Camilla Bruno Di-Nizo

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Dedicatória

Aos meus pais, Romano e Solange
Ao Prin

Epígrafe

"Das Leben gehört den Lebenden an, und wer lebt, muβ auf Wechsel gefaβt sein"

Johann Wolfgang von Goethe

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Índice

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.

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Figure and Table

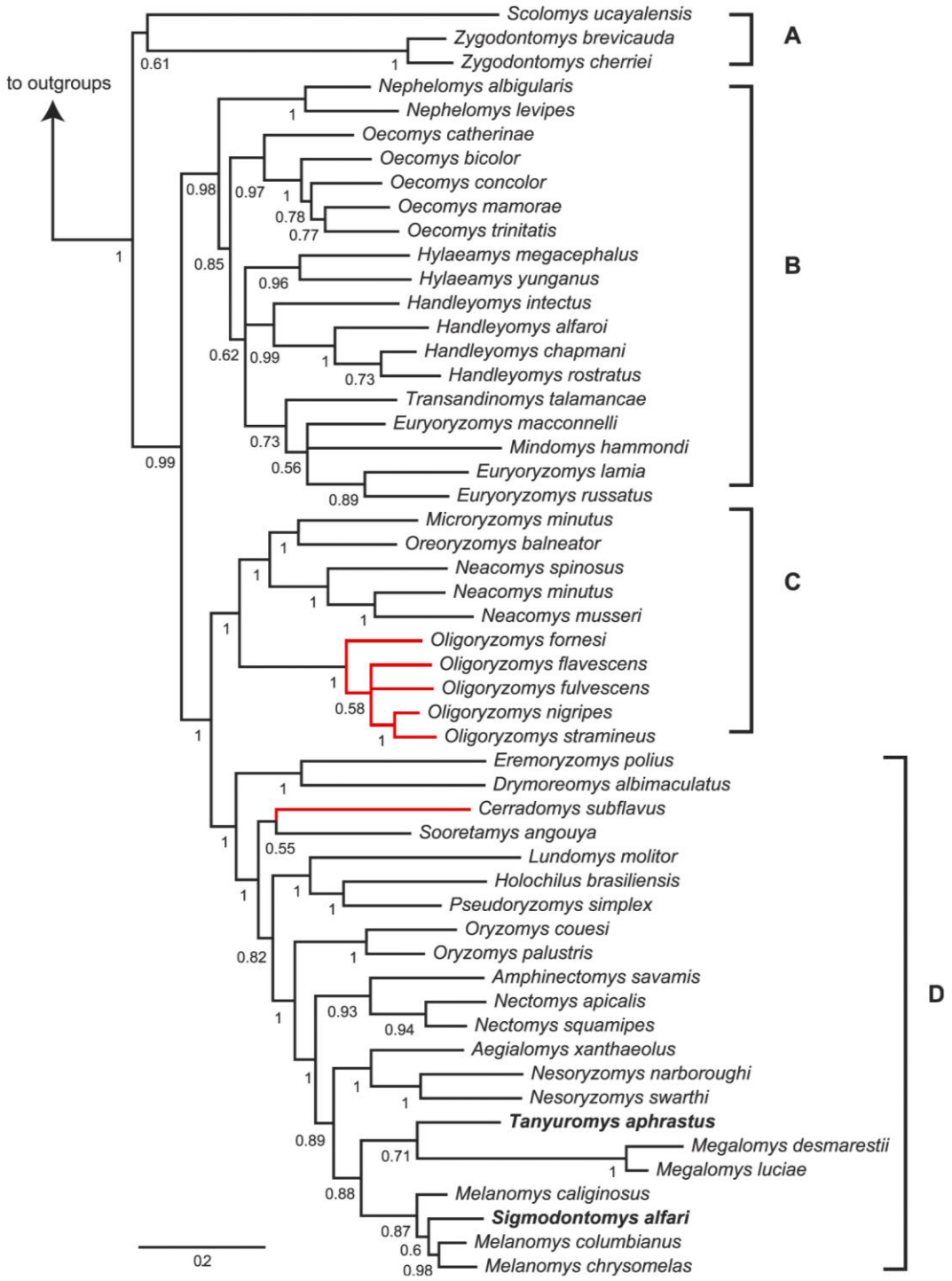

Fig. 1: Bayesian phylogenetic relationships of Oryzomyini using morphological and molecular (12S, cytochrome-*b* and interphotoreceptor retinoid–binding protein) characters. Numbers represent posterior probabilities. Outgroups include *Delomys sublineatus, Nyctomys sumichrasti, Peromyscus maniculatus, 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.

Chapter 2
Integrative taxonomy reveals species limits and patterns of diversification in Cerradomys (Sigmodontinae: Oryzomyini)

Abstract

Cerradomys is a genus of the Tribe Oryzomyini with eight species described so far. Representatives are mainly distributed in the diagonal of open formations, but some species reach through Atlantic Forest and *C. goytaca* is an endemic species of the Restinga formation. Integrative taxonomy is the use of multiple methods to investigate species limit to address taxonomic issues. In this study, cytogenetic and molecular data using four loci that evolves at different rates (two mitochondrial and two nuclear, being one exon and one intron) were performed in a sample that includes all the *Cerradomys* species reported, from 66 localities. Results revealed that cytogenetic is a marker to characterize all species. Molecular phylogeny recovered *Cerradomys subflavus* paraphyletic and DNA barcoding, population genetics, single-locus coalescent-based species delimitation did not recognized *C. subflavus* and *C. goytaca* as two distinct species. The multi-locus species delimitation detected two species within *C. langguthi* and four species within *C. subflavus*. Based on integrative taxonomy, we recognize at least the eight *Cerradomys* species described and we encourage to perform a taxonomic revision in *C. langguthi* and *C. subflavus*, in order to evaluate hypothesis of species-complexes or incipient species in both situation. Times of divergence revealed that *Cerradomys* is a genus extremely recent and the diversification occurred mainly during the Pleistocene. Climate shifts, together with the Quaternary geotectonic factors, are probably the main responsible processes for diversifications in this genus. This work corroborates that interdisciplinary approaches are mandatory to identify species properly.

Keywords: Interdisciplinarity, species delimitation, cytotaxonomy, molecular dating

1. Introduction

Oryzomyini is a complex and speciouse tribe of the Subfamily Sigmodontinae that underwent many taxonomic changes during the last two decades (Weksler *et al.*, 2006; Weksler and Percequillo, 2011; Percequillo *et al.*, 2011; Pine *et al.*, 2012).

Cerradomys used to belong to the genus *Oryzomys* but, together with other species or species group, it was erected to the genus category after molecular and morphological analyses (Weksler *et al.*, 2006).

Cytogenetic studies performed during the 1980s and 1990s were the first indications that "*Oryzomys subflavus*" could in fact contain more than one species, since

four different karyotypes were attributed to a single taxonomic entity (Maia and Hulak, 1981; Almeida and Yonenaga-Yassuda, 1985; Zanchin, 1988; Svartman and Almeida, 1992; Silva, 1994; Bonvicino *et al.*, 1999).

