UNIVERSIDADE DE BRASÍLIA

INSTITUTO DE CIÊNCIAS BIOLÓGICAS

Programa de Pós Graduação em Biologia Animal

FILOGENIA, DATAÇÃO MOLECULAR E BIOGEOGRAFIA DE ROEDORES TETRALOFODONTES DA TRIBO ORYZOMYINI (CRICETIDAE: SIGMODONTINAE)

Leonardo Ferreira Machado

Brasília-DF

Março/2012

UNIVERSIDADE DE BRASÍLIA

INSTITUTO DE CIÊNCIAS BIOLÓGICAS

Programa de Pós Graduação em Biologia Animal

FILOGENIA, DATAÇÃO MOLECULAR E BIOGEOGRAFIA DE ROEDORES TETRALOFODONTES DA TRIBO ORYZOMYINI (CRICETIDAE: SIGMODONTINAE)

Orientadora: Dr. Lilian Gimenes Giugliano

Co-orientador: Dr. Yuri Luiz Reis Leite

Dissertação apresentada ao Instituto de Ciências Biológicas da Universidade de Brasília como parte dos requisitos para obtenção do título de mestre em Biologia Animal

Brasília-DF

Março/2012

ÍNDICE

AGRADECIMENTOS

A presente dissertação é resultado de um esforço tremendo. Abri mão de várias coisas importantes na minha vida, como estar com a família, amigos de longa data, minha cidade natal e, portanto, distante do mar, do parque da Redenção, da boemia da Cidade Baixa, do Rio Guaíba, carne (bovina e ovina) de alta qualidade, erva mate pura, cerveja artesanal do Paulo e tantas outras que faço questão de fazerem parte da minha rotina novamente algum dia.

Para vencer esses obstáculos contém com o apoio irrestrito da minha família: Pai (Júlio), mãe (Bete) e irmão (Paulo), muito obrigao pelo apoio.

Agradeço enormemente a Ju Ribeiro que amo muito. Passamos por várias dificuldades e aprendizados juntos e se não fosse por ela talvez nem tivesse me mudado para Brasília.

À minha orientadora, Lilian, estou muito agradecido pela oportunidade de trabalhar com ela. Aprendi muito em seu laboratório e me sinto um biólogo mais competente depois desse período. Da mesma forma ao co-orientador, Yuri, parceiro e sempre presente na construção da dissertação.

Ainda bem que conheci muita gente legal que faz a vida em Brasília ser um prazer. Vou citar várias, cada uma contribuindo de alguma forma, e me desculpem se esqueci de alguém: Fábio, Tati, Marcella, Flávia, Fernando, Antônio, Renan, Alison, Mariana Mira, Daniel, Dannyel, Aelton, Julinho, Maurício, André, Vanessa, Luane, Neander, Xexa, Clarisse, Samuca, Pedro, Marina, Babi, Nicholas, Kid, equipe CHUNB em geral, Jonas, Sabrina, Antônio, Ricardo, Raimundo, Teteu, ...e tantas outras pessoas mais.

Agradeço ao Guarino e o Marcelo Weksler que contribuíram para melhorar a dissertação, desde a época da qualificação do projeto.

Agradeço também a equipe do Laboratório de Mastozoologia e Biogeografia e do Núcleo de Genética Aplicada à Conservação da Biodiversidade da UFES, principalmente a Carol Loss e Juliana Justino, pela disponibilidade em me ceder tecidos de espécies estudadas, primers, ajuda em laboratório e pelo "tour" em Vitória.

Muito obrigado aos curadores das coleções que cederam tecidos e permitiram acesso aos exemplares analisados: Robert Baker (Texas Tech University), Leonora Costa (UFES), Alexandre Uarth Christoff (ULBRA) e Jader Soares Marinho-Filho (UnB).

A Allison Gaynes, muitíssimo obrigado pela ótima revisão do inglês.

Colegas mastozoólogos de Porto Alegre que sempre serão parceiros de trabalho e grandes amigos: Alemão, Diegão, Duda, Roth e o Alexandre, que também contribuiu para a execução da dissertação: muito obrigado.

Por último, agradeço ao CNPq pela concessão da bolsa de mestrado.

APRESENTAÇÃO

O presente documento faz parte dos requisitos para obtenção do título de mestre em Biologia Animal pela Universidade de Brasília. O estudo se refere às relações evolutivas de roedores da tribo Oryzomyini, um agrupamento de mamíferos altamente rico em espécies e muito comum nos ambientes naturais de toda América do Sul. O mesmo está organizado da seguinte forma: inicialmente é apresentada uma introdução, que situa o leitor acerca do assunto abordado e dos objetivos propostos. Em seguida são apresentados os materiais e métodos, resultados e conclusões. Estes estão escritos em português e em forma reduzida, aonde o leitor consegue extrair as informações mais relevantes.

Em anexo é apresentado o manuscrito que será submetido para publicação no periódico Molecular Phylogenetics and Evolution. O mesmo foi redigido em inglês e contêm uma descrição minuciosa de todos os detalhes da pesquisa que realizamos, além de uma discussão situando a relevância do artigo frente ao estado atual do conhecimento sobre o tema.

RESUMO

Os objetivos da presente dissertação foram construir árvores filogenéticas e aplicar técnicas de datação molecular e reconstrução de áreas ancestrais para os roedores Oryzomyini do Clado "D", com ênfase nas formas tetralofodontes, um grupo pouco compreendido na literatura especializada. Métodos de máxima parcimônia, máxima verossimilhança e inferências bayesianas foram aplicados em 98 caracteres morfológicos e 4515 pares de bases de cinco fragmentos de DNA. As análises mostraram que táxons tetralofodontes não formam um grupo monofilético. Dentre os táxons viventes, *Pseudoryzomys* é irmão de *Holochilus* e *Lundomys* mais basal em relação à maioria das espécies do clado "D". Análises de parcimônia e bayesiana demonstraram que o fóssil *H. primigenus* não agrupa com os demais *Holochilus* e é basal em relação aos fósseis *Carletonomys* e *Noronhomys*, fazendo com que *Holochilus* seja parafilético. A maioria das divergências entre os táxons estudados ocorreram durante o Plioceno e em menor número no Pleistoceno e Mioceno. O ancestral comum mais recente do clado "D" possuía distribuição Cis e Trans-Andina e gêneros com distribuição atual ao norte do Panamá possuem ancestrais com distribuição Cis-Andina. Propomos que a identidade taxonômica de *H. primigenus* deve ser revista e que molares tetralofodontes surgiram mais de uma vez no clado "D". Alternativamente, discutimos que caracteres morfológicos tradicionalmente tidos como homólogos entre os tetralofodontes devem ser reavaliados. As divergências estimadas indicam que as grandes planícies da América do Sul durante o Plioceno foram favoráveis à diversificação principalmente dos gêneros tetralofodontes do clado "D". Além disso, eventos de soerguimento da porção norte dos Andes podem ter desempenhado um papel vicariante na diversificação das espécies dos gêneros estudados, ou que estes se diversificaram na América do Sul e migraram para a América do Norte após a formação do istmo do Panamá.

ABSTRACT

Our objectives were to construct phylogenetic trees, to apply molecular dating techniques and ancestral area analyses to Oryzomyini clade "D", emphasizing the poorly known tetralophodont forms. We applied maximum parsimony, maximum likelihood and Bayesian inference to 98 morphological characters and 4,515 base pairs from five DNA fragments. We found that tetralophodont genera do not form a monophyletic group. Among living taxa, *Pseudoryzomys* is sister to *Holochilus*, and *Lundomys* is basal to most species in clade "D". The fossil *H. primigenus* does not group with living *Holochilus* and is basal to the fossils *Carletonomys* and *Noronhomys*, making *Holochilus* paraphyletic. Most divergences occurred during the Pliocene, and others during the Miocene and Pleistocene. The most recent common ancestor of clade "D" had Cis and Trans-Andean distribution, while genera currently distributed to the north of Panama show ancestral Cis-Andean distribution. We propose that the taxonomic identity of *H. primigenus* should be reviewed, and that tetralophodont molars appeared more than once in clade "D". Alternatively, molar characters traditionally identified as homologies among tetralophodont genera should be reassessed. The most estimated divergences were placed in the Pliocene. Therefore, we hypothesized that great Plains in South America during the Pliocene may have especially favored the diversification of tetralophodont genera from clade "D". In addition, uplift events in northern Andes may have played a vicariant role in the diversification of the species in the studied genera, or that the lineages diversified in the South America ant dispersed to North and Central America after the Panama land bridge formation.

INTRODUÇÃO

Sigmodontinae é uma subfamília de roedores da família Cricetidae distribuída em maior parte na região Neotropical. É a subfamília mais rica em espécies desta porção do continente americano, ocorrendo em todos os biomas da América do Sul, Central e sul da América do Norte (Musser e Carleton, 2005). Os gêneros desta subfamília são comumente alocados em tribos, reconhecidamente: Abrotrichini, Akodontini, Ichthyomyini, Oryzomyini, Phyllotini, Reithrodontini, Sigmodontini, Thomasomyini and Wiedomyini (Smith e Patton, 1999; Musser e Carleton, 2005; D'Elía et al., 2007). Em menor número, alguns gêneros (e.g. *Wilfredomys*, *Abrawayaomys* e *Phaenomys*) são incertos quanto às suas afiliações tribais e tratados como Sigmodontinae *incertae sedis* (Smith e Patton, 1999). Dentre as tribos reconhecidas, Oryzomyini é a mais rica em espécies, abrangendo 31 gêneros e um expressivo número de novos táxons recentemente descritos (Weksler et al., 2006; Pardiñas, 2008; Turvey et al., 2010; Percequillo et al., 2011). Os Oryzomyini formam um clado amplamente distribuído na América Neotropical, ocorrendo desde o sul dos Estados Unidos até a região da Patagônia, no extremo sul da América do Sul. São encontrados em praticamente todos os biomas, incluindo florestas úmidas, savanas, campos e banhados, onde as espécies podem ser desde semi-aquáticas até arborícolas e com inúmeras adaptações morfológicas (Musser e Carleton, 2005; Weksler, 2006).

O progresso do conhecimento sobre a composição taxonômica dos Oryzomyini foi marcado por diferentes arranjos e incertezas sobre o monofiletismo da tribo (Hershkovitz, 1962; Steppan, 1995; Smith e Patton, 1999). A visão clássica de Hershkovitz (1955, 1962) estipulava que todos Oryzomyini compartilhavam molares pentalofodontes, excluindo desse grupo os gêneros com molares tendendo a laminação e com redução do número de dobras, reconhecidamente os molares tetralofodontes. Uma hipótese alternativa baseada na morfologia peniana defendida por Hooper e Musser

(1964) propunha que o gênero tetralofodonte *Holochilus* seria uma forma relacionada a Oryzomyini ao invés de associado à radiação de Sigmodontini. Este último ponto de vista foi sendo melhor compreendido em contribuições posteriores (Voss, 1991; Voss, 1992; Steppan, 1995), promovendo a hipótese de que os gêneros com mesolofo reduzido *Pseudoryzomys*, *Holochilus* e *Zygodontomys* compartilham sinapomorfias com Oryzomyini. Dessa forma, molares pentalofodontes foram identificados como plesiomorfias para Oryzomyini *sensu* Hershkovitz (1962). Recentemente, utilizando dados morfológicos e moleculares e com uma amostragem taxonômica ampla, Weksler (2003, 2006) corroborou a hipótese de monofiletismo dos Oryzomyini proposta por Voss e Carleton (1993), além de descrever outras sinapomorfias para esta tribo.

Apesar desse progresso, as relações filogenéticas entre vários gêneros de Oryzomyini permanecem incertas. Por exemplo, os tetralofodontes viventes e habitantes de áreas abertas *Pseudoryzomys*, *Holochilus*, *Lundomys* e os fósseis *Noronhomys*, *Carletonomys* e *Holochilus primigenus* possuem relações genealógicas mal resolvidas, apesar de serem comumente considerados próximos evolutivamente. Com base em dados morfológicos, Voss e Carleton (1993) obtiveram duas árvores igualmente parcimoniosas aonde *Pseudoryzomys* ou *Lundomys* aparecem como irmão de *Holochilus*. Steppan (1996) encontrou *Lundomys* como irmão de *Holochilus*, enquanto Carleton e Olson (1999) encontraram *Noronhomys* como irmão de *Holochilus* e *Lundomys* mais relacionado com estes do que com *Pseudoryzomys*. Com base em sequências do exon 1 do gene que codifica a proteína ligante do fotoreceptor retinóide (IRBP), Weksler (2003) encontrou *Pseudoryzomys* próximo a *Holochilus* e *Lundomys* irmão dos dois. Contudo quando dados morfológicos foram analisados em conjunto com a informação molecular, novamente *Pseudoryzomys* foi encontrado como irmão do clado formado por *Holochilus* e *Lundomys* (Weksler, 2006). Após as contribuições de Weksler (2003; 2006), a tribo Oryzomyini passou a ser dividida basicamente em quatro

grandes clados: “A”, “B”, “C” e “D”, sendo este último onde a maioria dos gêneros tetralofodontes (*Pseudoryzomys*, *Lundomys*, *Holochilus*) estão incluídos (Weksler, 2006; Turvey et al., 2010; Percequillo et al., 2011).

O registro fóssil de Oryzomyini é bem representado por gêneros tetralofodontes (Pardiñas, 2008; Pardiñas e Teta, 2011). O fóssil mais completo e bem preservado é *Noronhomys vespucci*, uma forma encontrada exclusivamente na Ilha de Fernando de Noronha, na costa nordeste do Brasil. Enquanto este táxon possui uma posição filogenética proposta (Carleton e Olson, 1999), outras formas relacionadas carecem de tal informação ou tem sua denominação específica discutível. Por exemplo, os fragmentos cranianos e dentários encontrados na província de Buenos Aires na Argentina, nomeado *Carletonomys cailoi*, apesar de ser indiscutivelmente semelhante com tetralofodontes viventes, nunca foi analisado sob uma perspectiva filogenética e dentro de uma amostragem taxonômica representativa (Pardiñas, 2008). Outro táxon, denominado *Holochilus primigenus* da Bolívia, foi posicionado como irmão de *Holochilus* viventes baseado principalmente nas similaridades de suas mandíbulas, embora as estruturas de seus molares sejam praticamente indistinguíveis de *Lundomys*. Tal ambiguidade acarreta diferentes interpretações e coloca incertezas sobre o reconhecimento específico de *H. primigenus* (Steppan, 1996; Carleton e Olson, 1999; Pardiñas, 2008; Musser e Carleton, 2005). Nesse contexto, os Oryzomyini tetralofodontes são um exemplo claro da necessidade de investigações em sistemática, assim como um interessante foco de estudo sobre a história biogeográfica da América do Sul.

