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Diversificação e caracterização de espécies em dois gêneros da tribo Oryzomyini (Rodentia: Cricetidae: Sigmodontinae) reveladas por abordagens moleculares e citogenéticas

Diversification and species limits in two genera of the tribe Oryzomyini (Rodentia: Cricetidae: Sigmodontinae) revealed by combined molecular and cytogenetic approaches

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Chapter 1
Introduction

1. Integrative Taxonomy

Delimiting species boundaries is the core in many subjects of evolutionary biology (Sites and Marshall, 2004). Associating scientific names unequivocally with species is essential for a reliable reference system. However, reaching a scientific consensus on the concept of species is one of the major challenges, since there are more than 20 concepts described (de Queiroz, 2005; 2007; Padial *et al*., 2010). An unified concept of species, based on the common fundamental idea among all the concepts, was proposed by de Queiroz (1998), in which species are lineages composed of metapopulations that evolve separately.

Currently, several methods have been used in order to delimit and / or describe species, since any character can be used for this purpose, as long as they are inheritable and independent (Schlick-Stein *et al*., 2010).

Traditionally, the primary identification of species is morphological. The advantage is that morphology is applicable to living, preserved or fossil specimens (Padial *et al.*, 2010). However, delimitation of taxa based only on morphology has some limitations: (i) it can hide lineages in which quantitative and qualitative morphological characteristics overlap, (ii) lineages that differ only in ecological or behavioral characteristics, (iii) species that exhibit large phenotypic plasticity or (iv) cryptic species (Bickford *et al.*, 2007; Padial *et al.*, 2010). Therefore, alternative methods to recognize biodiversity have increased considerably in recent decades and have contributed to the discovery of cryptic species or lineages with low interspecific morphological variation (Dayrat, 2005).

Cytogenetic proved to be useful in species identification in cases which species present morphological similarities and species-specific karyotypes, particularly in rodents. A recent cytogenetic review of Brazilian rodents showed chromosome information as an essential marker for recognizing species in 13 genera, including *Oligoryzomys* and *Cerradomys* (Di-Nizo *et al.,* 2017), both genera studied herein. By contrast, karyotype can not be used for species identification for many taxa that share the same diploid and fundamental numbers (number of autosome arms). More recently, molecular cytogenetic, using Fluorescence *in situ* Hybridization (FISH), allowed a refined comparison among karyotypes of different species (Ferguson-Smith *et al.*, 1998; Chowdhay and Raudsepp, 2001).

DNA sequences can also be used to delimit species, through different approaches. Mitochondrial DNA has been widely used in closely related taxa because of its

properties, such as: rapid evolutionary rate, small size, circular format, matrilineal inheritance, absence of recombination and rarely possess repetitive sequences, pseudogenes and introns (Avise *et al.*, 1987; Harrison, 1989). Comparatively, as nuclear DNA presents slower evolutionary rates, this marker is more used to infer phylogenetic relationships in suprageneric categories, although some studies showed that it is also effective for species-level inferences (Jansa and Voss, 2000; Prychitko and Moore, 2000).

Limitations of using molecular data includes: disagreement between species and gene trees (*i.e.*: whether the gene tree reflects the phylogeny of the organism), incomplete lineage sorting, gene duplication, recombination, retention of ancestral polymorphism (which leads to underestimation of number of species) and heteroplasmy (leading to overestimation of number of species) (Funk and Omland, 2003; Moritz and Cicero, 2004; Padial *et al*., 2010). Combining different loci can overcome these effects and help to solve taxonomic problems with greater robustness (Moritz and Hillis, 1996; Fabre *et al.*, 2016).

Some molecular methods are not based on phylogenetic trees (*e.g.*: DNA barcoding) (Hebert *et al*., 2003) while other methods generate phylogenetic hypotheses using some optimization criteria (such as Maximum Parsimony, Maximum Likelihood, Bayesian Inference), in order to search for monophyletic groups that may represent potential species (Moritz and Hillis, 1996; Sites and Marshall, 2004). Recently, methods using probabilistic coalescent framework have helped delimiting species in complex groups (Pons *et al*., 2006; Zhang *et al.*, 2013). In addition, phylogeographic studies can also reveal patterns of genetic diversity, aiding in species recognition (Avise *et al.*, 1987).

