UNIVERSIDADE FEDERAL DO PARÁ
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM NEUROCIÊNCIAS
E BIOLOGIA CELULAR

Estudos Citogenéticos em Roedores do Gênero *Oecomys* (Rodentia: Cricetidae)

Celina Coelho da Rosa

BELÉM - PARÁ
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Dissertação de Mestrado submetido ao curso de Programa de Pós-graduação em Neurociências e Biologia Celular da Universidade Federal do Pará (UFPA), como requisito parcial para a obtenção do grau de Mestre em Neurociências e Biologia Celular.

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“Todo estado atual de uma substância simples é naturalmente conseqüência de seu estado anterior, de tal modo que seu presente está impregnado no seu futuro.”
(Leibniz)

Aos meus melhores amigos, Clélia e Perilo

À minha orientadora Cleusa Nagamachi

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RESUMO

Os roedores representam o grupo de mamíferos viventes mais diversificados e com ampla diversidade de adaptações ecológicas. Os roedores, devido às características populacionais que apresentam, desenvolveram-se como o grupo mais especioso de mamíferos em florestas neotropicais e um dos mais interessantes para estudos da variabilidade genética e de evolução entre os vertebrados. Os roedores do gênero *Oecomys* compreendem aproximadamente 16 espécies que habitam floresta tropical e subtropical do Centro e do Sul da América. Destas, apenas seis têm ocorrência esperada para a Amazônia Oriental Brasileira. De acordo com a literatura, o gênero *Oecomys* apresenta uma grande diversidade cariotípica, com o número diplóide variando entre 58 e 86. Neste estudo, espécimes de *Oecomys paricola* Thomas, 1904 de Belém e da Ilha do Marajó foram estudadas usando analises citogenética, molecular e morfológica. Três cariótipos foram encontrados, dois de Belém (2n=68, NF=72 e 2n=70, NF=76) e um da Ilha do Marajó (2n=70, NF=74). Não foi encontrada diferença molecular e morfológica entre indivíduos dos diferentes citótipos de Belém e da Ilha do Marajó. Espécies da cidade de Belém representam duas espécies crípticas, pois dois cariótipos diferentes estão presentes na ausência de diferenças significativas nas características morfológicas e moleculares. Populações da Ilha do Marajó e Belém representam espécies distintas que foram separadas há algum tempo, e estão em processo de diferenciação morfológica e molecular, como consequência do isolamento reprodutivo a nível geográfico e cromossômico.

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ABSTRACT

The rodents are one of the most diversified groups of living mammals and also have a large range of ecological adaptations. The rodents, because of yours population characteristics, developed as the most specious group of mammals in Neotropical forests and one of the most interesting for studies of genetic variation and evolution among vertebrates. The genus *Oecomys* (Sigmodontinae) comprises approximately 16 species that inhabit tropical and subtropical forests in Central and South America. Six of these species are expected to occur in eastern Brasilian Amazon. In literature, the genus *Oecomys* has a large karyotypic variation, where the diploid number ranges from 58 to 86. In this study specimens of *Oecomys paricola* Thomas, 1904 from Belém and Marajó Island, northern Brazil, were investigated using cytogenetic, molecular and morphological analyses. Three karyotypes were found, two from Belém (2n=68, FN=72 and 2n=70, FN=76) and a third from Marajó Island (2n=70, FN=72). No molecular or morphological differences were found between the individuals with differing cytotypes from Belém and Marajó Island. Specimens from the Belém City region may represent two cryptic species because two different karyotypes are present in the absence of significant differences in morphology and molecular characteristics. The Marajó Island and Belém populations represent distinct species that have been separated for some time, and are in the process of morphological and molecular differentiation as a consequence of reproductive isolation at the geographic and chromosomal levels.

1. INTRODUÇÃO

1.1. ORDEM RODENTIA

Os roedores são membros importantes de quase todas as faunas, sendo cosmopolitas e nativos na maioria das áreas terrestres, exceto em algumas ilhas árticas e oceânicas, Nova Zelândia e Antártica. Os representantes são, em sua maioria, de porte pequeno, compreendendo desde animais de poucos gramas até a grande capivara (Nowak, 1994). Esses animais apresentam corpo versátil e esguio, bem adaptado a diversos modos de vida e variados climas. A enorme variação na morfologia, na diversidade de habitats e climas e na alimentação, os tornou a mais numerosa e melhor sucedida evolutivamente entre as ordens de mamíferos (Emmons & Feer, 1997).

Os roedores são animais predominantemente herbívoros, porém apresentam uma grande variedade de hábitos alimentares, podendo ser insetívoros, piscívoros ou carnívoros. Esta versatilidade alimentar tem sido considerada um dos principais fatores no notável sucesso das radiações adaptativas obtidos por estes mamíferos (Landry, 1970).

Uma característica marcante dos roedores são os seus dentes. Esses animais possuem dois pares de dentes incisivos, um superior e um inferior, sendo o superior de crescimento ilimitado, que se sobrepõe ao par inferior. Não possuem outros incisivos ou caninos e apresentam poucos molares ou pré-molares, sendo os incisivos separados dos demais dentes por um espaço chamado de diastema (Nowak, 1994).

Inicialmente os roedores foram classificados em três grandes categorias subordinais: Sciuromorpha, Hystricomorpha e Myomorpha (Simpson, 1945; Anderson, 1967). Esta classificação levou em consideração as diferenças na estrutura craniana e nos padrões de especialização do músculo masseter e principalmente nas relações deste com o conduto infraorbitário. Entretanto, atualmente acredita–se que as especializações deste músculo possam ter surgido independentemente mais de uma vez, não sendo, desta forma, relevantes para a classificação dos roedores. A grande diversidade morfológica, o número considerável de ramos de descendência e a evolução paralela de caracteres muito similares em alguns grupos de roedores geraram controvérsias quanto à sua classificação taxonômica. A classificação mais atual baseia-se nas diferenças da musculatura craniana e formas da mandíbula e crânio, que agrupa os roedores em duas subordens: Hystricognathi e Sciurognathi (Woods, 1976; Hartenberger, 1981; Wood, 1955).

Muroidea Miller & Gidley, 1918, superfamília da subordem Sciurognathi, é um táxon monofilético de roedores composto por seis famílias: Calomyscidae, Cricetidae, Muridae,

Nesomyidae, Platacanthomyidae e Spalacidae (Musser & Carleton, 2005). Os cricetídeos são considerados a família mais diversificada do Brasil.

Diversos estudos morfológicos mostraram uma série de diferenças entre os Cricetideos Norte e Sul– Americanos, como por exemplo, diferenças na morfologia peniana, na anatomia das glândulas acessórias do sistema reprodutor masculino, na morfologia do estômago, na microestrutura do pelo e parasitologia (Reig, 1984). Baseado nestas divergências Reig (1981) propôs elevar as tribos Sigmodontini e Neotomini (Hershkovitz, 1966, 1969, 1972) à condição de subfamílias da Família Cricetidae.

Cricetidae apresenta seis subfamílias, dentre as quais, Sigmodontinae é a segunda maior em número de espécies e representantes na biodiversidade de roedores (Swier et al., 2009). A subfamília Sigmodontinae (Figura 1) tem distribuição geográfica restrita às Américas, com grande maioria na América do Sul e abriga atualmente 377 espécies em 74 gêneros e nove tribos: Abrotrichini, Akodontini, Ichthyomyini, Oryzomyini, Phyllotini, Reithrodontini, Sigmodontini, Thomasomyini e Wiedomyini (Reig, 1980; Smith & Patton, 1999; Musser & Carleton, 2005; D'Elía *et al.*, 2005).

Figura 1. Roedores pertencentes à Subfamília Sigmodontinae; (a) *Delomys collinus* (b) *Cerradomys subflavus* (c) *Euryoryzomys russatus* (d) *Hylaeamys sp.* (e) *Juliomys pictipes* (f) *Necromys lasiurus*. Fonte: Bonvincino et *al.*, 2008

A tribo Oryzomyini foi cladisticamente definida por Voss e Carleton (1993) quando estabelecem cinco sinapomorfias para a tribo: ausência de vesícula biliar, presença de um par peitoral de mamas, ausência de cobertura timpânica, ausência da barra do alisfenóide e presença de um palato longo. Esta tribo compreende cerca de 35% das espécies de Sigmodontinae.

Atualmente existe uma grande controvérsia quanto ao número e quais os gêneros pertencentes à tribo Oryzomyini. Dependendo do autor, o número de gêneros varia entre 11 e 27 e de espécies descritas para a tribo Oryzomyini varia de 90 a 120 (Reig, 1986; McKenna e Bell, 1997; Smith e Patton, 1999; Musser e Carleton, 2005; Weksler *et al.*, 2006). Esta discrepância no número de gêneros é devida, principalmente, à inclusão ou retirada de gêneros de tribos mais ou menos relacionadas, especialmente Thomazomyini, Sigmodontini e Phyllotini. Porém, alguns gêneros sempre fizeram parte da tribo Orizomyini, independente do autor. São eles: *Neacomys, Nectomys, Nesoryzomys, Oecomys, Oryzomys e Oligoryzomys*, este último não está nominado na classificação de Reig (1986) pelo fato de que na época ele era considerado subgênero de *Oryzomys* (Tabela 1).

