
Gerbillinae	*Taterillus emini*	DQ019085
Murinae	*Miacelamys namaquensis*	DQ019089
Murinae	*Anisomys imitator*	DQ019090
Murinae	*Apodemus agrarius*	DQ019092
Murinae	*Apodemus semotus*	DQ019093
Murinae	*Arvicanthis neumanni*	DQ019094
Murinae	*Batomys granti*	DQ019095
Murinae	*Berylmys bowersi*	DQ019096
Murinae	*Conilurus penicillatus*	DQ019097
Murinae	*Dacnomys millardi*	DQ019098
Murinae	*Hybomys univittatus*	DQ019099
Murinae	*Hylomyscus parvus*	DQ019100
Murinae	*Leggadina forresti*	DQ019101
Murinae	*Lemniscomys barbarus*	DQ019102
Murinae	*Leopoldamys sabanus*	DQ019103
Murinae	*Malacomys longipes*	DQ019104
Murinae	*Maxomys bartelsii*	DQ019106
Murinae	*Maxomys surifer*	DQ019107
Murinae	*Niviventer culturatus*	DQ019108
Murinae	*Niviventer cremoriventer*	DQ019109
Murinae	*Oenomys hypoxanthus*	DQ019110
Murinae	*Parotomys* sp.	DQ019111
Murinae	*Phloeomys* sp.	DQ019112
Murinae	*Praomys jacksoni*	DQ019113
Murinae	*Praomys delectorum*	DQ019114
Murinae	*Praomys tullbergi*	DQ019115
Murinae	*Rhabdomys pumilio*	DQ019118
Murinae	*Rhynchomys isarogensis*	DQ019119
Murinae	*Stochomys longicaudatus*	DQ019120
Murinae	*Sundamys muelleri*	DQ019121
Murinae	*Uromys caudimaculatus*	DQ019122
Murinae	*Zelotomys hildegardeae*	DQ019123
Murinae	*Apomys datae*	EU349702
Murinae	*Archboldomys luzonensis*	EU349703

Murinae	*Bunomys adspersa*	EU349704
Murinae	*Chiromyscus chiropus*	EU349705
Murinae	*Chiruromys vates*	EU349706
Murinae	*Dasymys incomtus*	EU349707
Murinae	*Hydromys chrysogaster*	EU349709
Murinae	*Hyomys goliath*	EU349710
Murinae	*Leporillus conditor*	EU349711
Murinae	*Leptomys elegans*	EU349712
Murinae	*Lorentzimys nouhuysi*	EU349713
Murinae	*Macruromys major*	EU349714
Murinae	*Mallomys rothschildi*	EU349715
Murinae	*Mammelomys lanosus*	EU349716
Murinae	*Mastacomys fuscus*	EU349717
Murinae	*Mastomys erythroleucus*	EU349718
Murinae	*Melomys rufescens*	EU349720
Murinae	*Mesembriomys gouldii*	EU349721
Murinae	*Otomys* sp.	EU349722
Murinae	*Parahydromys asper*	EU349723
Murinae	*Paramelomys levipes*	EU349724
Murinae	*Paruromys dominator*	EU349725
Murinae	*Pogonomys macrourus*	EU349727
Murinae	*Rattus leucopus*	EU349728
Murinae	*Rattus norvegicus*	J01434
Murinae	*Mus musculus*	NC005089

TABLE 4. Locality information for individuals sequenced for this study.