As each character (morphology, chromosome, DNA, etc) evolves at different rates, efforts to join different disciplines to provide more consistent subsidies on species delimitation are increasing. Dayrat (2005) and Will *et al*. (2005), concomitantly, coined the term "integrative taxonomy" which is the science that aims to delimit species from multiple and complementary perspectives. According to Dayrat (2005), the confidence level increases when the delimitation is supported by different types of data. A premise of the integrative taxonomy is the absence of superiority of any character over another for species recognition (Dayrat, 2005).

When integrating different disciplines, the literature shows that there may be congruence between these different approaches regarding the number of species and

their identification (Schlick-Steiner *et al*., 2010). In contrast, other studies show disagreement among the different methods (morphology, molecular, cytogenetic, ecology, etc.) used. Disagreement can be solved by looking for an evolutionary explanation for such discrepancy (Schlick-Steiner *et al*., 2010). One possible evolutionary explanations to these differences is that the speciation process is not always accompanied by character changes at all levels and, the relative rate of changes during lineage divergence is heterogeneous (Padial *et al.*, 2010). In this way, the integrative usage of several disciplines is necessary to help the failure that a single discipline can show, increasing the rigor in the delimitation.

2. Tribe Oryzomyini (Rodentia: Cricetidae: Sigmodontinae)

2.1. Background

The subfamily Sigmodontinae comprises one of the most complex Neotropical mammalian lineages and it is widespread from southern North America to southernmost South America (Carleton and Musser, 2005).

Historically, this subfamily was subdivided into tribes, primarily based on morphological characters. Nevertheless, numbers of tribes and its content have been modified throughout the last 20 years, as morphological and molecular phylogeny became available and integrated. Currently, ten tribes are considered (Abrotrichini, Akodontini, Euneomyini, Ichthyomyini, Oryzomyini, Phyllotini, Reithrodontini, Sigmodontini, Thomasomyini and Wiedomyini), in addition to several *incertae sedis* genera that could not be affiliated to any of these tribes (Reig, 1984; Smith and Patton, 1999; Musser and Carleton, 2005; D'Elía *et al.*, 2007; Pardiñas *et al*., 2015).

Tribe Oryzomyini is undoubtedly the most diverse Sigmodontinae radiation, distributed ubiquitous at the Neotropics, in a variety of environments and at elevations from 4.000 m in Andes to the sea level (Weksler, 2006; 2015). This diversity is reflected in morphological, ecological, molecular and chromosomal variations, leading to taxonomic problems that can only be solved with multidisciplinary approaches.

The ancient genus *Oryzomys*, for example, came to consist of almost half of all Oryzomyini species. Chromosomal data were an indicative of the great diversity within this group and helped to clarify some relationships (Gardner and Patton, 1976). The current genera *Melanomys, Microryzomys, Nesoryzomys, Oecomys* and *Oligoryzomys* were for a long time considered subgenera of *Oryzomys*, but multidisciplinary studies

erected them at the generic level (Carleton and Musser, 1989; Myers *et al.*, 1995; Smith and Patton, 1999). Even so, phylogenetic analyses recovered *Oryzomys* as paraphyletic (Myers *et al.*, 1995; Bonvicino and Moreira, 2001; Weksler, 2003).

In order to recover the monophyly of oryzomyine rodents, Weksler *et al.* (2006), based on morphological and molecular data, elevated some *Oryzomys* species and species groups at genus category, recognizing ten new genera for this tribe (Table 1).

Phylogenetic relationships reiterates de monophyly of oryzomyine and recovered four major clades (A-D), but the relationships within each clade are not strongly supported (Weksler *et al.*, 2006; Percequillo *et al*., 2011; Pine *et al*., 2012; Machado *et al.*, 2014).

In the last years, after more comprehensive taxon sampling and multidisciplinary approaches, associating morphological and molecular data, new genera were established, so that the Oryzomyini tribe is nowadays composed of 29 extant genera (Table 1) (Musser and Carleton, 2005; Weksler *et al*., 2006; Percequillo *et al*., 2011; Pine *et al*., 2012). In addition, the *Oryzomys alfaroi* group is provisionally positioned in the genus *Handleyomys*, but they are considered a new genus, pending description (Weksler *et al.*, 2006). The most recent phylogeny, based on morphological and molecular (nuclear and mitochondrial) characters, is shown in Figure 1.

Times of divergence were estimated for the subfamily Sigmodontinae and the diversification of the tribe Oryzomyini varied according to different authors and methods of analyses (Parada *et al.,* 2013; Vilela *et al.,* 2013; Leite *et al.*, 2014). Parada *et al*. (2013) estimated crown age of oryzomyine at 7.72 Mya while Leite *et al.* (2014) estimated 6.6 Mya and 7.6 Mya, using BEAST and Multidivtime, respectively. Older divergence times were observed by Vilela *et al.* (2013) in which oryzomyine lineage dated approximately 11.9 Mya (using BEAST) and 11.5 Mya (using MCMCTree).