Interdisciplinary approaches, which included morphology and molecular phylogeny, posteriorly confirmed the previous cytogenetic information that the taxon was not monotypic (Bonvicino and Moreira, 2001; Bonvicino, 2003; Percequillo *et al.*, 2008). Currently, eight species are described: *C. akroai, C. goytaca, C. langguthi, C. maracajuensis, C. marinhus, C. scotti, C. subflavus* and *C. vivoi* (Bonvicino and Moreira, 2001; Bonvicino, 2003; Langguth and Bonvicino, 2002; Percequillo *et al.*, 2008; Tavares *et al.*, 2011; Bonvicino *et al.*, 2014).

Cerradomys' representatives inhabit mainly the diagonal of open vegetation areas in South America, from northeastern Brazil to southeastern Bolivia and northwestern Paraguay (Carleton and Musser, 2005; Percequillo *et al.*, 2008; Tavares *et al.*, 2011; Percequillo, 2015). Interestingly, *C. langguthi, C. subflavus* and *C. vivoi* alongside their diagonal distribution can penetrate in the Atlantic Forest, and *C. goytaca* is the only species endemic to the sandy coastal soils of Restinga formation (Percequillo *et al.*, 2008; Tavares *et al.*, 2011).

Phylogenetically, based in one marker (cytochrome *b*), *C. goytaca* and *C. subflavus* were not reciprocally monophyletic, suggesting that both species are conspecific (Bonvicino *et al.*, 2014). In the same work, *C. vivoi* was not reciprocally monophyletic in relation to *C. subflavus* and *C. goytaca* on Maximum Likelihood and Bayesian Inference analyses, although Maximum Parsimony recovered the monophyly of *C. vivoi*.

More recently, Tavares *et al.* (2016) using molecular phylogeny (with cytochrome *b*) and morphometric analyses demonstrated that, despite the polytomy of *C. goytaca* and *C. subflavus*, the former had undergone an extreme accelerated rate of phenotypic evolution, showing particular craniometric attributes.

'Integrative taxonomy' is an approach to delimit species using multiple and complementary perspectives such as phylogeny, comparative morphology, population genetics, ecology, development, behavior, etc. (Dayrat, 2005; Will *et al.*, 2005), providing consistent hypothesis in recognition of biological units. The confidence level increases when different types of data are corroborated and there is no superiority of one character over another (Dayrat, 2005; Sukumaran and Gopalakrishnan, 2015).

Interdisciplinary studies proved to be essential to understand this complex group, since different methods have been provided conflicting results on species limit. In order to investigate species limits, phylogenetic relationships and evolutionary history of *Cerradomys*, cytogenetic and phylogenetic inference, population genetics analyses, coalescent-based species delimitation, DNA barcoding and divergence times estimation were performed in a large sample comprising all the eight species described and almost the entire distribution of the genus, according to an integrative taxonomy approach.

2. Material and Methods

2.1. Samples

Samples from 93 individuals belonging to all the eight *Cerradomys* species described so far were analyzed under molecular approaches. From this amount, cytogenetic data were performed in 35 individuals (Table 1). Samples were collected in 41 localities from 10 Brazilian states. A map containing localities from the present work plus localities from samples downloaded from GenBank is shown in Figure 1, totalizing 65 localities from Brazil and one from Paraguay.

Surveys were carried out under ICMBio licences (numbers 11603-1 and 24003-4) of Instituto Chico Mendes de Conservação da Biodiversidade. Some specimens were captured by collaborators under their respective licences. Animals were euthanized according to the protocol of the "Animal experimentation ethics" (Carpenter *et al*., 1996) and under permission of Instituto Butantan Ethics Committee (1151/13) (Appendix 1). The skins, skulls and partial skeletons were deposited in the Museu de Zoologia da Universidade de São Paulo (MZUSP), Museu Nacional da Universidade Federal do Rio de Janeiro (MN), Museu de História Natural da Universidade Federal de Alagoas (MUFAL), Núcleo de Pesquisa em Ecologia e Desenvolvimento Sócio-Ambiental de Macaé (NUPEM), Museu de Zoologia João Moojen da Universidade Federal de Viçosa (MZUFV) and Coleção de Mamíferos da Universidade Federal do Espírito Santo (UFES) (Table 1).

Specimens were named based on geographic distribution and comparisons of our molecular phylogeny (including holotypes and paratypes) and chromosome data with previous studies (Maia and Hulak, 1981; Almeida and Yonenaga-Yassuda, 1985; Bonvicino and Moreira, 2001; Bonvicino, 2003; Langguth and Bonvicino, 2002; Percequillo *et al.*, 2008; Tavares *et al*., 2011; 2016; Bonvicino *et al.*, 2014). Samples

assigned as *Cerradomys goytaca* were also identified by morphology (Pablo Rodrigues Gonçalves, personal communication, April, 2014).

2.2. DNA extraction, amplifications and sequencing

DNA was extracted from liver or muscle using Chelex100 (Walsh *et al.*, 1991). Partial cytochrome *b* (cyt-*b*), cytochrome oxidase subunit 1 (COI), interphotoreceptor retinoid binding protein (IRBP), and intron 7 of β-fibrinogen (i7FBG) sequences were amplified using Polymerase Chain Reaction (PCR). Primers and cycle conditions are presented in Table 2. PCR were conducted in a thermalcycler (Eppendorf Mastercycle) and mixture consisted of a final volume of 18 μL containing 0.216 μL of Platinum® Taq DNA Polymerase (Invitrogen), 1.8 μL of DNA buffer, 0.72 μL of $MgCl_2$ (50mM), 0.36 μL of dNTP, 0.216 μL of each primer, 0.72 μL of DNA and 13.752 μL autoclaved water. Products were visualized using 1% agarose gel in TAE buffer, and purification was carried out with an ExoSAP-IT (Code number US78200, GE Healthcare).

Sequencing was performed with BigDye (Applied Biosystems) in ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). Electropherograms were visualized and aligned with Geneious 7.1.7 (Kearse *et al.*, 2012) using MUSCLE (Edgar, 2004). Sequences were submitted to a comparative similarity search on BLAST (*Basic Local Alignment Search Tool*), before the alignment. For nuclear sequences, double peaks were codified in both strands as ambiguous sites according to IUPAC code. Sequences will be submitted to GenBank after submission of the manuscript and acceptance.