Subfamily	Species	Tissue ID	Locality
Deomyinae	*Lophuromys sikapusi*	UVM 2525	Guinea, Guinée Forestière, Youmou, Forêt Claseé Diéké
Deomyinae	*Uranomys ruddi*	RWN 241	Guinea, Guinée Forestière, Forêt Claseé du Pic de Fon
Gerbillinae	*Desmodillus auricularis*	UVM 39	Stosba, South Africa
Gerbillinae	*Gerbilliscus kempi* (#1)	UVM 1515	Ghana, Volta Region, Kalakpa Resource Reserve, 3.25 km S Abutia Kloe, near Zitoe Camp
Gerbillinae	*Gerbilliscus kempi* (#2)	RWN 315	Guinea, Guinée Forestière, Forêt Claseé du Pic de Fon
Gerbillinae	*Gerbillurus paeba paeba*	CM 93305 SP 4465	Namibia, Keetmanshoop District
Gerbillinae	*Gerbillurus setzeri*	CM 93201 SP 4346	Namibia
Gerbillinae	*Gerbillurus vallinus seeheimi*	CM 93203 SP 4377	Namibia, Keetmanshoop District
Gerbillinae	*Gerbillus andersoni andersoni*	CM 113811 SP 10259	Egypt, Beheira Governate
Gerbillinae	*Gerbillus gerbillus gerbillus* (#1)	CM 113820 SP 10258	Egypt, Beheira Governate
Gerbilinae	*Gerbillus gerbillus gerbillus* (#2)	CM 113822 SP 10208	Egypt, Giza Governate
Gerbillinae	*Gerbillus pyramidum pyramidum*	CM 113835 SP 10239	Egypt, Giza Governate
Gerbillinae	*Meriones crassus*	TK 25633	Jordan
Gerbillinae	*Meriones shawi*	TK 25553	Jordan, Al Halabat
Gerbillinae	*Meriones tristrami*	TK 25532	Jordan, Al Ghor
Murinae	*Dephomys defua*	UVM 2502	Guinea, Guinée Forestière, Youmou, Forêt Claseé Diéké
Murinae	*Grammomys buntingi*	RWN 283	Guinea, Guinée Forestière, Forêt Claseé du Pic de Fon
Murinae	*Malacomys edwardsi*	UVM 2527	Guinea, Guinée Forestière, Youmou, Forêt Claseé Diéké
Murinae	*Mus setulosus*	UVM 2538	Guinea, Guinée Forestière, Lola, Forêt Claseé Déré
Murinae	*Mylomys dybowskii*	RWN 240	Guinea, Guinée Forestière, Forêt Claseé du Pic de Fon

Murinae	*Praomys daltoni*	RWN 259	Guinea, Guinée Forestière, Forêt Claseé du Pic de Fon
Murinae	*Rattus rattus*	UVM 1275	Pakistan, FATA, S. Waziristan, Rakmak Alexandra Fort

TABLE 5. Locality information for individuals sequenced for this study but excluded due to similarity in haplotype with other individuals.

Subfamily	Species	Tissue ID	Locality
Gerbillinae	*Gerbilliscus kempi*	RWN 278	Guinea, Guinée Forestière, Forêt Claseé du Pic de Fon
Gerbillinae	*Gerbilliscus kempi*	UVM 2572	Guinea, Guinée Forestière, Kpinita Village near Forêt Claseé Mt. Béro
Gerbillinae	*Gerbillurus paeba broomi*	CM 93199 TM 37465	South Africa, Transvaal Province
Gerbillinae	*Gerbillurus paeba exilis*	CM 93315 SP 4306	South Africa, Cape Province
Gerbillinae	*Gerbillurus paeba exilis*	CM 93200 TM 37502	South Africa, Cape Province
Gerbillinae	*Gerbillurus paeba mulleri*	CM 95021 SP 6287	South Africa, Cape Province
Gerbillinae	*Gerbillurus paeba paeba*	CM 98566 SP 4307	South Africa, Cape Province
Gerbillinae	*Gerbillurus vallinus*	TK 25669	South Africa, Cape Province
Gerbillinae	*Gerbillurus vallinus seeheimi*	CM 93204 TP 4379	Namibia, Keetmanshoop District
Gerbillinae	*Gerbillus andersoni andersoni*	CM 113810 SP 10257	Egypt Beheira Governate
Gerbillinae	*Gerbillus pyramidum pyramidum*	CM 113835 SP 10240	Egypt, Giza Governate
Gerbillinae	*Meriones tristrami*	TK 25525	Jordan, Al-Muwaggar

TABLE 6. Fossil calibration points evaluated. PBDB refers to the Paleobiology Database (paleodb.org) and includes a reference number.

