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THE PHYLOGENY AND BIOGEOGRAPHY OF THE MONITO DEL MONTE (DROMICIOPS GLIROIDES) AND ITS RELATIVES

by

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A Thesis Submitted in Partial Fulfillment
of the Requirements for a Degree with Honors
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Abstract

Marsupials are a group of mammals that give birth to young that are not fully developed. These offspring must complete the remainder of their development outside of the womb attached to their mother's teat. Marsupials only occur in South America and Australasia, with one species extending into North America. The marsupial known as the monito del monte, which is Spanish for 'little monkey of the mountain,' (*Dromiciops gliroides*) is a South American marsupial; however, it shares a key morphological feature of ankle bone morphology with Australasian marsupials. This shared, derived trait is what defines the cohort Australidelphia, to which the four Australasian orders of marsupials and the monito del monte belong. Because of this placement, the monito del monte appears to be a link between the South American and Australasian marsupials. By reviewing phylogenetic studies on marsupials, fossil evidence, biogeography, and extinct and extant marsupial and placental mammals of Australasia and South America, it is anticipated that the evolutionary history of marsupials will be better understood. The role of the monito del monte is pivotal in this story. The close relationship between the monito del monte and the Australasian marsupials was strongly supported by molecular, biogeographic, and fossil evidence, in addition to morphology. This thesis hypothesized that an ancient American marsupial dispersed across the supercontinent Gondwana and gave rise to the Australidelphians. Subsequently, dispersal is what brought the monito del monte to South America. Therefore, the Australidelphians, including the monito del monte, originated in Australasia.

Acknowledgements

The author would like to thank Irv Kornfield for all of his help and support throughout the entire thesis process. Thanks are due to the committee members for their patience and helpful suggestions. The author would also like to thank friends and family members that offered support and encouraging words at all times.

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1. *Introduction*

Mammals are one of the most diverse groups of vertebrates. There are many differences among species within the Class Mammalia in terms of life span, body size, reproduction, behavior, locomotion, and metabolic rate (Springer and Murphy 2007). They have adapted to live in nearly every habitat in the world. However, not every group within Mammalia is so widespread. The group of mammals known as marsupials is only located in South America and Australasia, which consists of Australia and New Guinea. However, one species, the Virginia opossum (*Didelphis virginiana*), extends up into North America. Living marsupials are only 7% of all extant mammals alive today (Yu et al. 2012). There are seven orders of marsupials and about two hundred and ninety-two species (Amrine-Madsen et al. 2003). Many marsupials are similar to placental mammals in their appearance and lifestyles due to convergent evolution, which is the process by which organisms that are not closely related have acquired similar traits independent of one another. Even within marsupials there is convergent evolution between the marsupials of Australasia and the marsupials of South America.

The first documented marsupial was found in 1500 in Brazil and brought to King Ferdinand and Queen Isabella of Spain (Armati et al. 2006). It was an opossum with young, and it made quite an impression on people. When Europeans began settling Australia in the 1700s, the first real expeditions and descriptions of marsupials were initiated (Armati et al. 2006). Marsupials peaked people’s interest because they seem so primitive, have a largely unknown history, and only occur on two continents. Although marsupials have attracted the curiosity of many, there has never been a consensus on the relationships among them or their biogeographic history.

When scientists endeavor in determining the relationships of marsupials, they almost always look to the monito del monte (*Dromiciops gliroides*) for answers. The monito del monte is a marsupial that lives in South America, but it has been hypothesized to actually be more closely related to Australasian marsupials (Szalay 1994). This relationship suggests a complex biogeography. The monito del monte is the key to understanding the link between South American and Australasian marsupials. By reviewing published phylogenetic data, fossil evidence, and biogeography, an attempt will be made to understand the evolutionary history of the monito del monte and its fellow marsupials.

2. *Review of Taxa*

The class Mammalia is split into two sub-classes: Monotremata and Theria. Monotremata includes the egg-laying mammals, platypuses and echidnas. Theria is broken down into two infraclasses Eutheria, the placental mammals, and Marsupialia, the pouched mammals. From there, each infraclass is broken down into orders, families, genera, and species. A simple mistake is made by defining marsupials as ‘pouched mammals’ because not all marsupials have pouches. For example, the numbat of Australasia does not have a pouch, but instead it simply has a fold of skin which helps hold the offspring to their mother. A better way to describe marsupials is that they give birth to young that are immature, still in an embryonic state, and must finish development outside of the womb. Young stay attached to the mother’s teat until they have developed sufficiently enough to move around on their own.

2.1 Extant Marsupials

There are currently seven orders of marsupials. These orders are categorized into two cohorts: Ameridelphia and Australidelphia. These two cohorts are defined by morphology of the ankle joint. Ameridelphian species are united by possession of a separate lower ankle joint pattern and a single-faceted calcaneocuboid joint (Szalay 1994). Australidelphians are united by having a continuous lower ankle joint pattern and a triple-faceted calcaneocuboid joint (Szalay 1994), which will be discussed later in this paper. There are two orders in the cohort Ameridelphia: Didelphimorphia and Paucituberculata (Szalay 1994), which are both currently located in South America. The five orders of Australidelphia are Microbiotheria, Peramelemorphia, Dasyuromorphia, Notoryctemorphia, and Diprotodontia (Szalay 1994). Of these Australidelphian orders, the only one that does not currently have species that live in Australasia is Microbiotheria.

Table 1. Taxonomy of Living Marsupials*

- Class Mammalia
  - Subclass Marsupialia
    - Cohort Ameridelphia (distribution: North and South America)
      - Order Didelphimorphia (1 family, 73 species)
      - Order Paucituberculata (1 family, 6 species)
    - Cohort Australidelphia (distribution: South America, Australia, and New Guinea)
      - Order Microbiotheria (1 family, 1 species)
      - Order Peramelemorphia (2 families, 12 species)
      - Order Dasyuromorphia (2 families, 71 species)
      - Order Notoryctemorphia (1 family, 2 species)
      - Order Diprotodontia (11 families, 137 species)

*Adapted from Nilsson et al. 2004

2.1.1 Ameridelphia

The order Didelphimorphia contains the true opossums of the Americas. They are mostly confined to South America, but the range of the Virginia opossum (*Didelphis virginiana*) stretches up and across North America (Emmons 1997). There are nearly one-hundred species of opossum (Lord 2007), and they are mostly omnivorous and semi-arboreal (Armati et al. 2006). However, there are exceptions, like the semi-aquatic yapok (*Chironectes minimus*), a species that possesses webbed hind feet to aid in swimming. Didelphid size ranges from that of a mouse to that of a house cat (Armati et al. 2006). The diversity of the didelphids can be seen in Figure 1. Members of the order Paucituberculata are known as shrew opossums or caenolestids (Armati et al. 2006). There are only six species of shrew opossums, and they are confined to the Andes Mountains (Lord 2007). They are mostly carnivorous and resemble shrews and other small rodents in appearance (Lord 2007).

2.1.2 Australidelphia

The order Peramelemorphia, which contains the rodent-like bandicoots and the bilby, contains only about a dozen species (Armati et al. 2006). They are about the size of a rabbit and are mostly insectivorous and omnivorous (Parish and Cox 2008). Dasyuromorphia contains over 50 species of carnivores that have a size ranging from that of a small rodent to that of a medium-sized dog (Parish and Cox 2008). Well-known dasyurids include numbats, quolls, dunnarts, and the Tasmanian devil (Parish and Cox 2008). Only two species of marsupial moles are in the order Notoryctemorphia (Parish and Cox (2008). They are insectivorous and are very similar to the placental African golden mole (Armati et al. 2006). The most diverse and numerous order of marsupials is Diprotodontia (Armati et al. 2006). There are over one-hundred species in eleven families, and most of them are herbivores (Parish and Cox 2008). The largest family is Macropodidae, the kangaroos, wallabies and their relatives, with over fifty species (Parish and Cox 2008). Other diprotodonts include the koala, wombats, and possums (Parish and Cox 2008). The smallest diprotodonts are the pygmy-possums, some weighing only six grams; the largest diprotodonts are the kangaroos that can be five to six feet tall (Parish and Cox 2008).