2.2. Diversity of Cerradomys and Oligoryzomys

Two oryzomyine genera (*Cerradomys* and *Oligoryzomys*) were studied in this work based on integrative approaches of molecular and chromosome data. *Oligoryzomys* is recovered in clade C and *Cerradomys* belongs to clade D in the Oryzomyini phylogeny (Fig. 1).

While *Oligoryzomys* is distributed throughout almost all biomes, from Tierra del Fuego, in southernmost South America, through northeast Mexico, *Cerradomys* has a more restricted distribution in the open diagonal belt of South America, from

northeastern Brazil to southeastern Bolivia and northwestern Paraguay, with some species penetrating Atlantic Forest and one endemic from Restinga (sandy plains with low index of annual rainfall, soil with low water retention, high salinity and extensive temperature fluctuation during the day) (Musser and Carleton, 2005; Percequillo, 2015; Weksler and Bonvicino, 2015).

Cerradomys was considered monotypic for a long time, although cytogenetic information pointed that its diversity was underestimated, once different karyotypes were attributed to the same name (formerly *Oryzomys subflavus*) (Maia and Hulak, 1981; Almeida and Yonenaga-Yassuda, 1985; Zanchin, 1988; Svartman and Almeida, 1992; Silva, 1994; Bonvicino *et al.*, 1999). Posteriorly, based on morphological, molecular phylogenetic and cytogenetic studies, new species were described, resulting in the ancient *Oryzomys* gr. *subflavus*. Nowadays, after being elevated to genus, eight species are recognized (Bonvicino and Moreira, 2001; Percequillo *et al.*, 2008; Tavares *et al.*, 2011; Bonvicino *et al.*, 2014). Nevertheless, Bonvicino *et al.* (2014) suggested that *Cerradomys goytaca* is a junior synonymous of *C. subflavus*, since this species is not reciprocally monophyletic based on cytochrome *b*.

Oligoryzomys is the richest oryzomyine genus in terms of species number (23 described species, although one of them is probably extinct). Besides, it is evident that the current taxonomy of this genus does not reflect its diversity since cytogenetic and molecular phylogeny revealed cryptic lineages (da Cruz and Weksler, 2017).

Morphological homogeneity with overlapping of quantitative and qualitative characters hamper diagnose of species within both genera (Carleton and Musser, 1989; Bonvicino and Moreira, 2001; Weksler and Bonvicino, 2005; 2015; Percequillo *et al.*, 2008). In this sense, cytogenetic proved to be an important tool for species recognition.

Diploid numbers range from 2n=44 to 72 in *Oligoryzomys*, and from 2n=46 to 60 in *Cerradomys* (Maia and Hulak, 1981; Silva and Yonenaga-Yassuda, 1997; Agrellos *et al.*, 2012; Bonvicino *et al.*, 2014). Besides, both genera proved to be models of chromosomal evolution, since several rearrangements were described. Chromosome painting in *Oligoryzomys* showed a huge genomic reshuffling in closely related species (Di-Nizo *et al.*, 2015). Regarding *Cerradomys*, classic cytogenetic showed several rearrangements, especially Robertsonian and pericentric inversions (Maia and Hulak, 1981; Almeida and Yonenaga-Yassuda, 1985). In fact, chromosome change may have had an important role in reproductive isolation during the explosive radiation of Sigmodontinae rodents.

Molecular dating recovered recent divergence times for both genera, with *Cerradomys* dated in the late Pliocene (Tavares *et al.*, 2016; Chapter 2) and *Oligoryzomys* in early Pleistocene (da Cruz and Weksler, 2017). These results corroborate the rapid radiation of both genera, accompanied by the great chromosome variability and ability of occupying diverse phytophysiognomies.

