**Introduction**

The subclass Theria of Mammalia is made up of marsupials and placentals. Placental mammals develop their unborn offspring to a mature embryo stage in an organ called the placenta which provides nourishment and resides within the mother’s uterus. In contrast, marsupial mammals develop their newborn offspring within an external pouch that is attached to the mother’s abdomen. There are five to seven extant orders of marsupial mammals and 18 extant orders of placental mammals (Szalay, 1994; Marshall et al., 1990; Aplin & Archer, 1987). Past studies on fossil and anatomy have suggested phylogenetic trees for the 18 placental orders (Novacek, 1992; Shoshani & McKenna, 1998). DNA sequences have suggested a few of the proposed superordinal groups, which are Paenungulata (elephants, sea cows and hyraxes) and Cetartiodactyla (artiodactyls and whales)(de Jong, 1998). Traditional clades such as Archonta (primates, tree shrews, flying lemurs and bats) have been rejected by statistical tests on DNA sequence analysis (Springer et al., 1997). Afrotheria, which is a group that includes paenungulates, aardvarks, elephant shrews, golden moles and tenrecs, has been suggested by mitochondrial ribosomal RNA and nuclear protein-coding genes data (Springer et al., 1997; Stanhope et al., 1998). Little is known about the complex phylogenetic relationships among placental mammals. This project aims to answer the questions of the relationships among placental mammals and whether parallel adaptive radiations were present among placental mammals.

Concatenations of individual genes can be employed to achieve high resolving power when trying to elucidate phylogenetic relationships (Teeling et al., 2000). A dataset that consists of concatenated DNA sequences for mitochondrial RNA genes and three nuclear genes (A2AB, IRBP, vWF) for 26 placental taxa was employed for phylogenetic analysis in this study. This dataset includes all eutherian orders and two marsupial outgroups. In addition, this dataset represents two insectivore families, Chrysochloridae (golden mole) and Tenrecidae (tenrec) for every gene except IRBP, which was necessary to elucidate the phylogenetic relationship of the insectivores and Afrotheria.

An independent data set from 3 kilobases (kb) of exon 11 of the single-copy breast and ovarian cancer susceptibility gene 1 (*BRCA1*) for 33 taxa was used for hypothesis testing. The BRCA1 data set contains all placental orders and one marsupial outgroup. Phylogenetic trees (maximum likelihood) for both datasets with bootstrap support values are given in Fig. 4 and 8. Phylogenetic trees for different phylogenetic methods are shown in the results section.

**Methods:**

Data collection

The two independent datasets were obtained from a paper that investigated parallel adaptive radiations in placental mammals (Madsen et al., 2001). The first dataset is a 5708 bp aligned dataset that is made up of concatenated DNA sequences for A2AB (1,164 bp), IRBP (1,292 bp), vWF (1,251 bp) and 12S rRNA/tRNA valine-16S rRNA (2,001 bp). The second data set is a 2947 bp aligned data set that is made up of BRCA1 DNA sequences.

Phylogenetic analysis

Maximum parsimony and bootstrap analysis using heuristic search were conducted in PAUP for both datasets. The heuristic search was conducted using a random stepwise addition sequence with TBR branch swapping for 10 replicates. Bootstrap support values are based on 200 replications.

Distance analysis for both datasets was conducted using PHYLIP. Jukes-Cantor and Kimura models of sequence evolution were used to calculate distances from both DNA sequence data. Neighbor joining trees were created using the two distance matrices and a consensus tree was created to infer if different matrices result in different trees. Minimum evolution analysis was carried out for both distance matrices. Maximum likelihood and bootstrap analysis were carried out for both datasets using the GTR+G model of sequence evolution in RAxML-NG.

MCMC analysis was carried out for the 5708bp dataset. Since the dataset used for MCMC analysis with MrBayes contains four distinct gene regions (mitochondrial RNA genes, A2AB, IRBP, vWF), the analysis was run by specifying the data partitions corresponding to these four gene regions and defining a substitution model for each data partition in order to understand if the substitution process differs between these different gene regions. The analysis was run by assuming that all four gene regions evolved under the evolved under the GTR+Γ model and setting the prior for the rates heterogeneity to an exponential distribution with a rate parameter, λ = 0.05.

**Results and Discussion**

Maximum likelihood trees for both dataset showed that placental orders are made up of Xenarthra, Afrotheria, Laurasiatheria (shrews, hedgehogs, pangolins, bats, whales), and Glires (rodents and lagomorphs) + Euarchonta (flying lemurs, tree shrews and primates) (Fig. 4 and 8). Xenarthra, Afrotheria and Laurasiatheria are consistently monophyletic with different methods of analysis whereas Euarchonta plus Glires is monophyletic or paraphyletic depending on the phylogenetic method.