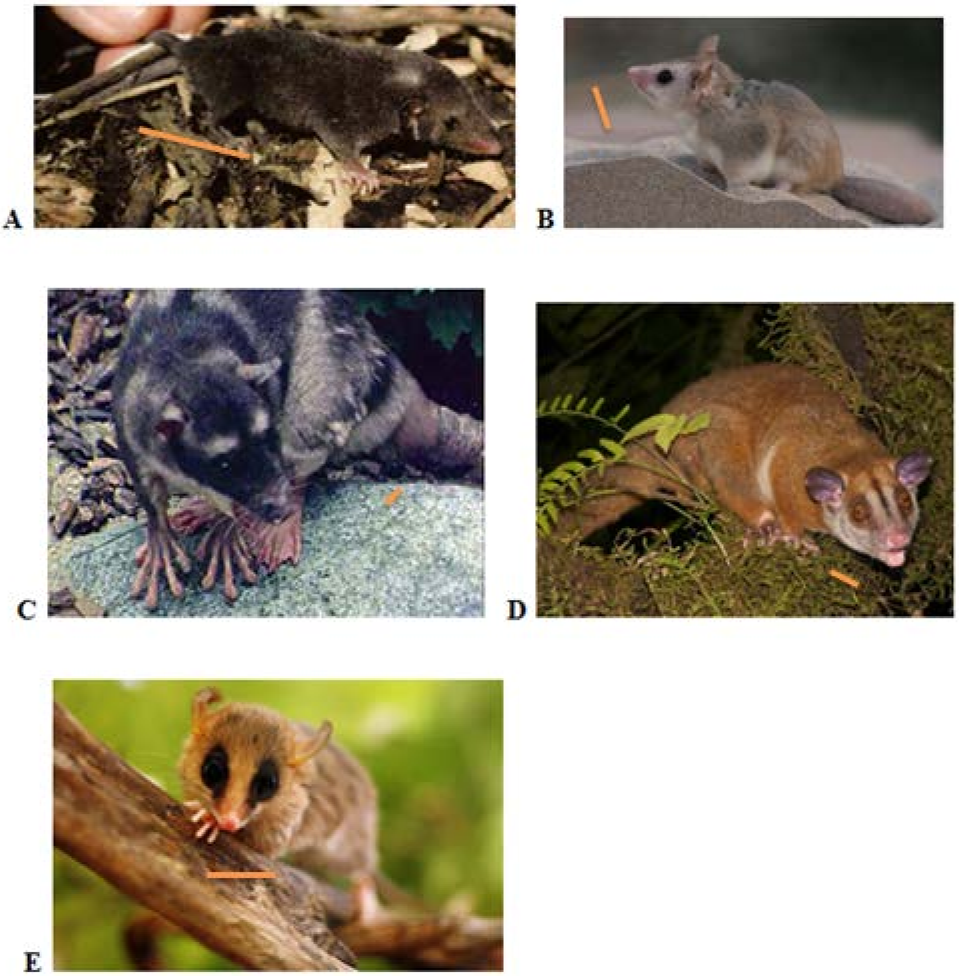


Figure 1. A sampling of five marsupials of the order Didelphimorphia. All are South American opossums with prehensile tails. The orange line in each image represents approximately one inch. **A.** *Monodelphis adusta* is terrestrial and eats invertebrates. **B.** *Lestodelphys halli* is terrestrial and carnivorous. **C.** *Chironectes minimus* is semi-aquatic and eats aquatic animals. **D.** *Caluromys lanatus* is arboreal and omnivorous. **E.** *Gracilinanus microtarsus* is arboreal and insectivorous. Images and information from Emmons 1977; bbc.co.uk; inaturalist.org; arkive.org; flickr.com; and monodelphis-adusta.blogspot.com.

Microbiotheria is an Australidelphian order that is particularly interesting since its only living species, the monito del monte (*Dromiciops gliroides*), only occurs in South America. The monito del monte is a small, arboreal, didelphid-looking marsupial that lives in cool, moist beech and bamboo forests of southern Chile and adjacent Argentina (Armati et al. 2006). It is an omnivore and eats mostly insects and other arthropods (Armati et al. 2006) (Figure 2).

Figure 2. *Dromiciops gliroides* is 11-13cm in length and has a prehensile tail. Image and information from Emmons 1997; animaladay.blogspot.com.

2.2 Extinct Marsupials of South America

It may seem as though the diversity of South America's marsupial fauna is poor, with just under one hundred species whereas Australasia has over two-hundred species, but what is seen today is only a fraction of what once was. The family Borhyaenidae is known to have existed in South America from the Late Paleocene to the Middle Pliocene (Simpson 1980). These dog-like marsupials, which arose from a didelphid ancestor, were most likely the largest carnivores in South America at the time (Beck et al. 2008). Another group of marsupials similar to the Borhyaenidae were the Thylacosmilidae. They existed for a brief time in the Pliocene (Simpson 1980). Their morphology has strong similarities to placental saber-tooth tigers, and they are also thought to have arisen from didelphids (Simpson 1980). The family Polydolopidae is known from the Late Paleocene to Early Oligocene and evolved from the order Paucituberculata (Simpson 1980). They were somewhat small and were probably omnivores (Simpson 1980). The family Groeberiidae also evolved from Paucituberculata and is known from the Early Oligocene (Simpson 1980). They were rodent-like, being highly specialized ‘gnawers’ (Simpson 1980). The family Argyrolagidae’s members were bipedal with large hind legs for leaping and a long, heavy tail for counterbalance (Simpson 1980). Rodent-like and similar to kangaroo rats of today, they evolved from Paucituberculata and are known from the Early Pliocene to Early Pleistocene (Simpson 1980). A very different marsupial belongs to the family Necrolestidae from the Early Miocene (Simpson 1980). It was a small, highly specialized insectivore that was much like the marsupial mole present in Australia today (Simpson 1980).

These extinct marsupials evolved similar body forms to placental mammals and marsupials that exist today. It can be speculated that competition with similar placental mammals had an influence on the extinction of these six South American marsupial families.

Table 2. Geologic Time*

Era	Period	Epoch	Time (MYA: millions of years ago)
Cenozoic	Quaternary	Recent	0.01-0
		Pleistocene	2-0.01
		Pliocene	5-2
		Miocene	22-5
		Oligocene	37-22
		Eocene	55-37
		Paleocene	65-55
Mesozoic	Cretaceous	Late Cretaceous	99-65
		Early Cretaceous	144-99
	Jurassic		206-144
	Triassic		248-206

*Adapted from Szalay (1994).

2.3 Extinct Placental Mammals of South America

Placental mammals that existed in the Paleocene of South America included xenarthrans, which are the sloths, anteaters, and armadillos, and the ungulates, which are animals with hooves (Simpson 1980). Xenarthrans of the Paleocene were not that different than those of today, but in some cases, they were much bigger. The ungulates are organized into seven categories. Condylarthra was a group of unspecialized mammals that eventually evolved to become omnivores and herbivores; they lived during the Late Paleocene to Middle Miocene (Simpson 1980). Litopterna was a group of long-necked mammals that had elongated snouts and lived from the Paleocene to the Pliocene (Simpson 1980). Astrapotheria was possibly an amphibious mammal with a short proboscis and tusks and lived from the Eocene to the Miocene (Simpson 1980). Trigonostylopoidea was similar to Astrapotheria, but had smaller tusks and lived from the Paleocene to the Eocene (Simpson 1980). Pyrotheria was a group that was elephant-like in size and appearance that lived from the Eocene to the Oligocene (Simpson 1980). Notoungulata were rhino-like and hippo-like in size and appearance; they emerged in the Paleocene and died off gradually until the Early Pleistocene (Simpson 1980). Xenungulata was tapir-like and was the largest ungulate; it went extinct earlier than the rest, in the Late Paleocene (Simpson 1980).

In the Early Oligocene, when South America was an island continent, primates and rodents entered South America, probably due to island hopping from Africa (Simpson 1980). The primates radiated into the New World monkeys, and the rodents radiated into the caviomorphs seen today: porcupines, guinea pigs, capybaras, chinchillas, and agoutis (Simpson 1980).

This is a quick glimpse of who the marsupials were sharing their space and resources with in early mammalian history. The differences from marsupials of that time suggest that there was minimal niche overlap, which allowed them to live alongside one another for a long period of time.

2.4 Extant Placental Mammals of South America

When North America and South America became connected in the Pliocene, an event known as the Great American Biotic Interchange occurred (Simpson 1980). Species dispersed across this new land connection, and so new species entered South America. Placental mammals that dispersed to South America included tapirs, deer, camels, rabbits, squirrels, mice, dogs, bears, raccoons, weasels, cats, horses, and mastodons (Simpson 1980). All but the last two currently inhabit South America today. There is speculation that the introduction of these placental mammals caused the native South American mammals to go extinct. However, there is no material evidence to support this.

2.5 Extant Placental Mammals of Australasia

Australasia's placental mammal population is much less known compared to its marsupial population. However, there are quite a few placentals now located in Australasia. There are sixty-four species of rats and mice that dispersed to Australasia, presumably by island hopping from Asia 4MYA (Parish and Cox 2008). The dingo was introduced to Australasia three thousand years ago and used by aboriginal people as companions (Parish and Cox 2008). Within the last two hundred years, twenty-three species of placental mammals have been introduced to Australasia including house mice, rabbits, deer, fox, cows, sheep, goats, horses, camels, and cats (Parish and Cox 2008). The recent placental introductions have been thought to have been associated with the decrease in population sizes and increases in extinctions of many Australasian marsupials (Parish and Cox 2008).

After separation from Antarctica, South America was isolated for about thirty-five million years. During this time, both marsupial and placental mammals evolved and radiated without any pressure from newly immigrating mammals, except monkeys and some rodent species. Upon connecting with North America, many of South America's native mammals went extinct, and many new species from North America took their places. It has seemed to be the assumption that this is the reason that the diversity of South American marsupials decreased to such a great extent compared to Australasian marsupial diversity, since Australia has remained isolated since its separation from Antarctica. However, it could be argued that Australia is no different than South America in regard to total marsupial diversity of extant and extinct species. Australia has been an isolated continent for thirty-eight million years, giving the marsupials time to radiate without disturbance into what is seen today, except for the introduced taxa discussed earlier. The introduction of placental mammals that has occurred over the past two hundred years could be compared to the dispersal of placental mammals from North America to South America during the Great American Biotic Interchange. Australia has not seen a large decrease in its marsupial diversity yet, but with the number of marsupials that have already gone extinct since European settlement (18) (Johnson et al. 2007) and the number that are now endangered, this could be an event similar to the marsupial extinctions of South America.

Importantly, the basal metabolic rate of marsupials is 70% that of placental mammals of equivalent size, although their field metabolic rates are the same (Tyndale-Biscoe 2001). This means that energy, nutrient, and water requirements for marsupials are less than similar-size placental mammals. Also, the longer period of time that marsupials lactate and feed their young compared to placentals allows the mother to survive better in harsh environments when resources are less available (Tyndale-Biscoe 2001). This is an advantage, especially in semi-arid environments that are nutrient-poor and do not have a lot of available water.