Objectives

The aim of this study is to investigate diversification and species limits in two genera of oryzomyine rodents: *Cerradomys* and *Oligoryzomys*. To achieve these goals, multiple approaches were performed: mitochondrial and nuclear markers were used for phylogenetic, species delimitation and phylogeographic analyses, and classic and molecular cytogenetic were performed to aid cytotaxonomy and to infer chromosomal evolution. The specific objectives is described in each chapter of this work as follows:

- ✓ Chapter 2: Investigate phylogenetic relationships, species limits and evolutionary history of *Cerradomys* using integrative taxonomy with cytogenetic, molecular systematics, DNA-barcoding, coalescent-based species delimitation and population genetics for inferring species limits. Additionally, divergence time estimation together with geographic distribution were performed to investigate diversification in this genus.
- ✓ Chapter 3: Understanding the processes involved in the karyotype evolution of *Cerradomys* based on differential staining, FISH with telomeric probes and chromosome painting in a phylogenetic context obtained in the Chapter 2.
- ✓ Chapter 4: Reconstruct hypotheses of relationships within *Oligoryzomys*, using a large number of samples from different biomes and associate them to karyotypic data and geographical distribution to aid species limit. Additionally, phylogeographic studies in *O. nigripes* were performed, in order to understand the genetic structure between populations of northeastern Brazil and populations of the central-south-southeast region.