Tabela 1. Cinco agrupamentos diferentes de gêneros propostos para a tribo Oryzomyini. Em negrito os gêneros comuns a todas as propostas

Reig (1986)	McKenna e Bell (1997)	Smith e Patton (1999)
Aepeomys	*Amphinectomys*	*Holochilus*
Chilomys	*Holochilus*	*Lundomys*
Delomys	*Melanomys*	*Melanomys*
Neacomys	*Lundomys*	*Microryzomys*
Nectomys	*Microakodontomys*	***Neacomys***
Nesoryzomys	*Microryzomys*	***Nectomys***
Oecomys	***Neacomys***	***Nesoryzomys***
Oryzomys	***Nectomys***	***Oecomys***
Phaenomys	***Nesoryzomys***	***Oligoryzomys***
Rhipidomys	***Oecomys***	***Oryzomys***
Scolomys	***Oligoryzomys***	*Pseudoryzomys*
Thomazomys	***Oryzomys***	*Sigmodontomys*
Wilfredomys	*Pseudoryzomys*	*Zygodontomys*
	Scolomys	
	Sigmodontomys	
	Zygodontomys	

Musser e Carleton (2005)	Mattevi e Andrades-Miranda (2006)
Amphinectomys	*Amphinectomys*
Handleyomys	*Handleyomys*
Holochilus	*Holochilus*
Lundomys	*Lundomys*
Melanomys	*Melanomys*
Microakodontomys	*Microryzomys*
Microryzomys	***Neacomys***
Neacomys	***Nectomys***
Nectomys	***Nesoryzomys***
Nesoryzomys	***Oecomys***
Oecomys	***Oligoryzomys***
Oligoryzomys	***Oryzomys***
Oryzomys	*Pseudoryzomys*
Pseudoryzomys	*Scolomys*
Sigmodontomys	*Sigmodontomys*
Zygodontomys	*Zygodontomys*

Atualmente a tribo Oryzomyini compreende 27 gêneros e cerca de 120 espécies que estão relacionadas na Figura 2, organizada segundo classificação básica de Voss e Carleton (1993) e Musser e Carleton (2005), incluindo propostas atuais que envolvem a descrição de novas espécies e, até mesmo, de novos gêneros. Weksler *et al*. (2006), através de estudos morfológicos e com marcadores moleculares reclassificaram espécies do polifilético gênero *Oryzomys* em dez novos gêneros: *Aegialomys*, *Cerradomys*, *Eremoryzomys*, *Euryoryzomys*, *Hylaeamys*, *Mindomys*, *Nephelomys*, *Oreoryzomys*, *Sooretamys* e *Transandinomys*. O gênero *Oryzomys* permaneceu com cinco espécies.

Ordem Rodentia
Subordem Sciurognathi
Família Cricetidae
Subfamília Sigmodontinae (80, 320)
Tribo Oryzomyini (27, 117)
Gênero *Amphinectomys* (1)
Gênero *Aegialomys* (2)
Gênero *Cerradomys* (4)
Gênero *Eremoryzomys* (1)
Gênero *Euryoryzomys* (6)
Gênero *Handleyomys* (8)
Gênero *Holochilus* (3)
Gênero *Hylaeamys* (7)
Gênero *Lundomys* (1)
Gênero *Melanomys* (3)
Gênero *Microryzomys* (2)
Gênero *Microakodontomys* (1)
Gênero *Mindomys* (1)
Gênero *Neacomys* (8)
Gênero *Nectomys* (5)
Gênero *Nephelomys* (7)
Gênero *Nesoryzomys* (4)
Gênero *Oecomys* (15)
Gênero *Oligoryzomys* (21)
Gênero *Oreoryzomys* (1)
Gênero *Oryzomys* (5)
Gênero *Pseudoryzomys* (1)
Gênero *Scolomys* (3)
Gênero *Sigmodontomys* (2)
Gênero *Sooretamys* (1)
Gênero *Transandinomys* (2)
Gênero *Zygodontomys* (2)

Figura 2. Classificação atual da Tribo Oryzomyini

Os orizomíneos habitam florestas, savanas, banhados, campos e ambientes semi-áridos, além de serem, na maioria das vezes, os mais abundantes pequenos mamíferos destes habitats. Muitos autores apontam os Oryzomyini como o grupo mais primitivo dentro dos Sigmodontinae baseado no estudo de caracteres dentários, cranianos, fálicos (Hershkovitz, 1972; Hooper e Musser, 1964; Reig, 1986; Voss e Linzey, 1981) e cromossômicos (Bianchi *et*

al., 1971; Gardner e Patton, 1976). Seus hábitos alimentares vão de onívoros a insetívoros e apresentam uma grande diversidade morfológica, variando no tamanho corporal, de pequenos com 10g até grandes com cerca de 300g. A maioria possui hábito escansorial, mas alguns podem desenvolver hábitos arbóreos ou até semi-aquáticos, constituindo assim um dos mais claramente definidos grupos multi-genéricos de muróides. A distribuição geográfica desta tribo é a mais ampla dentro dos Sigmodontinae, desde o extremo sul da América do Sul até o sudoeste dos Estados Unidos. Entre os 27 gêneros que a tribo Oryzomyini apresenta está o gênero *Oecomys* (Musser & Carleton, 2005).

1.2. GÊNERO *OECOMYS*

As espécies deste gênero (Figura 3) têm tamanho de pequeno a médio porte, sendo a cauda maior que o comprimento do corpo. O dorso varia de castanho-escuro a castanho-avermelhado. As laterais são mais claras que o dorso, com limite bem definido com o ventre. A pelagem do ventre pode ser completamente branca, creme com pêlos de base cinza, ou com este segundo padrão e manchas completamente brancas ou cremes na linha mediana do ventre. As patas são curtas, largas e claras e a cauda apresenta porção terminal pilosa, podendo ou não formar um pincel caudal (Reis, 2006).

Figura 3. Exemplar do gênero *Oecomys* (Bonvicino, 2008)

Este gênero apresenta roedores de hábitos noturnos e solitários. Comem frutas e sementes verdes e usa todos os níveis das florestas, inclusive o solo (Emmons e Feer, 1999).

De acordo com Musser & Carleton (2005) e Oliveira & Bonvicino (2006) o gênero *Oecomys* conta com dezesseis espécies atualmente reconhecidas como válidas: *Oecomys auyantepui* Tate, 1939, *O. bicolor* Thomas, 1860, *O. catherinae* Thomas 1909, *O. cleberi* Locks, 1981, *O. concolor* Wagner, 1845, *O. flavicans* Thomas, 1894, *O. mamorae* Thomas,

1906, *O. paricola* Thomas, 1904, *O. phaeotis* Thomas, 1910, *O. rex* Thomas, 1910, *O. roberti* Thomas, 1904, *O. rutilus* Anthony 1921, *O. speciosus* Allen & Chapman, 1893, *O. superans* Thomas, 1911, *O. sydandersoni* Carleton, 2009 e *O. trinitatis* Allen & Chapmam, 1893, distribuídas em áreas de floresta tropical e sub-tropical das Américas Central e do Sul, incluindo Costa Rica, Trinidad, Panamá, Venezuela, Colômbia, Equador, Peru, Bolívia, Guiana, Guiana Francesa, Suriname e Brasil (Hershkovitz, 1960). A Figura 4 mostra a distribuição das doze espécies encontradas no Brasil (Bonvicino *et al.*, 2008).

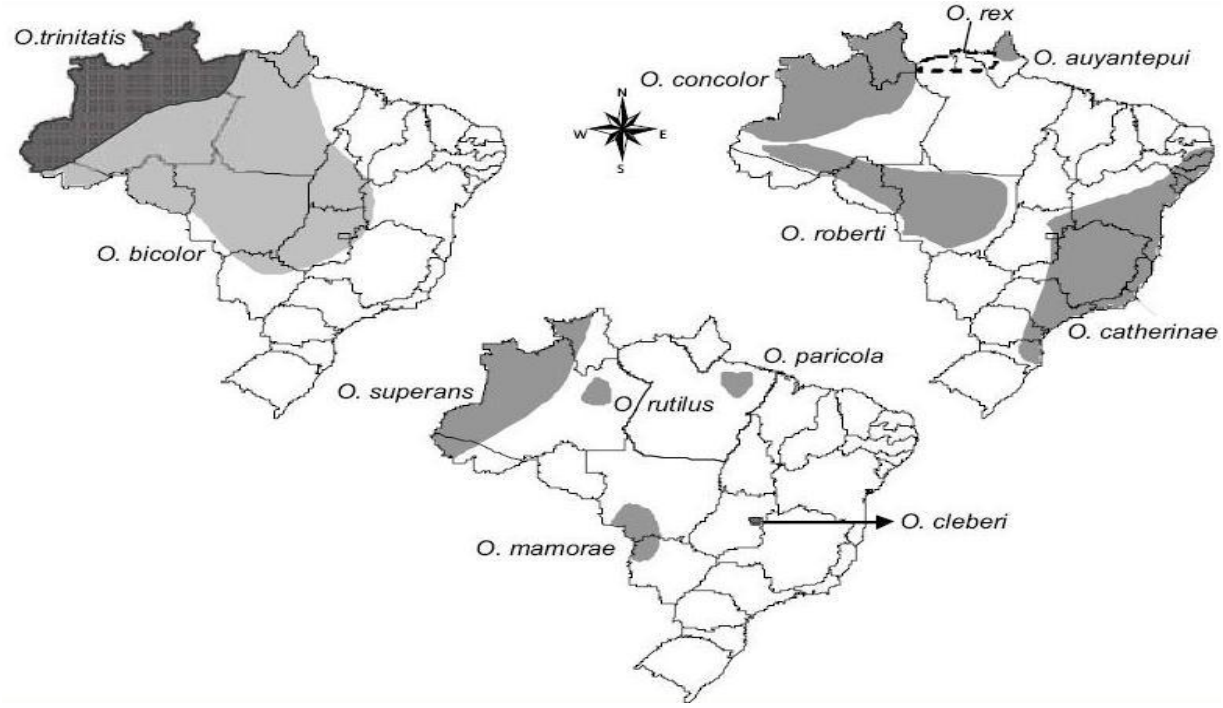

Figura 4. Distribuição das espécies de *Oecomys* no Brasil (Bonvincino *et al.*, 2008)

Para a Amazônia brasileira são conhecidas nove espécies de *Oecomys* assim distribuídas: *Oecomys auyantepui*, presente na Venezuela, Guianas e Brasil, no Estado do Amapá; *O. bicolor*, presente do Panamá à Colômbia, Equador, Peru, Bolívia, Venezuela, Guianas e no Brasil, nos Estados do Amapá, Roraima, Amazonas, Pará, Acre, Rondônia, Mato Grosso, Mato Grosso do Sul, Tocantins, Goiás, Bahia, Minas Gerais e no Distrito Federal; *O. concolor*, presente no Brasil, nos Estados do Amazonas e Roraima, e também na Venezuela, Colômbia e Bolívia; *O. paricola*, registrado apenas para o Estado do Pará, nas proximidades de Belém; *O. rex*, presente no norte dos estados do Amapá e Amazonas; *O. roberti*, presente no Brasil, no Estado do Mato Grosso, Amazonas e Rondônia, e na Bolívia, Peru, Venezuela e Guianas; *O. rutilans*, presente na Venezuela, Guianas e no Brasil, no Estado do Amazonas; *O.*

superans, com ocorrência na Colômbia, Equador, Peru e Brasil, nos Estado do Acre, Amazonas e Roraima e *O. trinitatis*, estendendo-se da Costa Rica até o Brasil, incluindo Guianas, Trinidad e Tobago, Colômbia até o Perú, e no Brasil os Estados do Acre, Amazonas, Roraima e Pará (Bonvicino *et al.*, 2008).