As hipóteses biogeográficas sobre os Oryzomyini sugerem que a tribo tenha se originado entre 5 e 9 milhões de anos atrás e que se dispersou em um sentido norte-sul na América do Sul, sendo a porção norte considerada ancestral (Engel et al., 1998; Smith e Patton, 1999; Steppan et al., 2004). Em sentido mais restrito, as hipóteses

biogeográficas sobre os tetralofodontes são escassas, e se confundem com suas questões taxonômicas. O arranjo tradicional de Hershkovitz (1962) e Reig (1984) advoga que *Pseudoryzomys* é uma linhagem do Phyllotini, que por sua vez é derivada de um estoque de Sigmodontini (onde *Holochilus* estaria incluso), sendo ambas as tribos tetralofodontes e habitantes de áreas abertas. Por outro lado, a noção atual de que *Pseudoryzomys*, *Holochilus* e *Lundomys* são de fato Oryzomyini, obrigatoriamente implica que os tetralofodontes descendem diretamente de Oryzomyini pentalofodontes que habitavam áreas florestais mais ao norte da América do Sul (Weksler, 2006; Pardiñas, 2008). Sendo assim, o cenário mais aceito é que os tetralofodontes descendem e um estoque selvático de Oryzomyini do norte da América do Sul. Uma linhagem ancestral teria atingido habitats em formações abertas do leste e sul do continente em estágios mais avançados de diversificação por volta do Plioceno. Neste momento eles teriam uma ampla distribuição no continente e as expansões e retrações das vegetações ocorridas no Pleistoceno teriam moldado suas distribuições recentes (Voss e Carleton, 1993; Pardiñas, 2008; Pardiñas e Teta, 2011).

Neste contexto de incertezas taxonômicas e cenários biogeográficos, o presente estudo teve como objetivo geral investigar hipóteses filogenéticas para os Oryzomyini do clado "D", enfatizando os tetralofodontes viventes e extintos e incluindo uma ampliação do conjunto de caracters analisados. Com base nestas análises, nossos objetivos específicos foram avaliar as hipóteses sobre o posicionamento filogenético de *Carletonomys cailoi* e *Holochilus primigenus* e testar a hipótese de monofiletismo dos Oryzomyini tetralofodontes do clado "D". Além disso, os tempos de divergência dos clados e áreas de distribuição ancestral foram estimados utilizando abordagens bayesianas e, com base nos resultados dessas análises, foram ropostos cenários evolutivos e biogeográficos.

MATERIAIS E MÉTODOS

AMOSTRAGEM TAXONÔMICA

As espécies do grupo interno são basicamente as mesmas presentes no clado "D" definido em Weksler (2006). As espécies *Drymoreomys albimaculatus*, descrita em Percequillo et al. (2011), *Holochilus sciureus*, *H. primigenus*, *N. vespucii* e *C. cailoi* também foram analisadas. O grupo externo foi composto pelas espécies mais distantes *Neacomys spinosus*, *Oligoryzomys nigripes*, *O. flavescens*, *Oecomys catherinae* e *Hylaeamys megacephalus* (Weksler, 2003, 2006).

Os números de referência das coleções e de acesso no GenBank (NCBI, 2011), assim como as espécies utilizadas em cada análise estão apresentados na Tabela 1.

CARACTERES MORFOLÓGICOS

Foram utilizados 98 caracteres morfológicos, propostos em Weksler (2006) e com as modificações realizadas em Turvey et al. (2010) e Percequillo et al. (2011). A matriz destas informações morfológicas é de livre acesso e disponibilizada no MorphoBank (O'Leary e Kaufman, 2007).

As informações dos fósseis foram retiradas das descrições originais (Steppan, 1996; Carleton e Olson, 1999; Pardiñas, 2008). Além desses, adicionamos informações de *Holochilus sciureus*, proveniente de dois exemplares depositados na coleção de mamíferos da Universidade de Brasília (Tabela 1). Os estados dos caracteres analisados foram adicionados na matriz disponibilizada no MorphoBank e pode ser visualizada no Apêndice 1.

TÉCNICAS MOLECULARES

O DNA genômico foi extraído de tecidos musculares preservados em etanol utilizando o kit de extração DNeasy (Qiagen). Fragmentos dos seguintes genes foram amplificados: exon 1 do gene nuclear que codifica a proteína ligante do fotoreceptor

retinóide (IRBP) (1265 pares de base) e citocromo-b (cyt-b) (800 pb). Estes dois foram alinhados com fragmentos do IRBP disponibilizados por Weksler (2003) e com 1136 pb do cyt-b disponibilizados em Percequillo et al.(2011) e disponíveis no GenBank. Esse procedimento possibilitou um incremento no número espécies por gene utilizado. Adicionalmente, amplificamos fragmentos do exon 28 do fator de von Willebrand (vWF) (1198 pb) e do íntron 7 da subunidade ! do gene fibrinogênio (Fgb-7) (643 pb) de parte das espécies presentes no clado "D". Por último, utilizamos sequências do íntron 2 do gene nuclear álcool deidrogenase (Adh1-I2) (609 pb) de dados não publicados (Hanson, 2008) disponíveis no GenBank. Os iniciadores e detalhes das referências dos fragmentos de DNA utilizados estão descritos na Tabela 2.

As reações em cadeia da polimerase foram realizadas com misturas de reagentes e ciclos de temperatura com pequenas modificações das referências indicadas na Tabela 2. O sequênciamento dos fragmentos de DNA foram realizados em empresa terceirizada (Macrogen Inc).

ALINHAMENTO E ANÁLISES FILOGENÉTICAS

As sequências obtidas de cada gene foram alinhadas no programa T-Coffee (Notredame et al., 2000) e o melhor modelo de evolução nucleotídica foi selecionado pelo critério de informação de Akaike implementado no software JModelTeset (Posada, 2008).

O programa DAMBE (Xia e Xie, 2001) foi utilizado para verificar sinais de saturação nucleotídica das sequências. O programa Mega (Tamura et al., 2011) foi utilizado para obter o número de sítios polimórficos, estimar a proporção de divergências entre as sequências dos gêneros estudados e descrever a média de freqüência de bases das sequências analisadas.

As análises filogenéticas de máxima parcimônia (MP) foram realizadas para os dados morfológicos e cada região gênica, independentemente, e de forma concatenada no programa PAUP* v4.0b10 (Swofford, 1999). A melhor árvore foi encontrada por busca heurística pelo algoritmo tree bissection reconnection de branch swapping e o suporte dos ramos acessados usando 1000 replicações de bootstrap (Felsensein, 1985) e índice de Bremer (Bremer, 1994), obtido com os programas MacClade (Maddison e Maddison, 1999) e PAUP*.

As análises filogenéticas também foram realizadas por método bayesiano (MB) e de máxima verossimilhança (MV). Tais análises foram realizadas para cada conjunto de dados individuais (gene por gene) e com todos os genes concatenados. A primeira análise foi realizada no programa MrBayes v.3.0b4 (Huelsenbeck e Ronquist, 2001), enquanto a segunda foi realizada no ambiente e na linguagem R (R Development Core Team, 2011) com o pacote phangorn (Schliep, 2011).

Apenas as espécies com mais de três regiões com sequências disponíveis dos genes utilizados fizeram parte das análises concatenadas, em vista de se obter uma proporção adequada do número de espécies e dados faltantes (para detalhes de dados faltantes em análises filogenéticas ver Wiens (2006); Wiens e Moen (2008), Wiens e Morrill (2011).

DATAÇÃO MOLECULAR E ANÁLISE BIOGEOGRÁFICA

As sequências dos genes concatenados foram submetidas a uma análise de tempo de divergência por datação molecular, utilizando o critério de relógio molecular relaxado no programa BEAST v.1.4.8 (Drummond et al., 2006) por meio de uma abordagem bayesiana. Para isso foram utilizados três pontos de calibração. O primeiro com base na divergência das linhagens de Oligoryzomys nigripes e O. flavescens há 1,54 ± 1 (média ± desvio padrão) milhões de anos atrás (mya) (Palma et al., 2010). O

segundo na idade estimada do fóssil *Carletonomys cailoi* (1,0 ± 0,5 mya) e o terceiro fundamentado na formação das ilhas de Galápagos (4 mya) (Geist, 1984; Greham, 2001), tendo em vista a ocorrência de duas espécies do gênero *Nesoryzomys* endêmicas desse arquipélago.

Tendo em vista as incertezas associadas com cada método de calibração, executamos uma análise para cada ponto de calibração separadamente e com as três juntas. Dessa maneira, as congruências encontradas possibilitaram inferir hipóteses biogeográficas com menor risco associados aos diferentes métodos.

As áreas de ocorrência dos ancestrais das espécies do clado "D" de Oryzomyini foram estimadas utilizando o software RASP (Reconstruct Ancestral State in Phylogenies) (Yu et al., 2011), que utiliza métodos bayesianos de reconstrução de caracteres ancestrais. Com base na distribuição das espécies disponibilizadas pela International Union for Conservation of Nature (IUCN), foram estipuladas três áreas de distribuição geográfica dos gêneros utilizados no presente estudo, Cis-Andina (leste e sul dos Andes), Trans-Andina (oeste e norte dos Andes), seguindo Weksler (2006), e América Central ao norte do Istmo do Panamá. Em seguida, foi construído um mapa apresentando a distribuição atual dos gêneros e as áreas de ocorrência ancestral estimadas pelo método de reconstrução de estados ancestrais.

RESULTADOS

CARACTERÍSTICAS DAS SEQUÊNCIAS

O número total de sítios polimórficos foi 179 para Adh1-I2, 446 para Cyt-b, 117 para Fgb-7, 210 for IRBP e 137 para vWF. Nenhum destes fragmentos mostrou sinal de saturação (Figura 1). O alinhamento dos dados concatenados demonstrou uma média de composição de bases de A = 26.31%, C = 24.91%, G = 22.86% e T = 25.81%. O teste do qui-quadrado (χ^2) rejeitou a hipótese de heterogeneidade entre as frequência das

bases: $x^2 = 44.59$ $p = 0.93$ e graus de liberdade = 60. A distância genética par – a – par entre os gêneros estudados demonstrou *Amphinectomys* e *Hylaeamys* como os mais distantes entre os táxons do grupo interno e externo (10.07%). Dentre o grupo interno, os gêneros mais distantes foram *Eremoryzomys* e *Amphinectomys* (8.16%), enquanto os mais próximos foram *Nesoryzomys* e *Aegialomys* (2.74%).

ANÁLISES FILOGENÉTICAS

A análise de MP com base em dados morfológicos resultou em dez árvores com 289 passos, índice de consistência (CI) = 0,40 e índice de retenção (RI) = 0,63. A árvore consenso da maioria (Figura 2A) apresenta apenas seis clados com valores de bootstrap acima de 60%. Esta topologia apresenta dois clados principais: um incluindo espécies tetralofodontes semi-aquáticas mais os fósseis *Noronhomys*, *Carletonomys* e *Holochilus primigenus*, irmão do clado composto por *Oryzomys couesi* e *O. palustris*. Nesta árvore, *Noronhomys* é irmão de *Holochilus*, mas com baixo apoio de bootstrap, e o fóssil *Carletonomys* é irmão deste clado fazendo com que *Holochilus* fique parafilético, visto que *Holochilus* viventes estão mais próximos de *Carletonomys* do que *H. primigenus*. Neste clado, *Pseudoryzomys* e *Lundomys* são os táxons mais basais. O segundo clado interno mostra uma politomia basal nos ramos de *Cerradomys* e um clado formado por *Sooretamys*, *Eremoryzomys* e *Drymoreomys*. O único grupo com apoio de boostrap (> 90%) neste clado são os ramos das duas espécies de *Nesoryzomys*, endêmicas de Galápagos.

A análise de MP com os dados concatenados resultou em uma árvore com 2484 passos, CI = 0,54 e RI = 0,47. O A topologia desta filogenia (Figura 2B) apresenta *Eremoryzomys* seguido de *Cerradomys* e *Sooretamys* como mais basais do grupo interno. O monofiletismo dos tetralofodontes semi-aquáticos não é sustentado. *L. molitor* forma um clado com as espécies de *Oryzomys* e compartilham o ancestral mais

recente com táxons do noroeste da América do Sul, Central e América do Norte, como *Aegialomys* e *Sigmodontomys*. *Amphinectomys* e *Nectomys* formam um clado irmão de *Aegialomys*, *Nesoryzomys*, *Melanomys* e *Sigmodontomys*. A relação entre as espécies viventes de *Holochilus* é diferente dos dados morfológicos, com *H. brasiliensis* basal ao clado formado por *H. sciureus* e *H. chacarius*, ambos com apoio de bootstrap. *Noronhomys* e *Carletonomys* são basais às espécies viventes de *Holochilus*, e a posição de *H. primigenus* faz com que *Holochilus* fique parafilético.

As análises por MB e MV recuperaram topologias diferentes das obtidas por MP, porém apresentando apoios de ramos sensivelmente mais altos (Apêndice 2). Essa tendência foi ainda mais evidente quando esses dois critérios de otimização foram feitos com os fragmentos de DNA concatenados, elevando ainda mais os apoios dos ramos (Figuras 3A, B). Estas duas análises não sustentam a hipótese de monofiletismo dos Oryzomyini tetralofodontes, com ambas colocando *Lundomys* como um táxon mais basal em relação ao restante do clado "D" e com apoio elevado dos ramos. As mesmas apresentaram algumas incongruências, essencialmente em pontos com baixo valor de bootstrap: *S. angouya* aparece agrupando com *C. subflavus* na MB, enquanto estão distantes na MV; o gênero de Galápagos *Nesoryzomys* se insere basal aos irmãos *Melanomys* e *Sigmodontomys* na MV, enquanto na MB este primeiro forma um clado com *Aegialomys*, o clado de tetralofodontes aparece em uma politomia basal na MB e um clado resolvido na MV. *Eremoryzomys polius* aparece como táxon mais basal do grupo interno na MB e formando politomia na MV.

A MB com dados morfológicos + moleculares (Figura 4) recuperou uma topologia que também não sustenta o monofiletismo de *Holochilus*, com *H. primigenus* não agrupando com as espécies viventes desse gênero. Da mesma forma que as demais análises, a MB com este conjunto de dados coloca *Pseudoryzomys* irmão de *Holochilus* e *Lundomys* distante destes gêneros.