Six matrices were constructed: (i) cyt-*b* matrix (733 pb; 135 terminal taxa) composed of 88 sequences obtained herein plus 41 sequences downloaded from GenBank (used for phylogenetic inference, single-locus coalescent-based species delimitation and pairwise distance); (ii) COI matrix (616 pb; 91 terminal taxa) composed only of sequences obtained in this work, since there is no sequence available on GenBank/BOLD (used for phylogenetic inference and DNA barcoding); (iii) IRBP matrix (790 pb; 92 terminal taxa) composed of 90 sequences obtained herein plus two sequences from GenBank (used for phylogenetic inference and phylogeography); (iv) i7FBG matrix (676 bp; 93 sequences) with only sequences obtained herein, since there is no sequence available on GenBank (used for phylogenetic inference and phylogeography); (v) concatenated mitochondrial matrix (cyt-*b*+COI) (1349 bp; 53 terminal taxa) (used for phylogeography) and (vi) concatenated multi-locus matrix (cyt-

b+COI+IRBP+i7FBG) (2815 pb; 95 sequences) (used for phylogenetic inference, coalescent-based species delimitation and molecular dating) (Table 1).

2.3. Phylogenetic reconstruction and evolutionary distance

The phylogenies were reconstructed based on Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI). All analyses were performed using the four genes independently and concatenated multi-gene data set using only sequences obtained in this work, with few missing data (Table 1). Outgroup was composed of *Oligoryzomys flavescens*, *Holochilus brasiliensis* and *Calomys tener* (*sensu* Weksler, 2006) (Table 3).

Maximum Parsimony was performed in TnT 1.1 (Goloboff *et al.*, 2008). For ML and BI, the best-fit partitioning schemes and models of nucleotide substitution were selected using the Bayesian information criterion (BIC) implemented in PartitionFinder version 2.1.1 (Lanfear *et al.*, 2016). ML was carried out with GARLI 0.951 (Bazinet *et al.*, 2014). Statistical support for the nodes was estimated by nonparametric bootstrapping (Felsenstein, 1985), with 1000 pseudoreplicates. Bayesian inference was carried out in MrBayes 3.2.6 (Ronquist and Huelsenbeck, 2003). Markov chains were started from a random tree and run for 1.0 x 10^7 generations with sampling every 1000th generation. The stationary phase was checked using Tracer 1.6 (Rambaut *et al.*, 2014). Sample points prior to the plateau phase were discarded as burn-in, and the remaining trees were combined to find the maximum *a posteriori* estimated probability of the phylogeny. Branch supports were estimated with Bayesian posterior probabilities. Two simultaneous analyses were performed to ensure convergence on topologies.

Evolutionary distance between pairs of cyt-*b* sequences was estimated with MEGA 7 (Kumar *et al.*, 2016) using Kimura 2-parameter (K2P).

2.4. Population genetics

Due to sample size, *C. vivoi, C. subflavus* and *C. goytaca* were analyzed under population genetics approaches.

Nucleotide (π) and haplotype (Hd) diversity as well as neutrality test indexes Tajima's D (Tajima, 1989) and Fu's (Fu, 1997) were estimated in DnaSP 5.10 (Librado and Rozas, 2009), with 10.000 permutations. The haplotypes were identified using DnaSP 5.10 (Librado and Rozas, 2009), and for nuclear genes, haplotypes from heterozygous individuals were resolved using the PHASE algorithm implemented with

default settings, for 1.000 iterations and burn-in 100. Haplotype network was obtained in Network 4.610 (http://www.fluxus-engineering.com/sharenet.htm), using median-joining (MJ) method (Bandelt *et al*., 1999). Analysis of molecular variance (AMOVA, Excoffier *et al.*, 1992) was performed using ARLEQUIN 3.5 (Excoffier *et al.*, 2005) with 10.000 permutations.

2.5. Coalescent-based species delimitation methods

Different methods of species delimitation using single-locus (cyt-*b*) and multi-locus (cit-*b*, COI, IRBP and i7FBG) were performed in order to identify the point when population genetics begin to produce phylogenetic patterns (Carstens *et al.*, 2013). For single-locus analyses, Bayesian implementation of Poisson Tree Processes (bPTP) and Bayesian General Mixed Yule Coalescent model (bGMYC) were used (Pons *et al*., 2006; Fujisawa and Barraclough, 2013; Zhang *et al*., 2013).

For bPTP analysis, the ML topology was used as input on the web server of the Exelixis Lab (http://species.h-its.org/ptp) (Zhang *et al*., 2013). To perform bGMYC analysis, an ultrametric tree reconstructed and calibrated by the Bayesian method using BEAST 1.8.3 (Drummond *et al*., 2012) was used, with the same nucleotide substitution model as in the phylogenetic analyses with an uncorrelated log-normal relaxed clock and Yule prior. The tree was implemented in GMYC web service (http://species.h-its.org/gmyc/) assuming a single threshold (Pons *et al*., 2006; Fujisawa and Barraclough, 2013; Zhang *et al*., 2013).

Multi-locus coalescent-based analysis was applied in Bayesian Phylogenetics and Phylogeography (BPP) software 3.3 (Yang and Rannala, 2010; Yang, 2015). Population size parameters (θs) were assigned by gamma prior G(2, 1000), with mean 2/2000 = 0.001. The divergence time at the root of the species tree (τ0) was assigned by the gamma prior G(2, 1000), while the other divergence time parameters were assigned by the Dirichlet prior (Yang and Rannala, 2010: equation 2).

2.6. DNA Barcoding

Each sequence was compared to the database Barcode of Life Data System (BOLD) using the tool BOLD identification system (IDS). Genetic distances using K2P molecular evolution model and NJ tree with 1000 bootstrap replication were implemented using MEGA 7 (Kumar *et al.*, 2016). Distance values were analyzed in

Microsoft Office Excel, in order to construct the distribution graph of intra and interspecific variations of all data set.

2.7. Cytogenetics

Metaphases were obtained *in vivo* from spleen and bone marrow or *in vitro* from fibroblast cell culture, according to Ford and Hamerton (1956) and Freshney (1986), respectively, with modifications.

Conventional Giemsa staining was used to determine the diploid (2n) and the number of autosome arms (FN). CBG-banding was performed according to Sumner (1972). Metaphases were captured with visible light on Axioskop 40 microscope (Carl Zeiss) using AxioVision software or with Zeiss Axiphot microscope (Carl Zeiss) using Ikaros Metasystems sofware.

2.8. Molecular dating

Divergence times were estimated using multi-gene data set. Relaxed Bayesian molecular clock with uncorrelated lognormal rates was implemented in BEAST 1.8.3 (Drummond *et al*. 2012). Four analyses were run with 10 million generations, sampled every 1000 generations. A Birth and Death incomplete sampling with lognormal priors, random starting tree, and a GTR+I+G model for each partition were implemented. The resulting BEAST log files were viewed in Tracer 1.6 (Rambaut *et al.*, 2014) to check convergence. Mutation rate was set with an exponential distribution prior according to Nabholz *et al.* (2008). Eleven calibration points based on fossil records were used as minimum constraints, according to Leite *et al*. (2014) and Tavares *et al.* (2016) (Table 4). These calibrations were performed with lognormal prior so that 5% corresponds to the minimum age of the fossil and 95% interval allows for the uncertainty of the maximum fossil age.