1.2.1. História taxonômica do gênero *Oecomys*

O gênero *Oecomys* foi primeiramente incluído no gênero *Rhipidomys* e posteriormente considerado um subgênero de *Oryzomys* (Musser & Carleton, 1993). Porém, por ser fortemente diferenciado sob o ponto de vista cariotípico, o subgênero *Oecomys* foi elevado ao nível de gênero (Gardner e Patton, 1976). Entretanto, alegando similaridades entre as espécies de *Oecomys* e *Oryzomys*, Ellerman (1941) questionou a validade deste táxon, até mesmo como subgênero.

Hershkovitz (1960) realizou a única revisão taxonômica ampla para o gênero *Oecomys*, o considerando subgênero de *Oryzomys*. Este autor, tomando por base as características como tamanho do corpo, proporção do tamanho do pé e da cauda em relação ao do corpo e grau de desenvolvimento da crista temporal, alocou as 25 espécies reconhecidas na época para apenas duas: *O. bicolor* e *O. concolor*. Somente a partir do trabalho de Musser & Carleton (1993) tornou-se consenso o status genérico de *Oecomys*.

1.2.2. Diversidade Cromossômica em *Oecomys*

Os roedores, devido às características populacionais que apresentam, desenvolveram-se como o grupo mais especioso de mamíferos em florestas neotropicais e um dos mais interessantes para estudos da variabilidade genética e de evolução entre os vertebrados. De acordo com a literatura, o gênero *Oecomys* apresenta uma grande diversidade cariotípica, com o número diplóide variando entre 58 e 86, sugerindo um alto grau de rearranjo cromossômico. O primeiro estudo citogenético nesse gênero foi realizado por Gardner & Patton (1976), identificando três populações dentro de *Oecomys*, sendo uma relacionada a *Oecomys bicolor* (2n=80, NF=134-136) e duas relacionadas a *Oecomys concolor* (2n=80, NF=108; 2n=60, NF=62). Dentro do gênero *Oecomys* apenas oito espécies contam com o cariótipo descrito, sendo dois destes citótipos descritos para espécimes sem classificação final em nível de espécie. A análise citogenética comparativa entre espécies distintas permite detectar diferenças cromossômicas e entender os mecanismos de rearranjos cromossômicos que atuaram na diversificação das espécies. Além disso, constitui uma ferramenta bastante útil para auxiliar na

identificação taxonômica e na compreensão das relações filogenéticas entre os diferentes grupos.

3. OBJETIVOS

3.1. Geral:

O presente trabalho tem como objetivo geral estudar os cariótipos de espécies *Oecomys paricola* de duas localidades, visando diagnosticar quais os possíveis rearranjos cromossômicos envolvidos na evolução cariotípica das espécies deste gênero e auxiliar na elucidação de possíveis problemas taxonômicos.

3.2. Específicos:

Os objetivos específicos são:

a) Caracterizar os cariótipos (2n e NF) de roedores da espécie *Oecomys paricola*, organizando os cromossomos de acordo com a classificação morfológica e em ordem decrescente de tamanho;

b) Identificar homologias cromossômicas por bandeamento G;

c) Analisar a distribuição da heterocromatina constitutiva por bandeamento C;

d) Identificar os sítios de rDNA ativos por meio de impregnação por Nitrato de Prata (marcação Ag-NO_3);

e) Fornecer dados citogenéticos para auxiliar na identificação das espécies coletadas através do número diplóide (2n) e número fundamental (NF), bem como comparar os resultados obtidos com os já descritos na literatura;

f) Comparar dados citogenéticos com os resultados moleculares e morfológicos da espécie *O. paricola*.

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4. MANUSCRITO DO ARTIGO CIENTÍFICO

Genetic and morphological variability in *Oecomys paricola* (Sigmodontinae, Rodentia): evidences for a complex of species.

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Running title: Evidence for cryptic species in *Oecomys paricola*.

ABSTRACT

The rodent genus *Oecomys* (Sigmodontinae) comprises approximately 16 species that inhabit tropical and subtropical forests in Central and South America. In this study specimens of *Oecomys paricola* Thomas, 1904 from Belém and Marajó Island, northern Brazil, were investigated using cytogenetic, molecular and morphological analyses. Three karyotypes were found, two from Belém (2n=68, FN=72 and 2n=70, FN=74) and a third from Marajó Island (2n=70, FN=74). No molecular or morphological differences were found between the individuals with differing cytotypes from Belém, but differences were evident between the individuals from Belém and Marajó Island. *O. paricola* is a complex of species. Specimens from the Belém City region may represent two cryptic species because two different karyotypes are present in the absence of significant differences in morphology and molecular characteristics. The Marajó Island and Belém populations represent distinct species that have been separated for some time, and are in the process of morphological and molecular differentiation as a consequence of reproductive isolation at the geographic and chromosomal levels.

Key words: Cytogenetic, Molecular, *Oecomys*, Rodentia, Cryptic species.

INTRODUCTION

Rodents are important members of most faunal communities as they are cosmopolitan and native to most terrestrial areas. Their wide variation in morphology, diversity of habitats, climatic tolerance and food sources make them the most numerous and evolutionarily successful among the orders of mammals (Emmons and Feer, 1997).

The subfamily Sigmodontinae, which includes most of the species of South American rodents, occurs only in the Americas. It includes 386 species in 81 genera and nine tribes: Abrotrichini, Akodontini, Ichthyomyini, Oryzomyini, Phyllotini, Reithrodontini, Sigmodontini, Thomasomyini and Wiedomyini (Reig, 1980, 1984; Smith and Patton, 1999; Musser and Carleton, 2005; Weksler *et al*., 2006; D'Elía *et al*., 2007). The tribe Oryzomyini comprises 28 genera (Weksler *et al*., 2006) and includes the genus *Oecomys*, which was first described by Thomas as a subgenus of *Oryzomys* (1906), and later accepted as a full genus (Thomas, 1909). In the only taxonomic review of *Oecomys*, Hershkovitz (1960) followed Thomas (1906) in considering the group as a subgenus of *Oryzomys*, but others disagreed with this taxonomic arrangement. Only after the study of Musser and Carleton (1993) the generic status of the genus *Oecomys* was largely accepted. Recent phylogenetic studies including species of *Oecomys* have confirmed the monophyly of this genus (Patton and Da Silva, 1995; Smith and Patton, 1999; Patton *et al*., 2000; Andrade and Bonvicino, 2003; Weksler, 2003, 2006), which now includes 16 valid species (Musser and Carleton, 2005; Carleton *et al*., 2009).

The genus *Oecomys* occurs in tropical and subtropical rainforests in Central and South America. In the Brazilian Amazon region there are nine species, of which only five have had their karyotype described (Andrade and Bonvicino, 2003; Langguth *et al*., 2005). Cytogenetic studies of *Oecomys* show that the diploid number ranges from 58 to 86 (Table 1), suggesting a high level of chromosomal rearrangement among the karyotypes of these species (Gardner and Patton, 1976; Patton *et al*., 2000). This karyotypic variability demonstrates the importance of chromosomal studies in the taxonomic identification of the species (Langguth *et al*., 2005).

Oecomys paricola Thomas, 1904 is an Amazonic species with an incompletely defined distribution south of the Amazon River and east of the Tocantins River (Voss *et al*., 2001; Musser and Carleton, 2005). No karyotype information has been reported for the species. According to Voss *et al*. (2001), *O*. *paricola* has predominantly gray-based ventral fur, except for the chin and throat, which are usually self-colored (all pale), sometimes with a ventral selfcolored midline extending caudally to the groin. The tail is uniformly dark, with a terminal

tuft of hairs 6–10 mm long. Cranially, *O. paricola* has a primitive pattern of carotid arterial supply, separated accessory oval and buccinator−masticatory foramina; the subsquamosal fenestra are absent.

The identification of rodent species using morphological traits is very complex (Patton and Gardner, 1972; Gardner and Emmons, 1984), leading to uncertainties in the taxonomy of many genera of this group. Studies combining morphological, molecular and karyotype analyses have the potential to demonstrate the occurrence of a greater diversity of *Oecomys* species, resulting in the identification of new species, and revalidation of previously described species (Patton and Da Silva, 1995; Smith and Patton, 1999; Andrade and Bonvicino, 2003; Weksler, 2006). Molecular studies using the cytochrome b gene as a marker in phylogenetic studies of Sigmodontinae rodents are particularly relevant (Smith and Patton, 1991, 1993, 1999; Patton *et al*., 2000; Andrade and Bonvincino, 2003; D'Elia, 2003; Miranda *et al*., 2007; D'Elia *et el*., 2008; Catzeflis and Tilak, 2009). This gene has been reported to have arrangements consistent with the species boundaries based on classic taxonomic studies (Johns and Avise, 1998; Avise and Walker, 1999), making it highly appropriate for biodiversity studies (Bradley and Baker, 2001).

In this study we undertook chromosomal, molecular and morphological analyses of *O. paricola* from the Belém City region and Marajó Island, in Pará State, Brazil. This is the first description of the karyotype of the species, and provides new information on molecular and morphologic variation in the species.

MATERIAL AND METHODS

Karyotype analysis

We karyotyped 6 specimens of *Oecomys paricola* (Table 2), of which 4 were collected in the Environmental Park in Belém City (01°27′ S, 48°29′ W) and 2 (males) were collected at Marajó Island (01°00′S, 49°30′W), in the region of the Amazon River mouth (Figure 1). The specimens were collected using pitfall traps (buried 60 l buckets) connected by plastic tapes, and with conventional traps including the Sherman trap and cages on the ground containing baits of peanuts, flour and canned fish. Samples for metaphase chromosome analysis were taken in the field using the bone marrow technique (Ford and Hamerton, 1956). We also made laboratory culture of fibroblasts.

The chromosome analysis involved conventional staining, G- and C-banding, and Ag-

NOR staining, according to Seabright (1971), Sumner (1972), and Howell and Black (1980), respectively. The karyotypes were organized by size and morphology of the chromosome pairs.

Molecular analysis

We extracted 738 bp sequences of the cytochrome b gene from 17 ethanol-preserved samples of muscle tissue of *O. paricola*, including 4 of the 6 specimens we used for karyotype analysis (Table 2). We also used nine *Oecomys* sequences available in GenBank (several species, Table 3), two sequences of *O. auyantepui* and *O. rutilus* (CN120 and CN123, respectively) obtained in our laboratory, and one GenBank sequence of *Hylaeamys megacephalus* (AY275124), as the outgroup in the phylogenetic analysis.