DATAÇÃO MOLECULAR E ÁREAS ANCESTRAIS

Todas as abordagens de calibrações recuperaram topologias idênticas. Igualmente, estas diferentes abordagens estimaram tempos de divergência muito próximos (Figura 5), aonde apenas a divergência de *Eremoryzomys* ocorre no Mioceno e a maioria das outras divergências durante o Plioceno. Nesta época, os tetralofodontes divergiram por volta de 3,80 mya. *Pseudoryzomys* divergiu da linhagem de *Holochilus* por volta de 2,75 mya, as espécies de *Holochilus* divergiram ha 1,72 mya e as espécies de *Nesoryzomys* divergiram por volta de 1,87 mya. *Melanomys* e *Sigmodontomys* divergiram mais recentemente, durante o Pleistoceno ha 1,25 mya, assim como as duas espécies de *Oryzomys* ha 0,45 mya.

A análise de áreas ancestrais encontrou o ancestral do clado "D" com distribuição Cis e Trans-Andina. O ancestral de *Lundomys* apresentou distribuição Cis-Andina, assim como os ancestrais dos dois clados mais internos (Figura 6). Distribuição Trans-Andina foi encontrada para os ancestrais do clado composto por *Oryzomys*, *Nesoryzomys*, *Aegialomys*, *Melanomys* e *Sigmodontomys*. Contudo, apesar de possuir gêneros com distribuição atual acima do Panamá, este clado tem ancestral mais recente compartilhado pelo clado irmão composto por *Nectomys* e *Amphinectomys* com distribuição Cis-Andina.

CONCLUSÕES

- Nossos dados não sustentam a hipótese de que *Holochilus primigenus* deva ser alocado a esse gênero. Isto já foi sugerido em outros estudos (Steppan, 1996; Carleton e Olson, 1999; Pardiñas, 2008) e a alocação genérica desta entidade taxonômica deve ser revista.

- *Carletonomys cailoi* é uma espécie relacionada a *Noronhomys* e espécies viventes de *Holochilus*;

- A hipótese proposta neste estudo de que *Lundomys*, *Holochilus* e *Pseudoryzomys* não formam um agrupamento monofilético consiste em uma nova tendência sobre o entendimento da evolução desses táxons. Nesse contexto, argumentamos que a redução do mesolofo (ou ausência do mesmo – característica compartilhada pelos gêneros supracitados) ocorreu duas vezes no clado "D". De forma alternativa, argumentamos que podem haver equívocos no tratamento dessa estrutura, e que as estruturas semelhantes dos molares apresentadas por essas espécies tetralofodontes não seriam homólogas (Pardiñas, 2008).

- A análise de tempo de divergência estimada no presente estudo estipula que as espécies tetralofodontes divergiram muito antes do que o esperado pelo registro fóssil. Ao mesmo tempo, discutimos que o limite mínimo de 5 mya para a diversificação de Oryzomyini é implausível (Engel et al., 1998; Smith e Patton, 1999; Steppan et al., 2004), dado que estimamos a radiação do clado "D" em um tempo médio por volta de 5,39 mya.

- Com base nos tempos de divergências e áreas ancestrais, inferimos que a distribuição de ancestrais das espécies estudadas era em maior parte Cis-Andina e que eventos de dispersão de uma linhagem do sul dos Andes em direção ao Norte dos Andes devem ter ocorrido anteriormente aos últimos eventos de soerguimento dos Andes do Norte. Além disso, linhagens que ficaram restritas à porção norte da América do Sul dispersaram em direção ao norte após a formação do istmo do Panamá;

- A topografia da América do Sul foi constituída por grandes planícies com vegetação aberta durante o Plioceno e foi representada por uma grande riqueza de espécies de mamíferos pastadores de áreas abertas (Donato et al., 2003;Ortiz-Jaureguizare e Cladera, 2006). Esta conformação ambiental pode ter favorecido a diversificação e dispersão de linhagens ancestrais dos tetralofodontes do clado "D", visto que este grupo é fortemente associado com áreas abertas e possui espécies de dieta baseada em folhas (Weksler, 2006);

- Os resultados apresentados no presente estudo oferecem uma nova compreensão da história evolutiva das espécies estudadas, principalmente os gêneros tetralofodontes. Além disso, apresentamos relações filogenéticas do clado "D" com sustentação mais alta do que comumente encontrado na literatura. Neste sentido, ressaltamos a necessidade de outros estudos que avaliem as hipóteses propostas por nós, assim como com maior número de espécies e com outras sequências de DNA.

BIBLIOGRAFIA

Bremer K. 1994. Branch support and tree stability. Cladistics **10**: 295-304.

Carleton M, Olson L. **1999**. Amerigo Vespucci and the Rat of Fernando de Noronha: a new genus and species of rodentia (Muridae: Sigmodontinae) from volcanic island off Brazil's continental shelf. American Museum Novitates **3256**: 1-59.

D'Elía G, Pardiñas UFJ, Teta P, Patton JL. **2007**. Definition and diagnosis of a new tribe of Sigmodontinae rodents (Cricetidade: Sigmodontinae), and a revised classifications of the subfamily. Gayana **71**: 187-194.

Donato M, Posadas P, Miranda-esquivel DR, Jaureguizar EO, Cladera G. **2003**. Historical biogeography of the Andean region: evidence from Listroderina(Coleoptera# Curculionidae: Rhytirrhinini) in the context of the South American geobiotic scenario. Biological Journal of the Linnean Society **80**: 339-352.

Drummond AJ, Ho SYW, Phillips MJ, Rambaut A. **2006**. Relaxed phylogenetics and dating with confidence. PLoS biology **4**: 699-710.

Engel SR, Hogan KM, Taylor JF, Davis SK. **1998**. Molecular systematics and paleobiogeography of the South American Sigmodontine Rodents. Molecular Biology **15**: 35-49.

Felsenstein J. 1985. Confidence-limits on phylogenies - an approach using bootstrap. Evolution **39:** 783-791.

Geist D. **1984**. On the emergence and submergence of the Galápagos islands. Noticias de Galapagos **56**: 5-9.

Grehan J. **2001**. Biogeography and evolution of the Galapagos: integration of the biological and geological evidence. Biological Journal of the Linnean Society **74**: 267-287.

Hanson JD. 2008. Molecular Phylogenetics of the tribe Oryzomyini: does a multi-gene approach help resolve a systematic conundrum? D. Phil. Thesis, Texas Tech University.

Hershkovitz P. **1955**. South American marsh rats, genus Holochilus, with summary of Sigmodont rodents. Fieldiana Zoology **37**: 639-687.

Hershkovitz P. **1962**. Evolution of Neotropical cricetine rodents (Muridae) with special reference to the phyllotine group. Fieldiana Zoology **46**: 1-524.

Hershkovitz P. 1966. Mice, land bridges and Latin American faunal interchange. In: Ectoparasites of Panama (R Venzer RL, Tipton VJ eds.). Field Museum of Natural History, Chicago 725-751.

Hooper E, Musser GG. **1964**. The glans penis in Neotropical Cricetines (Family Muridae) with comments on classification of Muroid rodents. Miscellaneous Publications Museum of Zoology, University of Michigan **123**: 1-64.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics **17**: 754-755.

IUCN. 2011. IUCN Red List of Threatened Species. Version 2011. http://www.iucnredlist.org. Downloaded on December 2011.

Maddison W, Maddison DR. 1999. MacClade: Analysis of phylogeny and character evolution. Sinauer Associates, Inc. Sunderland, MA.

Musser GG, Carleton MD. 2005. Superfamily Muroidea. In: Wilson DE, Reeder DA, eds. Mammal species of the world: a taxonomic and geographic reference. Baltimore, MD: Johns Hopkins University Press, 894–1531.

NCBI. 2011. http://www.ncbi.nlm.nih.gob/genbank/.

Notredame C, Higgins DG, Heringa J. **2000**. T-Coffee: A novel method for fast and accurate multiple sequence alignment. Journal of molecular biology **302**: 205-17.

O'Leary, M. A., and S. G. Kaufman. 2007. MorphoBank 2.5: Web application for morphological phylogenetics and taxonomy. http://www.morphobank.org.

Ortiz-Jaureguizar E, Cladera G. **2006**. Paleoenvironmental evolution of southern South America during the Cenozoic. Journal of Arid Environments **66**: 498-532.

Palma RE, Rodríguez-Serrano E, Rivera-Milla E, Hernandez CE, Salazar-Bravo J, Carma MI, Belmar-Lucero S, Gutierrez-Tapia P, Zeballos H, Yates TL. **2010**. Phylogenetic relationships of the pygmy rice rats of the genus *Oligoryzomys* Bangs, 1900 (Rodentia: Sigmodontinae). *Zoological Journal of the Linnean Society* **160**: 551-566.

Pardiñas UFJ. **2008**. A new genus of oryzomyine rodent (Cricetidae: Sigmodontinae) from the Pleistocene of Argentina. *Journal of Mammalogy* **89**: 1270-1278.

Pardiñas UFJ, Teta P. **2011**. Fossil history of the marsh rats of the genus *Holochilus* and *Lundomys* (Cricetidae, Sigmodontinae) in southern South America. *Estudios Geol—gicos* **67**: 111-129.

Percequillo AR, Weksler M, Costa LP. **2011**. A new genus and species of rodent from the Brazilian Atlantic Forest (Rodentia: Cricetidae: Sigmodontinae: Oryzomyini), with comments on oryzomyine biogeography. *Zoological Journal of the Linnean Society* **161**: 357-390.

Posada D. 2008. jModelTest: Phylogenetic Model Averaging. *Molecular Biology and Evolution* **25:** 1253-1256.

Rambaut A, Drummond AJ. 2007. Tracer v1.4, Available from http://beast.bio.ed.ac.uk/Tracer

R Development Core Team. 2011. R: A language and Enviroment for Statistical Computing. *R Foundation for Statistical Computing* Vienna, Austria.

Reig O. **1984**. Distribuição geográfica e história evolutiva dos roedores muroideos sulamericanos (Cricetidae: Sigmodontinae). *Revista Brasileira de GenŽtica* **7**: 333-365.

Schliep KP. **2011**. phangorn: phylogenetic analysis in R. *Bioinformatics* **27**: 592-593.

Smith MF, Patton JL. **1999**. Phylogenetic relationships and the radiation of Sigmodontine rodents in South America# Evidence from Cytochrome b. *Journal of Mammalian Evolution* **6**: 89-128.

Steppan S. **1995**. Revision of the tribe Phyllotini (Rodentia: Sigmodontinae), with a phylogenetic hypothesis for the Sigmodontinae. Fieldiana Zoology **80**: 1-112.

Steppan SJ. **1996**. A new species of Holochilus (Rodentia: Sigmodontinae) from the middle Pleistocene of Bolivia and its phylogenetic significance. Journal of Vertebrate Paleontology **16**: 522-530.

Steppan SJ, Adkins RM, Anderson J. **2004**. Phylogeny and divergence-date estimates of rapid radiations in Muroid rodents. Systematic Biology **53**: 533-553.

Swofford DL. 1999. PAUP*: Phylogenetic Analysis using Parsimony (and other methods), version 4.0b1. Sinauer Associates, Inc. Sunderland, MA.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution **28**: 2731-2739.

Turvey ST, Weksler M, Morris EL, Nokkert M. **2010**. Taxonomy, phylogeny, and diversity of the extinct Lesser Antillean rice rats (Sigmodontinae: Oryzomyini), with description of a new genus and species. Zoological Journal of the Linnean Society **160**: 748-772.

Voss R. **1991**. An introduction to the Neotropical muroid rodent genus Zygodontomys. Bulletin of the American Museum of Natural History **210**: 1-210.

Voss R. **1992**. A revision of the South America species of Sigmodon (Mammalia: Muridae) with notes on their natural history and biogeography. American Museum Novitates **3050**: 1-56.

Voss RS, Carleton MD. **1993**. A New Genus for Hesperomys molitor Winge and Holochilus magnus Hershkovitz (Mammalia, Muridae) with an analysis of its phylogenetic relationships. Nature **225**: 1-40.

Weksler M, Percequillo AR, Voss RS. **2006**. Ten New Genera of Oryzomyine Rodents (Cricetidae: Sigmodontinae). American Museum Novitates **3537**: 1.

Weksler M. **2006**. Phylogenetic relationships of Oryzomine rodents (Muroidea: Sigmodontinae): separate and combined analyses of morphological and molecular data. Bulletin of the American Museum of Natural History **296**: 1-149.

Weksler M. **2003**. Phylogeny of Neotropical oryzomyine rodents (Muridae: Sigmodontinae) based on the nuclear IRBP exon. Molecular Phylogenetics and Evolution **29**: 331-349.

Wiens JJ. **2006**. Missing data and the design of phylogenetic analyses. Journal of Biomedical Informatics **39**: 34-42.

Wiens JJ, Moen DS. **2008**. Missing data and the accuracy of Bayesian phylogenetics. Journal of Systematics and Evolution **46**: 307-314.

Wiens JJ, Morrill MC. **2011**. Missing Data in Phylogenetic Analysis: Reconciling Results from Simulations and Empirical Data. Systematic biology **60**: 1-13.

Xia X, Xie Z. 2001. DAMBE: data analysis in molecular biology and evolution. Journal of Heredity **92**:371–373.

Yu Y, Harris AJ, He X-J. 2011. RASP (Reconstruct Ancestral State in Phylogenies) 2.0 beta. Available at http://mnh.scu.edu.cn/soft/blog/RASP

Phylogeny, molecular dating and biogeographyof tetralophodont Oryzomyini rodents (Cricetidae: Sigmodontinae)

Leonardo Ferreira Machado

ABSTRACT

Our objectives were to construct phylogenetic trees, to apply molecular dating techniques and ancestral area analyses to Oryzomyini clade "D", emphasizing the poorly known tetralophodont forms. We applied maximum parsimony, maximum likelihood and Bayesian inference to 98 morphological characters and 4515 base pairs from five DNA fragments. We found that tetralophodont genera do not form a monophyletic group. Among living taxa, *Pseudoryzomys* is sister to *Holochilus*, and *Lundomys* is basal to most species in clade "D". The fossil *H. primigenus* does not group with living *Holochilus* and is basal to the fossils *Carletonomys* and *Noronhomys*, making *Holochilus* paraphyletic. Most divergences occurred during the Pliocene, and others during the Miocene and Pleistocene. The most recent common ancestor of clade "D" had Cis and Trans-Andean distribution, while genera currently distributed to the north of Panama show ancestral with Cis-Andean distribution. We propose that the taxonomic identity of *H. primigenus* should be reviewed, and that tetralophodont molars appeared more than once in clade "D". Alternatively, molar characters traditionally identified as homologies among tetralophodont genera should be reassessed. The most estimated divergences were placed in the Pliocene. Therefore, we hypothesized that great Plains in South America during the Pliocene may have especially favored the diversification of tetralophodont genera from clade "D". In addition, uplift events in northern Andes may have played a vicariant role in the diversification of the species in the studied genera, or that the lineages diversified in the South America ant dispersal to North and Central America after the Panama land bridge formation.