Figure and Table

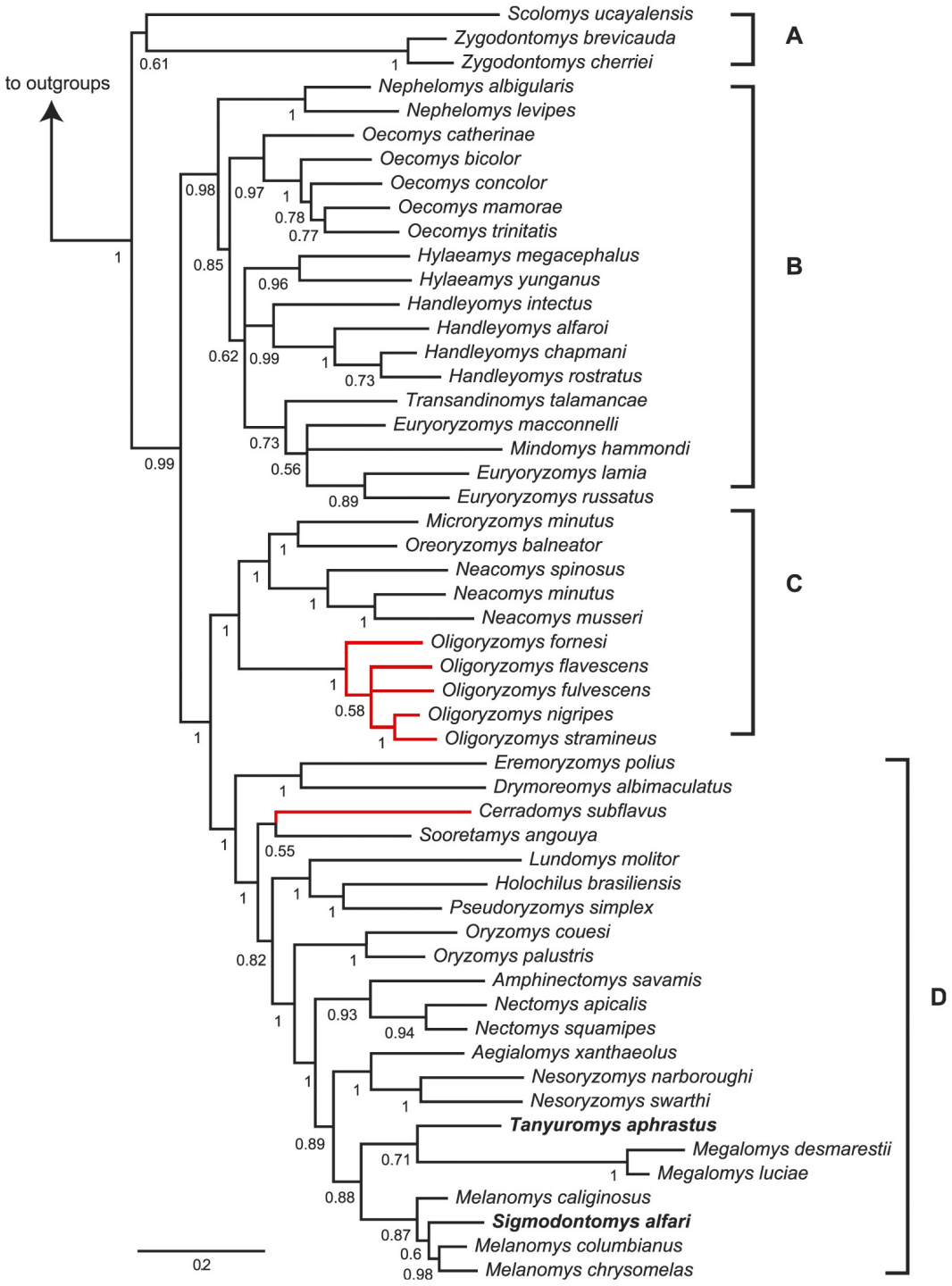

Fig. 1: Bayesian phylogenetic relationships of Oryzomyini using morphological and molecular (12S, cytochrome-*b* and interphotoreceptor retinoid–binding protein) characters. Numbers represent posterior probabilities. Outgroups include *Delomys sublineatus, Nyctomys sumichrasti, Peromyscus maniculatus, Rhipidomys nitela, Thomasomys baeops*, and *Wiedomys pyrrhorhinos*. The two genera studied in this work are highlighted in red. Extracted and modified from Pine *et al.* (2012).

Table 1: Current classification of extant genera of the tribe Oryzomyini (with previous group each new genus used to be allocated), according to Weksler *et al*. (2006), Percequillo *et al*. (2011) and Pine *et al*. (2012).

Order Rodentia
Suborder Sciurognathi
Family Cricetidae
Subfamily Sigmodontinae
Tribe Oryzomyini
Genus *Aegialomys* (previous "*Oryzomys* gr. *xanthaeolus*")
Genus *Amphinectomys*
Genus *Cerradomys* previous ("*Oryzomys* gr. *subflavus*")
Genus *Drymoreomys*
Genus *Eremoryzomys* (previous *Oryzomys polius*)
Genus *Euryoryzomys* (previous "*Oryzomys* gr. *nitidus*")
Genus *Handleyomys*
Genus *Holochilus*
Genus *Hylaeamys* (previous "*Oryzomys* gr. *megacephalus*")
Genus *Lundomys*
Genus *Melanomys*
Genus *Microakodontomys*
Genus *Microryzomys*
Genus *Mindomys* (previous *Oryzomys hammondi*)
Genus *Neacomys*
Genus *Nectomys*
Genus *Nephelomys* (previous "*Oryzomys* gr. *albigularis*")
Genus *Nesoryzomys*
Genus *Oecomys*
Genus *Oligoryzomys*
Genus *Oreoryzomys* (previous *Oryzomys balneator*)
Genus *Oryzomys* (previous "*Oryzomys* gr. *palustris*")
Genus *Pseudoryzomys*
Genus *Scolomys*
Genus *Sigmodontomys*
Genus *Sooretamys* (previous "*Oryzomys* gr. *angouya*")
Genus *Tanyuromys*
Genus *Transandinomys* (previous *O. bolivaris* and *O. talamancae*)
Genus *Zygodontomys*

Genera studied in present work are underlined.

Final discussion and conclusions

In this work, we performed cytogenetic and molecular data using loci that evolves at different rates, integrated to geographic distribution, previous morphologic and phylogenetic studies, based on an integrative taxonomy approach, in order to investigate species limits and patterns of diversification in *Cerradomys* and *Oligoryzomys,* two genera of the tribe Oryzomyini.

New sequences of mitochondrial and nuclear markers were generated, increasing representativeness for each one of the genera. For *Cerradomys*, 88 sequences of cyt-*b*, 91 of COI, 90 of IRBP and 93 of i7FBG, from all species described so far, were generated. For *Oligoryzomys*, 102 cyt-*b* and 97 IRBP sequences were obtained, for 13 species or candidate species.

This work brings inedited results of DNA barcoding, population genetics, coalescent-based species delimitation and karyotype evolution in *Cerradomys*. A new phylogenetic hypothesis for *Oligoryzomys* is being proposed as well as inedited phylogeographic data for *O. nigripes*.

Molecular phylogeny recovered well support internal relationships among *Cerradomys* species, but *C. subflavus* and *C. goytaca* were not reciprocally monophyletic. The other molecular approaches (DNA barcoding and K2P) did not distinguish both species. Nevertheless, cytogenetic, morphology, allopatric distribution and multi-locus coalescent-based species delimitation support that *C. subflavus* from *C. goytaca* are distinct and detected four species within the latter. In addition, the multi-locus species delimitation, detected two species within *C. langguthi*.

Following the integrative taxonomy, this work recognizes that the previously eight described species under *Cerradomys* are valid and reinforces that a revision should be performed in *C. langguthi* and *C. subflavus*, in order to evaluate if they represent species-complex.