3. *Biogeography*

The South American marsupial order Microbiotheria lives across the world from its closest relatives, the Australasian marsupials. However, this is not the only case of organisms living across the world from close relatives. Many plants and animals separated by vast distances and oceans are closely related. How has this happened? Continental drift seems to have caused this situation. The continents are located on tectonic plates that are constantly shifting, albeit very slowly. Continents collide, sharing shores for a period of time, and then eventually they shift and separate again. This process goes on even today and is one of the strongest influences on geographic distribution of organisms.

3.1 Continental Drift

The supercontinent known as Pangaea began its separation near the end of the Triassic (Fooden 1972). It split into two landmasses known as Laurasia and Gondwana. North America and Eurasia remained connected, composing the land mass Laurasia in the northern hemisphere. South America, Africa, Antarctica, and Australia formed the land mass in the southern hemisphere known as Gondwana. Africa separated from Gondwana in the Late Triassic-Early Cretaceous (Fooden 1972), leaving the continental formation seen in Figure 3A.

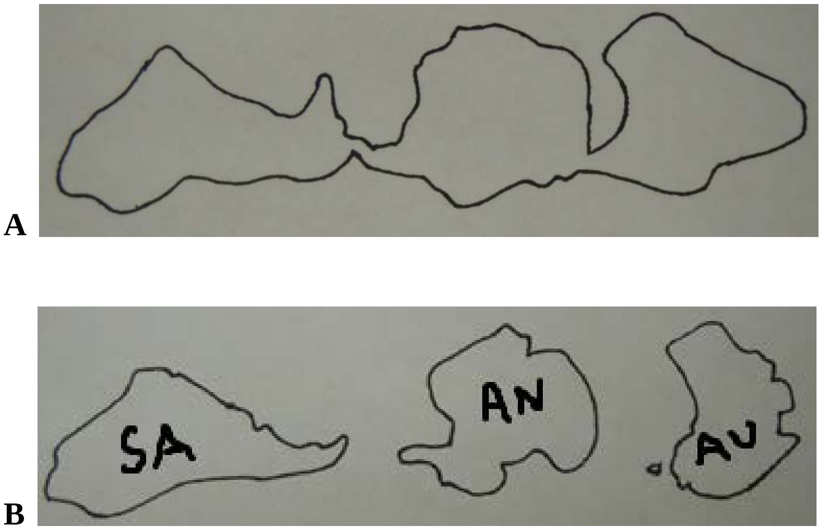


Figure 3. Continental Drift. **A.** The supercontinent Gondwana approximately 60MYA. **B.** After the break-up of Gondwana approximately 28MYA. From left to right: South America (SA), Antarctica (AN), Australia (AU).

The climate at this time was very different than it is today. Temperatures were much warmer, allowing even Antarctica to be tolerant of rainforests (Woodburne and Zinsmeister 1982). The number of angiosperms present across the world increased by 80% in the Cretaceous (Meredith et al. 2011). Warmer climate allowed tropical species of plants to be present across the whole of Gondwana. The mistletoe family Loranthaceae dates back to the Middle Cretaceous and was widespread in Gondwana (Vidal Russell et al. 2007). Today, it occurs in tropical and temperate habitats of Australia, New Zealand, New Guinea, and South America (Vidal Russell et al. 2007). One of the most primitive genera of the family Loranthaceae is *Tristerix* (Amico and Aizen 2000). Since the monito del monte is currently the sole seed disperser for *Tristerix corymbosus* (Amico and Aizen 2000), this could shed light on the monito del monte’s dispersal across Gondwana, proving this to be an ancient mutualistic relationship

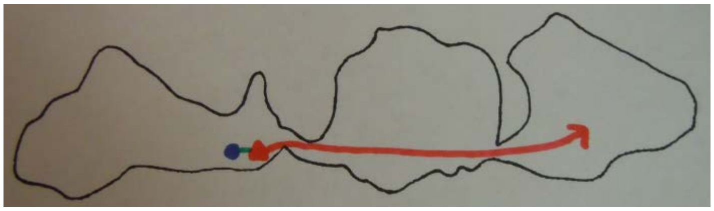
(Amico and Aizen 2000). Another plant found across Gondwana is *Nothofagus*, or southern beech (Pielou 1979). It originated in the southern hemisphere during the Late Cretaceous (HaoMin and ZheKun 2007). Fossils of *Nothofagus* have been found in Antarctica and dated to the Oligocene (Pielou 1979) and the Eocene (HaoMin and ZheKun 2007). *Nothofagus* is not only currently found in southern South America, where the monito del monte calls its forests home, but it is also currently found in Australia, New Guinea, and New Zealand (HaoMin and ZheKun 2007). Forests such as these created a continuous biogeographical region that stretched across South America, Antarctica, and Australia and would have aided movement and dispersal (Beck 2012). During the Late Cretaceous and Early Cenozoic, the Antarctic Peninsula served as the main filter for movement between South America and Australasia (Woodburne and Zinsmeister 1982).

There is a wide range of estimates as to when Australasia separated from Antarctica: 38-56 MYA (Palma and Spotorno 1999; Nilsson et al. 2004; Springer et al. 1998; Nilsson et al. 2003; Beck 2012; Godthelp et al 1992; Woodburne and Zinsmeister 1982; Pielou 1974; Szalay 1994). Separation began 56MYA, and final separation occurred 38MYA. What is agreed upon is that the separation of Australasia from Antarctica was before the separation of South America and Antarctica, which took place 28-41MYA (Beck 2012; Palma and Spotorno 1999). As Australasia separated from Antarctica, shallow water environments emerged; however, organisms could still traverse them until the distance between the two continents became too great and the water became too deep (Godthelp et al. 1992). This happened with the separation of South America from Antarctica, as well where evidence suggests a former beach dating to 40MYA where Antarctica was previously attached to South America (Woodburne and Zinsmeister 1982). Upon separation from Antarctica, Australasia began moving northward, as did South America (Pielou 1979) (Figure 3B). South America and Australia remained isolated continents through most of the Tertiary (Tyndale-Biscoe 1973), giving life on each continent a long period of time in which to radiate. Antarctica was now home to, not only the organisms left after the splitting of South America and Australia, but to newly forming glaciers as oceanic circulation began cooling waters around the continent (Springer et al. 1998). As this cooling spread, Cenozoic cooling of the world began (Nilsson et al. 2004). This cooling of the climate has caused increased aridity in Australia (Tyndale-Biscoe 2001). Although life on isolated Antarctica survived the separation from Australasia and South America, organisms eventually went extinct in the Early Miocene due to the decreasing temperatures and fully regional glacial conditions (Woodburne and Zinsmeister 1982).

Although Australia and Antarctica have remained isolated continents since their separation, South America did not. North and South America became connected in the Pliocene or Early Pleistocene via the Isthmus of Panama (Tyndale-Biscoe 1973). Prior to this connection, neither continent had any families of terrestrial mammals in common with the other (Pielou 1979). This is when the Great American Biotic Interchange began, and this event is what has helped shape the South American fauna into what it is today. As for Australia, it has remained isolated since its split from Antarctica 38MYA. The Australian marsupials have had 38 million years to evolve and radiate into what is seen there today almost completely alone. It is almost exclusively travel by man that has introduced new species of competitors to Australia.

3.2 Dispersal Scenarios of Microbiotheria

It is evident that during the time when South America, Antarctica, and Australasia were connected, organisms were moving around all three continents. With evidence suggesting that the monito del monte is more closely related to Australasian marsupials than marsupials in South America, a natural conclusion is that some form of dispersal occurred across Gondwana. This is especially true because there is fossil evidence, as will be discussed later. There are many different scenarios of dispersal that could have occurred. Figure 4 represents a simplified summary of possible dispersal scenarios.



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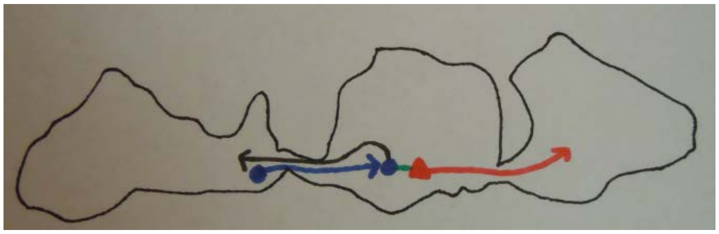

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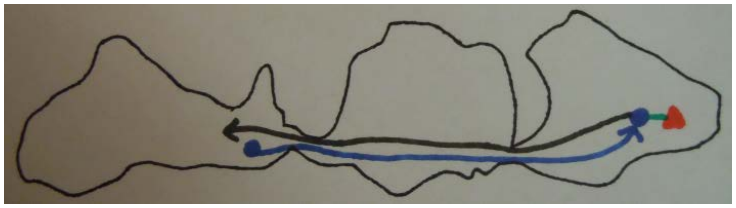

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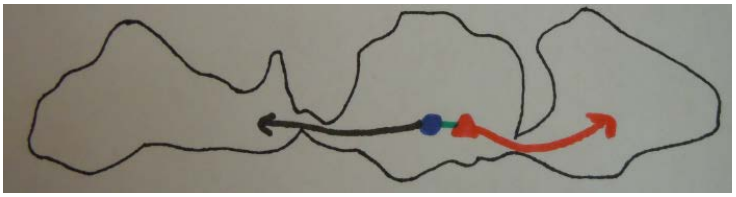

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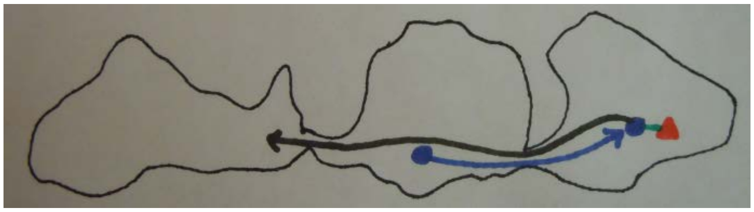