DNA extraction involved the phenol–chloroform and proteinase K−RNase protocol (Sambrook *et al*., 1989). Cytochrome b fragments were isolated and amplified using the polymerase chain reaction (PCR) with primers MVZ05 and MVZ16 (Smith and Patton, 1993). The amplification protocol consisted of initial denaturation at 94°C for 3 min, 35 denaturation cycles of 30 s at 94°C, 1 min of annealing at 45°C, 2 min of extension at 72°C, and a final extension at 72°C for 7 min.

Sequences were edited and aligned in BioEdit 7.0.5.2 (Hall, 1999) using ClustalW (Larkin *et al*., 2007), following the parameters proposed by Schneider (2006). We verified sequence saturation using DAMBE 5.2.34 software (Xia and Xie, 2001), and undertook phylogenetic analyses. Using MrModeltest 2.3 (Nylander, 2004) in PAUP* 4.0 we found the best evolutionary GTR+I+G model fit to our sequences with a substitution rate of 6, a gamma distribution parameter of 0.5449, and an invariable sites proportion of 0.4145. Maximum likelihood (ML) was estimated from the above model using the online PhyML (Guindon and Gascuel, 2003) site to construct a BioNJ initial tree. Maximum parsimony (MP) was determined using PAUP* 4.0 with a heuristic search; the starting tree was obtained by stepwise addition and branch-swapping using a tree–bisection–reconnection (TBR) algorithm. Clade support was performed for both analyses using bootstrap with 1000 replicates. Estimates of evolutionary divergence of sequence pairs between and within *Oecomys* species and populations were conducted using the GTR model in DAMBE 5.2.34 (Xia and Xie, 2001). All codon positions were included.

Morphological and morphometric analyses

We examined the morphological characteristics of 35 specimens of *O. paricola* from Pará State, held by the Museu Paraense Emílio Goeldi (MPEG); these included skulls, dried skins and preserved fluids (Table 2). Specimens temporarily housed in the Universidade

Federal do Pará (UFPA), identified in this study by the abbreviation BAR (Barcarena Project), will be deposited in the MPEG. All six specimens for which we obtained karyotypes were included in the morphological and morphometric analyses (Table 2). For comparison, we also examined the following 15 MPEG specimens of *O. auyantepui*: 7169, 13132, 39793, 39794, 39798, 39804, 39816, 39817, 39825, 39831, 39836, 40076, 40078, 40084 and 40085.

To enable more accurate morphological and morphometric comparisons the specimens were classified into three age classes based on the eruption pattern and the differential wear of the occlusal surface of the superior molars. These classes, modified from Voss (1991), and Brandt and Pessôa (1994), were: (i) *Age class 1* (young)—M3 incompletely erupted or unworn, (ii) *Age class 2* (adult)—occlusal surface exhibiting slight to moderate wear, but still tubercular; mesoflexus and paraflexus of M1 and M2 sometimes as enamel islands; all M3 flexus, except paraflexus, obliterated and sometimes as enamel islands, (iii) *Age class 3* (old adult)—occlusal surface flat or concave; only paraflexus, metaflexus, protoflexus and hypoflexus are present in M1 and M2; others, when present, are just enamel islands; paraflexus of M3 always as an enamel island, or totally absent.

We evaluated morphological characteristics of the skin and skull of two populations of *O. paricola* (one from the Belém region and the other from Marajó Island) for use in separating groups of individuals. Comparisons among specimens were made with respect to sex and age classes. To avoid misinterpreting ontogenetic variation as taxonomic variation we did not restrict comparisons to the specimens for which we have karyotypes, as they belonged to different age classes. The anatomical nomenclature used followed Pocock (1914), Hershkovitz (1962, 1977), Carleton and Musser (1984, 1989) and Voss (1988) for external morphology, and McDowell (1958), Hershkovitz (1962), Wahlert (1974), Carleton and Musser (1984, 1989), Voss (1988), Steppan (1995) and Weksler (2006) with respect to cranial morphology.

The following external measurements were obtained directly from the specimen labels, and were used for descriptive statistics only: head and body length (HBL), tail length (TL), foot length (FL), ear length (EL), weight (W). We also obtained 10 craniodental measurements (to the nearest 0.01 mm) from each of the 32 skull specimens using a digital caliper during skull examination with a stereomicroscope. These included: (i) *Condyloincisive Length (CIL)*—from the greater curvature of one upper incisor to the articular surface of the condyle on the same side, (ii) *Length of Diastema (LD))*—from the crown of the first cheek tooth to the lesser curvature of the incisor on the same side, (iii) *Length of Molars (LM))*—the crown length from M1 to M3, (iv) *Breadth of M1 (BM1))*—the greatest crown breadth of

the first upper molar (M1), (v) *Length of Incisive Foramen (LIF)* —the greatest anterior posterior dimension of one incisive foramen, (vi) *Breadth of Incisive Foramen (BIF)*—the greatest transverse dimension across both incisive foramina, (vii) *Breadth of Palatal Bridge (BPB)*—measured between the protocones of the right and left M1, (viii) *Breadth of Zygomatic Plate (BZP)*—the least distance between the anterior and posterior edges of the zygomatic plate, (ix) *Least Interorbital Breadth (LIB)*—the least distance across the frontal bones between the orbital fossa, and (x) *Zygomatic Breadth (ZB)*—the greatest transverse dimension across the squamosal zygomatic processes.

We evaluated the presence of sexual dimorphism in our largest sample, from the Belém region (6 females and 7 males), using Hotelling's T-square test. We also used this test to evaluate morphometric differences between specimens from the Belém region and Marajó Island. The morphometric analyses were conducted on specimens of age class 2 only using PAST 2.02 (Hammer *et al*., 2001), as this class contained the greatest number of samples, and because skull size appeared to vary greatly among different age classes.

RESULTS

Karyotype analysis

(a) Environment Park, Belém

The specimens MPEG 39703 (male) and MPEG 39699 (female) had the karyotype 2n=68, FN=72, comprising 30 one-armed pairs and 3 bi-armed pairs. The X chromosome was large and bi-armed, and the Y chromosome was small and submetacentric. Figure 2 shows the G-banded karyotype of this cytotype. Constitutive heterochromatin was found at the centromeric region of all chromosomes. The short arm of the X chromosome was almost all heterochromatic, and almost all the Y chromosome was heterochromatic (Figure 2). The NOR was found on the small short arm of 3 one-armed pairs (one mid-sized and two small; Figure 3).

Specimens MPEG 39701 (male) and MPEG 39705 (female) had the karyotype 2n=70, FN=76, comprising 30 one-armed chromosome pairs and 4 bi-armed pairs. The X chromosome was large and bi-armed and the Y chromosome was small and submetacentric (Figure 4). Constitutive heterochromatin was found in the centromeric region of all chromosomes. The short arm of the X chromosome was mostly heterochromatic, and almost the entire Y chromosome was heterochromatic (Figure 4). The NOR was found on the small short arm of 2 one-armed pairs (one mid-sized and one small; Figure 5).

(b) Amazon River mouth region

The specimens MPEG 40846 and 40851 (both males), which were collected from Marajó Island, had a karyotype of 2n=70, FN=74, comprising 31 one-armed chromosome pairs and 2 bi-armed pairs. The X chromosome was large and bi-armed, and the Y chromosome was mid-sized and submetacentric (Figure 6). Constitutive heterochromatin was found at the centromeric region of all chromosomes. The X chromosome had a heterochromatic block at the distal region of the short arm, and the Y chromosome was almost entirely heterochromatic (Figure 6). The NOR was found on the proximal long arm of 2 pairs of mid-sized acrocentric autosomes (Figure 7).

Molecular analysis

All phylogenetic analyses based on the Cytochrome b gene (ML and MP; Figure 11) indicated the same topologies for the *O. paricola* populations. In both analyses the specimens from Marajó Island grouped together in a clade that was distinct from those collected in Belém, and there was high nodal support (96% for MP and 87% for ML for the Belém clade; 99% for MP and 97% for ML for the Marajó Island clade). Nodal support for the monophyletic clade that corresponded to *O. paricola* was 100% for the MP analysis and 99% for the ML analysis. Relationships among *Oecomys* species were not supported by bootstrap statistics.

The intrapopulation genetic divergences were 0.8% for the Belém clade and 0.7% for the Marajó Island clade, while the divergence between the populations was 4.3%. The intrageneric divergences ranged from 8.2% to 17.6%, with divergences between *O. paricola* and other congeneric species varying from 10.6% to 15.4% (Table 4).

Morphological and morphometric analyses

We found no morphological characters that could be used to discriminate the two Belém cytotypes, other than the smaller size of the specimens of karyotype 2n=68, NF=72 relative to those of karyotype 2n=70, NF=76 (Table 5). However, the size difference may be explained by ontogenetic variation, as the former cytotype was based on young specimens from age class 1, while the latter was based on adult and older specimens belonging to age classes 2 and 3.

In contrast, the specimens from Belém had several characters distinguishing them from the Marajó Island specimens. With respect to external features, the specimens from Marajó Island were very similar to those from Belém. However, in the former the color of the ventral pelage was gray with a broad midline of cream or white hairs, but was completely gray in the latter. In both populations the chin and throat were always self-colored (cream or

white). Feet coloration and the plantar surface were also different in the two populations. In the Marajó Island specimens the feet were dark brown with a darker spot on the dorsum, while in the Belém specimens the feet were cream or light brown with a brown spot on the dorsum. The plantar surface was smooth or had several squamae near the plantar pads in specimens from Marajó Island, but was always smooth in Belém specimens. We also observed that the tail length was approximately 116% of the head and body length in Marajó Island specimens, whereas it was only about 106% in the Belém specimens (Table 5).

The skulls were also notably different between the two populations. In the Marajó Island population the nasal bone was short and did not surpass the lachrymal–frontal maxillary suture, whereas in specimens from Belém this bone was long, and aligned with or surpassing the lachrymal–frontal–maxillary suture. In the specimens from Marajó Island the supra-orbital ridges were well developed, projecting dorsally from the border of the frontal bone at the orbital fossa and extending slightly onto the parietal bone, whereas in the specimens from Belém they were poorly developed and restricted to the frontal bone. The frequency of subsquamosal fenestra, which was used by Voss *et al*. (2001) to distinguish *O. auyantepui* from *O. paricola*, was highly variable in the specimens from Marajó Island. Among the 16 specimens from Belém it was bilateral in 15 specimens and unilateral in one, whereas in specimens from Marajó Island it was bilaterally absent in four specimens and bilaterally present in other four. The mandible also differed between the two populations with respect to the capsular process of the lower incisor alveoli, which varied from slightly to moderately curved in the specimens from Marajó Island, but was slightly curved in the specimens from Belém.