Internal relationships were poorly resolved in *Oligoryzomys*. Four putative new species that could not be related to any name were recovered (*Oligoryzomys* sp. A to D). Additionally, several samples were recovered related to some species, and they may represent species-complex or incipient species, as follows: *O. microtis*, *O. flavescens* and *O. nigripes*, reiterating that taxonomy does not match to all the evolutionary lineages described so far.

Results evidenced that conventional cytogenetic is important for species recognition in both genera. Extensive genomic reshuffle was observed for both genera after chromosome painting studies (cytogenomics). Nevertheless, *Cerradomys* showed a tendency in reduction of diploid number, whereas chromosomal evolution has been associated with both decrease and increase in diploid numbers in *Oligoryzomys*.

Comparison of molecular phylogeny and chromosome painting revealed that the interstitial telomeric sites (ITS) observed for some *Cerradomys* species is associated to fusions events. However, as several rearrangements were detected in chromosomes that lack ITS, karyotype evolution in *Cerradomys* showed both retention and loss of interstitial telomeric sequences, while telomeric repeats may have been eliminated by chromosome breakage in all species of the genus *Oligoryzomys* studied so far.

Chromosomal rearrangements may have played an importante role in speciation of both genera due to mal-segregation and origin of unbalanced gametes or suppression of the recombination, reducing gene flow. The pericentric inversions observed between *O. nigripes* and *Oligoryzomys aff. nigripes*; and *O. microtis* and *Oligoryzomys aff. microtis* as well as the Robertsonian rearrangements described for *C. langguthi* (CLA 2/7 and CLA 3/5)*, C. subflavus* (CSU 5/6) and *Oligoryzomys aff. rupestris* (2n=44 and 2n=46) may be illustrating an incipient process of speciation. Thus, what is nowadays being called as polymorphisms, may be a reflection of the process of speciation, in which we are detecting only a initial slice of this evolutionary process.

New distributional records are being described for both genera, reiterating that survey efforts are important for the knowledge of biodiversity.

Early divergence times were observed, with the majority of splits in Pleistocene, showing the importance of Quaternary events in shaping diversity and corroborating the rapid adaptive radiation of *Cerradomys* and *Oligoryzomys*.

Complex patterns of differentiation were observed for these two genera. *Cerradomys* may have originated in Cerrado domain, with some species occupying Caatinga and transitional areas of Atlantic Forest, reaching Restinga formations. The Cerrado corridor that possibly connected northern Rio de Janeiro to central Minas Gerais during the late Quaternary, may have favored to the expansion of one lineage through Restinga. Thereafter, climatic conditions expanded Atlantic Forest, creating a vicariant barrier blocking gene flow between populations from Restingas of north Rio de Janeiro and south Espírito Santo states and populations from Cerrado (Minas Gerais).

Our results, together with previous studies, showed that *Cerradomys goytaca* differentiated from other species both morphologically and cytogenetically.

For *Oligoryzomys*, it was not possible to state if this genus originated in Amazonia or Cerrado, since the ancestral clade within the genus is represented by species of both biomes. Results showed that lineages dispersed through South America reaching Central and North America through Panama isthmus.

One single mechanism of diversification could not explain the complex pattern observed for both genera. Instead, we hypothesize that several events may have shaped their diversity, such as rivers formation, glacial cycles of Plio-Pleistocene and Quaternary geotectonic.

Because each character evolves at different rates, this work corroborates the importance of interdisciplinary studies in order to better understand such complexes groups. The combined approaches used herein provided great robustness for diversification and species delimitation and showed that the diversity of *Cerradomys* and *Oligoryzomys* is underestimated.