Key words: Oryzomyini, tetralophodont, phylogeny, genes, morphology, paraphyletic

INTRODUCTION

Sigmodontinae is a subfamily of Cricetidae rodents distributed mainly in the Neotropical region. It is by far the most species rich group among Neotropical mammals, and present in all biomes of South, Central and southern North America (Musser and Carleton, 2005). Sigmdontinae genera have been allocated into nine tribes: Abrotrichini, Akodontini, Ichthyomyini, Oryzomyini, Phyllotini, Reithrodontini, Sigmodontini, Thomasomyini and Wiedomyini (Smith and Patton, 1999; Musser and Carleton, 2005; D'Elía et al., 2007), but some genera (e.g. *Wilfredomys*, *Abrawayaomys*, *Phaenomys*) are of uncertain tribal affiliation, and are commonly treated as Sigmodontinae *incertae sedis* (Smith and Patton, 1999). Oryzomyini is the richest tribe, encompassing 31 genera and including an increasing number of new species and genera recently described (e.g. Weksler et al., 2006; Costa et al., 2007; Turvey et al., 2010; Percequillo et al., 2011). The Oryzomyini form a widely distributed clade, present throughout de Neotropical region, ranging from southern United States to extreme southern South America in the Patagonian region. Moreover, the Oryzomnyini are present in a variety of biomes, including rain forests, savannas, grasslands and swamps where a plethora of morphological adaptations have been documented (Musser and Carleton, 2005; Weksler, 2006).

The progress concerning the taxonomic composition of Oryzomyini is marked by different arrangements that brougth uncertainty regarding the monophyly of the tribe (Hershkovitz, 1962; Steppan, 1995; Smith and Patton, 1999). Hershkovitz's (1962) classical view is that all Oryzomyini must have pentalophodont molars, therefore excluding genera that display a tendency to lamination of molar cusps and a reduction on number of molar folds, known as tetralophodont molars, a view corroborated by karyotypic evolution (Gardner and Patton, 1976). The alternative hypotheses advocated by Hooper and Musser (1964) based on glans penis morphology, proposed an

evolutionary scenario where the tetralophodont genus *Holochilus* is closest to Oryzomyini, instead of part of the Sigmodontini radiation (Hershkovitz, 1955, 1962), a similar hypothesis proposed based on parsimony analysis with chromosomal arrangements as characters (Baker et al., 1983). This later point of view gained support in subsequent contributions (Voss, 1991; 1992; Steppan, 1995) and provided the current view that the tetralophodont genera *Pseudoryzomys*, *Holochilus* and *Zygodontomys* share a series of synapomorphies with Oryzomyini. In this scenario, the presence of the mesoloph and pentalophodont molar morphology are plesiomorphies within Oryzomyini *sensu* Hershkovitz, and *Sigmodon* is not part of this group (Voss and Carleton, 1993). More recently, in light of modern phylogenetic techniques using morphological and molecular characters and denser taxonomic sampling, Weksler (2003, 2006) corroborated the Oryzomyini monophyletic hypothesis (Voss and Carleton, 1993) including tetralophodont genera, and provided additional information supporting the tribe's synapomorphies.

Despite such progress, many phylogenetic relationships within Oryzomyini remain unclear. For example, the living tetralophodont open area dwellers *Pseudoryzomys*, *Holochilus*, *Lundomys* and the fossil *Noronhomys*, *Carletonomys* and *Holochilus primigenus* are usually placed together in phylogenetic reconstructions, although their genealogical relationships are unclear or in disagreement. Based on morphological characters, Voss and Carleton (1993) found two equally parsimonious trees where either *Pseudoryzomys* or *Lundomys* is sister to *Holochilus*. Steppan (1996) found *Lundomys* as sister to *Holochilus* and Carleton and Olson (1999) found *Noronhomys* as sister to *Holochilus* and *Lundomys*, and therefore more related to them than to *Pseudoryzomys*. Based on Interphotoreceptor Retinoid Binding Protein gene (IRBP) sequences, Weksler (2003) found *Pseudoryzomys* closest to *Holochilus*, and *Lundomys* sister to both, but when morphological and molecular characters were

analyzed together, *Pseudoryzomys* was sister to a clade formed by *Holochilus* and *Lundomys* (Weksler, 2006; Percequillo et al., 2011). After the most recent contributions (Weksler, 2006; Turvey et al., 2010; Percequillo et al., 2011), the Oryzomyini have been divided into four main clades: "A", "B", "C" and "D", and clade "D" including most tetralophodont genera (*Pseudoryzomys*, *Holochilus* and *Lundomys*).

The Oryzomyini fossil record is primarily represented by tetralophodont genera (Pardiñas, 2008; Pardiñas and Teta, 2011). The most complete and preserved specimen of extinct tetralophodont is *Noronhomys vespucci* from Fernando de Noronha, an archipelago located ca. 350 km offshore, in northeastern Brazil. This taxon has been considered closer related to *Holochilus* than to *Lundomys* or *Pseudoryzomys* (Carleton and Olson, 1999), while other related forms have not been analyzed phylogenetically, or have debatable taxonomic status. For example, *Carletonomys cailoi* – a South American species from the province of Buenos Aires, Argentina - possesses indubitable morphological affinities with *Holochilus* and *Noronhomys*, although this hypothesis has never been assessed under broad taxonomic sampling and phylogenetic tools (Pardiñas, 2008). In addition, *Holochilus primigenus* from Bolivia, described by Steppan (1996), is placed in this genus based mainly on morphological mandibular similarities, despite the resemblance of molar structures shared with *Lundomys*. This ambiguity allows for different interpretations and provides uncertainty of taxonomic relationships regarding this taxon (Steppan, 1996; Carleton and Olson, 1999; Pardiñas, 2008; Musser and Carleton, 2005). Thus, new studies on the phylogeny of the tetralophodont genera from Oryzomyini clade "D" are needed to clarify taxonomic and systematic issues, and they can provide new information for understanding the South America biogeographic history (Pardiñas and Teta, 2011).

The time and biogeographic origins of Oryzomyini is considered previous to the Panama land bridge formation (Reig, 1984), placed between 5 and 9 millions years ago

(mya) (Smith and Patton, 1999; Steppan et al., 2004) and with the northern South America considered as ancestral area (Reig, 1984; Smith and Patton, 1999; Steppan et al., 2004). After the South America invasion by the ancestral of Sigmodontinae lineage during Miocene (Hershkovitz, 1966; Reig, 1984; Steppan et al., 2004), the Oryzomyini differentiated in multiple lineages that dispersed to eastern and southern South America and Central and North America after the Panama land bridge formation (Hershkovitz, 1966; Reig, 1984; Smith and Patton, 1999; Steppan et al., 2004). However, biogeographic hypotheses concerning the tetralophodont Oryzomyini are scarce and confused with their taxonomic history. Their placement as Sigmodont and Phyllotini lineages (Hershkovitz, 1962; Reig, 1984) implies in a different evolutionary trajectory compared to when they are recognized as Oryzomyini (Voss and Carleton, 1993; Weksler, 2003, 2006). The traditional hypotheses of Hershkovitz (1962) and Reig (1984) advocated the allocation of *Pseudoryzomys* within Phyllotini and *Holochilus* within the Sigmodont lineage, implying that these genera descended from a pastoral and tetralophodont ancestor. Alternatively, the current notion that *Pseudoryzomys* *Lundomys* and *Holochilus* are members of the Oryzomyini tribe (Voss and Carleton, 1993; Weksler 2003, 2006; Percequillo et al., 2011) brings the compelling idea of direct descent hypothesis of non-sylvan forms from core Oryzomyini forest dwellers (Weksler 2006; Pardiñas, 2008). Based on this hypothesis, the more acceptable scenario available to date is that tetralphodont lineages descended from a sylvan stock of Oryzomyini, with the ancestral lineage ranging primarily in the northern portion of South America, and reaching eastern and southern habitats in latter stages of diversification (Hershkovitz, 1962; Voss and Carleton, 1993). In this case, the tetralophodont genera must have had a broad distribution into the Pleistocene, where retractions and expansions of natural vegetation probably mold their more recently distribution (Voss and Carleton, 1993; Pardiñas 2008; Pardiñas and Teta, 2011).

In this complex context of systematic uncertainty and biogeographic scenarios, the goal of the present study is to investigate phylogenetic hypotheses concerning the Oryzomyini clade "D", with emphasis on extant and extinct tetralophodont genera. Based on a large taxonomic sample, morphological and multi-gene approach, we specifically address the phylogenetic position of *Carletonomys cailoi*, *Holochilus primigenus* and test the hypothesis of monophyly of tetralophodont from clade "D". Additionally, we infer times of divergence based in the bayesian relaxed molecular clock, and estimate ancestral areas of occurrence to propose biogeographic historical scenarios.

MATERIAL AND METHODS

TAXONOMIC SAMPLING

The ingroup species analyzed were primarily those from Oryzomyini clade "D" defined by Weksler (2006). Additionaly taxa placed in this group in subsequent papers (e. g. *Drymoreomys* Precequillo et al., 2011) and *Holochilus sciureus* were also included. The fossil taxa *Holochilus primigenus*, *Noronhomys vespucci* and *Carletonomys cailoi* were integrated into the analyses (Table 1). The outgroup was represented by five species found in distinct clades in previous studies (Weksler, 2006; Turvey et al., 2010; Percequillo et al., 2011): *Neacomys spinosus*, *Oligoryzomys nigripes*, *O. flavescens*, *Oecomys catherinae* and *Hylaemays megacephalus* (Table 1).

MORPHOLOGICAL CHARACTERS

The morphological characters used have been proposed by Weksler (2006), with the modifications defined by Turvey et al. (2010) and Percequillo et al. (2011), and available from the Morphobank repository (O'Leary and Kaufman, 2007). Data from fossils were extracted from the original descriptions (Steppan, 1996; Carleton and

Olson, 1999; Pardiñas, 2008), and *Holochilussciureus* character states were scored based on specimens housed at the Mammal Collection at the Universidade de Brasília (UnB), Brazil (see Table 1 for voucher details). These data were incorporated into the morphological character matrix available (see Appendix 1 for details of the final matrix and character scoring).

MOLECULAR TECHNIQUES

Genomic DNA was isolated from tissue samples preserved in ethanol using the DNeasy (Qiagen) kit. Fragments of the nuclear Interphotoreceptor Retinoid Binding Protein (IRBP) (1265 base pairs) and mitochondrial cytocrome *b* (cyt-*b*) (800bp) genes were amplified and analyzed with IRBP sequences from Weksler (2003) and cyt-*b* sequences from Percequillo et al.(2011), available in GenBank (NCBI, 2011). Additionally, we amplified fragments of exon 28 of the nuclear von Willebrand Factor gene (vWF) (1198bp) and intron 7 of beta-fibrinogen (Fgb-7) (643bp) from part of the taxa sampled from Weksler's (2006) Oryzomyini clade"D". These gene fragments were showed with well resolution in mammals studies (Huchon et al., 1999; Wickliffe et al., 2003). Finally, we used 609 bp sequences of intron 2 of the alcohol dehydrogenase gene (Adh1-I2) from Hanson's (2008) unpublished thesis available in GenBank. Specific pairs of primers available from the literature were used for each fragment (Table 2).

The polymerase chain reactions (PCRs) were performed using Platinum-Taq DNA polymerase (Invitrogen). Each fragment was amplified with specific mix of reagents (Table 3). The IRBP, cyt-*b*, Fgb-7 and the first fragment of vWF were amplified in 30$l volume mix, while the second fragment of vWF in 25 $l volume mix.

All PCR thermocycling protocols consisted of an initial denaturation step of 94 °C for 5 min and final extension at 72 °C for 10 min. Between these steps, 35-40 cycles were performed at 94 °C for 30 sec, 62 °C for 90 sec and 72 °C for 3 min (for IRBP); 94

°C for 45 sec, 54 °C for 60 sec and 72 °C for 90 sec (for cyt-b); 94 °C for 30 sec, 63 °C for 50 sec and 72 °C for 45 sec (for Fgb-7); 94 °C for 30 sec, 64 °C for 90 sec and 72 °C for 90 sec (for vWF with v1+w2 primers); 94 °C for 30 sec, 60 °C for 60 sec and 72 °C for 60 sec (for vWF with v4+w1 primers). The PCR products were directly purified with USB ExoSAP-IT kit (Affymetrix, Inc.) with two steps at 37 °C for 90 min and 80 °C for 30 min. Finally, the purified products were submitted to sequencing reactions at the Macrogen Inc., Korea.

SEQUENCE ALIGNMENT AND PHYLOGENETIC ANALYSES

The consensus sequences of light and heavy strands were obtained with the software Gap and PreGap of the Staden package (Bonfield et al., 1995), and aligned using T-Coffee (Notredame et al., 2000). The best evolutionary models of nucleotide substitution were estimated in JModeltest (Posada, 2008) using the Akaike information criterion, and the following models were chosen: TIM1 with proportion of invariants sites (I) and gamma distribution (G) for IRBP, GTR+I+G for cyt-b, TPM2uf+G for Adh1-I2, HKY+G for Fgb-7 and k80+G for vWF.

The level of nucleotide substitutions saturations were analyzed in DAMBE software (Xia and Xie, 2001), by plotting transitions and transversions against F84 model distance for each gene alignments. The software MEGA version 5 (Tamura et al., 2011) was used to obtain the pairwise sequence divergence between all genera analyzed, number of polymorphic sites, base frequencies and to perform a χ^2 test concerning the hypothesis of homogeneity base composition.

The morphological and molecular data were submitted to maximum parsimony (MP) analysis using PAUP* v.4.0b10 (Swofford, 1999). These analyses were performed on the combined morphological and molecular matrix and on the morphological data matrix only. A heuristic search was performed using 100 replicates of random taxon

addition with tree bisection-reconnection (TBR) branch swapping. Nodal support was inferred with bootstrap resampling using 1000 pseudoreplicates (Felsenstein, 1985) and Bremer support (Bremer, 1994) was calculated with decay commands provided in MacClade (Maddison and Maddison, 1999) and performed in PAUP*. The molecular characters were treated as unordered, while some multistate morphological characters were treated as ordered with the polymorphic coding of Wiens (1995) implemented by Weksler (2006), Turvey et al. (2010) and Percequillo et al. (2011).