Hotelling's T-square test for sexual dimorphism showed that there was no statistically significant difference between the sexes (p = 0.4684) in the Belém population. The same test was applied to evaluate morphometric differences between the Belém and Marajó Island specimens. This showed that there were no statistically significant differences between the specimens from each location (p = 0.2825), although the mean values for almost all external measurements and all skull dimensions of the Marajó Island specimens examined were greater (Table 5).

DISCUSSION

We have provided here the first description of the karyotype of *O. paricola*. We found three cytotypes, two in the specimens from the Belém City region (2n=68, FN=72 and 2n=70, FN=76) and a third from the Marajo Island (2n=70, NF=74). The karyotypes with 2n=68 and

2n=70 are new for the genus *Oecomys*. The differences in the diploid number and chromosome morphology of the three karyotypes of *O. paricola* can be explained by rearrangements including fusion/fission and pericentric inversions. Because of the number of chromosomes and differences on their condensation, comparative analyses of the G-banding patterns was not possible, and consequently we were not able to precisely identify the chromosomes involved in the karyotype differentiation, or the chromosome rearrangements that occurred. No hybrid karyotypes were found in the Belém sample, indicating that there has been no gene flow between the karyotypes.

Bradley and Baker (2006) assessed whether the degree of cytochrome b sequence divergence in mammals can be used for species-level differentiation within the genetic species concept. They observed that sister species recognized on the basis of morphology often have cytochrome b distance values > 5%. With respect to rodents they found that intrapopulation divergence values ranged from 0.0 to 1.4%, intraspecific divergence values ranged from 0.0 to 4.7%, and intrageneric divergence values ranged from 1.3 to 16.9%. In molecular studies of sigmodontine rodents not considered by Bradley and Baker (2006) values have been reported that range from 0 to 3.87% for intrapopulation divergence, 0 to 11.37% for intraspecific divergence, and 1.23 to 21% for intrageneric divergence (Smith and Patton, 1991, 1993, 1999; Patton *et al*., 2000; D'Elia, 2003; Miranda *et al*., 2007; D'Elia *et al*., 2008; Catzeflis and Tilak, 2009). For *Oecomys*, no intrapopulation divergence values have been reported, but intraspecific divergence ranges from 0 to 10.3%, and intrageneric divergence ranges from 7 to 12% (Smith and Patton, 1999; Patton *et al*., 2000; Andrade and Bonvincino, 2003).

The intrapopulation divergences of *O. paricola* from Marajó Island (0.7%) and the Belém region (0.8%) are in agreement with those reported by Bradley and Baker (2006) for rodents in general, and for sigmodontine species (citations above). Similarly, the intraspecific evolutionary divergence we found (4.3% in *O. paricola*) was relatively high, close to the 5% limit of morphologically diagnosable species of mammals stated by Bradley and Baker (2006). The intrageneric divergences (8.5 to 16.7%) we found are greater than those previously reported for *Oecomys*, but are comparable to those of other sigmodontine rodents.

Direct morphological comparisons among the specimens for which we had karyotype data were not possible, as one of the Belém cytotypes was represented by young individuals. However, our morphological analyses, which included several specimens from the Belém region, showed no significant differences consistent with the presence of more than one taxonomic unit. Similarly, our molecular analyses showed low genetic divergence (0.8%) and

no genetic structure within this population, even between specimens representing two of the three cytotypes described (Fig. 11).

In contrast, we found consistent morphological differences between the populations from the Belém region and Marajó Island. These differences, allied to the relatively high genetic divergence between the populations (4.3%), the genetic structure indicated by the molecular analyses (Fig. 11), and the unique karyotype of the specimens from Marajó Island (Figs 8–10) suggest that these two populations are distinct species. Morphologically, the specimens from Marajó Island are similar to specimens of *O. auyantepui*, with which they share the same pattern of ventral pelage color, length of the nasal bones, and development and extension of the supraorbital ridges, despite the genetic divergence of 12.9% between these species. It is possible that the two cytotypes from Belém represent cryptic species, whereby recent speciation mediated by chromosome rearrangements has occurred, but there has been insufficient time for the fixation of molecular and morphological differences.

This shows that chromosome rearrangements can have a very important role as a postmating reproductive isolation mechanism. According to King (1987, 1993), chromosome rearrangement can be the main cause of speciation when they cause a negative heterosis effect, by reducing the fertility of heterozygous bearers. This is a postmating reproductive isolation (rearrangements that segregate badly in meiosis in structural hybrids, producing unbalanced gametes and a consequent reduction in fertility). There is some evidence of a correlation between population structure and karyotypic variability, with greater variability occurring in species that have small inbreeding populations, such as occurs in rodents (Bush *et al*., 1977; Bengtsson, 1980; Maruyama and Imai, 1981), relative to species with outbreeding populations, such as cats and whales (Bush *et al*., 1977). Population structure has a crucial role in fixing rearrangements, as this only happens if the rearranged chromosome can rapidly become homozygous. In small inbreeding populations the probability of this homozygous condition arising is greater, making the rearranged chromosome more common. A similar situation was reported by Granjon and Dobigny (2003), who undertook a morphological and cytogenetic study of rodents from the Lake Chad region, Africa. They demonstrated that a significant portion of the species are cryptic (morphologically indistinguishable), and can only be distinguished by karyotype analysis. This situation occurs in many vertebrates groups in the Neotropical region. For instance, Milhomem *et al*. (2008) used classical cytogenetics to describe 2 cryptic species in the species complex of *Gymnotus carapo* (a Neotropical electric fish); the species are morphologically identical, and have diverged only in the karyotype (2n=40 and 2n=42), apparently by a fusion and some

inversions. Nagamachi *et al*. (2010) used chromosome painting to demonstrate that the level of chromosome reorganization is greater than previously thought, confirming that *Gymnotus carapo* with different karyotypes are truly different species.

The chromosome differentiation between the Marajó Island and Belém cytotypes must have occurred some time ago, because our results indicate differences at the molecular and morphologic levels, which must have occurred since the interruption of gene flow. A similar situation has been reported in the genus *Akodon* (Geise *et al*., 2005), where *A. montensis*, *A. cursor* and *A.* aff *cursor* form a species complex with other undescribed species (Silva and Yonenaga-Yassuda, 1998). These species are so similar that they cannot be distinguished at the morphological level (Christoff *et al.*, 2000). However, mitochondrial (Geise *et al*., 2001) and karyological data (Geise *et al*., 1998; Silva and Yonenaga-Yassuda, 1998) show that they are distinct species.

The occurrence of morphologically identical taxa with different diploid and fundamental numbers is common among rodents. In *Oecomys*, for instance, Gardner and Patton (1976) described the karyotype of *O. bicolor* as 2n=80 and FN=134−136. Patton *et al*. (2000) subsequently found another karyotype in *O. bicolor*; this had the same diploid number, but had three bi-armed chromosomes instead of the three one-armed chromosomes in the initial karyotype, (2n=80, FN=140; Table 1). The phylogenies described by Smith and Patton (1999), and Andrade and Bonvicino (2003) suggest that *O. bicolor* is a species complex. Two karyotypes have also been described for *O. roberti*, one (2n=80, FN=114) having 21 one-armed chromosome pairs and 18 bi-armed pairs (Patton *et al*., 2000), and the other (2n=82, FN=106) having 27 one-armed chromosome pairs and 13 bi-armed pairs (Langguth *et al*., 2005).

We conclude that *O. paricola* from Belém may represent two cryptic species because two different karyotypes are present in the population in the absence of significant differences in morphology and molecular characteristics. No hybrid karyotypes were found, which indicates the absence of gene flow among the cryptic species. Additionally, the Marajó Island and Belém populations represent distinct species that have been separated for some time and are undergoing morphological and molecular differentiation following reproductive isolation at the geographical and chromosomal levels. This is possibly an example of speciation where the morphological and molecular differences are accumulating following reproductive isolation.

As currently defined, *O. paricola* represents a complex of species. Specimens from the

Belém City region appear to be an example of cryptic species with no significant differences in morphology and molecular characters, but with different karyotypes. The populations of Marajó Island and Belém are distinct species that have been separated for some time and are in the process of morphological and molecular differentiation following reproductive isolation at the geographic and chromosomal levels.

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Table 1. Chromosomal characterization of the genus *Oecomys*. 2n=diploid number. FN=Fundamental number.

Species	**2n**	**FN**	**Reference**
Oecomys sp.	86	98	Gardner and Patton, 1976
Oecomys bicolor	80	134-136	Gardner and Patton 1976
Oecomys bicolor	80	140	Patton et *al.*, 2000
Oecomys roberti	80	114	Patton et *al.*, 2000
Oecomys roberti	82	106	Langguth et *al.*,2005
Oecomys superans	80	108	Gardner and Patton, 1976
Oecomys sp.	72	90	Andrade and Bonvincino, 2003
Oecomys paricola	70	76	Present work
Oecomys paricola	68	72	Present work
Oecomys concolor	60	62	Patton et *al.*,2000
Oecomys bahiensis	60	62	Langguth et *al.*,2005
Oecomys trinitatis	58	96	Patton et *al.*, 2000

Table 2 – Specimens analyzed, with localities, specimen preparation type, collection and genbank number. **Kar** - Karyotype; **MPEG** – Museu Paraense Emilio Goeldi; **BAR** – Specimens from Universidade Federal do Pará (Barcarena Project).

Locality	Material					Collection Number	Genbank Number
	Skin	Skull	Fluid	Cyt-b	Kar		
Barcarena (01º30'S 48º40'W)	X	X				BAR006	
	X	X		X		BAR013	
	X	X		X		BAR017	
	X	X		X		BAR023	
	X	X		X		BAR029	
Belém, Parque Ambiental de Belém (Utinga) (01º27'S 48º29'W)	X	X				MPEG2477	
	X	X				MPEG2584	
	X	X				MPEG2593	
	X	X				MPEG2605	
	X					MPEG2610	
	X	X				MPEG2614	
	X	X				MPEG2615	
	X					MPEG2632	
	X					MPEG2635	
	X	X				MPEG2647	
	X	X		X		MPEG38659	
	X	X		X		MPEG38664	
	X	X		X	X	MPEG39699	
	X	X		X	X	MPEG39701	
	X	X			X	MPEG39703	
	X	X		X	X	MPEG39705	
	X	X		X		MPEG39708	
	X	X				MPEG39709	
		X				MPEG40732	
BR-010, km 87-97 (01º40'S 47º47'W)	X	X				MG8382	
	X	X				MG8438	
Marajó Island, Chaves, Fazenda Tauarí (00º39'S 50º11'W)	X	X		X		MPEG40842	
	X	X		X		MPEG40843	
	X	X		X		MPEG40844	
	X	X		X		MPEG40845	
	X	X			X	MPEG40846	
	X	X		X		MPEG40848	
	X	X		X		MPEG40849	
		X	X			MPEG40850	
	X	X		X	X	MPEG40851	

Table 3 – GenBank sequences used in our phylogenetic analysis.