Resumo

Neste trabalho, utilizou-se a abordagem de taxonomia integrativa para compreender os limites das espécies e padrão de diversificação em dois gêneros de roedores orizominos (*Cerradomys* e *Oligoryzomys*). Para tanto, marcadores moleculares com taxas evolutivas distintas foram utilizados em diferentes abordagens (filogenia, delimitação de espécies baseada em coalescência, DNA *barcoding*, filogeografia, datação). Análises de citogenética clássica e molecular foram realizadas, contribuindo como um marcador citotaxonômico e revelando padrões de evolução cromossômica. Os dados moleculares e citogenéticos, combinados à dados de distribuição geográfica, tornaram esse trabalho interdisciplinar. Esta tese está dividida em quatro capítulos, incluindo uma breve introdução (Capítulo 1). No capítulo 2, a abordagem de taxonomia integrativa foi utilizada para estudar o gênero *Cerradomys*, a partir dos dados citogenéticos e moleculares. Os resultados revelaram que a citogenética é importante no reconhecimento de todas as espécies descritas (citotaxonomia). A reconstrução filogenética mostrou que as relações internas são bem suportadas, com exceção de *C. subflavus* e *C. goytaca*, que não são reciprocamente monofiléticos. De acordo com a taxonomia integrativa, em que a delimitação de espécies é baseada na congruência entre a maioria dos dados, esse trabalho reconhece e reitera as oito espécies de *Cerradomys* descritas até o momento. Sugerimos uma revisão taxonômica em *C. langguthi* e *C. subflavus*, uma vez que ambas podem representar complexos de espécies ou casos de especiação em curso. Os tempos de divergência mostram que *Cerradomys* é um gênero recente, cujos eventos de especiação ocorreram preponderantemente no Pleistoceno. No capítulo 3, estudos de citogenética clássica e molecular (hibridação *in situ* fluorescente – FISH com sondas teloméricas e cromossomo-específicas de *Oligoryzomys moojeni*) foram realizados para compreender a evolução cromossômica de *Cerradomys,* com base na filogenia obtida no capítulo anterior. A pintura cromossômica mostrou que um grande número de rearranjos ocorreu ao longo da evolução cariotípica de *Cerradomys*. As espécies com os maiores números diplóides mostraram sinais exclusivamente teloméricos enquanto que sinais teloméricos intersticiais (ITS) foram observados nas espécies com menores números diplóides. Comparações dos dados de pintura cromossômica com os dados de filogenia molecular corroboram a hipótese de que as ITS, neste caso, são remanescentes de telômeros. No entanto, outros rearranjos cromossômicos foram detectados com ausência de ITS, de modo que essas sequências

podem ter sido perdidas no processo das quebras cromossômicas, evidenciando que houve tanto retenção quanto perda das ITS ao longo da evolução cariotípica do gênero. Além disso, rearranjos complexos foram detectados entre os cariótipos de *C. goytaca* e *C. subflavus*, reiterando que essas duas espécies são distintas, uma vez que provavelmente os híbridos não seriam viáveis devido a problemas meióticos. No capítulo 4, com o objetivo de recuperar a história evolutiva e os limites das espécies de *Oligoryzomys*, estudos de filogenia molecular foram integrados a dados citogenéticos. O gênero mostrou-se monofilético, mas as relações internas tiveram baixo suporte. A compilação dos dados filogenéticos, cromossômicos e de distribuição geográfica (interdisciplinaridade) foram importantes para compreender os limites das espécies. Quatro linhagens não puderam ser relacionadas a nenhum nome, sendo prováveis espécies novas (*Oligoryzomys* A-D). *Oligoryzomys flavescens* foi recuperado parafilético em relação à *O. fornesi*. *Oligoryzomys stramineus, O. microtis* e *O. nigripes* foram recuperados em dois clados bem estruturados cada. No caso das duas últimas espécies, os subclados provavelmente estão relacionados à cariótipos exclusivos. Em *O. microtis*, um dos clados é composto por exemplares do oeste da região amazônica e o outro, por exemplares distribuído ao sul da região amazônica, transição com Cerrado (2n=64, NF=64). Em *O. nigripes*, um dos clados é composto por exemplares do nordeste do Brasil (2n=62, NF=78) e o outro por exemplares da região centro-sul-sudeste do Brasil, Argentina, Paraguai e Uruguai (2n=62, NF=80-82). Os dados filogeográficos suportam os dados filogenéticos e cromossômicos, revelando dois filogrupos em *O. nigripes*, sugerindo que essas populações estejam em processo de especiação. Os dados cromossômicos corroboram as informações da literatura e puderam ser associados aos seguintes nomes: *O. mattogrossae*, *O. moojeni*, *O. chacoensis*, *O. stramineus, O. flavescens* e *O. nigripes*, embora as duas últimas devam ser reavaliadas. Adicionalmente, um novo cariótipo está sendo reportado para *Oligoryzomys aff. utiaritensis* (2n=70, NF=72), assim como novos dados de distribuição no Brasil para quatro espécies. Sugerimos uma revisão taxonômica em *O. microtis, O. flavescens* e *O. nigripes,* pois estas espécies provavelmente representam complexos de espécies ou estão em processo de especiação. Além disso, os exemplares relacionados à *Oligoryzomys aff. delicatus*, *Oligoryzomys aff. chacoensis, Oligoryzomys aff. rupestris* e *Oligoryzomys aff. utiaritensis* devem ser avaliados morfologicamente para confirmar suas identidades. Os resultados desse trabalho corroboram a importância dos estudos interdisciplinares, uma vez que as taxas de evolução para cada caráter são heterogêneas.