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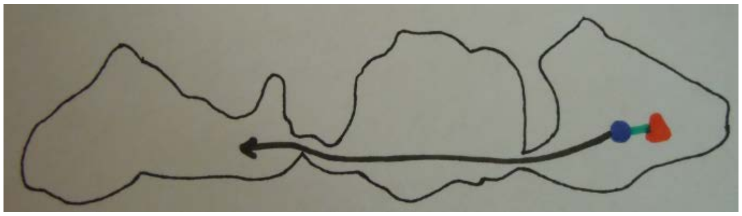

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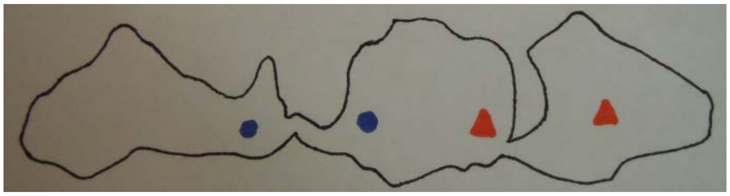


Figure 4. Dispersal Scenarios for Microbiotheria. The blue circle represents Microbiotheria. The red triangle represents Australasian marsupials. The blue arrow represents the dispersal of Microbiotheria. The green line represents the radiation of Australasian marsupials. The red arrow represents the dispersal of Australasian marsupials. The black arrow represents the back-dispersal of Microbiotheria to South America. **A-C** depicts the three scenarios for the South American origin of Microbiotheria. **D-E** depicts the two scenarios for the Antarctic origin of Microbiotheria. **F** depicts the scenario for the Australasian origin of Microbiotheria. **G** depicts the scenario of confluent marsupial fauna across Gondwana where vicariance resulted in the two marsupial faunas of South America and Australasia. All scenarios require the extinction of marsupials from Antarctica. Scenarios that place Microbiotheria in Australasia require the order to have gone extinct there. The scenario that places Australasian marsupials in South America requires them to have gone extinct there.

3.2.1 South American Origin of Microbiotheria

One way that dispersal may have begun was if Microbiotheria originated in South America. Microbiotheria could have radiated in South America, and the species that originated from that radiation dispersed across Antarctica to Australasia and gave rise to the Australasian marsupials seen today (Figure 4A). Alternatively, Microbiotheria could have originated in South America, dispersed to Antarctica, and radiated there. The species that radiated would have to then disperse to Australasia, and Microbiotheria would have had to disperse back to South America (Figure 4B). Another version for the South American origin of

Microbiotheria entails its dispersal from South America to Australasia via Antarctica, radiating in Australasia, and dispersing back to South America (Figure 4C). These scenarios imply movement via sweepstakes dispersal (Beck 2012), meaning that there was in place some sort of barrier that prevented most other mammals from dispersing, but, by chance, a few made their way through. For each scenario, any Microbiotherids that were present in Australasia and/or Antarctica and any Australasian marsupials that were present in Antarctica and/or South America had to have gone extinct, as they are not currently seen in these localities.

3.2.2 Antarctic Origin of Microbiotheria

Another scenario begins with the origin of Microbiotheria in Antarctica. One version of this involves the Australasian marsupials radiating from Microbiotheria in Antarctica and dispersing to Australasia, with Microbiotheria dispersing to its current location in South America (Figure 4D). Another version involves Microbiotheria originating in Antarctica, dispersing to Australasia, radiating there, and then dispersing to South America (Figure 4E). These scenarios all require that marsupials go extinct in Antarctica, and Microbiotheria go extinct in Australasia. These dispersal scenarios also would rely on some level of sweepstakes dispersal because, according to these scenarios, there is not a large flow of organisms moving across Gondwana (Beck 2012).

3.2.3 Australasian Origin of Microbiotheria

The Australasian origin scenario places the radiation of a clade containing Microbiotheria and the Australasian marsupials in Australasia (Figure 4F).

Again, this scenario implies sweepstakes dispersal where an Ameridelphian, or some form of Australidelphian ancestor, dispersed from South America, across Antarctica, and into Australasia. Microbiotheria would then have dispersed across Antarctica and into South America, subsequently going extinct in Australasia and Antarctica.

3.2.4 Confluent Marsupial Faunal Spread

One last scenario suggests that there was a broad stretch of marsupials across all of Gondwana (Beck 2012) (Figure 4G). There would not have been any barriers to restrict the flow of organisms across the three continents, which means movement occurred by corridor dispersal. This would mean that the differences between South American and Australasian marsupials are due to vicariance, meaning the geographical range of taxa is split because of the formation of a physical barrier, rather than by sweepstakes dispersal. In this case, the differences in South American and Australasian marsupials are due to the separation of the continents and the formation of oceans between them. Oceanic barriers would have inhibited gene flow, causing each continent's marsupials to evolve separately into the ones seen today.

Deciding which of these dispersal scenarios was most likely depends on fossil evidence, morphology, and phylogenetic data.

4. Fossil Evidence

The best way that the movements of marsupials can be tracked is through paleontology. Fossils can offer proof that a certain species was in one place at a certain time, and it can be compared to the same species found in another place or another animal found elsewhere. This kind of information can be sewn together to begin to tell a story about marsupials. Fossils are dated by a method called radiometric dating. The substrate that the fossil was found in is dated by the level of decay of its radioactive elements. Since the fossils themselves are not being dated, the actual age of the substrate may be slightly older or slightly younger than the fossil itself.

4.1 Comparative Morphology in Determining Taxonomy of a Marsupial Fossil

Comparative morphology is essential for placing an organism into the proper taxonomic group. By examining a fossil, similarities and differences are noted, and if an organism has all of the features of a certain group, it will be placed within it; if not, a new group may be made and its relationships to other groups considered. There are many ways for a paleontologist to categorize a newly found fossil, especially when dealing with complex organisms like mammals. There are a lot of bones involved, and there may be many different arrangements and morphologies of a single bone type. The most crucial determinants in marsupial fossil taxonomy are the ankle joints (Szalay 1994). The continuous lower ankle joint pattern (CLAJP) is one in which the ectal and sustentacular facets are continuous (Beck 717) (Figure 5). The separate lower ankle joint pattern (SLAJP) has discontinuous ectal and sustentacular facets, otherwise separate (Beck 2012) (Figure 5). The CLAJP and SLAJP are the characters upon which the cohorts Ameridelphia and Australidelphia are based. Australidelphians possess the CLAJP, and Ameridelphians display the SLAJP (Beck 2012). Another feature exclusively seen in Australidelphia is the triple-faceted calcaneocuboid joint (Beck 2012) (Figure 5). This joint is separated into three parts in Australidelphia, whereas it is one continuous piece in Ameridelphia. These are the easiest characters to analyze in order to tell the difference between Ameridelphia and Australidelphia, but, of course, other methods are used to sort fossils into further, more specific taxonomic groups.

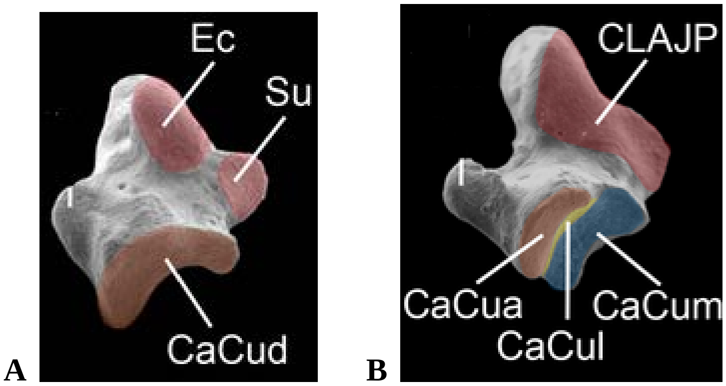


Figure 5. Ankle joints of Ameridelphian and Australidelphian marsupials. **A.** Ameridelphian. Separate lower ankle joint with Ec (ectal facet) and Su (sustentacular facet), red. Uniform calcaneocuboid joint CaCud (distal facet), orange. **B.** Australidelphian. Continuous lower ankle joint, red. Triple-faceted calcaneocuboid with CaCua (auxiliary facet), orange; CaCul (lateral facet), yellow; and CaCum (medial facet), blue. Adapted from Beck et al. (2008).

4.2 Early Marsupial Fossils

The oldest known stem-marsupial fossil is *Sinodelphys szalayi*, which was found in China and dated at 125MYA, in the Cretaceous (Springer and Murphy 2007). Along with it was the oldest known eutherian, *Eumaia*, also dated at 125MYA (Springer and Murphy 2007). These two are thought to be the stem taxa that eventually gave rise to marsupials and placental mammals. Since there are no marsupials currently inhabiting Asia, it can be seen that marsupials have had a complex biogeographic history. Skeletal remains have been found in North America from the Oligocene and given the name Herpetotheriids (Horovitz et al. 2009). These specimens have been categorized as marsupials, but they are placed as the sister taxon to all extant marsupials (Horovitz et al. 2009). Even older Herpetotheriids have been found in North America. A Herpetotheriid, *Nortedelphys magnus*, was found in North America and dated at 75MYA; this establishes the minimum age for the split between marsupials and their sister group Herpetotheriidae (Horovitz et al. 2009). Another North American fossil is in the marsupial family Peradectidae and was dated to 55MYA (Horovitz et al. 2009). It has been placed as the sister group to the family Didelphidae, which includes living American opossums, and both groups are in the order Didelphimorphia (Horovitz et al. 2009). A great example that gives evidence of the dispersal of marsupials is the family Polydolopidae, which belongs to the cohort Ameridelphia (Goin et al. 2006). Polydolopids are known from the Late Cretaceous in North America and from the Paleocene to Eocene in South America (Goin et al. 2006). The oldest known Polydolopid is *Cocatherium*, which was found in South America and dated to the Paleocene (Goin et al. 2006). Another Polydolopid was found in Antarctica and was dated at 40MYA (Woodburne and Zinsmeister 1982). The locations of these fossils would suggest that marsupials originated in Laurasia, specifically in North America. Marsupials then dispersed to South America where they were able to move freely across Gondwana, from South America into Antarctica.