Species	GenBank Entry	Locality
Oecomys bicolor	AF108699	Peru[1] (Smith & Patton 1999)
Oecomys bicolor	OBU58382	Brazil, Acre, Sobral, left bank Rio Juruá. 08º22'S 72º49'W (Patton & Da Silva 1995; Patton *et al.* 2000)
Oecomys roberti	ORU58384	Brazil, Amazonas, Penedo, right bank Juruá. 06º50'S 70º45'W (Patton & Da Silva 1995; Patton *et al.* 2000)
Oecomys sp.	AY072772	Brazil, Mato Grosso do Sul, Corumbá. 19º00'S 57º36'W (Andrade & Bonvincino 2003)
Oecomys sp.	OSU58388	Brazil, Amazonas, Lago Vai-Quem-Quer, right bank Rio Juruá. 03º19'S 66º01'W (Patton & Da Silva 1995; Patton *et al.* 2000)
Oecomys superans	OSU58385	Brazil, Amazonas, Penedo, right bank Juruá. 06º50'S 70º45'W (Patton & Da Silva 1995; Patton *et al.* 2000)
Oecomys trinitatis	OTU58390	Brazil, Acre, Opposite Igarapé Porongaba, left bank Rio Juruá. 08º40'S 72º47'W (Patton & Da Silva 1995; Patton *et al.* 2000)
Hylaeamys megacephalus	AY275124	Not specified (D'Elia, 2003)

[1]There are two localities in Peru associated to two specimens of *Oecomys bicolor* in Smith & Patton (1999), but it is not clear from which one is the sequence available on GenBank.

Table 4 – Estimates of evolutionary divergences over sequence pairs within and between *Oecomys* species based on 738 base pairs of cytochrome-b gene. For *Oecomys paricola*, divergences within and between populations are shown. Bold numbers represents the evolutionary divergences within species or populations calculated using the Kimura-2 parameter method in MEGA4 (Kimura, 1980; Tamura et al., 2007). The rate variation among sites was modeled with a gamma distribution (shape parameter = 0.5449). The presence of n/c in the results denotes cases in which it was not possible to estimate evolutionary distances. All estimates are shown in percentage.

	1	2	3	4	5	6	7	8	9	10	11
1. *Oecomys paricola* (Belém region)	**0.8**										
2. Oecomys paricola (Marajó Island)	4.3	**0.7**									
3. Oecomys auyantepui	11.8	12.9	**n/c**								
4. Oecomys rutilus	15.4	16.7	14.3	**n/c**							
5. Oecomys bicolor	12.6	13.7	13.7	13.8	**6.4**						
6. Oecomys roberti	11.8	13.9	13.4	16.6	9.7	**n/c**					
7. *Oecomys* sp. (Corumbá.MT)	13.5	13.9	13.4	14.4	9.5	11.7	**n/c**				
8. *Oecomys* sp. (Juruá River)	13.3	14.0	13.1	13.2	9.4	11.4	8.5	**n/c**			
9. Oecomys superans	10.6	12.2	14.5	13.4	9.7	8.2	10.5	10.7	**n/c**		
10. Oecomys trinitatis	15.4	16.7	15.2	17.6	13.4	13.0	11.9	12.7	12.4	**n/c**	
11. Hylaeamys megacephalus	21.4	21.0	19.0	10.3	18.0	18.7	18.2	18.1	18.0	19.4	**n/c**

Table 5 - Selected external and cranial dimensions for *Oecomys paricola* from Belém region and Marajó Island. Mean, standard deviation and range (in parenthesis) of measurements are given in mm, followed by sample size. Measurements of specimens with karyotypes discussed on text are shown in different columns for comparisons.

Variable	Belém region					Marajó Island		
	Descriptive statistcs[1]	2n=68, NF=72		2n=70, NF=76		Descriptive statistcs[6]	2n=70, NF=72	
		MPEG 39699[2]	MPEG 39703[3]	MPEG 39701[4]	MPEG 39705[5]		MPEG 40846[4]	MPEG 40851[5]
HBL	103±12.4 (90 – 129) 9	78	71	99	104	105 ± 3.7 (97 – 106) 4	103	125
TL	108 ± 13.8 (80 – 125) 9	98	89	97	124	119 ± 5.9 (110 – 124) 4	120	132
FL	23 ± 0.8 (22 – 24) 9	22	22	23	22	21 ± 3.0 (21 – 28) 4	23	22
EL	15 ± 2.1 (12 – 19) 9	13	14	15	14	15 ± 1.4 (13 – 16) 4	14	16
CIL	23.30 ± 1.11 (21.5 – 25.0) 10	20.77	19.91	23.03	24.68	25.6 ± 0.36 (24.4 – 25.3) 4	25.02	26.81
LD	6.77 ± 0.53 (5.8 – 7.6) 13	5.82	5.43	6.66	7.20	7.52 ± 0.21 (7.10 – 7.56) 4	7.10	7.90
LM	4.02 ± 0.10 (3.8 – 4.2) 13	-	-	3.95	3.89	4.18 ± 0.18 (3.98 – 4.37) 4	3.99	4.29
BM1	1.12 ± 0.06 (1.01 – 1.18) 13	1.14	1.12	1.12	1.12	1.19 ± 0.03 (1.14 – 1.20) 4	1.20	1.19
LIF	4.36 ± 0.33 (3.9 – 5.0) 13	4.14	3.63	4.08	4.51	4.89 ± 0.23 (4.44 – 4.95) 4	4.44	5.03
BIF	2.15 ± 0.11 (2.0 – 2.3) 12	1.83	1.95	2.11	2.18	2.30 ± 0.06 (2.16 – 2.28) 4	2.17	2.48
BPB	2.57 ± 0.14 (2.4 – 2.9) 12	2.23	2.21	2.44	2.66	2.75 ± 0.23 (2.47 – 2.93) 4	2.47	2.80
BZP	2.35 ± 0.25 (1.0 – 2.6) 13	2.02	1.73	2.07	2.62	2.52 ± 0.05 (2.37 – 2.46) 4	2.37	2.81
LIB	4.84 ± 0.17 (4.6 – 5.2) 12	4.50	4.71	4.77	4.88	4.97 ± 0.12 (4.68 – 4.95) 4	4.68	5.23
ZB	13.58 ± 0.61 (12.1 – 14.5) 11	12.1	12.11	12.87	13.64	14.83 ± 0.5 (13.8 – 15.0) 4	14.26	15.63

[1] Based on the follow specimens of age class 2: MPEG 2477, 2605, 2614, 2615, 8438, 38659, 39701, 39708, 40732; BAR 006, 013, 023, 029.
[2] Female, age class 1.
[3] Male, age class 1.
[4] Male, age class 2.
[5] Female, age class 3
[6] Based on the follow specimens of age class 2: MPEG 40844, 40846, 40848, 408

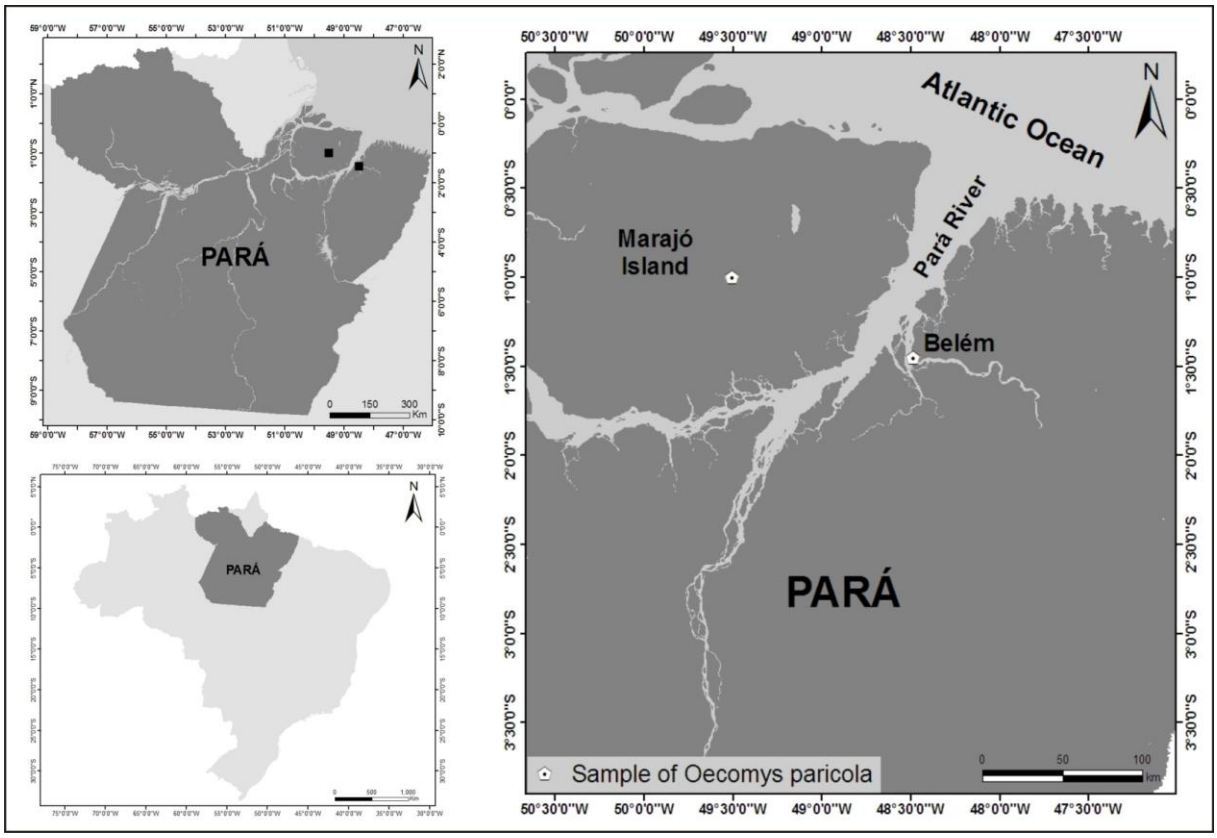

Figure 1: Map with the collect locations.