The sequence alignments were subject to Bayesian inference (BI) in MrBayes v.3.0b4 (Huelsenbeck and Ronquist, 2001). These analyses were performed for each gene, on a combined matrix of genes, and total evidence (genes + morphology). Two runs of BI were conducted for 5 million generations, with sample frequency every 1000 generations for each gene, and four runs of 10 million generation with sample frequency every 10000 generations for concatenated genes and total evidence. The software Tracer 1.3 (Rambaut and Drummond, 2007) was used to check for convergence between runs and 25% of samples trees was discarded as "burnin". The combined and individual gene matrices were also submitted to maximum likelihood (ML) analyses with the phangorn (Schliep, 2011) package of software R (R Development Core Team, 2011). Nodal support was inferred with 500 pseudoreplicates of bootstrap.

In all analyses using concatenated matrices, only species with three or more sequenced genes were used (exceptions for fossil taxa), in order to provide reasonable balance between taxa and missing values. For details of the impact of missing data on phylogenetic reconstructions see Wiens (2006), Wiens and Moen (2008) and Wiens and Morrill (2011).

MOLECULAR DATING AND BIOGEOGRAPHIC ANALYSIS

We used a concatenated matrix to estimate molecular date divergences under the relaxed molecular clock using BEAST v.1.4.8 (Drummond et al.; 2006). Data partition was set for each gene and analyzed under independent evolutionary models estimated in JModeltest (Posada, 2008). This analyses implemented the Yule speciation processes model and the randomly generated starting tree as priors. The Markov Chain Monte Carlo was run for 10 million generations with sampling every 10000 generations and "burnin" of 25% trees.

We used three calibrations points. The first was based on divergence of *Oligoryzomys nigripes* and *O. flavescens* lineages estimated to 1.54 ± 0.031 (mean ± SD) million years ago (mya) (Palma et al., 2011). However, for this calibration, we used a high SD (= 1.0) given the uncertainty associated to this kind of secondary calibration (Ho, 2007). The second calibration point was based on the fossil *Carletonomys cailoi*, dated to middle Pleistocene (1.0 ± 0.5 mya) at the Ensenadan age (Pardiñas, 2008). We found that *C. cailoi* is basal to the extant *Holochilus* in MP analyses, then we hypothesized that the divergence of *Pseudoryzomys* and *Holochilus* must occur prior to 1 mya. The last calibration point was based on a biogeographic event, the Galapagos archipelago formation at 4 mya (Geist, 1984; Grehan, 2001). We set the divergence of *Nesoryzomys*, endemic to the Galapagos islands, at 4.0 ± 2.0 mya. The first calibration was set as a normal distribution, the second as lognormal and the third were set as truncated normal distribution, following the recommendations of Ho (2007). Finally, we ran independent analysis for each calibration (except for the secondary calibration), combining fossil + biogeographic calibrations and fossil + biogeographic + secondary calibration points to check for congruence in terms of divergence times.

The ancestral geographic distribution of clade "D" was inferred using the Bayesian approach in the software RASP (Reconstruct Ancestral State in Phylogenies)

(You et al., 2011). Based on the distribution data available in the International Union for Conservation of Nature (IUCN), we defined three main distribution patterns for the analyzed genera: Cis-Andean, Trans-Andean (*sensu* Weksler, 2006) and to the north of Panama Isthmus. This analysis was run under F81+G nucleotide substitution model for 5 million generations, with sample frequency every 1000 trees and the first 1000 trees discarded as "burnin".

Based on the current distribution and the ancestral ranges estimated, we construct a map showing the present and the ancestral ranges, and proposes evolutionary scenarios and biogeographic hypotheses.

RESULTS

GENE FRAGMENTS CHARACTERISTICS

The total polymorphic sites were 179 for Adh1-I2, 446 for Cyt-b, 117 for Fgb-7, 210 for IRBP and 137 for vWF. None of these fragments show signal of saturation, as seen in the nucleotide saturation plots (Figure 1). The concatenated alignment show mean base composition of A = 26.31%, C = 24.91%, G = 22.86% and T = 25.81%, and the x^2 test reject the hypothesis of heterogeneous base frequencies: $x^2 = 44.59$ $p = 0.93$ and $df = 60$. The pairwise sequence divergences show *Amphinectomys* and *Hylaeamys* as the more distant genera between ingroup and outgroup (10.07%). Within ingroup, the more distance genera were *Eremoryzomys* and *Amphinectomys* (8.16%), while the closest genera were *Nesoryzomys* and *Aegialomys* (2.74%).

PHYLOGENETIC ANALYSES

The topology based on morphological data resulted in ten MP trees with 289 steps, consistency index (CI) = 0.40 and retention index (RI) = 0.63. The majority rule consensus tree (Figure 2A) presents only six clades with bootstrap support above 60%.

There are two main, but poorly supported basal clades: one comprising semi-aquatic tetralophodont plus the fossil *Noronhomys*, *Carletonomys* and *Holochilus primigenus*, sister to a clade composed of *Oryzomys couesi* and *O. palustris*. In this tree, *Noronhomys* is weakly supported as sister to extant *Holochilus* and the fossil *Carletonomys* is sister to this clade, making *Holochilus* paraphyletic, since extant *Holochilus* are closer to *Carletonomys* than to *H. primigenus*. *Pseudoryzomys* and *Lundomys* are the most basal taxa. The second internal clade presents a basal polytomy consisting of *Cerradomys* and a clade formed by *Sooretamys*, *Eremoryzomys* and *Drymoreomys*. The only well-supported (bootstrap > 90%) groups in this clade are two species of *Nesoryzomys*, endemic to the Galapagos.

The concatenated data set resulted in one MP trees with 2484 steps, CI = 0.54 and RI = 0.47, and shows a different topology from the morphological tree. The MP tree of this dataset (Figure 2B) shows *Eremoryzomys* as the most basal of the ingroup, followed by *Cerradomys* and *Sooretamys* clade. The monophyly of semi-aquatic tetralophodont genera is not supported, with *L. molitor* forming another clade with *Oryzomys*, and sharing a more recent common ancestor with taxa mainly from northwestern South America and Central and North America, such as *Aegialomys* and *Sigmodontomys* than with the remaining tetralophodont South American semi-aquatic genera, like *Holochilus* and *Pseudoryzomys*. *Amphinectomys* and *Nectomys* form a supported clade, sister to *Aegialomys*, *Nesoryzomys*, *Melanomys* and *Sigmodontomys* clade. The relationships among extant species of *Holochilus* are slightly different from morphological data alone, with *H. brasiliensis* basal to a clade formed by *H. sciureus* and *H. chacarius*, both well supported. *Noronhomys* and *Carletonomys* are basal to extant *Holochilus*, and the position of *H. primigenus* also makes *Holochilus* paraphyletic.

The other optimization criteria resulted in different topologies compared to MP, but well supported clades found in MP were also recovered in both BI and ML analyses

(Figures 3A and B). Moreover, node supports were tipically higher in BI and ML than in MP. When genes were analyzed individually, BI and ML showed some incongruences at the base of the trees (Appendix 2), but such incongruences occur mainly on weakly supported nodes. An important observation is that our analyses supports *Pseudoryzomys* as sister taxon to extant *Holochilus* in all analyses based on individual genes, and away from *Lundomys*. This pattern is also revealed with strong support in the concatenated BI (Figure 3A) and ML (Figure 3B) trees, where *Lundomys* is basal in clade "D" described by Weksler (2003, 2006). The concatenated BI and ML topologies are very similar and supports are high on several nodes, contrasting with analyses of individual genes. The BI and MV topologies disagree in some weakly supported nodes: *Sooretamys* and *Cerradomys* are sisters in BI, but not in ML; *Nesoryzomys* is sister to the *Melanomys* and *Sigmodontomys* in ML, but to *Aegialomys* in BI; *Pseudoryzomys* and *Holochilus* species forms a basal polytomy in BI; and *E. polius* is the most basal ingroup in BI, but not in ML analysis.

The total evidence of BI analysis recovered a less resolved tree (Figure 4) comparing to the other analyses. However, it also found *Pseudoryzomys* sister to *Holochilus* and *H. primigenus* did not group with extant *Holochilus*.

MOLECULAR DATING AND ANCESTRAL AREAS

All analyses with different calibration approaches recovered identical topologies, and with very close estimate divergence times. The analysis with fossil + biogeographic + secondary calibration approach (Figure 5) found the split of Eremoryzomys ancestor as the only divergence during Miocene, with others divergence events mostly found in at the Pliocene. During this time, the main basal divergences between the two internal clades occurred and the tetralophodont lineage starting to diverge around 3.80 mya. *Pseudoryzomys* diverged from the *Holochilus* lineage around 2.75 mya, while

Holochilus species split in the Pleistocene. The basal tetralophodont, *Lundomys*, is marked by a very early divergence around 4.23 mya. *Amphinectomys* and *Nectomys* diverge around 2.58 mya and the Galapagos species of *Nesoryzomys* split around 1.87 mya. *Melanomys* and *Sigmodontomys* diverged from each other more recently, during the Pleistocene at 1.25 mya, as well as the two species of *Oryzomys* at 0.45 mya.

The ancestral area analysis found the ancestral of clade "D" with South American distribution (Cis and Trans-Andean). The ancestral of *Lundomys* show Cis-Andean, as well as the ancestral of two internal clades (Figure 6). Trans-Andean distribution is found only in the ancestral of *Oryzomys*, *Nesoryzomys*, *Aegialomys*, *Melanomys* and *Sigmodontomys* clade. This clade, altough presents genera with current distribution above the Panama Isthmos, show the most recent ancestral sharing Cis-Andean distribution with the sister clade composed by *Nectomys* and *Amphinectomys*

DISCUSSION

The generic recognition of *Holochilus primigenus*, which is based primarily on mandible morphology, is questionable because it shares several molar features with *Lundomys* (Steppan, 1996; Carleton and Olson, 1999; Pardiñas, 2008). Although we found seven morphological characters shared between *H. primigenus* and some of the extant *Holochilus* species, and six shared with *Lundomys*, the speculations that *H. primigenus* represents a transitional form from *Lundomys* to *Holochilus* (Carleton and Olson, 1999) is unlikely. *Carletonomys* and *Noronhomys* are more closely related to the extant *Holochilus* than *H. primigenus*, as shown in the MP and BI phylogenies, and ML and BI analyses do not support the sister group relationship between *Lundomys* and *Holochilus*. In addition, the mandible characters uniting *H. primigenus* and the extant *Holochilus* (Steppan, 1996) are known to be polymorphic in some Oryzomyini genera, such as the capsular process of lower incisor alveolus varying with acute projection or

slightly elevated, or to represent continuous traits without clear definition of character variation, like the position of the coronoid process (Weksler, 2006). In this view, the acceptance of *H. primigenus* as another *Lundomys* species cannot be discarded (Steppan, 1996; Carleton and Olson, 1999; Pardiñas, 2008), and taking these evidence into account, we concluded that the taxonomic position of *H. primigenus* must be revised.

The first molecular phylogenetic analysis with large taxon sampling of Oryzomyini was performed by Weksler (2003), and followed by a morphological and molecular analysis by Weksler (2006). This last contribution forms the base for the current acceptance of relationships among Oryzomyini, which have been basically subdivided into four major clades "A", "B", "C" and "D". This allowed additional studies to access the phylogenetic position of many other species, as well as the descriptions of new taxa (Weksler et al., 2006; Voss and Weksler, 2009; Turvey et al., 2010; Percequillo et al., 2011). Out of these studies, Percequillo et al. (2011) proposed the sister group relationship of *Cerradomys* and *Sooretamys*, although they found low support for it. We found high support for this clade in both BI and ML analyses of the vWF gene and in BI using concatenated genes and total evidence. This is congruent with their adjacent geographic distributions, with *Cerradomys* in the open vegetation belt across South America and *Sooretamys* in the Atlantic forest of eastern Brazil (Musser and Carleton, 2005; Percequillo et al., 2008; Weksler, 2006), suggesting ancestry and closer geographic associations. In addition, the phylogenetic position of *Oryzomys*, represented here by *O. palustris* and *O. couesi*, has been uncertain based on node support as well genealogical relationships (Weksler, 2003, 2006, Voss and Weksler, 2009; Turvey et al., 2010; Percequillo, 2011). But, in our BI and ML analyses *Oryzomys* was placed in a well supported clade with *Melanomys*, *Sigmodontomys* and *Nesoryzomys*, genera with Trans-Andean distributions (Weksler, 2006). Regarding the

tetralophodont genera, the basal position of *Lundomys*, well supported in BI and ML trees, is a novel hypothesis for the Oryzomyini and the paraphyly of tetralophodont taxa has critical implications on the current systematics of these species.

The classification of Sigmodontinae rodents has been historically based on different characters systems, such as external morphology, glans penis, stomach morphology, chromosomes, skeleton and dentition (e g. Hershkovitz, 1944, 1962, 1993; Hooper and Musser, 1964; Carleton, 1973; Reig, 1980; Baker et al., 1986; Myers and Patton, 1989). The homologies of molar structures has played a central role in the taxonomic arrangement of the tetralophodont Oryzomyini genera, where the reduced mesoloph was a fundamental criterion uniting these genera. Nevertheless, all well-supported topologies presented in this paper attest that this feature may possibly have appeared twice in clade "D", the first in the *Lundomys* branch, the second in the *Pseudoryzomys*+*Holochilus* lineage. Another hypothesis for tetralophodonty evolution is that the reduced mesoloph of *Holochilus brasiliensis* is, in fact, a pseudomesoloph (Hershkovitz, 1993; Pardiñas, 2008) resulting from the high interpenetrations of labial and lingual flexi and the median mure extending almost to the lingual margin of the teeth with the participation of the paralophule, which resulted in a mesoloph-like structure (see fig 4. In Pardinãs, 2008). Conversely, *Lundomys* presents a true, but reduced, mesoloph which arises directly from the median mure without paralophule capture. It is therefore possible that homologies of molars structures have not been correctly assessed in these species. This scenario compels us to hypothesize that *Holochilus* species are derived from lineage with reduced true mesoloph (seen in *Pseudoryzomys*, *Carletonomys* and *Noronhomys*) and then completely lost this structure, as clearly seen in *H. sciureus* and *H. chacarius*

The initial diversification of Oryzomyini is placed between 5 and 9 mya in studies with more inclusive taxonomic categories of Muroid species (Engel et al., 1998; Smith

and Patton, 1999; Steppan et al., 2004). This is compatible to the early arrival hypothesis of South America by ancestral lineage of Sigmodontinae before the land connection between Central and South America (Smith and Patton, 1999; Steppan et al., 2004), and posterior dispersions to north of already diversified lineage after the formation of Panama land bridge (Reig, 1984; Steppan, 2004; Weksler, 2006). This hypothesis is corroborated by our analysis of ancestral area and molecular date divergence, which shows that the ancestral lineage of Oryzomys was distributed mainly in South America and the molecular time divergence was closest to the Panama land bridge formation. However, a lower bound of 5 mya for Oryzomyini diversification is implausible based on our mean estimate of 5.39 mya for the main diversification within clade "D". If our date estimates are correct, the diversification of tetralophodont genera is much older than the fossil records indicates (Steppan, 1996; Pardiñas et al., 2002; Pardiñas, 2008), which is a common feature in the literature regarding the evolution of muroid rodents (Engel et al., 1998; Steppan et al., 2004; Palma et al., 2010). Concerning the Oryzomyini, the fossil record is even scarcer than sigmodontines as a whole, because most species are sylvan dwellers (Weksler, 2006; Pardiñas and Teta, 2011), therefore occupying sites of poor preservation potential. This gives more uncertainty to whether tetralophodonty had origins in forest or open areas.