4.3 Microbiotherid Fossils

For this study, Microbiotherids are of particular interest, so finding fossils that belong to this order is of utmost importance for tracking its biogeographic history. The oldest Microbiotherid in South America is *Khasia cordillerensis*, which was dated to 59.2-64.5MYA (Beck et al. 2008). Another Microbiotherid found in South America was *Mirandatherium alipoi*, which dated to 59MYA (Beck et al. 2008). *Microbiotherium tehuelchum* belongs to the order Microbiotheria and was found in South America and dated to 16MYA (Beck et al. 2008). Other Microbiotherids have been found in Antarctica that date to the Late Eocene (Goin and Carlini 1995). Since Microbiotheria is an order that belongs to the cohort Australidelphia, these fossil organisms found in South America and Antarctica are closely related to the extant species of marsupials in Australasia. Here is evidence that Microbiotherids dispersed across Gondwana.

4.4 Early Australasian Marsupial Fossils

Two of the most important and informative marsupial fossils found are *Djarthia murgonensis* and a specimen known only by its designator QM F30060. *Djarthia* is known from Early Eocene Australia and is the oldest known Australidelphian (Beck et al. 2008). It possesses the CLAJP and the triple-faceted calcaneocuboid, which explains its placement in the cohort Australidelphia. However, *Djarthia* is placed outside of the clade containing extant Australasian marsupials, placed instead as a sister group to them (Beck et al. 2008). This is significant because *Dromiciops gliroides* is also placed within Australidelphia but as a sister to Australasians, suggesting a close relationship between *Djarthia* and

Dromiciops (Beck et al. 2008). *Djarthia*'s dental and tarsal remains suggest that it was an insect-eating, scansorial (climbing) or arboreal animal (Beck et al. 2008). This is also a significant connection between *Djarthia* and *Dromiciops*, since the latter is insect-eating and arboreal. The specimen QM F30060 was also found in Australia and dates to the Early Eocene about 54.6MYA (Beck 2012). What is unique about this specimen is that it lacks the CLAJP, instead having the SLAJP, but it possesses the tripartite calcaneocuboid joint morphology (Beck 2012). Since QM F30060 has one of the derived features of Australidelphians but not both, it is not an Australidelphian, but instead, a possible Ameridelphian (Beck 2012). QM F30060 is a sister taxon to Australidelphia, in a group containing Australidelphia+*Djarthia*. This is the first non-Australidelphian found in Australasia. QM F30060 seems to be an intermediate between the American Didelphid morphology and the Australidelphian condition, by which the monito del monte, *Djarthia*, and extant Australasian marsupials are defined (Beck 2012). Like *Djarthia* and the monito del monte, QM F30060 seems to have been arboreal or scansorial rather than terrestrial (Beck 2012). The combination of the SLAJP and the triple-faceted calcaneocuboid morphology suggests that this marsupial diverged before the CLAJP evolved in marsupials (Beck 2012). QM F30060 and *Djarthia* exemplify transitions from Ameridelphian to Australidelphian morphology. The fact that they are both found in Australasia suggests that Australidelphia originated in Australasia.

4.5 Non-Marsupial Fossils

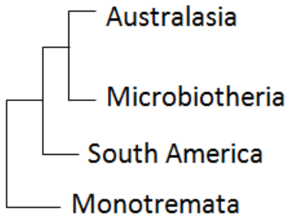
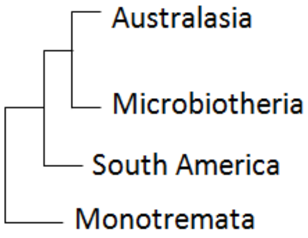
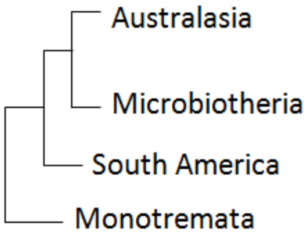
Marsupial fossils are not typically found isolated from other types of fossils. A wide range of other animals are found at the same sites. A site in Australia dated to 54.6MYA yielded remains of bats, snakes, turtles, frogs, birds, and placental and marsupial mammals (Godthelp et al. 1992). The marsupials at this site are somewhat ambiguous, bearing resemblance to peramelids, dasyurids, and didelphids (Godthelp et al. 1992). What is of significance at this site is the presence of placental Condylarths, which have been previously known from South America and thought to have originated in the Early Paleocene of North America (Godthelp et al. 1992). Another case of a non-marsupial mammal found in an area where it does not exist today is the monotreme *Monotrematum sudamericanum*, which was found in South America and dated to the Early or Middle Paleocene (Beck 2012). Monotremes are found today only in Australasia. This gives more support to the dispersal of mammals across Gondwana during this time. These fossils suggest that marsupials dispersed across Gondwana and were not alone when they arrived in Australasia, as has been previously assumed by their successful radiation into the diversity of forms presently seen.

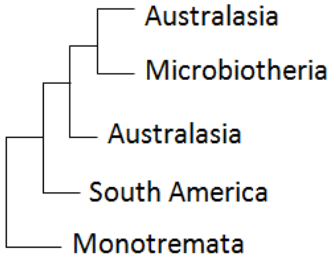
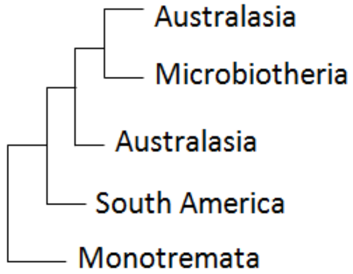
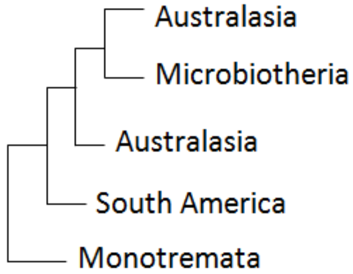
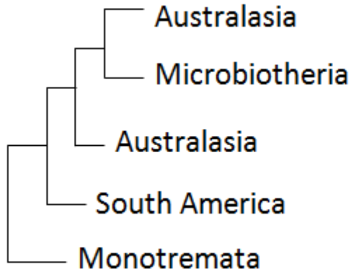
Fossils tell paleontologists that not only marsupials, but all three mammalian forms moved across Gondwana. Their transitionary phases allow their progression to be tracked. Marsupials were not alone when they dispersed into Australasia. They were accompanied by both non-mammalian vertebrates and placental mammals, the latter of which they most likely engaged with in competition. Fossils give a solid foundation of information that can be used as a base for all phylogenetic and biogeographic hypotheses.

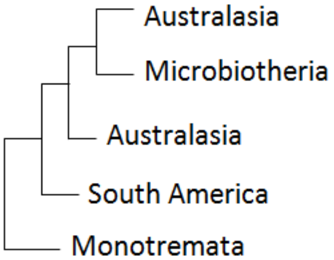
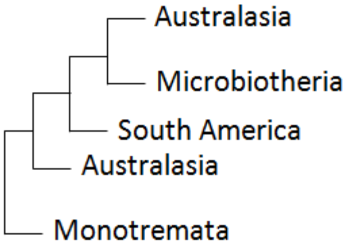
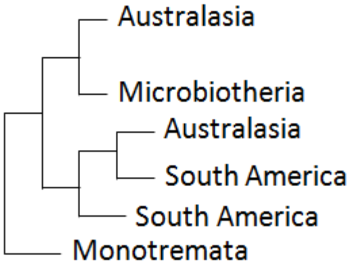
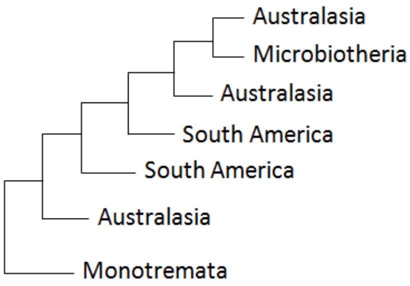
5. Molecular Phylogeny

In a survey of eleven phylogenetic studies, there were quite a few different ways in which results placed the seven orders of marsupials into cladograms. What is of special interest is the location of the monito del monte. Keeping the placement of the monito del monte in mind, three simplified phylogenetic trees were produced. One tree resulted in an Australasian order at the base of marsupial divergence, with the monito del monte somewhere within the rest of the Australasian and American marsupials (Table 3, Trees 3-7). Another tree had the monito del monte nested within the Australasian marsupials, with American orders at the base of marsupial divergence (Table 3, Trees 2A-2E). The third tree placed the monito del monte as the sister group to all Australasian marsupials, with American orders at the base of marsupial divergence (Table 3, Trees 1A-1C).