3. Results

3.1. Phylogenetic reconstruction and pairwise distance of cyt-b

For cyt-*b* sequences, the best-fit models selected to perform phylogenetic analyses were GTR+I+G for the first and third codon positions and GTR+G for the second codon position. For COI matrix, the models selected were GTR+G for the first and second positions and GTR for third codon position. The models selected for IRBP were GTR+I+G for the first and second positions and GTR+G for the third position. Finally,

GTR+I+G was the model selected for i7FBG matrix. These models were also used for concatenated analyses.

All analyses (single and concatenated data sets) recovered *Cerradomys* as monophyletic. Phylogenetic reconstructions using cyt-*b* and concatenated matrix recovered seven main clades (Clades A-G). The analyses using COI sequences recovered clades A-E reciprocally monophyletic while clades F and G were recovered as polytomies (data not shown). The nuclear genes IRBP and i7FBG only recovered clades A and B reciprocally monophyletic in all analyses (data not shown).

For cyt-*b*, the seven main clades recovered in MP, ML and BI analyses were: **Clade A** represented by *C. marinhus,* including the paratype (99MP/100ML/1.0BI); **Clade B** by *C. maracajuensis,* including the holotype (100MP/94ML/1.0BI); **Clade C** by *C. akroai*, also including sequence of the holotype (99.8MP/99ML/1.0BI); **Clade D** by sequences from individuals treated as *C. scotti* (98.3MP/96ML/0.91BI); **Clade E** is composed of *C. langguthi*, including sequence of the holotype (97MP/<70ML/1.0BI); **Clade F** *C. vivoi*, including the holotype (99.9MP/<70ML/1.0BI) and **Clade G** of sequences identified by *C. goytaca* and *C. subflavus* (<70MP/ML/<0.7BI) (Appendix 2).

Multi-gene matrix recovered the same seven clades and topology as cyt-*b* in all analyses (MP, ML and BI), with higher support. Thus, herein, we are describing multi-gene results. MP recovered four equally parsimonious trees (length: 1460, CI: 0.63 and RI: 0.89) and the best ML tree for concatenate matrix had a –ln likelihood score of -11876.52395 (Fig. 2).

Relationships among clades were the same in MP, ML and BI analyses and recovered *Cerradomys marinhus* and *C. maracajuensis* (Clades A and B) as sister clades with high support (96MP/97.9ML/1.0BI). Clade A is composed of sequences from Cerrado of Minas Gerais and Bahia and Clade B of sequences from Cerrado of Goiás (Fig. 2).

Clades A and B were recovered sister of all other *Cerradomys* species. Clade C (*C. akroai*) is composed of individuals from the Cerrado of Piauí state and is closely related to the widespread *C. scotti* (clade D) that included sequences from the Brazilian states of Goiás, Minas Gerais and Mato Grosso do Sul, with high support (100MP/100ML/1.0BI). Clades C and D are sisters of a group composed of clades E, F and G (100MP/100ML/1.0BI), from Eastern Brazil (Fig. 2).

Clade E is composed of *C. langguthi*, with individuals from northeast Brazil, distributed in Atlantic Forest, Cerrado and Brejos (mountain ranges of humid forests in areas of Caatinga). Two well structured clades were recovered within Clade E, one subclade with sequences exclusively from Atlantic Forest and Brejos (<70MP/ 77.1ML/ 0.97BI) and the other clade with sequences from Brejos and Cerrado (<70MP/ 92.5ML/0.99BI). Samples from Pacoti (Ceará state, Brazil, region of Brejos) are shared in both subclades (Fig. 2).

The sister-clades F and G have high support (99MP/100ML/0.93BI) and is composed of *C. vivoi* (clade F), which is distributed in Cerrado, Caatinga, Atlantic Forest and transitional areas of Minas Gerais, São Paulo and Bahia; *C. subflavus* and *C. goytaca* (clade G), the first one distributed in Atlantic Forest and Cerrado and the last one endemic to Restingas. None of the phylogenetic analyses (MP, ML and BI) recovered *C. subflavus* and *C. goytaca* reciprocally monophyletic.

Within Clade G, *C. goytaca* clustered in a clade with moderate to high support (86MP/99.7ML/1.0BI) (Fig. 2). *Cerradomys subflavus* were recovered in four main subclades with low to high support: (i) composed of sequences from Cerrado and transitional areas of Atlantic Forest of Minas Gerais and São Paulo states (<70MP/94ML/<0.7BI); (ii) composed of sequences from Atlantic Forest and Cerrado of Minas Gerais, Espírito Santo and Bahia states (<70MP/99.3ML/0.99BI); (iii) composed of sequences from Atlantic Forest and Cerrado of Espírito Santo and Minas Gerais states (<70MP/93ML/1.0BI) and (iv) composed of sequences from Atlantic Forest and Cerrado of Minas Gerais and São Paulo states (88MP/97.7ML/1.0BI) (Fig. 2).

Intraespecific distances varied from 0 (zero) in *C. vivoi* to 1.2% in *C. langguthi*. The lowest interespecific distance was observed between *C. subflavus* and *C. goytaca* (0.7%) and the highest was observed between *C. maracajuensis* and *C. goytaca* (15%) (Table 5).

3.2. Phylogeographic data

Phylogeographic analyses were performed in populations of *C. vivoi* (Clade F), *C. subflavus* and *C. goytaca* (Clade G).

For the mitochondrial data set, twenty-five haplotypes were observed for the total sample, from which seven correspond to *C. vivoi*, one to *C. goytaca* and 17 to *C. subflavus* (Table 6, Fig. 3). The haplotype network was colored according to the species

and showed two major haplogroups separated by 18 evolutionary steps that correspond to *C. vivoi* (black) and *C. subflavus* (yellow) and *C. goytaca* (blue) (Fig. 3b). Within this last haplogroup, *C. goytaca* is represented by one haplotype (blue), separated from *C. subflavus* (yellow) by six evolutionary steps (Fig. 3b). *Cerradomys subflavus* showed a substructure corresponding to the subclades (i), (ii and iii) and (iv) of the molecular phylogeny (Fig. 3b). Haplotype diversity (Hd) was high in *C. vivoi* and *C. subflavus* and zero in *C. goytaca*. Nucleotide diversity (π), instead, was low in all three species (Table 6).

Haplotype networks using nuclear genes were not structured in both IRBP and i7FBG data sets (Fig. 3c and 3d, respectively). IRBP recovered seven haplotypes (one shared between the three species and one shared between *C. subflavus* and *C. vivoi*) (Fig. 3c). Haplotype diversity (Hd) was moderate in *C. vivoi,* low in *C. goytaca* and high in *C. subflavus* and nucleotide diversity (π), low in all three species (Table 6). i7FBG recovered 22 haplotypes, two of them shared by the three species and one shared by *C. subflavus* and *C. vivoi* (Fig. 3d). For this sequence, the three species showed high haplotype (Hd) and low nucleotide diversity (π) (Table 6).