In the Miocene, South America was under the influence of a great marine introgression that dominated the central part of the continent from south to north, adjacent to the Andes land mass (Donato et al., 2003;Ortiz-Jaureguizar and Cladera, 2006; Hoorn et al., 2010). This water-dominated environment was substituted by spread plains of open vegetations during the Pliocene, and was a period of great diversification of grazers mammals (Donato et al., 2003; Ortiz-Jaureguizar and Cladera, 2006). This environment may have favored the diversification of tetralophodont genera, since our date estimates are closest to the Pliocene, their ancestral distribution is Cis-Andean and

these taxa are strongly associated to grasslands and other open areas (Hershkovitz, 1955; Weksler, 2006). If tetralophodont genera were widespread distributed in the grasslands during Pliocene, it could explain the presence of the ancestor of *Noronhomys* on coastal Brazil, which posteriorly rafted out in vegetation platform to the Fernando de Noronha archipelago (Carleton and Olson, 1999). Additionally, the broad distribution of *Lundomys* in the Pleistocene, which is confirmed by fossil records in central Brazil, Uruguay and Argentina (Voss and Carleton, 1993; Pardiñas and Teta, 2011), may also be associated with the South American "Age of Southern Plains" during the Pliocene (Ortiz-Jaureguizar and Cladera, 2006). During this time, an ancestral lineage could have reached the southern plains of South America and extended its distribution to central Brazil during cooler periods of the Pleistocene, considering the possible association of *Lundomys* with moderate temperatures (Pardiñas, 2008, Pardiñas and Teta, 2011). Additionally, recent models of paleo-distribution found retractions of central Brazilian vegetations during cooler periods (Werneck et al.,in press). The open habitats from southern Brazil and Uruguay, where *Lundomys* is currently found, may have extended through central Brazil during cooler periods (Werneck et al., in press), replacing the Cerrado vegetation found today. This would explain the presence of *Lundomys* at its type locality (Lagoa Santa, Minas Gerais) in the Pleistocene, but not today (Voss and Carleton, 1993).

The diversification effects caused by the Andes mountains are observed for many taxonomic groups, like birds (Ribas et al., 2007) and butterflies (Hall, 2005), whereas reciprocal monophyletic groups are found within Andes mountains and adjacent extra-Andean mountains. Concerning the Sigmodontinae, the importance of the Andes in terms of harboring endemic lineages is documented for some groups. For example, the Abrotrichini tribe is mainly distributed in the southern and central Andes (D'Elía et al., 2007), while the *Microryzomys* genus is typical from higher altitudinal zones in the

central and northern Andes (Carleton and Musser, 1989). In the same way, classical biogeographic hypotheses also reveal the Andes importance to the early diversifications of the Sigmodontinae (see Reig, 1984). In order to accommodate the Oryzomyini genera under a biogeographic distributional model, Weksler (2006) propose three general patterns: taxa primarily distributed in lowland or lower montane biomes east of the Andes (Cis-Andean), west of the Andes (Trans-Andean), and taxa found only in Andean habitats such as montane forest and Paramos, usually on both sides of the cordillera and above 1500–2000 m (Andean). We recognize these patterns in clade "D", with *Oryzomys*, *Aegialomys*, *Nesoryzomys*, *Melanomys* and *Sigmodontomys* as the only clade with support for a Trans-Andean distribution and sister to a Cis-Andean clade.

In light of Weksler (2006) distributional proposal and based in our ancestral area and time divergence analyses, is possible to infer that an ancestral of Trans-Andean clade could have dispersed from Cis-Andean region. The route of these migration is probably the extreme northern South America, a region where the latest uplift events of northern Andes occurred during Pliocene (Hoorn et al., 2010), closest to the divergence time estimated for the Trans and Cis-Andean lineages. In this view, the Andes formation could actuate as a barrier post-dispersal event.

In summary, the data presented in this study brings novel hypotheses concerning the systematics and evolutionary history of tetralophodont Oryzomyini genera. Additionally, the biogeographic scenario proposed provides a better understanding of the historical distributions of Oryzomyini genera from clade "D". In this context, additional studies focusing on multi-gene approach with broader taxonomic coverage and with more biogeographic areas as analytical units are welcome to test the hypotheses considered in this paper.

LITERATURE CITED

Baker RJ. 2003. Intron 7 (Fgb-i7) of the Fibrinogen, B Beta Polypeptide (Fgb): A nuclear DNA phylogenetic marker for mammals. Occasional Papers, Museum of Texas Tech University **219**: 1-6.

Baker RJ, Koop BF, Haiduk MW. **1983**. Resolving systematic relationship with G-Bands: a study of five genera of South American Cricetine rodents. Systematic Zoology **32**: 403-416.

Bonfield JK, Smith KF. 1995. Staden, R. A new DNA sequence assembly program. Nucleic Acids Research **23:** 4992-4999.

Bremer K. 1994. Branch support and tree stability. Cladistics **10**: 295-304.

Carleton MD. **1973**. A survey of gross stomach morphology in new world Cricetinae (Rodentia, Muroidea), with comments on functional interpretations. Miscellaneous Publications Museum of Zoology, University fo Michigan **146**: 1-43.

Carleton MD, Musser GG. 1989. Systematic studies of Oryzomyini rodents (Muridae, Sigmodontinae): a synopsis of Microryzomys Bulletin of the American Museum of Natural History **191: 1-83.**

Carleton M, Olson L. **1999**. Amerigo Vespucci and the Rat of Fernando de Noronha: a new genus and species of rodentia (Muridae: Sigmodontinae) from volcanic island off Brazil's continental shelf. American Museum Novitates **3256**: 1-59.

Costa LP, Pavan SE, Leite YLR, Fagundes V. 2007. A new species of Juliomys (Mammalia, Rodentia, Cricetidae) from the Atlantic Forest of southeastern Brazil. Zootaxa **1463:** 21–37.

D'Elía G, González EM, Pardiñas UFJ. **2003**. Phylogenetic analysis of sigmodontine rodents (Muroidea), with special reference to the akodont genus Deltamys Mammalian Biology **68**: 351-364.

D'Elía G, Pardiñas UFJ, Teta P, Patton JL. **2007**. Definition and diagnosis of a new tribe of Sigmodontinae rodents (Cricetidade: Sigmodontinae), and a revised classifications of the subfamily. Gayana **71**: 187-194.

Donato M, Posadas P, Miranda-esquivel DR, Jaureguizar EO, Cladera G. **2003**. Historical biogeography of the Andean region: evidence from Listroderina (Coleoptera# Curculionidae: Rhytirrhinini) in the context of the South American geobiotic scenario. Biological Journal of the Linnean Society **80**: 339-352.

Drummond AJ, Ho SYW, Phillips MJ, Rambaut A. **2006**. Relaxed phylogenetics and dating with confidence. PLoS Biology **4**: 699-710.

Engel SR, Hogan KM, Taylor JF, Davis SK. **1998**. Molecular systematics and paleobiogeography of the South American Sigmodontine Rodents. Molecular Biology **15**: 35-49.

Felsenstein J. 1985. Confidence-limits on phylogenies an approach using bootstrap. Evolution **39:** 783-791.

Gardner, A.L., and J.L. Patton. 1976. Karyotypic variation in Oryzomyine rodents (Cricetinae) with comments on chromosomal evolution in the Neotropical cricetine complex. Occasional Papers, Museum of Zoology, Louisiana State University **49**: 1–48.

Geist D. **1984**. On the emergence and submergence of the Galápagos islands. Noticias de Galapagos **56**: 5-9.

Gibbard PL, Head MJ, Walker MJC. 2010. Formal ratification of the Quaternary system/period and the Pleistocene series/epoch with a base at 2.58 ma. Journal of Quaternary Science **25**: 96-102.

Grehan J. **2001**. Biogeography and evolution of the Galapagos: integration of the biological and geological evidence. Biological Journal of the Linnean Society **74**: 267-287.

Hall JPW. 2005. Montane speciation patterns in Ithomiola butterflies (Lepidoptera: Riodinidae): are they consistently moving up in the world? Proceeding of Royal Society B **272: 2457-2466.**

Hanson JD. 2008. Molecular Phylogenetics of the tribe Oryzomyini: does a multi-gene approach help resolve a systematic conundrum? D. Phil. Thesis, Texas Tech University.

Hershkovitz P. 1944. Systematic review of the Neotropical water rats of the genus Nectomys (Cricetinae). Miscellaneous Publications, Muse um of Zoology, University of Michigan **58:** 1-101.

Hershkovitz P. **1955**. South American marsh rats, genus Holochilus, with summary of Sigmodont rodents. Fieldiana Zoology **37**: 639-687.

Hershkovitz P. **1962**. Evolution of Neotropical cricetine rodents (Muridae) with special reference to the phyllotine group. Fieldiana Zoology **46**: 1-524.

Hershkovitz P. 1966. Mice, land bridges and Latin American faunal interchange. In: Ectoparasites of Panama (Wenzer RL, Tipton VJ eds.). Field Museum of Natural History, Chicago 725-751.

Hershkovitz P. 1993. A new central Brazilian genus and species of Sigmodontine rodent (Sigmodontinae) transitional between Akodonts and Oryzomyines, with a discussion of Muoid molar morphology and evolution. Fieldiana Zoology new series **75:** 1-18.

Ho SYW. **2007**. Calibrating molecular estimates of substitution rates and divergence times in birds. Journal of Avian Biology **38**: 409-414.

Hoorn C, Wesselingh FP, Steege H ter, Bermudez M a, Mora A, Sevink J, Sanmartín I, Sanchez-Meseguer A, Anderson CL, Figueiredo JP, Jaramillo C, Riff D, Negri FR, Hooghiemstra H, Lundberg J, Stadler T, Särkinen T, Antonelli A. **2010**. Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. Science **330**: 927-931.

Hooper E, Musser GG. **1964**. The glans penis in Neotropical Cricetines (Family Muridae) with comments on classification of Muroid rodents. Miscellaneous Publications Museum of Zoology, University of Michigan **123**: 1-64.

Huchon D, Catzeflis FM, Douzery EJP. **1999**. Molecular evolution of the nuclear von Willebrand Factor gene in Mammals and the phylogeny of rodents. Molecular Biology **16**: 577-589.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics **17**: 754-755.

IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2. http://www.iucnredlist.org. Downloaded on November 2011

Maddison W, Maddison DR. 1999. MacClade: Analysis of phylogeny and character evolution. Sinauer Associates, Inc. Sunderland, MA.

Musser GG, Carleton MD. 2005. Superfamily Muroidea. In: Wilson DE, Reeder DA, eds. Mammal species of the world: a taxonomic and geographic reference. Baltimore, MD: Johns Hopkins University Press, 894–1531.

Myers P, Patton JL. **1989**. Akodon of Peru and Bolivia - revision of the Fumeus group (Rodentia: Sigmodontinae). Occasional Papers of the Museum of Zoology, The University of Michigan **721**: 1-35.

NCBI. 2011. http://www.ncbi.nlm.nih.gob/genbank/.

Notredame C, Higgins DG, Heringa J. **2000**. T-Coffee: A novel method for fast and accurate multiple sequence alignment. Journal of molecular biology **302**: 205-17.

O'Leary, M. A., and S. G. Kaufman. 2007. MorphoBank 2.5: Web application for morphological phylogenetics and taxonomy. http://www.morphobank.org.

Ortiz-Jaureguizar E, Cladera G. **2006**. Paleoenvironmental evolution of southern South America during the Cenozoic. Journal of Arid Environments **66**: 498-532.

Palma RE, Rodríguez-Serrano E, Rivera-Milla E, Hernandez CE, Salazar-Bravo J, Carma MI, Belmar-Lucero S, Gutierrez-Tapia P, Zeballos H, Yates TL. **2010**. Phylogenetic relationships of the pygmy rice rats of the genus Oligoryzomys Bangs, 1900 (Rodentia: Sigmodontinae). Zoological Journal of the Linnean Society **160**: 551-566.

Pardiñas UFJ, D'Elía G, Ortiz P. **2002**. Sigmodontinos fósiles (Rodentia, Muroidea, Sigmodontinae) de América del Sur: estado actual de su conocimiento y propectiva. Mastozoologia Neotropical **9**: 209-252.

Pardiñas UFJ. **2008**. A new genus of oryzomyine rodent (Cricetidae: Sigmodontinae) from the Pleistocene of Argentina. Journal of Mammalogy **89**: 1270-1278.

Pardiñas UFJ, Teta P. **2011**. Fossil history of the marsh rats of the genus Holochilus and Lundomys (Cricetidae, Sigmodontinae) in southern South America. Estudios Geol—gicos **67**: 111-129.

Percequillo AR, Hingst-Zaher E, Bonvicino CR. **2008**. Systematic Review of Genus Cerradomys Weksler, Percequillo and Voss, 2006 (Rodentia: Cricetidae: Sigmodontinae: Oryzomyini), with Description of Two New Species from Eastern Brazil. American Museum Novitates **3622**: 1-46.

Percequillo AR, Weksler M, Costa LP. **2011**. A new genus and species of rodent from the Brazilian Atlantic Forest (Rodentia: Cricetidae: Sigmodontinae: Oryzomyini), with comments on oryzomyine biogeography. Zoological Journal of the Linnean Society **161**: 357-390.

Posada D. 2008. jModelTest: Phylogenetic Model Averaging. Molecular Biology and Evolution **25:** 1253-1256.