Table 3. Simplified phylogenetic trees

Tree	Genes (base pairs)	Gene Type	# of Taxa	Source
Tree 1A 	ApoB, BRCA1, RAG1, IRBP, vWF (total=6,300)	all nuclear	22	Amrine-Madsen et al., 2003
Tree 1B 	53 retroposons	retroposon	20	Nilsson et al, 2010
Tree 1C 	A2AB, ApoB, BRCA1, BRCA2, DMP1, ENAM, GHR, IRBP, RAG1, vWF, TTN, CNR1, BCHE, EDG1, RAG2, ATP7A, TYR1, BDNF, ADRB2, APP, BMI1, CREM, FBN1, PLCB4, ADORA3, PNOC (total=35,603)	all nuclear	24	Meredith et al., 2011

Tree 2A 	P1 (243)	nuclear	18	Retief et al., 1995
Tree 2B 	IRBP (1,276)	nuclear	7	Springer et al., 1997
Tree 2C 	12S rRNA, tRNA Valine, 16S rRNA, cytb, IRBP (total=4,757)	4 mtDNA and 1 nuclear	10	Springer et al., 1998
Tree 2D 	complete mitochondrial genome (15,000-18,000)	mtDNA	10	Nilsson et al., 2003

Tree 2E 	complete mitochondrial genome (15,000-18,000)	mtDNA	19	Nilsson et al., 2004
Tree 3 	12S rRNA (818)	mtDNA	18	Palma and Spotorno, 1999
Tree 4 	12S rRNA (818)	mtDNA	18	Palma and Spotorno, 1999
Tree 5 	12S rRNA (818)	mtDNA	18	Palma and Spotorno, 1999

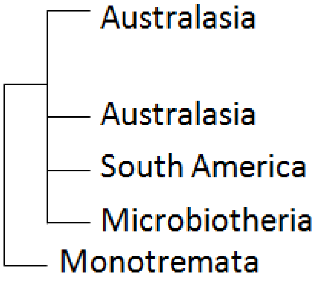
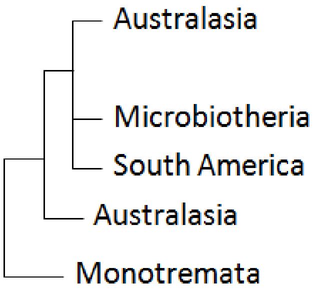
Tree 6 	PGK-1 (430)	nuclear	40	Colgan, 1999
Tree 7 	chrm-1, drd1a, c-mos, sox9, adra2b (total=2,793)	all nuclear	6	van Rheede et al., 2006

Table 3. Simplified phylogenetic trees from the literature. Monotremata is the outgroup. Australasia refers to Australasian marsupials. South America refers to South American marsupials. Microbiotheria refers to the marsupial order Microbiotheria i.e., *Dromiciops gliroides*. Wherever an Australasian marsupial occurred, the term 'Australasia' was put in its place. If there was a grouping of Australasian marsupials, the term 'Australasia' was put in its place. The same was done for South American and Microbiotherid species. mtDNA refers to mitochondrial DNA.

5.1 An Australasian Basal Species

Three studies are exemplified here that resulted in a cladogram with an order of Australasian marsupials at the base of the tree and the monito del monte placed within the remaining Australasian and American orders (Table 3, Trees 3-7).

In a single-gene study of the nuclear gene PGK-1, Colgan (1999) produced a tree which gave a polytomy, with Microbiotheria, Notoryctemorphia, Ameridelphia, and the remaining Australasian marsupials branching out from the same node (Table 3, Tree 6). Although Ameridelphian monophyly was supported (Colgan 1999), there is no support for any ordinal branches, leaving the relationships among the marsupials unresolved. In another study, van Rheede et al. (2006) used five nuclear genes to analyze mammalian relationships. For marsupials, the basal split between the group containing Dasyuromorphia and Peramelemorphia and the remaining marsupials was supported, yet another polytomy, between Diprotodontia, Microbiotheria, and the two orders of American marsupials, left the rest of the tree unresolved (van Rheede et al. 2006) (Table 3, Tree 7). Palma's and Spotorno's (1999) single-gene study of the mitochondrial gene 12S rRNA resulted in three different cladograms. One cladogram that placed an Australasian order Peramelemorphia at the base of the marsupial tree had Microbiotheria as the sister group to a clade containing the three remaining Australasian orders (Palma and Spotorno 1999) (Table 3, Tree 3). A second tree that resulted in Peramelemorphia being basal to all marsupials placed Microbiotheria within the remaining Australasian marsupials, making Notoryctemorphia the basal taxon of a clade composed of Microbiotheria and the Australasian orders Dasyuromorphia and Diprotodontia (Palma and Spotorno 1999) (Table 3, Tree 5). A third tree by Palma and Spotorno (1999) resulted in a basal split that grouped Peramelemorphia and the two American orders into one clade and the remaining marsupial orders in the other clade. The latter clade placed Microbiotheria as sister taxon to the group containing the rest of the Australasian marsupials (Palma and Spotorno 1999) (Table 3, Tree 4). There is support within the order Didelphimorphia for each tree, but there is no support for the placement of the other orders in any of the trees (Palma and Spotorno 1999). This study was completely unresolved at the ordinal level.

All of these studies recover an Australasian marsupial in a basal position on the tree, separate from the other Australasian marsupials, suggesting that Australasian marsupials are not monophyletic. The Australasian order Peramelemorphia was typically separated from the other Australasians. Although there is hardly any support for these placements, it is worth entertaining the notion that perhaps Peramelemorphia is different from the rest of the Australasian marsupials in ways that are unclear at this time.

5.2 Microbiotheria Nested Within an Australasian Clade

In the eleven published papers found in this survey, five of them placed Microbiotheria within the clade containing Australasian marsupials (Table 3, Trees 2A-2E). This means that the most recent ancestor of Microbiotheria is an Australasian marsupial, not an American marsupial.

The study by Retief et al. (1995) on the nuclear P1 gene resulted in a basal split between Ameridelphia and Australidelphia; of the Australidelphians, the order Peramelemorphia is in the basal position. Microbiotheria is nested deep in the tree, being sister to the clade containing Australasian orders Dasyuromorphia and Notoryctemorphia (Retief et al. 1995) (Table 3, Tree 2A). There was maximum support for the basal split between Ameridelphia and Australidelphia; however, the remaining ordinal branches have little support. The P1 gene was found to be orthologous in all seven marsupial orders (Retief et al. 1995), meaning the gene came from a shared, common ancestor of all extant marsupials. The 1997 study on the nuclear IRBP gene by Springer et al. (1997) does not give any more clarification of the relationships among marsupial orders. There was support for American marsupials being the basal group, but among Australidelphians, there was no resolution (Springer et al. 1997) (Table 3, Tree 2B). Springer et al. (1998) reported a study of four mitochondrial genes and one nuclear gene. The two orders of American marsupials were well-supported as being basal to the remaining marsupials (Springer et al. 1998). The monito del monte was placed within the clade of Australidelphians (Springer et al. 1998). In that clade, the most basal marsupial order was Peramelemorphia, which was well-supported (Springer et al. 1998). The monito del monte's placement as sister to the clade containing Diprotodontia, Notoryctemorphia, and Dasyuromorphia was well-supported, as were the remaining divergences among Australasian marsupials (Springer et al. 1998) (Table 3, Tree 2C). In a study by Nilsson et al. (2003) on the complete mitochondrial genome, the monito del monte was placed within Australasian marsupials. American marsupials were well-supported as being basal to all marsupials, and the split between Diprotodontia and the clade containing the monito del monte and Peramelemorphia had maximum support (Nilsson et al. 2003). As seen before, the monito del monte is supported as being closely related to Peramelemorphia, here, as sister taxa (Nilsson et al. 2003). However, other topologies from Nilsson et al. (2003) placed the monito del monte as sister to Diprotodontia, and these could not be rejected (Nilsson et al. 2003). The monito del monte as the sister to all Australasian marsupials was rejected

(Nilsson et al. 2003) (Table 3, Tree 2D). Nilsson et al. adds another piece of interesting information. American marsupials have a conserved anti-codon sequence of (UUU) for tRNA-Lys, whereas the other marsupial orders in this study, including the monito del monte, do not possess this feature (Nilsson et al. 2003). This suggests that the monito del monte is more closely related to Australasian marsupials than to American ones. In 2004, Nilsson et al. (2004) published another study of the complete mitochondrial genome, but added more species to the number of marsupial taxa represented. The resulting tree had a split between the Ameridelphian and Australidelphian marsupials, which was supported, and maximum support for the split between Diprotodontia and remaining Australidelphians (Nilsson et al. 2004). The monito del monte as sister to a clade containing Notoryctemorphia, Dasyuromorphia, and Peramelemorphia was strongly supported, as were the remaining ordinal divergences (Nilsson et al. 2004) (Table 3, Tree 2E). The monophyly of Australasian marsupials and the monito del monte being sister to all Australasian marsupials were both rejected (Nilsson et al. 2004). The monophyly of Australidelphia was strongly supported (Nilsson et al. 2004).

These studies all have Ameridelphian marsupials in the basal position of the marsupial tree. Also, they all place the monito del monte within the clade of Australasian marsupials and not as a sister taxon. This suggests that Australasian marsupials may not be monophyletic, since a taxon currently living in South America is nested within them.

5.3 Microbiotheria as Sister Group to an Australasian Clade

The results of the remaining studies of this survey place the monito del monte as the sister taxon to all of the Australasian marsupials. In these studies, American marsupials are basal to all other marsupials (Table 3, Trees 1A-1C).

In a study by Amrine-Madsen et al. (2003) using five nuclear genes, Ameridelphian marsupials are well-supported as being basal group of all marsupials, and there is maximum support for the placement of the monito del monte as sister to the Australasian marsupials (Amrine-Madesen et al. 2003) (Table 3, Tree 1A). The monophyly of both Australidelphia and Australasian marsupials were each supported (Amrine-Madesen et al. 2003). The marsupial tree being rooted by a split between Ameridelphia and Australidelphia was rejected, as was the monito del monte being the basal taxon of all marsupials (Amrine-Madesen et al. 2003). A study on twenty-six nuclear genes by Meredith et al. (2011) concluded that Ameridelphian marsupials are at the base of the tree, and the monito del monte is the sister taxon to all Australasian marsupials (Table 3, Tree 1C). All of the branches of the marsupial tree were strongly supported with bootstrap values at or above 90% (Meredith et al. 2011). The monophyly of Australidelphia and Australasian marsupials were both strongly supported.