Abstract

In this work, the integrative taxonomy approach was performed to understand species limits and patterns of diversification in two genera of orizomyine rodents (*Cerradomys* and *Oligoryzomys*). Therefore, molecular markers with distinct evolutionary rates were used with different approaches (phylogeny, coalescent-based species delimitation, DNA barcoding, phylogeography, molecular dating). Classic and molecular cytogenetic analyzes were performed, contributing to cytotaxonomy and revealing chromosomal evolution. This work is divided into four chapters, including a brief introduction (Chapter 1). In Chapter 2, the integrative taxonomy approach was used to study the genus *Cerradomys*, based on cytogenetic and molecular data. The results revealed that cytogenetics is important in the recognition of all described species (cytotaxonomy). Phylogenetic reconstruction showed that internal relationships are well supported, with the exception of *C. subflavus* and *C. goytaca*, which are not reciprocally monophyletic. Following the integrative taxonomy, in which species limits are based on the congruence of methods, this work recognizes and reiterates the eight *Cerradomys* species described so far. We suggest a taxonomic revision in *C. langguthi* and *C. subflavus*, since both may represent species-complex or in process of speciation. Times of divergence show that *Cerradomys* is a recent genus, with speciation events occurred mainly in the Pleistocene. In Chapter 3, classic and molecular cytogenetics (Fluorescence *in situ* hybridization - FISH with telomeric and *Oligoryzomys moojeni* probes) were used to study chromosomal evolution in *Cerradomys*, based on the molecular phylogeny obtained in Chapter 2. Chromosome painting revealed extensive chromosome reshuffling in *Cerradomys.* Species with the highest diploid numbers showed exclusively telomeric signals whereas interstitial telomeric signals (ITS) were observed in the species with the lowest diploid numbers. Comparisons of chromosome painting with molecular phylogeny data corroborate the hypothesis that ITS, in this case, are remnants of telomeres. Nevertheless, other chromosomal rearrangements were detected with absence of ITS, indicating that these sequences may have been lost in the process of chromosomal breakages, evidencing that there was both retention and loss of ITS along the karyotypic evolution of the genus. In addition, complex rearrangements were detected between the karyotypes of *C. goytaca* and *C. subflavus*, reiterating that these two species are distinct, since hybrids probably would not be viable due to meiotic problems. In Chapter 4, aiming to recover the evolutionary history and species limits of

Oligoryzomys, molecular phylogeny studies were integrated into cytogenetic data. The genus was monophyletic, but the internal relations had low support. The compilation of phylogenetic, chromosomal data and geographic distribution (interdisciplinarity) was important to understand species boundaries. Four lineages could not be related to any name and may be new species (*Oligoryzomys* A-D). *Oligoryzomys flavescens* was recovered paraphyletic in respect to *O. fornesi*. *Oligoryzomys stramineus*, *O. microtis* and *O. nigripes* were recovered in two well-structured clades each. In the case of the last two species, the subclades are probably related to exclusive karyotypes. In *O. microtis*, one subclade is composed of samples from the western Amazon region and the other with samples distributed in southern Amazon region, transition with Cerrado (2n=64, FN=64). In *O. nigripes*, one of the clades is composed of specimens from northeastern Brazil (2n=62, FN=78) and the other from central-south-southeast Brazil, Argentina, Paraguay and Uruguay (2n=62, FN=80-82). Phylogeographic results corroborate phylogenetic and cytogenetic data, revealing two distinctive phylogroups, consistent with incipient species. Chromosome data corroborate previous work and could be associated to the following names: *O. mattogrossae*, *O. moojeni*, *O. chacoensis*, *O. stramineus, O. nigripes* and *O. flavescens*, although the last two species should be reassessed. In addition, an undescribed karyotype is being reported for *Oligoryzomys aff. utiaritensis* (2n=70, FN=72), as well as new records in Brazil for four species. We suggest a taxonomic revision in *O. microtis*, *O. flavescens* and *O. nigripes*, as these species probably represent incipient or species-complex. In addition, samples related to *Oligoryzomys aff. delicatus*, *Oligoryzomys aff. chacoensis, Oligoryzomys aff. rupestris* and *Oligoryzomys aff. utiaritensis* should be evaluated morphologically to confirm their identities. The results of this work corroborate the importance of interdisciplinary studies, since the rates of evolution differ according to each character.

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