Rambaut A, Drummond AJ. 2007. Tracer v1.4, Available from http://beast.bio.ed.ac.uk/Tracer

R Development Core Team. 2011. R: A language and Enviroment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Reig O. **1980**. A new fossil genus of South American cricetid rodents allied to Wiedomys, with an assessment of the Sigmodontinae. Journal of Zoology **192**: 257-281.

Reig O. **1984**. Distribuição geográfica e história evolutiva dos roedores muroideos sulamericanos (Cricetidae: Sigmodontinae). Revista Brasileira de Genética **7**: 333-365.

Ribas CC, Moyle, RG, Miyaki CY, Cracraft J. 2007. The assembly of montane biotas: linking Andean tectonics and climatic oscillations to independent regimes of diversification in Pionus parrots. Proceedings of the Royal Society B **274: 2399-2408**.

Schliep KP. **2011**. phangorn: phylogenetic analysis in R. Bioinformatics **27**: 592-593.

Smith M, Patton JL. 1993. The diversification of South American murid rodents: evidence from mitochondrial DNA sequence data for the akodontine tribe. Biological Journal of the Linnean Society **50**: 159-177.

Smith MF, Patton JL. **1999**. Phylogenetic relationships and the radiation of Sigmodontine rodents in South America# Evidence from Cytochrome b. Journal of Mammalian Evolution **6**: 89-128.

Stanhope M, Czelusniak J, Si JS, Nickerson J, Goodman M. 1992. Perspective on Mammalian evolution from the gene Interphotoreceptor Retinoid Binding Protein, with convincing evidence for bat monophyly. Molecular Phylogenetics and Evolution **1**: 148-160.

Steppan S. **1995**. Revision of the tribe Phyllotini (Rodentia: Sigmodontinae), with a phylogenetic hypothesis for the Sigmodontinae. Fieldiana Zoology **80**: 1-112.

Steppan SJ. **1996**. A new species of Holochilus (Rodentia: Sigmodontinae) from the middle Pleistocene of Bolivia and its phylogenetic significance. Journal of Vertebrate Paleontology **16**: 522-530.

Steppan SJ, Adkins RM, Anderson J. **2004**. Phylogeny and divergence-date estimates of rapid radiations in Muroid rodents. Systematic Biology **53**: 533-553.

Swofford DL. 1999. PAUP*: Phylogenetic Analysis using Parsimony (and other methods), version 4.0b1. Sinauer Associates, Inc. Sunderland, MA.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution **28**: 2731-2739.

Turvey ST, Weksler M, Morris EL, Nokkert M. **2010**. Taxonomy, phylogeny, and diversity of the extinct Lesser Antillean rice rats (Sigmodontinae: Oryzomyini), with description of a new genus and species. Zoological Journal of the Linnean Society **160**: 748-772.

Voss R. **1991**. An introduction to the Neotropical muroid rodent genus Zygodontomys. Bulletin of the American Museum of Natural History **210**: 1-210.

Voss R. **1992**. A revision of the South America species of Sigmodon (Mammalia: Muridae) with notes on their natural history and biogeography. American Museum Novitates **3050**: 1-56.

Voss RS, Carleton MD. **1993**. A New Genus for Hesperomys molitor Winge and Holochilus magnus Hershkovitz (Mammalia, Muridae) with an analysis of its phylogenetic relationships. Nature **225**: 1-40.

Voss RS, Weksler M. 2009. On the taxonomic status of Oryzomys curasoae McFarlane and Debrot, 2001, (Rodentia: Cricetidade: Sigmodontinae) with remarks on the phylogenetics relationships of O. gorgasi Hershkovitz, 1971. Caribbean Journal of Science **45**: 73-79.

Weksler M, Percequillo AR, Voss RS. **2006**. Ten New Genera of Oryzomyine Rodents (Cricetidae: Sigmodontinae). American Museum Novitates **3537**: 1-29.

Weksler M. **2006**. Phylogenetic relationships of Oryzomine rodents (Muroidea: Sigmodontinae): separate and combined analyses of morphological and molecular data. Bulletin of the American Museum of Natural History **296**: 1-149.

Weksler M. **2003**. Phylogeny of Neotropical oryzomyine rodents (Muridae: Sigmodontinae) based on the nuclear IRBP exon. Molecular Phylogenetics and Evolution **29**: 331-349.

Werneck F, Nogueira C, Colli G, Sites J, Costa G. Climatic stability in the Brazilian Cerrado: implications for biogeographical connections of South American savannas, species richness, and conservation in a biodiversity hotspot. In press.

Wiens JJ. 1995. Polymorphic characters in phylogenetic systematics. Systematic Biology **44**: 482–500.

Wiens JJ. **2006**. Missing data and the design of phylogenetic analyses. Journal of biomedical informatics **39**: 34-42.

Wiens JJ, Moen DS. **2008**. Missing data and the accuracy of Bayesian phylogenetics. Journal of Systematics and Evolution **46**: 307-314.

Wiens JJ, Morrill MC. **2011**. Missing Data in Phylogenetic Analysis: Reconciling Results from Simulations and Empirical Data. Systematic biology **60**: 1-13.

Wicklife JK, Hoffman FG, Carroll DS, Dunina-Barkovskaya YV, Bradley RD, Xia X, Xie Z. 2001. DAMBE: data analysis in molecular biology and evolution. Journal of Heredity **92**:371–373.

Yu Y, Harris AJ, He X-J. 2011. RASP (Reconstruct Ancestral State in Phylogenies) 2.0 beta. Available at http://mnh.scu.edu.cn/soft/blog/RASP

Table 1. Table showing the taxonomic sampling, GenBank and catalog number of sequences analyzed. UNB are sample from the mammal collection of Universidade de Brasília; UFES are sample from mammal collection of Universidade do Espírito Santo; MCNU and Ijuí are samples from mammal collection of Museu de Ciências Naturais Universidade Luterana do Brasil; TK are samples from the tissue collection of Texas Tech University.

	GenBank acession number and/or catalog number				
Taxon	IRBP	Cyt-b	Adh1-I2	Fgb-7	vWF
Hylaemys megacephalus	AY163621	AY27059	EU648994	UNB3069	UNB3069
Oecomys catherinae	AY163605	UFES247	EU649009	UFES247	UFES247
Neacomys spinosus	AY163597	UFES1730	EU649002	UFES1730	UFES1730
Oligoryzomys flavescens	AY163609 MCNU1287	GU126528	EU649013	MCNU1297 MCNU1287	MCNU1287 MCNU1297
Oligoryzomys nigripes	AY163612 MCNU1963	GU126530	EU649015	MCNU1963 MCNU1950	MCNU1950
Eremoryzomys polius	AY163624	GU126540	EU648980		
Cerradomys subflavus	AY163626	UFES927	EU648979	UFES927	UFES927
Sooretamys angouya	AY163616	UFES1058	EU649029 EU649028	UFES1058	UFES1058
Pseudoryzomys simplex	AY163633	UFES457 UFES491	EU649024 EU649023	UFES491	UFES457 UFES491
Holochilus brasiliensis	AY163586 MCNU1946	MCNU1943 MCNU1946	EU648989	MCNU1946 MCNU1943	MCNU1943
Holochilus chacarius	UFES1539 UFES582 UFES590	UFES1539 UFES582 UFES590	DQ227456	UFES1539 UFES582 UFES590	UFES1539 UFES582
Holochilus sciureus	UFES135 UFES528	UFES526 UFES528	EU648990	UFES135 UFES526 UFES528	UFES135 UFES528
Lundomys molitor	AY163589 MCNU2308	MCNU2302 IJUI08	EU648994	MCNU2302 IJUI08	MCNU2302
Oryzomys couesi	TK27059	TK27059	EU649021 EU649020	TK136206 TK27059	TK136206 TK27059

Oryzomys palustris	AY163623	TK102016 TK93244	EU649022	TK93244 TK102016	TK102016 TK93244
Amphinectomys savamis	AY163579	EU579480	EU648977		
Nectomys squamipes	AY163598 UFES925	UFES928	EU649005	UFES928 UFES925	UFES925 UFES928
Aegialomys xanthaeolus	AY163628	GU126545	EU648997		
Nesoryzomys swarthi	AY163601	GU126524	EU649008		
Nesoryzomys narborough	AY163600	GU126523			
Melanomys caliginosus	AY163590	GU126518	EU648995		
Sigmodontomys alfari	AY163641	EU074635 GU126548	EU649027 EU649026		
Drymoreomys albimaculatus	GU126515	EU126516			

Table 2. The gene fragments and sequence of primers utilized for amplifications and references.

Gene	Primer	Sequence	Reference
CYT B	MVZ05	CGAAGCTTGATATGAAAAACCATCGTTG	Smith & Patton, 1993
CYT B	MVZ16	AAATAGGAARTATCAYTCTGGTTTRAT	Smith & Patton, 1993
IRBP	+IRBP217	ATGGCCAAGGTCCTCTTGGATAACTACTGCTT	Stanhope et al., 1992
IRBP	-IRBP1531	CGCAGGTCCATGATGAGGTGCTCCGTGTCCTG	Stanhope et al., 1992
vWF	V1	TGTCAACCTCACCTGTGAAGCCTG	Huchon et al., 1999
vWF	V4	AAGCAGGCCCCTGAAAACAA	Huchon et al., 1999
vWF	W1	TGCAGGACCAGGTCAGGAGCCTCTC	Huchon et al., 1999
vWF	W2	ACGTCCATGCGCTGGATCACCT	Huchon et al., 1999
Fgb-7	Fgb-17U	GGGGAGAACAGAACCATGACCATCCAC	Wickliffe et al., 2003
Fgb-7	Fgb-17L	ACCCCAGTAFTATCTGCCATTCGGATT	Wickliffe et al., 2003

Table 3. Table showing volume and concentrations of reagents used in the amplifications process per gene fragment. vWF (1) and vWF (2) represent independents amplification process, which were subsequently joined (see Material and Methods).

Gene	dNTPs (10 mM)	10x Buffer	$MgCl_2$ (50 mM)	Primer (10 $M)	Taq DNA polymerase (5 U/$l)	Template
	Volume	Volume	Volume	Volume	Volume	Volume
IRBP	0. 6 $l	3 $l	1. 2 $l	0. 36 $l	0. 24 $l	2. 4 $l
Fgb-7	0. 6 $l	3 $l	1. 2 $l	0. 36 $l	0. 36 $l	2. 4 $l
vWF (1)	0. 48 $l	3 $l	0. 6 $l	0. 96 $l	0. 6 $l	2. 4 $l
vWF (2)	0. 4 $l	2.5 $l	0. 5 $l	0. 8 $l	0. 25 $l	4 $l
cyt-b	0. 6 $l	3 $l	0. 9 $l	1. 44 $l	0. 24 $l	4 $l

Figure 1. Nucleotide transitions and tranversions plotted against F84 model distance of analyzed genes.

Figure 2. Majority rule consensus tree based on morphological (A) and most parsimonious tree based on morphological + molecular (B) characters. Near the nodes are bootstrap on left side of the bar, and Bremer support on right side. Nodes without values are bootstrap and Bremer support less than 50 and 1 respectivelly.

Figure 3. Bayesian inference (A) and maximum likelihood (B) phylograms based on concatenated molecular data-set. Numbers near nodes represent posterior probabilities (A) and bootstrap (B). Stars indicate lineages having only tetralophodont genera.

Figure 4. Bayesian inference based on molecular + morphological data. Numbers near nodes represent posterior probabilities.

Figure 5. Chronogram showing molecular date divergence estimated in clade “D” based on three calibration approaches. Numbers near nodes represents mean age and 95% confidence intervals in brackets. Epochs limits are based in Gibbard et al. (2010). Outgroups are omitted.

Figure 6. Map of current distribution of genera analyzed and cladogram showing the estimated ancestral distribution (outgroups omitted). Black squares, horizontal and diagonal lines near terminal taxa in the cladogram denote the respective current distribution on the map. Pie chart shows approximate probabilities of ancestral area distribution according to the areas in the legend.

$#

#

Figure 1$

!" #

#

(A) (B)

Figure 2.

!" #

#

(A)

(B)

Figure 3.

"

"

"

"

"

"

"

"

"

"

"

"

"

"

"

"

"

Figure 4.

!! "

"

#

#

#

#

#

#

#

#

#

#

#

#

#

#

#

#

#

Figure 5.

#

!" #

#

#

#

#

#

#

#

#

#

#

#

#

#

#

#

#

#

#

#

Figure 6.