Neutrality tests were not significant, except for *C. vivoi* in mitochondrial data set (Table 6).

Analysis of molecular variance (AMOVA) was performed with the mitochondrial data set for lineage G (*C. subflavus* and *C. goytaca*) and showed that the highest percentage of genetic variation was observed among populations (51.03%) and that the fixation index are 0.51028 ($p<0,001$) (Table 7).

3.3. Coalescent-based species delimitation methods

The bPTP method using cyt-*b* matrix recognized seven distinct lineages: *C. marinhus, C. maracajuensis, C. akroai*, *C. scotti, C. langguthi*, *C. vivoi* and *C. subflavus* + *C. goytaca*. *Cerradomys subflavus* and *C. goytaca* were considered a single putative species (Appendix 2).

bGMYC recovered six maximum likelihood entities with confidence interval from 2 to 7. The bGMYC model (ML=1266.595) was preferred over the null model (ML=1260.211). The likelihood-ratio test to determine if there was a change from interspecific to intraspecific processes was significant ($p = 0.0016$). The six species recognized in bGMYC were the same as in bPTP method, except that *C. vivoi* was not

considered a single putative species, clustering together with *C. subflavus* and *C. goytaca*, so that these three last species were considered one entity (Appendix 2).

Multi-locus species delimitation method (BPP) recovered 12 lineages as putative species, with high posterior probability. The analysis detected two species within *C. langguthi* and four within *C. subflavus* in addition to *C. marinhus*, *C. maracajuensis*, *C. akroai*, *C. scotti*, *C. vivoi* and *C. goytaca* (Fig. 2).

3.4. DNA Barcoding

Neighbor-Joining topology (NJ) recovered the genus as monophyletic with low support value (bootstrap = 47). *Cerradomys maracajuensis*, *C. marinhus*, *C. scotti*, *C. akroai*, *C. vivoi* and *C. langguthi* were monophyletic, with boostrap support equal to 100, 100, 100, 99, 54 and 49, respectively. *C. goytaca* and *C. subflavus* were not reciprocally monophyletic. This result is similar to the one observed for phylogenetic analyses with MP, ML and BI (NJ topology not shown).

Intraespecific distances varied from 0 (zero) to 1.2% and the mean distance is 0.37%. The highest intraespecific value was observed for *C. langguthi* (1.2% ± 0.36) and the lowest for *C. maracajuensis* and *C. goytaca* (zero) (Table 8).

Interespecific distance varied from 0.9% (*C. subflavus* and *C. goytaca)* to 14.4% (*C. maracajuensis* and *C. vivoi*) and the mean distance was 8.4% ± 1.5% (Table 8). Overlapping between intra and interespecific distance was observed (Fig. 4).

3.5. Cytogenetics

From the 35 individuals analyzed, karyotypes were attributed to eight names: (i) *C. marinhus* - 2n=56, FN=54 (Fig. 5a); (ii) *C. maracajuensis* - 2n=56, FN=58 (Fig. 5b); (iii) *C. akroai* - 2n=60, FN=76 (Fig. 5c); (iv) *C. scotti* - 2n=58, FN=72 (Fig. 5d); (v) *C. langguthi* - 2n=46, FN=56 (Fig. 5e); (vi) *C. vivoi* - 2n=50, FN= 64 (Fig. 5f); (vii) *C. goytaca* - 2n=54, FN=66 (Fig. 6a) and (viii) *C. subflavus* – 2n=54-56, FN=62-64 (Fig. 6b-d).

Chromosomal heteromorphisms and polymorphisms were observed in our sample. *Cerradomys marinhus* showed a variation in the size of X chromosome in one female (Fig. 5a). In *C. akroai*, CBG-banding showed C-positive signals in the distal regions of two autosomal pairs (possibly pairs 13 and 14), in addition to the subtle signal at the pericentromeric region of the autosomes (Fig. 5c).

Regarding *C. subflavus*, seven cytotypes and three different diploid numbers were observed (Fig. 6b-d): 2n=54, FN=62; 2n=55, FN=63 and 2n=56, FN=64. Differences in the karyotypes are related to pairs 5 and 6. Karyotype (i) showed one very large metacentric pair that corresponds to pairs 5 and 6 (Fig. 6b). Karyotype (ii) showed a very large submetacentric (5/6), one subtelocentric (5b) and one acrocentric (6) chromosomes (Fig. 6c). Karyotype (iii) showed pair 5 subtelocentric (5b) and pair 6 acrocentric. The pair 5 homomorphic subtelocentric is presented for the first time (Fig. 6d). Polymorphisms in sex chromosomes were also observed, being two types of X: large acrocentric (Xa) and large subtelocentric (Xb) and three types of Y: medium acrocentric (Ya), large acrocentric (Yb) and medium subtelocentric (Yc). The Yc is also being described for the first time (Fig. 6b). CBG-pattern was essential to discriminate Xa from Yb.

3.6. Molecular dating

Estimates of divergence times using concatenated data set placed the most recent common ancestor (MRCA) of the genus in the late Pliocene / early Pleistocene at 2.71 Mya (95% HPD: 1.39-3.81 Mya) (node 1) (Fig. 7) and the majority of splits occurred in early to medium Pleistocene (Fig. 7, Table 9). *C. marinhus* and *C. maracajuensis* (node 2) split at 1.27 Mya (95% HPD: 0.35-2.06 Mya) and the remaining species (node 3) were estimated at 1.96 Mya (95% HPD: 0.96-2.98 Mya). Node 4 gave rise to the sister species *C. akroai* and *C. scotti* at 1.09 (95% HPD: 0.39-1.93 Mya) followed by node 5 estimated at 1.09 (95% HPD: 0.45-1.80 Mya) composed of the Eastern Brazilian species *C. langguthi, C. vivoi, C. goytaca* and *C. subflavus*. During the late Pleistocene, *Cerradomys vivoi* diverged from *C. subflavus* and *C. goytaca* at 0.54 Mya (0.21-0.97 Mya) (node 6). The lineage composed of *Cerradomys subflavus* and *C. goytaca* was estimated at 0.31 (0.11-0.51 Mya) (Fig. 7).

4. Discussion

This is the first work that uses multi-gene data set with different approaches, allied to cytogenetic in all species described so far, leading to an integrative taxonomy to access species delimitation and patterns and processes of differentiation in *Cerradomys*. We increased the coverage of the genus by adding 88 sequences of cyt-*b*, 91 of COI, 90 of IRBP and 93 of i7FBG, from all species described so far.

4.1. Integrative taxonomy reveals species limits

Since integrative taxonomy suggests that final decision should be in favour of the majority of the disciplines attributed (Schlick-Steiner *et al.*, 2010; Carstens *et al.*, 2013), based on the integrative results of the present study and previous work, the eight *Cerradomys* species currently described can be considered valid.