The study by Nilsson et al. published in 2010 is different than all of the studies previously discussed (Table 3, Tree 1B). As those studies all analyzed either mitochondrial genes, nuclear genes, or both, Nilsson et al. (2010) constructed a study to analyze fifty-three retroposons. Retroposons emerge when fragments of DNA are copied and pasted randomly into other places in the genome. This study on the patterns of retroposon insertion in marsupials resulted in Ameridelphian marsupials being basal to all marsupials, and the monito del monte and Australasian marsupials being sister groups (Nilsson et al. 2010). The possession of at least three retroposons is necessary in order for a divergence to be significant, according to Nilsson et al (2010). For example, the American order Didelphimorphia lacks two retroposons that all other marsupials have, and because that number is less than three, it is not significant (Nilsson et al. 2010). This means that the split between Didelphimorphia and all other marsupials is not significant. All Australasian marsupials and the monito del monte possess thirteen retroposons that the American orders Didelphimorphia and Paucituberculata do not have (Nilsson et al. 2010). This is the longest branch, and it gives strong support to the monophyly of Australidelphia. Australasian marsupials all have four retroposons that American marsupials, including the monito del monte, do not have (Nilsson et al. 2010). This supports the monophyly of the Australasian marsupials. Diprotodontia has three retroposons that no other marsupial order has (Nilsson et al. 2010). The branch that leads to the clade containing the three Australasian orders Notoryctemorphia, Dasyuromorphia, and Peramelemorphia has only one retroposon marker (Nilsson et al. 2010). However, the orders Dasyuromorphia and Peramelemorphia have three and four unique retroposons, respectively (Nilsson et al. 2010), which is significant. According to this study, Microbiotheria, the order containing the monito del monte, does not have any unique retroposons that are not already possessed by any other order, which is also true for the order Notoryctemorphia (Nilsson et al. 2010).

All three of the previous studies discussed resulted in nearly identical phylogenetic trees with disagreement among the clade containing orders Notoryctemorphia, Peramelemorphia, and Dasyuromorphia. They all suggest that Australidelphia and Australasian marsupials are monophyletic.

5.4 Divergence Times

Just as there were many results and ideas about the relationships among the marsupials, there are also many different results about the divergence times of the marsupial orders.

Monotremata is the sister group to Theria, and they diverged 231-217MYA (van Reade 2006). Marsupials and placentals diverged 193-186MYA (van Reade 2006), but as will be seen shortly, these times vary, and Nilsson et al. (2010) placed Eutherian and Marsupial divergence at 130MYA. There are many different numbers published for the earliest marsupial divergence. Nilsson et al. (2010) says it occurred 65-85MYA, Meredith et al. (2011) says around 80MYA, Yu et al. (2012) says around 77MYA, and Nilsson et al. (2004) says 69MYA. Since the first date listed here includes all of these dates, the date of the earliest marsupial divergence is reasonably set at 65-85MYA. The strongest support for the most basal order has been from American taxa. There are two hypotheses about which American order diverged first. Some authors suggest that Didelphimorphia is the most basal marsupial order, and others say that

Paucituberculata is the most basal. With these two hypotheses come different dates, discussed below.

For the hypothesis that places Didelphimorphia as the most basal marsupial order, Nilsson et al. (2003) found that Didelphimorphia and all of the other marsupials diverged 64MYA. This study also found that Paucituberculata diverged from the Australidelphians 62MYA, Diprotodontia diverged 51MYA, and the monito del monte split from Peramelemorphia 43MYA (Nilsson et al. 2003). Another study by Nilsson et al. (2004) had similar results. The results of the study were that Didelphimorphia diverged 69MYA, Paucituberculata diverged 60MYA, Diprotodontia diverged 50MYA, and the monito del monte split from the clade containing the remaining three Australasian orders 46MYA (Nilsson et al. 2004). The Australasian orders Dasyuromorphia, Notoryctemorphia, and Peramelemorphia diverged sometime in the Middle Eocene to Early Oligocene (Nilsson et al. 2004).

A study by Meredith et al. (2011) resulted in Paucituberculata being the most basal marsupial order, diverging 82MYA. Didelphimorphia diverged 75MYA, the monito del monte diverged 64MYA, Diprotodontia diverged 62MYA, Notoryctemorphia diverged 61MYA, and the orders Peramelemorphia and Dasyuromorphia split 60MYA (Meredith et al. 2011).

These two phylogenies are not only different, but the dates are drastically different, as well. Both studies by Nilsson et al. (2003; 2004) placed the monito del monte within Australasian marsupials, and the resulting divergence time estimated to be 46-43MYA. The study by Meredith et al. (2011) placed the monito del monte as sister to all the Australasian marsupials, and their divergence time for the monito del monte was 64MYA. Disregarding the phylogeny of the studies, combined, the studies say that the earliest marsupial divergence was 82-64MYA and that the monito del monte diverged from the rest of marsupials 64-43MYA. This is a large time frame since, as will be discussed shortly, marsupials are thought to have radiated in a very short period of time, as compared to placental mammals (Yu et al. 2012).

5.5 Diversification Rates

Meredith et al. (2011) suggests that 24-45 million years elapsed between the splitting of Monotremata and Theria and the splitting of Marsupialia and Eutheria. There is not much known about mammals during this stretch of time. Marsupials also lagged behind placental mammals in diversification by 15-20 million years (Yu et al. 2012; Meredith et al. 2011). Meredith et al. (2011) found an increase in diversification rate in mammals 100MYA and 80MYA and a rate decrease 78MYA. Yu et al. (2012) found large increases in the net diversification rates of Euaustralidelphians, which are the marsupials currently living in Australasia, and of Boreoeutheria, which gave rise to more than 90% of extant placental mammals. The large increase in diversification for Boreoeutheria occurred in the Upper Cretaceous and for Euaustralidelphians in the Early Paleocene (Yu et al. 2012). The timing of the two diversification rate increases (Meredith et al. 2011; Yu et al. 2012) overlap, so 100-80MYA is when Boreoeutheria had increased diversification (Meredith et al. 2011; Yu et al. 2012), and 63MYA is when Euaustralidelphia had their rate increase (Yu et al. 2012).

Another big increase in the diversification rate of marsupials occurred in the family Macropodidae, which belongs to the order Diprotodontia, in the Middle Eocene (Yu et al. 2012).

As far as the orders of marsupials go, diversification within each occurred at different times, even happening long after their initial divergences from the other marsupial orders. The study by Yu et al. (2012) resulted in diversification within the order Didelphimorphia 34MYA, within Paucituberculata 10MYA, within Diprotodontia 50MYA, within Dasyuromorphia 27MYA, and within Peramelemorphia 25MYA (2012). According to Meredith et al. (2011), diversification within the order Paucituberculata took place 12MYA, and within the order Didelphimorphia, diversification began 31MYA. Diversification occurred within the Australasian orders Peramelemorphia 28MYA, within Dasyuromorphia 30MYA, and within Diprotodontia 53MYA (Meredith et al. 2011). Since Microbiotheria and Notoryctemorphia currently contain only one and two species each, respectively, their diversification times were not calculated. The results of Meredith et al. (2011) and Yu et al. (2012) are very similar, being different by only 2-3 million years. Most of the marsupial orders displayed intra-ordinal diversifications much later than when they diverged from the other marsupial orders. For example, diversification within the order Paucituberculata did not occur until 12MYA (Meredith et al. 2011), even though the results show that Paucituberculata was the first marsupial order to diverge 82MYA. Yet, some orders like Diprotodontia had intra-ordinal diversifications a short time, relatively speaking, after the inter-ordinal split from the rest of the marsupial orders. Even with this apparently large difference in diversification times, Nilsson et al. (2003) suggested that mitochondrial genome evolutionary rates are very similar between marsupials compared to placental mammals. The fastest evolving marsupial taxon, family Macropodidae, and the slowest evolving marsupial taxon, order Paucituberculata, display a maximum difference in evolutionary rates of 14% (Nilsson et al. 2003). The fastest evolving eutherian taxon, Perissodactyla, and the slowest evolving eutherian taxon, Cetacea, display a maximum difference in evolutionary rates of 200% (Nilsson et al. 2003).

5.6 Understanding Molecular Analyses

With so many different outcomes for the phylogeny of Microbiotheria, how does one gauge the accuracy of them and select the one that is correct? Sometimes the data that one is using to construct a tree can be misleading, even though the data are perfectly valid (van Rheede et al. 2006). For example, some of the studies discussed earlier were based on a single gene. If the sequence of the gene is short, one might not obtain good resolution in the resulting tree (Springer and Murphy 2007). By increasing the length of the sequences, and by using more than one gene, phylogenetic resolution increases (Springer et al. 1998). It is also important to have a broad taxonomic representation. If the number of species used is increased, and if the number of species reflects the diversity of a particular group, then the resolving power of the tree produced will be increased (Springer et al. 1997). Another reason that some phylogenies may be less accurate than others is because of variation in the third codon of an amino acid. The third nucleotide of an amino acid can vary and still produce the same amino acid. However, the differences of this third codon can influence the results of a phylogenetic tree if they are not corrected for by excluding it from the study (Beck et al. 2008). Another way to ensure better resolution in a tree is by using both mitochondrial and nuclear DNA. Alone, each of these forms of DNA can produce different phylogenetic results, but together, phylogenetic resolution increases (Phillips et al. 2013).

Retroposons have yielded more information to support the origin of the Microbiotheria and are one of the best ways to produce a phylogeny. This is because retroposons are inserted into the genome completely at random, so the odds of the same retroposon being inserted in the same place in another organism are very low (Nilsson et al 2010). Also, once a retroposon is inserted into the genome, it most likely will remain there, and because of this, if organisms share retroposons, it most likely means that they have a common ancestor that obtained that retroposon (Nilsson et al 2010). A large percent of the marsupial genome (52%) is made up of retroposons (Nilsson et al 2010). This is more than most other groups of animals have, and so this makes retroposons a good tool to use to produce marsupial phylogenies.