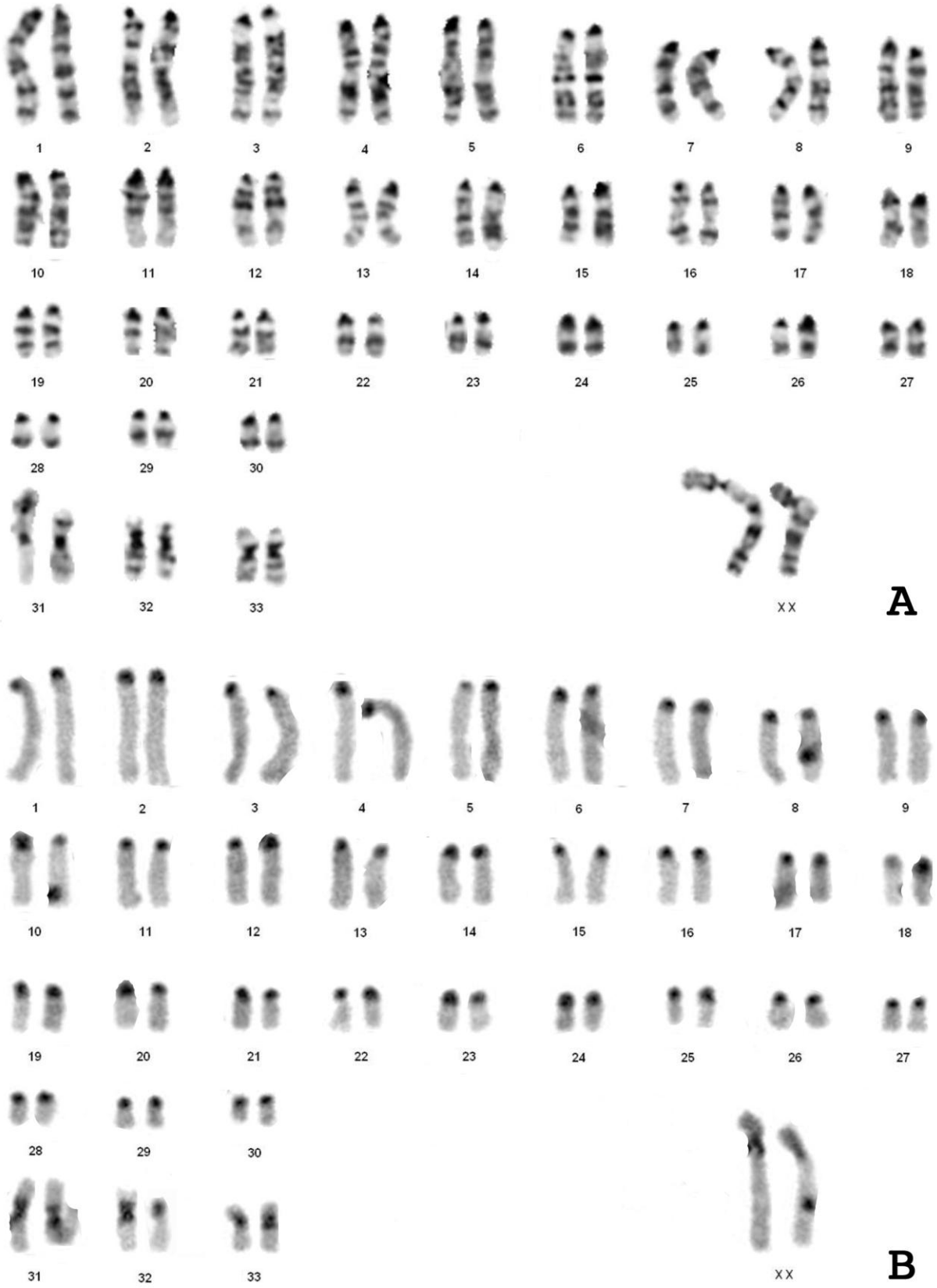

Figure 2: The karyotype with 2n=68 from Belém. A) G-banding B) C-banding.

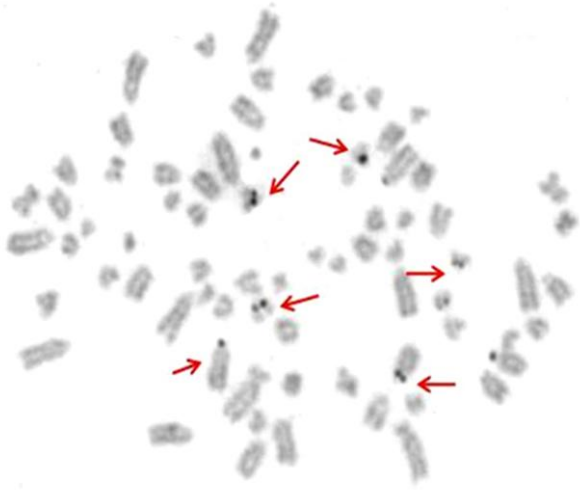

Figure 3: Ag-NOR staining (red arrows) on the karyotype with 2n=68 from Belém.

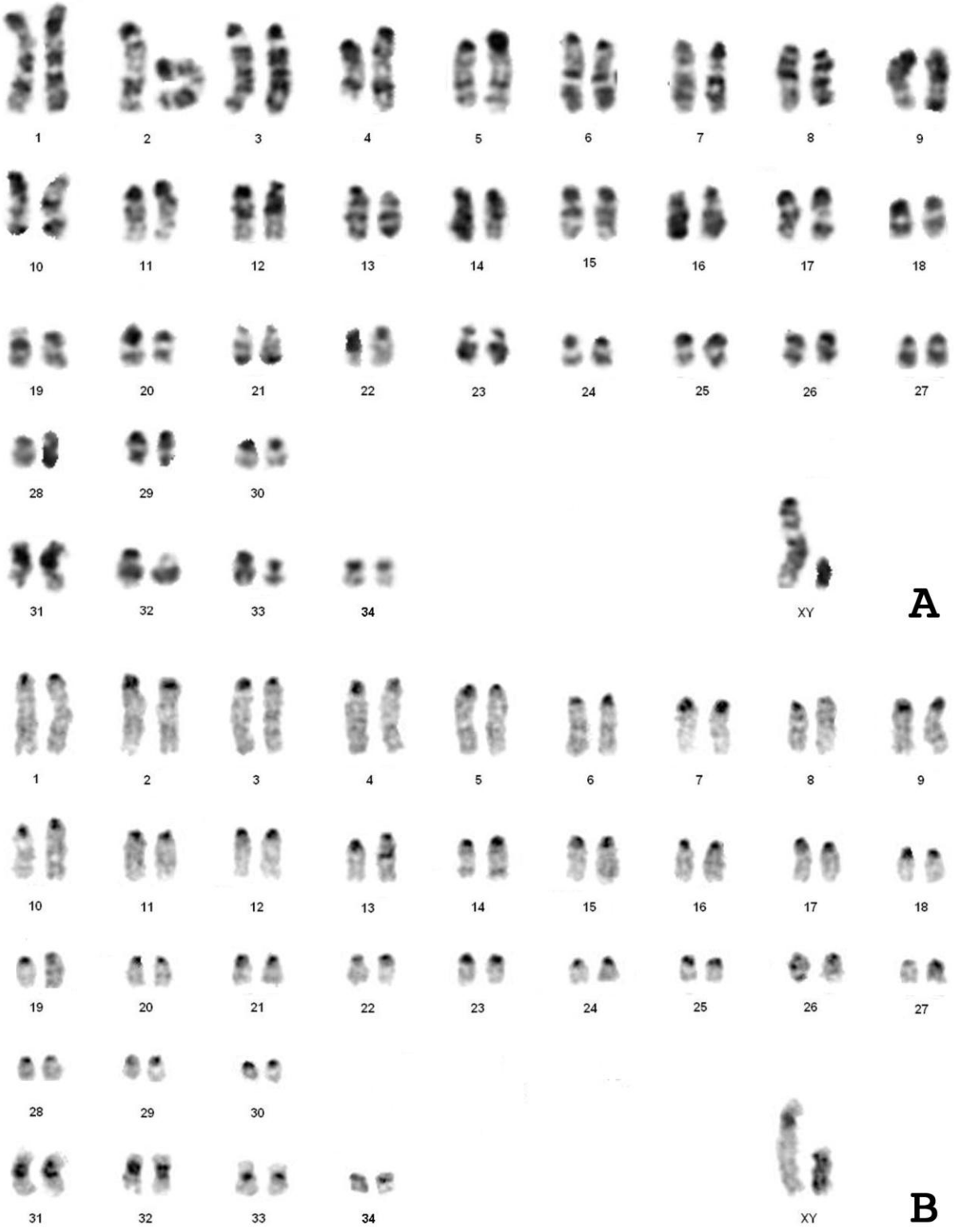

Figure 4: The karyotype with 2n=70 from Belém. A) G-banding. B) C-banding.

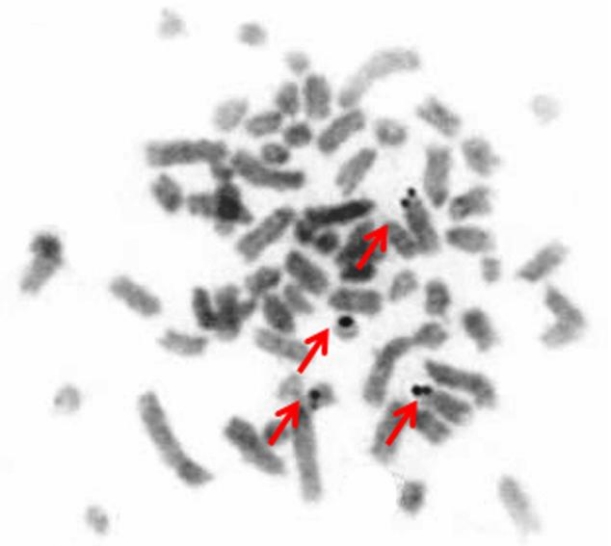

Figure 5: Ag-NOR staining (red arrows) on the karyotype with 2n=70 from Belém.