Based on the BRCA1 DNA sequence data, Afrotheria is further supported by a 9-bp deletion that is present in all 12 afrotherians but not in the other placental mammals and marsupials that were investigated (Fig. 9). This suggests that the 9-bp deletion could be due to shared common ancestry of Afrotheria. However, without any morphological evidence for Afrotheria (Asher, 1999; Shoshani & McKenna, 1998), it is challenging to explain this hypothesis. There is robust support for the Paenungulata in the Afrotheria clade. Afrotheria also includes golden moles and tenrecs, with 71% bootstrap support value for golden mole and 100% bootstrap support value for tenrec using maximum likelihood analysis (Fig. 8). Maximum parsimony analysis resulted in 91.8% support for golden mole and 100% support for tenrec (Fig. 6C). In addition, there is also strong support for the Cetartiodactyla and the monophyly of eulipotyphlan insectivores (hedgehogs, shrews and moles) in the Laurasiatheria clade.

There was inconsistent support for Euarchonta + Glires because the position of the root was not consistent across the different phylogenetic methods that were used to analyze the data. Maximum-likelihood analysis with the BRCA1 data set resulted in 100% bootstrap support for Glires + Euarchonta, whereas maximum parsimony analysis resulted in 79% support (Fig. 8 and 6C). Distance analysis using Kimura and Jukes Cantor models showed differences in the rooting for Euarchonta + Glires (Fig. 7A and 7B). Neighbor joining tree with Jukes Cantor model of sequence evolution roots on rat and mouse. The two shortest parsimony trees (11,018 steps; Fig. 6A and 6B) for BRCA1 root on Old World porcupine, resulting in Euarchonta + Glires, Glires and Rodentia being paraphyletic.

Maximum parsimony analysis using heuristic search on the 5708bp dataset provided one parsimony tree with 18891 steps (Fig. 1B). Parsimony bootstrap support value for Glires + Euarchonta was 72%, whereas maximum likelihood bootstrap support value was 97% (Fig. 4 and Fig. 8). There is 100% support for the Paenungulata in the Afrotheria clade in both maximum parsimony and likelihood analysis. The root for Xenarthra (Sloth) was inconsistent because the position of the root seemed sensitive to the phylogenetic method that was employed. The shortest parsimony tree roots on Sloth with 100% bootstrap support value, whereas maximum likelihood tree showed that Sloth shared common ancestor with Glires + Euarchonta and Laurasiatheria clades with 58% bootstrap support value. MCMC analysis mirrored maximum likelihood results for Sloth (Fig. 5).

Distance analysis neighbor joining trees using Kimura and Jukes Cantor models for the 5708bp dataset did not group Glires and Euarchonta together (Fig. 2A and 2B). There was no polytomy observed for the majority rule consense tree of both Kimura and Jukes Cantor models, suggesting that different distance matrixes did not result in different trees (Fig. 2C). Minimum evolution trees using Kimura and Jukes cantor models did not result in differences in topology, further supporting the hypothesis that different distance matrixes would lead to the same trees (Fig. 3A and 3B). Minimum evolution analysis also did not group Glires and Euarchonta together. Rabbit was not grouped with Rat and Capybara, rendering Glires being paraphyletic.

These phylogenetic analysis results support hypotheses such as Paenungulata, which is morphology-based, and other hypotheses such as Afrotheria and Laurasiatheria. It had been suggested that there were parallel adaptive radiations in placental and marsupial mammals, leading to robust examples of convergence (Madsen et al., 2001). The results from current study also provide support for parallel adaptive radiations in the placental clades Afrotheria and Laurasiatheria.

**Results:**

Shape

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Figure 1A: 50% majority-rule consensus tree of 200 bootstrap trees for maximum parsimony analysis on 5708bp dataset.

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Figure 1B: Maximum parsimony tree for 5708bp dataset using heuristic search

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Figure 2A: Neighbor joining tree for 5708bp dataset using Kimura model

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Figure 2B: Neighbor joining tree for 5708bp dataset using Jukes Cantor model

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Figure 2C: Majority rule consense tree for 5708bp dataset neighbor joining trees using Jukes Cantor and Kimura models

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Figure 3A: Minimum evolution tree for 5708bp dataset using Kimura model

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Figure 3B: Minimum evolution tree for 5708bp dataset using Jukes Cantor model

A picture containing graphical user interface

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Figure 4: Best maximum likelihood tree for 5708bp dataset with Felsenstein bootstrap support values

Diagram, schematic

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Figure 5: MCMC consense tree for 5708bp dataset

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Figure 6A: Maximum parsimony tree (1) using heuristic search for BRCA1 dataset

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Figure 6B: Maximum parsimony tree (2) using heuristic search for BRCA1 dataset

Diagram

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Figure 6C: 50% majority-rule consensus tree of 200 bootstrap trees for maximum parsimony analysis on BRCA1 dataset.

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Figure 7A: Neighbor joining trees using Kimura (left) and Jukes Cantor (right) models for BRCA1 dataset

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Figure 7B: Majority rule consense tree for BRCA1 dataset neighbor joining trees using Jukes Cantor and Kimura models

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Figure 8: Best maximum likelihood tree with Felsenstein bootstrap support values for BRCA1 dataset



Figure 9: The 9-bp deletion at positions 353–383 of the BRCA1 dataset that presents in all 12 afrotherians but not in other placental and marsupial mammals that were investigated.

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