!" #

#

Appendix 1

Final matrix used in the parsimony analyses. Polymorphic characters coded as: {01}=A, {12}=B, {23}=C. Information about Holochilus sciureus (UNB 0061, 0062). Holochilus primigenus, Noronhomys vespuccii and Carletonomys cailoi are added in comparisons to Weksler (2006), Turvey et al. (2010) and Percequillo et al. (2011):

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Drymoreomys albimaculatus	?	0	0	0	0	1	0	0	0	3	0	0	0	0	0	0	1	0	0	2	0	3	0	0	1	2	0	0	0	0
Pennatomys nivalis	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	1	0	3	0	0	?	?	?	1	1	?
Megalomys desmarestii	?	0	1	0	0	1	3	0	0	2	0	1	0	1	0	0	0	1	1	2	1	4	1	1	1	1	0	1	1	1
Megalomys luciae	?	0	1	1	0	1	3	0	0	2	0	1	0	0	2	0	0	1	1	2	1	4	1	1	1	1	0	1	1	1
Noronhomys vespuccii	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	1	?	1	1	?	1	1	0	1	1	2
Holochilus.primigenus	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	?
Carletonomys cailoi	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	?
Holochilus sciureus	?	0	1	1	2	1	3	1	2	1	B	1	0	0	1	0	0	0	0	1	1	4	1	1	1	1	0	2	0	1
Holochilus chacarius	?	0	1	1	2	1	3	1	2	1	0	1	0	0	1	0	0	0	0	1	1	2	1	1	1	1	0	2	0	1
Holochilus brasiliensis	2	0	1	1	2	1	3	1	2	1	0	1	0	0	1	0	0	0	0	1	1	2	1	1	1	1	0	2	0	1
Pseudoryzomys simplex	2	0	0	1	2	1	2	0	1	1	2	1	0	0	0	0	0	0	1	1	1	3	0	0	1	1	0	2	0	1
Amphinectomys savamis	?	?	?	?	?	?	?	1	2	1	?	1	0	0	0	0	0	0	0	2	1	4	?	0	0	2	0	1	1	0
Cerradomys subflavus	2	0	0	0	1	1	1	0	0	1	1	1	0	0	0	0	0	0	0	1	0	4	0	0	1	B	0	1	0	B
Eremoryzomys polius	2	0	0	0	1	1	0	0	0	0	2	1	0	0	1	0	0	0	0	1	0	4	0	0	1	2	0	1	1	0
Hylaeamys megacephalus	2	0	0	0	1	0	1	0	0	1	1	1	0	0	0	0	0	0	0	1	1	3	0	0	0	1	0	1	1	1
Lundomys molitor	2	1	1	1	2	1	3	1	2	1	0	1	0	0	1	0	0	0	1	1	0	1	0	0	1	1	0	2	1	1
Melanomys caliginosus	2	0	0	0	1	1	2	0	0	2	0	1	0	0	2	0	0	0	1	B	1	4	0	1	1	2	0	1	1	1
Neacomys spinosus	2	0	0	0	1	1	1	0	0	1	0	1	1	1	0	0	0	0	1	2	0	4	0	0	0	1	0	1	0	1
Nectomys squamipes	2	0	0	1	2	1	2	1	2	1	0	1	0	0	1	0	0	1	1	2	1	4	0	1	1	1	0	1	1	1
Nesoryzomys narboroughi	?	0	0	0	1	1	1	0	0	0	2	0	0	0	1	1	0	0	0	1	1	1	0	0	1	1	0	1	1	1
Nesoryzomys swarthi	?	0	0	0	1	1	1	0	0	0	1	0	0	0	1	1	0	0	0	1	1	1	0	0	1	1	0	1	1	0
Oecomys catherinae	2	0	0	0	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	1	0	4	0	0	1	1	0	0	1	1
Oligoryzomys flavescens	2	0	0	0	1	1	1	0	0	1	1	1	0	0	1	0	0	0	1	1	0	0	0	0	0	1	0	1	0	2
Oligoryzomys nigripes	2	0	0	0	1	1	1	0	0	1	1	1	0	0	0	0	0	0	A	1	0	0	0	0	0	1	0	1	0	2
Oryzomys couesi	2	0	0	1	2	1	2	0	0	1	1	1	0	0	1	0	0	0	0	1	1	4	0	0	1	2	0	1	0	1
Oryzomys palustris	2	0	1	1	2	1	2	0	1	1	2	1	0	0	0	0	0	0	1	1	1	4	0	0	1	2	0	1	0	1
Sigmodontomys alfari	2	0	0	1	2	1	2	0	1	2	0	1	0	0	0	0	0	0	A	B	1	4	0	1	1	1	0	1	0	B
Sigmodontomys aphrastus	2	0	0	1	2	0	3	0	0	2	0	1	0	0	2	0	0	1	1	2	1	4	0	1	1	1	0	0	0	0
Sooretamys angouya	2	0	0	0	1	1	0	0	0	1	0	1	0	0	0	0	0	0	A	1	A	1	0	0	1	B	0	1	1	1
Aegialomys xanthaeolus	2	0	0	0	1	1	1	0	0	1	1	1	0	0	0	0	0	0	A	B	1	4	0	0	1	B	0	1	A	1

Character matrix continued

Taxon	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Drymoreomys albimaculatus	A	A	0	2	1	B	2	0	0	0	1	1	1	0	A	0	1	1	0	?	0	0	0	0	0	1	0	0	1	1
Pennatomys nivalis	0	2	0	?	?	?	?	?	?	?	?	?	?	2	1	0	?	0	2	1	0	0	0	0	0	1	2	0	0	1
Megalomys desmarestii	1	1	0	3	1	2	2	1	0	1	0	1	0	2	0	0	?	1	2	1	0	0	0	0	0	1	0	0	0	1
Megalomys luciae	1	1	0	3	1	1	2	1	0	1	0	1	0	2	0	0	?	1	2	?	0	0	0	0	0	1	0	0	0	1
Noronhomys vespuccii	1	1	1	2	?	?	2	0	?	0	1	1	?	?	1	0	?	1	2	?	0	1	1	1	?	2	2	3	0	0
Holochilus.primigenus	1	1	?	?	?	?	?	?	?	?	?	?	?	2	1	1	?	1	2	?	?	?	0	1	?	1	0	3	0	0
Carletonomys cailoi	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	?	?	?	?	1	1	?	2	0	3	0	0
Holochilus sciureus	1	1	1	2	2	0	2	0	0	0	2	1	1	1	0	1	?	1	2	1	0	1	1	1	1	2	2	3	0	0
Holochilus chacarius	1	2	1	2	2	0	2	0	0	0	2	1	0	2	1	1	?	1	2	1	0	1	1	1	1	2	2	3	0	0
Holochilus brasiliensis	1	1	1	B	2	0	2	0	0	0	2	1	1	2	1	1	1	1	2	1	0	1	1	1	1	2	2	3	0	0
Pseudoryzomys simplex	0	2	0	2	1	0	2	A	0	0	1	1	1	2	0	0	1	1	2	1	0	0	0	0	0	0	1	3	0	1
Amphinectomys savamis	1	2	0	3	?	1	?	?	?	1	?	?	?	1	?	0	?	?	?	?	1	?	0	?	?	?	?	3	?	?
Cerradomys subflavus	0	1	0	3	1	0	2	1	0	1	1	1	A	2	0	0	1	1	2	0	0	0	0	0	0	0	1	0	0	A
Eremoryzomys polius	0	1	0	3	1	0	2	0	0	0	1	1	1	0	1	0	1	0	0	?	0	0	0	0	0	0	2	0	0	0
Hylaeamys megacephalus	1	2	0	B	1	B	1	1	0	0	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	2	0	0	0
Lundomys molitor	0	2	1	2	2	1	2	1	0	0	1	1	1	1	0	0	1	0	2	A	0	0	0	1	1	1	0	1	0	0
Melanomys caliginosus	1	1	0	2	1	2	2	1	0	1	1	1	0	1	1	0	?	1	2	0	1	0	0	0	0	0	2	2	0	1
Neacomys spinosus	1	2	0	2	1	1	0	1	0	0	0	1	A	2	0	0	?	1	0	0	0	0	0	0	0	0	2	0	0	1
Nectomys squamipes	1	1	0	3	1	B	2	1	0	1	1	1	0	1	1	0	1	1	2	0	0	0	0	0	0	1	2	2	0	0
Nesoryzomys narboroughi	0	2	0	3	1	0	2	1	0	1	2	1	0	2	1	1	1	1	2	0	0	0	0	0	0	0	0	0	0	1
Nesoryzomys swarthi	0	1	0	3	1	0	2	1	0	0	2	1	0	2	1	1	1	1	2	?	0	0	0	0	0	0	?	?	?	?
Oecomys catherinae	1	2	0	2	1	2	0	1	0	0	1	1	0	1	0	0	?	1	0	0	0	0	0	0	0	1	2	0	0	0
Oligoryzomys flavescens	1	2	0	2	0	0	1	1	0	0	0	1	1	2	0	1	?	0	0	0	0	0	0	0	0	0	0	0	0	1
Oligoryzomys nigripes	1	2	0	2	0	0	1	1	0	0	0	1	1	2	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1
Oryzomys couesi	0	2	0	3	1	2	2	1	0	0	1	1	1	2	0	0	?	1	2	?	0	0	0	0	0	0	2	0	0	1
Oryzomys palustris	0	2	0	3	1	0	2	1	0	0	1	1	1	1	0	0	1	1	B	A	0	0	0	0	0	0	1	0	0	1
Sigmodontomys alfari	1	1	0	2	1	1	2	1	0	1	1	1	0	2	A	0	1	1	2	1	0	0	0	0	0	1	2	2	0	0
Sigmodontomys aphrastus	1	1	0	1	1	1	2	1	0	1	1	1	0	A	0	0	?	1	2	?	1	0	0	0	0	2	2	2	0	0
Sooretamys angouya	0	1	0	3	1	0	2	1	0	0	1	1	1	2	0	0	?	0	1	0	0	0	0	0	0	0	2	0	1	1
Aegialomys xanthaeolus	0	2	0	3	1	0	2	1	0	1	1	1	A	1	1	0	1	1	2	1	0	0	0	0	0	0	2	2	0	0

Character matrix continued

Taxon	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
Drymoreomys albimaculatus	0	0	0	0	1	0	0	0	2	1	1	0	0	1	0	0	1	1	0	2	1	1	1	0	1	1	2	0	1	1
Pennatomys nivalis	0	0	1	0	0	0	1	1	1	1	0	0	0	1	0	0	?	?	?	?	1	1	1	?	?	?	?	?	?	?
Megalomys desmarestii	0	0	1	0	0	0	0	0	1	1	0	0	0	1	0	0	1	1	0	2	1	0	?	?	?	?	?	?	?	?
Megalomys luciae	0	0	?	0	0	0	0	0	1	1	0	0	0	?	?	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Noronhomys vespuccii	1	1	1	0	-	0	0	0	1	1	0	2	0	0	1	2	?	?	?	?	1	?	?	?	?	?	?	?	?	?
Holochilus.primigenus	1	0	0	0	-	1	0	0	1	1	0	1	0	0	0	2	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Carletonomys cailoi	1	1	?	?	-	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Holochilus sciureus	2	1	1	0	-	1	1	0	1	1	0	2	0	0	0	2	?	1	?	?	1	1	1	?	?	?	?	?	?	?
Holochilus chacarius	2	1	1	0	-	0	0	0	1	1	0	2	0	0	0	2	1	?	?	?	?	?	?	?	?	?	?	?	?	?
Holochilus brasiliensis	1	1	1	0	-	0	0	0	1	1	0	2	0	0	0	2	1	1	0	2	1	1	1	1	0	1	0	1	1	0
Pseudoryzomys simplex	1	0	0	0	?	1	0	0	1	0	0	2	0	0	0	1	1	1	1	2	1	1	1	1	1	?	0	0	1	?
Amphinectomys savamis	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Cerradomys subflavus	0	0	0	0	1	0	0	A	1	1	0	0	0	0	0	0	1	1	0	2	1	1	1	1	2	0	0	0	?	?
Eremoryzomys polius	0	0	0	0	0	0	0	1	1	1	0	0	0	1	0	0	1	?	?	?	?	?	?	?	?	?	?	?	?	?
Hylaeamys megacephalus	0	0	0	0	0	0	1	0	1	1	1	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	0	0	1	0
Lundomys molitor	1	0	0	0	?	1	0	0	1	1	0	1	0	0	0	2	1	1	0	2	1	1	1	1	0	0	0	0	?	?
Melanomys caliginosus	0	0	1	0	0	0	0	1	1	1	0	0	0	0	0	0	1	1	1	2	1	1	1	1	1	0	0	0	1	0
Neacomys spinosus	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	0	0	0	1	0
Nectomys squamipes	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1	?	2	1	?	1	1	0	?	?	?	1	0
Nesoryzomys narboroughi	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1	0	0	1	1	1	1	1	?	?	0	1	1
Nesoryzomys swarthi	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	1	0	2	1	1	1	?	?	?	?	?	?	?
Oecomys catherinae	0	0	0	1	0	0	0	0	1	1	1	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Oligoryzomys flavescens	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	1	1	1	?	1	1	?	1	0	0	1	0	1	?
Oligoryzomys nigripes	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1	1	2	1	1	?	1	0	0	1	0	2	?
Oryzomys couesi	0	0	0	0	1	0	0	0	1	1	0	0	0	1	0	0	1	1	1	2	1	1	1	1	0	0	1	1	?	?
Oryzomys palustris	0	0	0	0	1	0	0	0	1	1	0	0	0	1	0	0	1	1	1	2	1	1	1	1	0	1	1	1	1	0
Sigmodontomys alfari	0	0	1	0	0	0	0	1	1	1	0	0	1	0	1	0	1	1	0	2	1	1	1	1	1	1	2	0	1	0
Sigmodontomys aphrastus	0	0	1	0	0	0	0	1	1	1	0	0	0	1	1	0	?	?	?	?	?	?	?	1	1	1	0	0	?	?
Sooretamys angouya	0	0	0	0	1	0	0	0	1	1	1	0	0	0	0	0	1	1	0	2	1	0	1	1	0	1	0	0	1	0
Aegialomys xanthaeolus	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	1	1	0	2	1	1	?	1	1	0	0	0	?	?

Character matrix continued

Taxon	91	92	93	94	95	96	97	98
Drymoreomys albimaculatus	0	0	0	1	1	0	1	0
Pennatomys nivalis	?	?	?	?	?	?	?	?
Megalomys desmarestii	?	?	?	?	?	?	?	?
Megalomys luciae	?	?	?	?	?	?	?	?
Noronhomys vespuccii	?	?	?	?	?	?	?	?
Holochilus.primigenus	?	?	?	?	?	?	?	?
Carletonomys cailoi	?	?	?	?	?	?	?	?
Holochilus sciureus	?	?	?	?	?	?	?	?
Holochilus chacarius	?	?	?	?	?	?	1	1
Holochilus brasiliensis	0	0	0	1	1	0	1	1
Pseudoryzomys simplex	?	?	?	?	?	?	1	1
Amphinectomys savamis	?	?	?	?	?	?	?	?
Cerradomys subflavus	?	?	?	?	?	?	1	0
Eremoryzomys polius	?	?	?	?	?	?	?	?
Hylaeamys megacephalus	0	0	0	1	0	1	1	0
Lundomys molitor	?	?	?	?	?	?	1	0
Melanomys caliginosus	0	0	0	1	1	0	1	0
Neacomys spinosus	0	0	1	1	1	0	1	0
Nectomys squamipes	0	0	0	1	1	0	1	1
Nesoryzomys narboroughi	1	1	?	1	1	?	1	0
Nesoryzomys swarthi	?	?	?	?	?	?	?	?
Oecomys catherinae	?	?	?	?	?	?	?	?
Oligoryzomys flavescens	?	?	?	?	?	?	1	?
Oligoryzomys nigripes	?	?	?	?	?	?	1	0
Oryzomys couesi	?	?	?	?	?	?	1	0
Oryzomys palustris	0	0	0	1	1	1	1	0
Sigmodontomys alfari	0	0	?	2	1	0	1	?
Sigmodontomys aphrastus	?	?	?	?	?	?	1	1
Sooretamys angouya	0	0	0	1	1	0	1	?
Aegialomys xanthaeolus	?	?	?	?	?	?	1	0

APPENDIX 2

Philograms of individual genes analyses. On left side are showing the Bayesian analyses, and Maximum Likelihood (ML) on right. Numbers near nodes represents posterior probability for and bootstrap value for ML.

IRBP:

Cyt-b:

#

!" #

#

Adh1-I2:

!" #

#

Fgb-7:

#

!"

#

vWF:

"

!! "

"