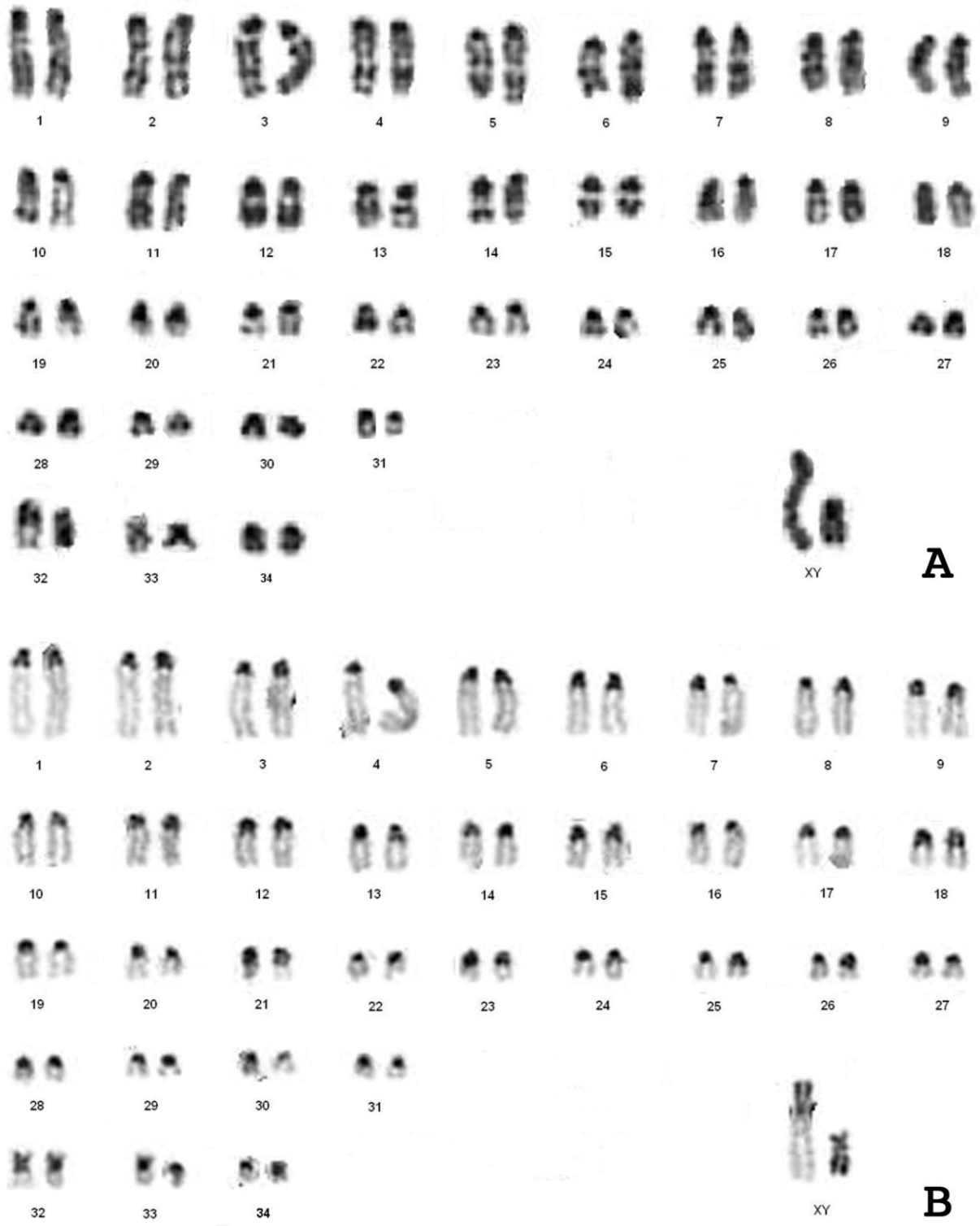

Figure 6: The karyotype with 2n=70 from Marajó Island. A) G-banding. B) C-banding.

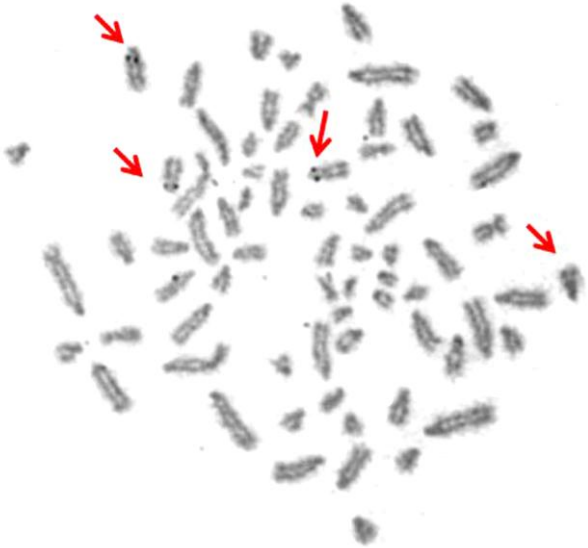

Figure 7: Ag-NOR staining (red arrows) on the karyotype with 2n=70 from Marajó Island.

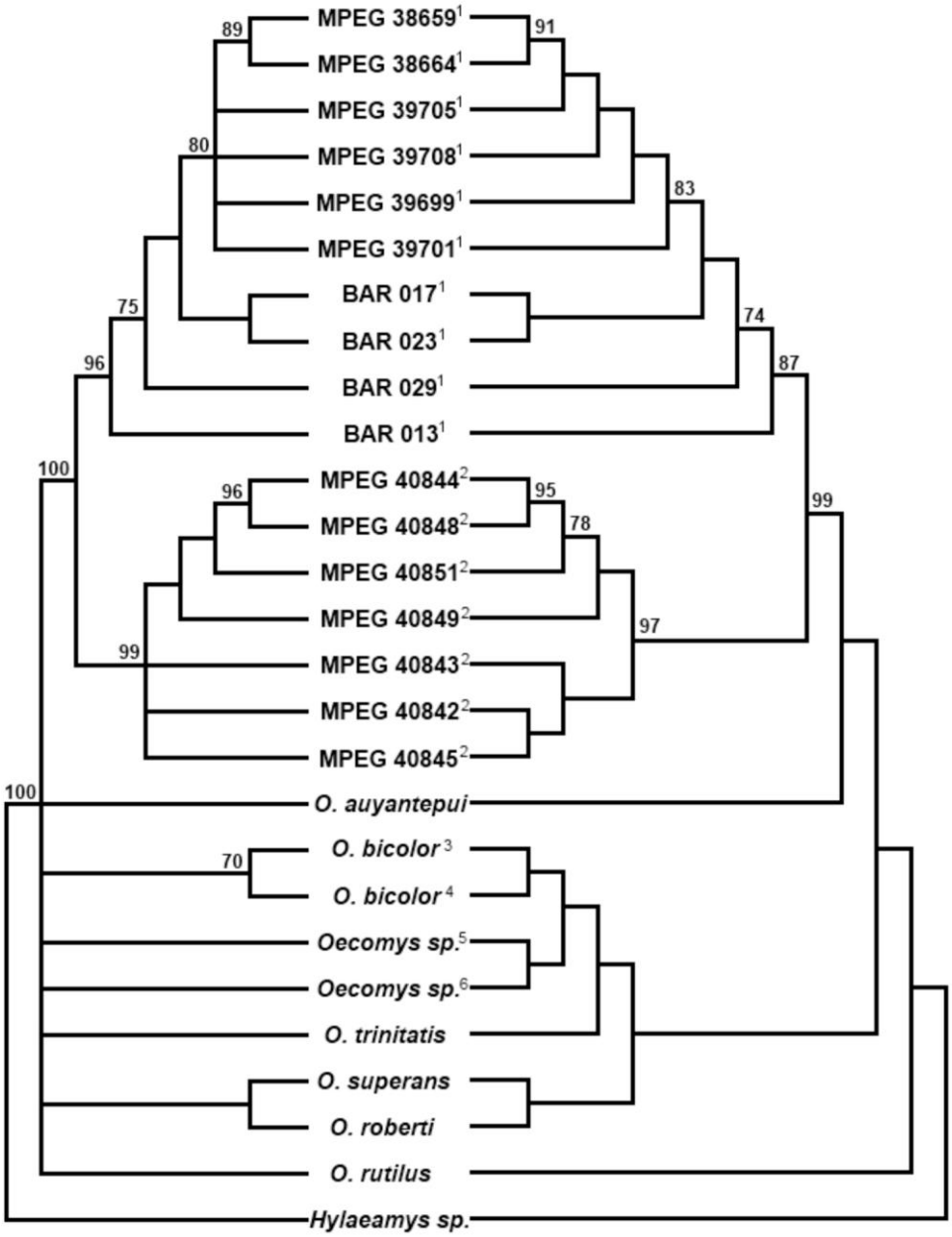

Figure 8: Phylogenetic analysis of *Oecomys paricola* from Belém region and Marajó Island. The data set of 738 bp of cytochrome-b contained 229 variable sites and 137 parsimony informative characters. Cladogram at the left shows the best tree found using the Maximum Parsimony criterion with 492 steps long, consistency index (CI) of 0.579268, homoplasy index (HI) of 0.420732, retention index (RI) of 0.677067, and rescaled consistency index (RC) of 0.392203. Cladogram at the right shows the 50% consensus tree of the best trees yielded by the Maximum Likelihood criterion using GTR model of nucleotide substitution with gamma shape parameter = 0.545; Log-*L* = -3234.53275; Unconstrained-*L* = -2669.36706; tree size = 1.02629; GTR relative parameters: A – C = 3.76984, A – G = 9.13837, A – T = 2.91617, C – G = 0.00021, C – T = 23.26447, G – T = 1.00000. Bootstrap support ≥ 70 % is shown above branchs. Legend: [1]Specimens from Belém region; [2]Specimens from Marajó Isnald; [3]Specimen AF108699; [4]Specimen OBU58382; [5]Specimen from Corumbá, MT; [6]Specimen from Juruá River.

5. CONCLUSÕES

1. Os resultados obtidos mostram a grande variabilidade cromossômica na espécie *Oecomys paricola* e no gênero *Oecomys*.
2. Os exemplares de *O. paricola* presentes em Belém são um exemplo de espécies crípticas, já que não apresentarem diferenças significativas em nível morfológico e molecular e são possuidores de cariótipos diferentes, indicando ausência de fluxo gênico entre elas.
3. As populações de Marajó e Belém são espécies já separadas há algum tempo e em processo de diferenciação morfológica e molecular, após ter havido isolamento reprodutivo ao nível cromossômico e geográfico. Deste modo, observa-se um exemplo de especiação já que o acúmulo de diferenças morfológicas e moleculares é posterior ao isolamento reprodutivo.
4. Os resultados mostram a necessidade de estudos com abordagens moleculares e citognéticos para auxiliar na resolução da composição taxonômica e das relações filogenéticas dentro deste grupo.