Calibration	Fossil taxon [lineage]	Minimum Date (Ma)	References	Used?
Dipodidae – Muroidea	*Pappocricetodon* [Muroidea]	45	Wang and Dawson, 1994; Norris et al. (chapter 2)	Y
Spalacidae – Eumuroida	*Tachyoryctoides* [Spalacidae]	23.03 (Chattian)	PBDB 64412: Lucas et al., 1998	N
Acomys – Lophuromys	*Acomys*	4.5	Denys, 1990a; Musser and Carleton, 2005	N
Desmodillus – Gerbilliscus robustus	*Desmodillus*	3.5	PBDB 59167: Muizon and Hendey, 1980	Y
Gerbillurus – Gerbilliscus kempi	*Gerbillurus*	1.81 (Late Pliocene)	Senut et al., 1992; Musser and Carleton, 2005	N
Meriones – Gerbillus	*Mascaremys* [*Meriones*]	3.5	Tong, 1989	N
Apodemus – Praomys	*Apodemus*	9.7	Martin-Suàrez and Mein, 1998; Freudenthal and Martin-Suàrez, 1999; Musser and Carleton, 2005	Y
Apodemus agrarius – A. semotus	*A. chevrieri* [*A. agrarius*]	0.781 (Early Pleistocene)	Zheng, 1993; Musser and Carleton, 2005	N
Mus terricolor – M. musculus	First African *Mus*	2.95	PBDB 21824: Brain, 1994	N
Otomys – Oenomys	*Euryotomys* [Otomyine]	4	Sénégas & Avery 1998; Sénégas 2001; Taylor et al., 2004	N
Rhabdomys – Mylomys	*Rhabdomys*	3.5	PBDB 59167: Muizon and Hendey, 1980	Y
Rattus – Leopoldamys	*Rattus*	3	Zheng, 1993; Chaimanee et al., 1996; Benton and Donoghue, 2007	N
Sundamys – Berylmys	*Berylmys*	0.781 (Early Pleistocene)	McKenna and Bell, 1997	N
Leopoldamys – Dacnomys	*Leopoldamys*	1.81 (Late	McKenna and Bell, 1997	Y

		Pliocene)		
Arvicanthis – Lemniscomys	*Arvicanthis* and *Lemniscomys*	2.95	PBDB 21546: Wesselman, 1984	Y
Mastomys – Hylomyscus	*Mastomys*	2.95	PBDB 21546: Wesselman, 1984	N
Dasymys – Stochomys	*Dasymys*	1.7	PBDB 21824: Brain, 1994	N
Zelotomys – Praomys delectorum	*Zelotomys*	1.81 (Late Pliocene)	Denys, 1999; Musser and Carleton, 2005	Y
Leporillus – Conilurus	*Leporillus*	1.81 (Late Pliocene)	Aplin, 2005; Musser and Carleton, 2005	N
Grammomys – Micaelamys	*Micaelamys*	1.81 (Late Pliocene)	Denys, 1990b; Musser and Carleton, 20005	N

TABLE 7. Divergence dates estimated for select nodes. BR represents the results of analysis of the basal rodent dataset. MU indicates the results of the analysis using the murid dataset. The results for the best tree sampled and the 95% confidence interval are shown for both analyses. The three hypotheses specifically tested against the *Antemus* (14 Ma) – *Progonomys* (12.3 Ma) – *Karnimata* (11.1 Ma) fossil series are indicated in bold.

Node	BR: Best	BR: 95% C.I.	MU: Best	MU: 95% C.I.
Cricetidae – Muridae	-	-	19.6	16.2-27.5
Muridae	**-**	**-**	**16.8**	**13.2-20.7**
Deomyinae – Gerbillinae	-	-	15.9	12.4-20.0
Deomyinae	-	-	11.6	9.8-17.7
Gerbillinae	-	-	10.9	8.0-13.3
Murinae	**-**	**-**	**15.8**	**11.7-17.8**
Mus* - *Rattus	**16.0**	**13.8-20.7**	**13.1**	**10.7-14.4**

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