6. *Conclusions*

What was attempted in this thesis was to synthesize different aspects of information known about marsupials to solve the problem of the unknown origin of Microbiotheria, and it was successful. In the totality of evolutionary history, there is no great difference between the American and Australasian marsupials in diversity. The diversity of South American marsupials, living and extinct, matches that of current Australasia. Across both continents marsupials comprised large carnivores, small, rodent-like forms, bipedal hoppers, and opossums. The only difference in these faunas was the time of arrival of introduced species. It can also be said that placental mammals did exist in the early isolation of Australasia but did not inhibit the radiation of Australasian marsupials. Since only one placental specimen has been found in Australasia, the number of placental mammals there cannot be known. Some marsupials have an advantage over placentals in certain environments and under stressful climatic changes. Combined with a possible small number of placentals compared to marsupials, this might have been a reason for the marsupials outcompeting placental mammals early in the history of isolated Australia, especially since, at this time, the climate was cooling, increasing the aridity of Australasia.

Geography was very influential for early marsupial radiations. Marsupials were able to exploit the land connections of South America, Antarctica, and Australasia, moving eastward to escape the crowds of South American animals and fill the open niches available across Antarctica and Australasia. With climatic cooling in the Eocene, Antarctica became glaciated, and marsupials there went extinct.

However, left across Antarctica are fossils of the marsupials that once existed there. These fossils support the hypothesis of initial dispersal from South America to Australasia, and then dispersal of Microbiotherids to South America. The fact that Microbiotherid fossils of South America are more derived than those found in Antarctica imply dispersal in the direction from Antarctica to South America. There are currently no known Microbiotherid fossils in Australasia. This does not mean that they do not exist, but perhaps that they may not have been found yet. Upon discovery of Microbiotherids in Australasia, more evidence could be added to support the hypothesis of dispersal from Australasia to South America. An Ameridelphian fossil being found in Australasia is one of the best pieces of evidence to support the Australasian origin of the monito del monte and the other Australidelphians because of its transitional morphology between the Ameridelphians of South America and the Australidelphians of Australasia. This suggests that an American marsupial dispersed across Gondwana to Australasia, evolving one of the two Australidelphian morphological characters along the way, the tripartite calcaneocuboid joint; then as the Australidelphian marsupials evolved in Australasia, the CLAJP trait arose.

As far as molecular phylogenetic evidence goes, the only thing that can be said for certain is that Microbiotheria is more closely related to Australasian marsupials than to American marsupials. Not one study placed them in a basal position on the phylogenetic tree, or as the sister group of either American marsupial order, which would be evidence that Microbiotheria was more related to South American marsupials. The retroposon study by Nilsson et al. (2010) is the best evidence for the close relationship between Microbiotheria and Australasian marsupials. The fact that retroposons occur randomly and stay in place once they are inserted makes this study’s results vital to the understanding of marsupial relationships. The synthesis of nuclear, mitochondrial, and retroposon analyses strongly supports the close relationship between the monito del monte and its Australidelphian relatives. Thus, these studies collectively support the Australasian origin of all Australidelphians, especially in the light of the other non-molecular pieces of evidence previously discussed.

It is now possible to paint a picture of marsupial history from the evidence provided in this thesis. It is generally accepted that marsupials originated in North America. This occurred in the Late Jurassic-Early Cretaceous. When South America and North America were still connected, early marsupials dispersed to South America. After North and South America split, marsupials radiated into didelphid and caenolestid forms in South America, and marsupials went extinct in North America. With the exception of the known monotreme fossils at this time, Antarctica and Australasia did not have many, if any, mammals. With South America being filled with placental and marsupial mammals, it was natural for some to disperse into Antarctica and make their way across to Australasia. It is assumed that this movement was via sweepstakes dispersal since only a few fossils have been found in Antarctica and Australasia during the time these continents were connected.

The ancestral marsupial that migrated from South America to Australasia is here argued as being an Ameridelphian. The strongest support for this is the fossil QM F30060 that is clearly not an Australidelphian, but a transitionary form between Ameridelphian and Australidelphians. The presence of this fossil and the fossil Australidelphian *Djarthia*, which are the oldest marsupial fossils of Australasia, give strong support to the hypothesis that Australidelphia, including

Microbiotheria, originated in Australasia. This is supported by phylogenetic studies. The retroposon study by Nilsson et al. (2010) gives the greatest support, by yielding a long branch of thirteen retroposon markers that belong to all extant Australidelphians, but not Ameridelphians. Other studies show exceptional support for this, as well (Meredith et al. 2011; Amrine-Madsen et al. 2003). Since there is evidence that Australidelphia originated in Australasia, this would require Microbiotheria dispersing to South America. There are Microbiotherid fossils in Antarctica and South America, but none have been found in Australasia. Although the Microbiotherids in South America are older than ones found in Antarctica, Microbiotherids in South America are more derived, implying dispersal from Antarctica to South America. All of these dispersals would have had to take place before 38MYA when Australasia was completely separated from Antarctica. The dating of these fossils agrees with this time frame. The movement of Microbiotheria from Antarctica into South America would have had to occur no later than 28MYA, and the Microbiotherid fossils of South America agree with this time frame, as well. The Microbiotherid fossils found in Antarctica that date to the Miocene are consistent with the climate change. The Miocene was when Antarctica started becoming fully glaciated and similar to what it is today, and so any marsupials left in Antarctica after the separation from South America would have gone extinct during this time.

Marsupials in Australasia then radiated, outcompeting the few placental mammals that migrated from South America. Nothing stood in their way, being an isolated continent with little to no immigrations possible—that is, until humans arrived and brought other placental mammals with them. This was the beginning of the Australasian marsupial decline.

South American marsupials radiated on an island continent similarly, with little to no immigration. When South and North America became reconnected in the Pliocene, many of the North American mammals expanded their range by dispersing south into South America. These North American placental mammals were adapted to eating the new forms of herbaceous material that the South American mammals were not adapted to. Because of this, North American mammals could outcompete the South American mammals for resources. Only a few South American mammals made their way into North America: the marsupial Virginia opossum, an armadillo, and a porcupine (Simpson 1980). After this Great American Biotic Interchange, the only marsupials left in South America were the didelphids, caenolestids, and the monito del monte, which are presently seen today.

There are two main factors that influenced marsupial evolution to produce the marsupials of today, isolation and climate change. Geographic isolation of land masses allowed marsupials to evolve without competition. Because of the separation of the continents, climate changed, allowing marsupials to take advantage of new habitats. These factors allowed marsupials to radiate rapidly. Rapid radiation is seen especially with the family Macropodidae because they had the opportunity to disperse into a new area that offered a respite from selection pressures associated with a changing climate, and so they were able to radiate rapidly and fill new niches (Yu et al. 2012).

It is most likely because of the geographic and climatic changes, and the fact that marsupials radiated rapidly, that it is difficult to find fossils. The sparse fossil record of marsupials is one of the reasons that their evolutionary history has been difficult to put together. More marsupial fossils from the Late Cretaceous-Paleocene are needed, especially from Australasia, in order to further evaluate hypotheses on marsupial dispersal.

Because of the condylarth fossil found in Australasia, it is now clear that the previous assumption that Australasian marsupials thrived because of the inability of placental mammals to reach Australasia is incorrect. Both marsupial and placental mammals were present in the early history of the island-continent Australasia, yet placental mammals did not survive.

The phylogenetic and fossil data suggests that Australidelphians did in fact originate in Australasia, and that the presence of the Microbiotherid monito del monte is the result of back-dispersal to South America. The biogeographic data support this hypothesis, as well. No matter what the dispersal route was, there is a clear separation between the two South American orders, Didelphimorphia and Paucituberculata, and the five Australidelphian orders, which includes Microbiotheria. The monito del monte is clearly more related to Australasian marsupials than to South American marsupials.

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Glossary

Amino acid- one of twenty naturally occurring basic compounds that make up a protein

Bootstrap value- a measure of the statistical support for a branch in a cladogram, the closer it is to 100, the more support there is for that branch

Cladogram- (phylogenetic tree), a diagram that shows relatedness of organisms based on synapomorphies

Codon- sequence of three nucleotides that codes for an amino acid

Convergent evolution- process where unrelated organisms have acquired the same traits independently of one another

Corridor dispersal- a dispersal route that allows unrestricted flow from one place to another

Mammal- a vertebrate that has hair, nourishes their young with milk from the mammary glands, and has live birth (except for monotremes)

Marsupial- a mammal with a shortened gestation period that gives birth to undeveloped young that continue development outside of the womb, and many have pouches

Mitochondrial DNA- DNA from the mitochondria of cells, inherited from the mother in mammals

Monotreme- egg-laying mammal

Nuclear DNA- DNA in the nucleus of cells

Nucleotide- a molecule that is one piece of the sequence of DNA

Parsimonious- a method that chooses the simplest explanation with the least number of assumptions (minimum number of steps)

Phylogeny- evolutionary history of a group of organisms

Retroposon- a piece of DNA that is copied and randomly inserted into another part of the genome

Sweepstakes dispersal- a dispersal route that will not likely be used by most organisms, usually because some kind of barrier exists; by chance, some organisms will make it through

Synapomorphy- shared, derived characteristic

Vicariance- the geographic range of organisms is split because of the formation of a geographic barrier

Author's Biography

Ariel Berthel was born in Manchester, NH in 1990. Her early years were spent in Derry, NH. She has spent the last 16 years of her life in Salem, NH where she graduated from Salem High School in 2009. Ariel's major is zoology, and she is a member of the National Society of Collegiate Scholars. She will graduate in May 2013. Ariel is unsure of exactly where her career will take her, but she does know it will involve wildlife in some way.