Molecular phylogeny recovered seven monophyletic clades (*C. marinhus, C. maracajuensis, C. akroai, C. scotti, C. langguthi, C. vivoi* and *C. goytaca*) while *C. subflavus* was paraphyletic, with samples recovered in four main subclades. Multi-locus species delimitation corroborates the phylogenetic data, and also suggested that *C. langguthi* and *C. subflavus* are polytypic.

The DNA barcoding was effective in identifying the majority of species. However, a clear barcoding gap, as suggested by (Hebert *et al.*, 2003) was not observed, resulting in overlapping of interespecific distance of *C. goytaca* and *C. subflavus*, and intraespecific distance of *C. langguthi*.

Cytogenetic data can distinguish the eight species currently described since each one of them has its own chromosome formula, although polymorphisms were described for *C. langguthi* and *C. subflavus* (Maia and Hulak, 1981; Almeida and Yonenaga-Yassuda, 1985; Zanchin, 1988; Bonvicino *et al.*, 1999; Langguth and Bonvicino, 2002; Tavares *et al.*, 2011; Bonvicino *et al.*, 2014; present work).

Morphology can also diagnose the eight *Cerradomys* species (Percequillo *et al.*, 2008; Tavares *et al.*, 2011; Bonvicino *et al.*, 2014; Tavares *et al.*, 2016), but qualitative and quantitative characteristics may overlap in populations that occur between *C. vivoi* and *C. langguthi* distributions (Caccavo and Oliveira, 2016).

Cerradomys vivoi, *C. goytaca* and *C. subflavus* were considered as a single putative lineage using bGMYC species delimitation and these species were recovered paraphyletic in previous study (Bonvicino *et al.*, 2014). Nevertheless, all of the other molecular analyses (multi-gene phylogeny based on three different criteria, bPTP and BPP species delimitation, DNA barcoding) in addition to karyotype and morphology recognize *C. vivoi* as a distinct species. Phylogeographic data (mithocondrial data set) support this information since two major haplogroups were recovered, one corresponding to *C. vivoi* and the other corresponding to *C. goytaca* and *C. subflavus* haplotypes.

Regarding *C. goytaca* and *C. subflavus*, molecular phylogeny, cyt-*b* distance and DNA barcoding pointed out that they belong to the same entity, while five evidences

support the distinction between them (morphology, cytogenetics, multi-locus coalescent-based species delimitation, mitochondrial haplotype network and allopatric distribution).

Morphology and chromosomes have already been differentiated between *C. goytaca* and *C. subflavus*, while haplotypes did not reach monophyletism in the case of samples assigned to *C. subflavus*, generating a mismatch between data, even using different and unlinked loci. As speciation is a gradual process, incongruent results would be expected, since each character evolves at different rates.

Trying to overcome this issue, the multi-locus coalescent-based species delimitation was applied, and results were congruent with hypothesis that *C. goytaca* is valid and *C. subflavus* may be polytypic. This method uses the multispecies coalescent model to compare different models of species delimitation and species phylogeny in a Bayesian framework, accounting for incomplete lineage sorting due to ancestral polymorphism and gene tree species tree conflicts (Yang and Rannala, 2010; Yang and Rannala, 2014).

Thus, previous hypothesis (based only on cyt-*b*) that suggests *C. goytaca* as junior synonym of *C. subflavus* (Bonvicino *et al.*, 2014) is incongruent with our results (based on multi-locus and integrative taxonomy approach) that recovered *C. goytaca* and *C. subflavus* as distinct.

Besides, two evidences based on four loci suggest that *C. subflavus* may be a cryptic species (its paraphyletism in relation to *C. goytaca* and the multi-locus coalescent-based analysis). Nevertheless, the single locus analyses of K2P distance (0.6%) and DNA barcoding did not support *C. subflavus* as polytypic. In addition, we do not have morphological and cytogenetic information from all samples and, albeit this species present chromosome polymorphisms, the three karyotypes were found in the same subclade.

Evidences advocate that *C. langguthi* may also represent species-complex or incipient species. Two evidences suggest its species-complex status (the presence of two subclades in the molecular phylogeny and multi-locus coalescent-based analysis) whereas K2P distance (1.2%) and DNA barcoding evidenced only one entity. Although chromosome polymorphism were observed in this species (Maia and Langguth, 1981), the only karyotype observed in our sample (2n=46, FN=56) was present in both subclades and morphological data is not available for the entire sample.

We suggest that taxonomic revision should be performed in *C. langguthi* and *C. subflavus*, with exhaustive sampling covering its entire distribution, in order to evaluate if these are cases of species-complex or incipient species. Thus, what is been called now as polymorphisms may, in the future, represent distinct fixed karyotypes.

4.2. Population genetics

Mitochondrial haplotype network was congruent to phylogenetic results revealing two major structured haplogroups, one composed of *C. vivoi* and the other of *C. subflavus* and *C. goytaca* samples. In addition, *C. goytaca* were recovered in a single haplotype, separated from haplotypes referred to *C. subflavus*.

Both nuclear haplotypes networks were not well structured, with haplotypes shared between the three species. It is expected that nuclear loci show less pronounced phylogeographic structuration compared to the mitochondrial one due to their different effective population sizes (N_e) (Hare, 2001). Hare (2001) stated that nuclear loci are feasible to phylogeographic studies, althoght its utility should be empirically tested.

The high haplotypic diversity (Hd) and low nucleotide diversity (π) observed in *C. vivoi* and *C. subflavus* is consistent with population expansion of these species. Besides, although *C. goytaca* showed high haplotypic diversity for i7FBG gene, the extremely low haplotypic diversity observed for IRBP and mitochondrial data set corroborates the hypothesis of recent separation of this population from *C. subflavus*. Neutrality testes also corroborate population expansion for *C. vivoi* and *C. subflavus*, although for this last species, values were not significant.

4.3. Cytotaxonomy and chromosome polymorphisms

According to cytogenetic data obtained in this work and previous studies, karyotypes were correlate to eight different names: *C. marinhus* (2n=56, FN=54), *C. maracajuensis* (2n=65, FN=58), *C. scotti* (2n=58, FN=70 and 72), *C. akroai* (2n=60, FN=74 and 76), *C. langguthi* (2n=50, 49, 48, and 46, FN=56), *C. vivoi* (2n=50, FN=64), *C. subflavus* (2n=54, 55 and 56, FN=62-64) and *C. goytaca* (2n=54, FN=66) (Maia and Hulak, 1981; Almeida and Yonenaga-Yassuda, 1985; Zanchin, 1988; Svartman and Almeida, 1992; Bonvicino *et al.*, 1999; Langguth and Bonvicino, 2002; Tavares *et al.*, 2011; Bonvicino *et al.*, 2014).

Karyotype proved to be an important tool in aid species limits because even in the species that share the same diploid number (for example, *C. vivoi* and *C. langguthi* –

2n=50; *C. subflavus* and *C. goytaca* – 2n=54), fundamental number and chromosome size can accurately discriminate them. In addition, although *C. marinhus* and *Pseudoryzomys simplex* (another species of the Tribe Oryzomyini), shares the same karyotype (2n=56, FN=54), when compared to other congeneric species, *C. marinhus* can be karyotypically distinguished.

Several polymorphisms were described in *Cerradomys* species. Almeida and Yonenaga-Yassuda (1985) described seven cytotypes for *C. subflavus*, due to Robertsonian rearrangement in pairs 5 and 6 and sex chromosome polymorphisms. Besides, pericentric inversion was observed in pair 5, which could be homomorphic acrocentric (5a) or heteromorphic acrocentric/subtelocentric (5a5b). Herein, seven cytotypes with three diploid numbers were observed: 2n=54 (individuals from São Paulo and Minas Gerais states), 2n=55 (from Rio Claro, São Paulo state) and 2n=56 (from Ribeirão Preto, São Paulo state). Autosomes were identical as described by Almeida and Yonenaga-Yassuda (1985) in both 2n=54 and 55, but the sample with 2n=56 showed pair 5 homomorphic subtelocentric (5b5b), a variation never described before.

Sex chromosome polymorphisms were detected by Almeida and Yonenaga-Yassuda (1985), that described two types of X and Y: Xa (large acrocentric), Xb (large subtelocentric), Ya (medium acrocentric) and Yb (large acrocentric). In this work we described a new morphology for Y, medium subtelocentric (Yc), probably due to pericentric inversion since the heterochromatic block is located at distal region of this chromosome. Sex chromosome polymorphisms were also detected for *C. scotti* (Svartman and Almeida, 1992) and *C. vivoi* (Silva, 1994).

Pericentric inversion, in addition to *C. subflavus* (Almeida and Yonenaga-Yassuda, 1985), was also described for *C. scotti* – 2n=58, FN=70,72 (Langguth and Bonvicino, 2002) and *C. vivoi* - 2n=50, FN=62-64 (Percequillo *et al.*, 2008). In this work, new fundamental number is being described for *C. akroai* (FN=76), probably due to pericentric inversion in one medium size acrocentric.

Karyotypic changes are very common in rodents and may be associated to speciation due to meiotic problems, generating unbalance gametes or suppression of the recombination in rearranged regions, creating a barrier to gene flow (Noor *et al.*, 2001; Rieseberg, 2001). Robertsonian changes is one of the main rearrangements that lead to species formation (Romanenko and Voloboeuv, 2012), corroborating that *C. langguthi*

and *C. subflavus* (species with Robertsonian rearrangement occurring in large pairs) may be incipient species.

4.4. New distribution records

The large number of samples obtained in this work enhanced the distributional records of four *Cerradomys* species. Although pending taxonomic revision, this is the first record of *Cerradomys langguthi* in Atlantic Forest and Brejos of Alagoas state (ESEC Murici and Mata Grande, respectively) and in the Cerrado of Piauí state. As a matter of fact, Caccavo and Oliveira (2016) have morphologically analyzed samples of *Cerradomys* from Alagoas, but they were not able to identify these individuals since some morphological attributes of *C. langguthi* and *C. vivoi* from this region overlap.

Cerradomys marinhus is only reported from four localities: Jaborandi and Fazenda Formoso (type locality), Bahia state and Parque Nacional Grande Sertão Veredas and Uberlândia, Minas Gerais state (Bonvicino *et al*., 2014; Percequillo, 2015). Herein, we record one more locality in Bahia: Cocos (locality 21).

Cerradomys akroai is a recently described species only known by its type locality (Novo Jardim, Tocantins state), so that this work adds ESEC Uruçuí-Una, in Piauí state, as a new locality.

In addition, new localities of *C. vivoi* presented in this work raised considerably its distribution to north of São Paulo state and upper São Francisco river (Ibiraba, Bahia state). The increase in sample coverage obtained in this study was important not only to understand the boundaries of species but also to reveal patterns of diversification precisely, as it was observed that *C. vivoi* is not restricted to the right bank of São Francisco River, but also occurs in the left bank.

In addition, *C. scotti* and *C. maracajuensis* were found simpatrically in Parque Nacional Emas (Goiás state), *C. marinhus* and *C. scotti* in Jaborandi (Bahia state) and *C. vivoi* and *C. subflavus* in two localities: Juramento (Minas Gerais state) and Andaraí (Bahia state).

4.5. Phylogenetic relationships and patterns of diversification

The monophyly of the genus was recovered which is consistent with several other studies in this group (Bonvicino and Moreira, 2001; Percequillo *et al.*, 2008; Bonvicino *et al.*, 2014; Tavares *et al.*, 2016). Relationships among species have a robust support

and are congruent with other recent phylogenies (Bonvicino *et al*., 2014; Tavares *et al.*, 2016).

Divergence time estimates were similar to the one described by Leite *et al*. (2014) for Sigmodontinae clades and Tavares *et al*. (2016) for *Cerradomys*, and corroborates that this is an extremely recent oryzomyine group. The mean age obtained herein was slightly more recent, probably because we use a multi-locus data set and sequences of *C. akroai*, not included in the molecular dating of Tavares *et al.* (2016).

The origin of the genus probably took place in Cerrado domain giving rise to the majority of species distributed in this biome and latter occupying Caatinga and Atlantic Forest, reaching to Restinga formation. Diversification occurred during Pleistocene, with the almost simultaneous split of Cerrado endemic species *C. marinhus* and *C. maracajuensis* about 1.27 Mya and the remaining species, approximately 1.96 Mya. The split of *C. akroai* and *C. scotti*, both species that also occupy Cerrado, occurred about 1.09 Mya.

The diversification of the East Brazilian species was also estimated in 1.09 Mya, leading to *C. langguthi* in Cerrado, Caatinga (including Brejos) and Atlantic Forest, in the left bank of São Francisco river, northeast Brazil. Two structured subclades were recovered within *C. langguthi*, one composed of samples from Cerrado and Brejos and the other with samples from Atlantic Forest and Brejos

Approximately 0.53 Mya, occurred the split of *C. vivoi*, which is distributed in Caatinga, Cerrado and transition areas of Atlantic Forest of Sergipe, Bahia, Minas Gerais and São Paulo (new recorded observed herein).

Finally, the lineage of *C. subflavus* and *C. goytaca* has recently diversified (about 0.31 Mya). *Cerradomys subflavus* occurs in Cerrado and Atlantic forest (states of São Paulo, Espírito Santo, Minas Gerais, Bahia and Goias) while *C. goytaca* is limited to Restinga of north Rio de Janeiro and south Espírito Santo states. Both species are in allopatry, separated by approximately 500 km of semideciduous Atlantic Forest (Veloso *et al.*, 1991).

Our results corroborate Tavares *et al.* (2016), showing that the expansion of this lineage through the Restinga formation may be facilitated by the Cerrado corridor that possibly conected the north of Rio de Janeiro to central Minas Gerais (Werneck *et al..*, 2012). Posteriorly, climatic oscillation favoured the expansion of Atlantic Forest, creating a barrier between the population of Restinga and the population of Minas Gerais.

Several hypotheses have been postulated to explain the great biodiversity of Neotropics. Theory of forest refugia claims that cycles of contraction and expansion of forests occurred during the Pleistocene, leading to isolation of populations and accumulating differences between them, with consequently interruption of gene flow (Haffer, 1969; Vanzolini and Williams, 1970). The role of rivers as a vicariant barrier was also hypothesized (Wallace, 1852) as well as ecological gradient theory (Smith *et al*, 2001) and geotectonic activity during Quaternary (Batalha-Filho *et al*., 2010; Thomé *et al*., 2010).

Percequillo *et al.* (2008) suggested that São Francisco river represented a barrier for *Cerradomys* species of the Eastern Brazilian clade, as *C. langguthi* is restricted to the left bank of this river while *C. vivoi, C. subflavus* and *C. goytaca* are distributed in the right bank. However, in this work, *C. vivoi* was found in the upper São Francisco River (Ibiraba, Bahia state, locality 58 – Fig. 1).

In addition, Tavares *et al.* (2016) postulated that geographic limit of *C. vivoi*, *C. subflavus* and *C. goytaca* lies between Jequitinhonha and Doce Rivers. Nevertheless, we analyzed a sample of *C. vivoi* from São Paulo state. Besides, although the taxonomic status of *C. subflavus* has to be confirmed, our samples were found in both sides of the Doce river (Fig. 1). Thus, rivers may not have been a primary barrier for speciation in *Cerradomys*.

Cerradomys species are predominantly associated with forested habitats of the open vegetation formation, including gallery forests, moist semideciduous forest patches (Brejos), and woodland savannas (cerradão) (Percequillo, 2015). Based on the recent divergence times, it is likely that climatic oscillations of the Pleistocene and the Quaternary geotectonic played a fundamental role in diversification of the genus.

Noteworthy, recent studies in Atlantic Forest challenged the Pleitocene refugia theory, showing that currently diversity is in fact associated with historically stable forest areas (Thomé *et al.*, 2010; Leite *et al.*, 2016). Leite *et al.* (2016) suggested that the interplay of sea level and land distribution was important for shapping diversity of Atlantic Forest small mammals. Thus, we can not discard the role of the emerged continental shelf in the differentiation of *Cerradomys* species that occupy Atlantic Forest.

4.6. Final conclusions

This work reiterates that interdisciplinary approaches are important to elucidate the diversity of *Cerradomys*, because of the heterogeneity nature of characters. Integrative taxonomy revealed that the eight described *Cerradomys* species are valid and also suggests that *C. langguthi* and *C. subflavus* may represent complex and incipient species.

Data obtained herein, including new distributional records, reiterates that *Cerradomys* and Brazilian fauna are still poorly known.

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Figures and Tables

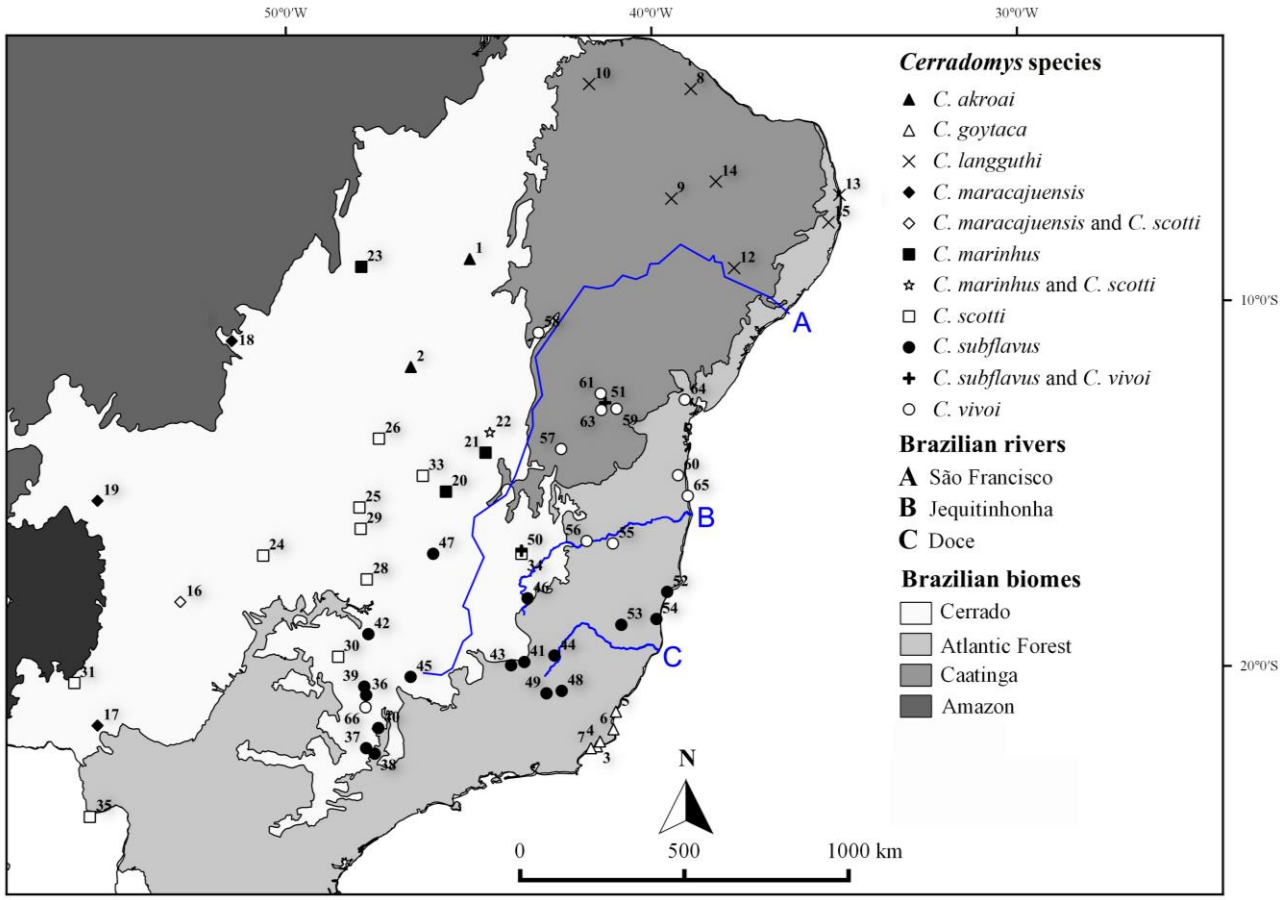

Fig. 1: Geographic distribution of *Cerradomys* sampled in this work plus localities from sequences extracted from GenBank. Numbers correspond to localities in Table 1.

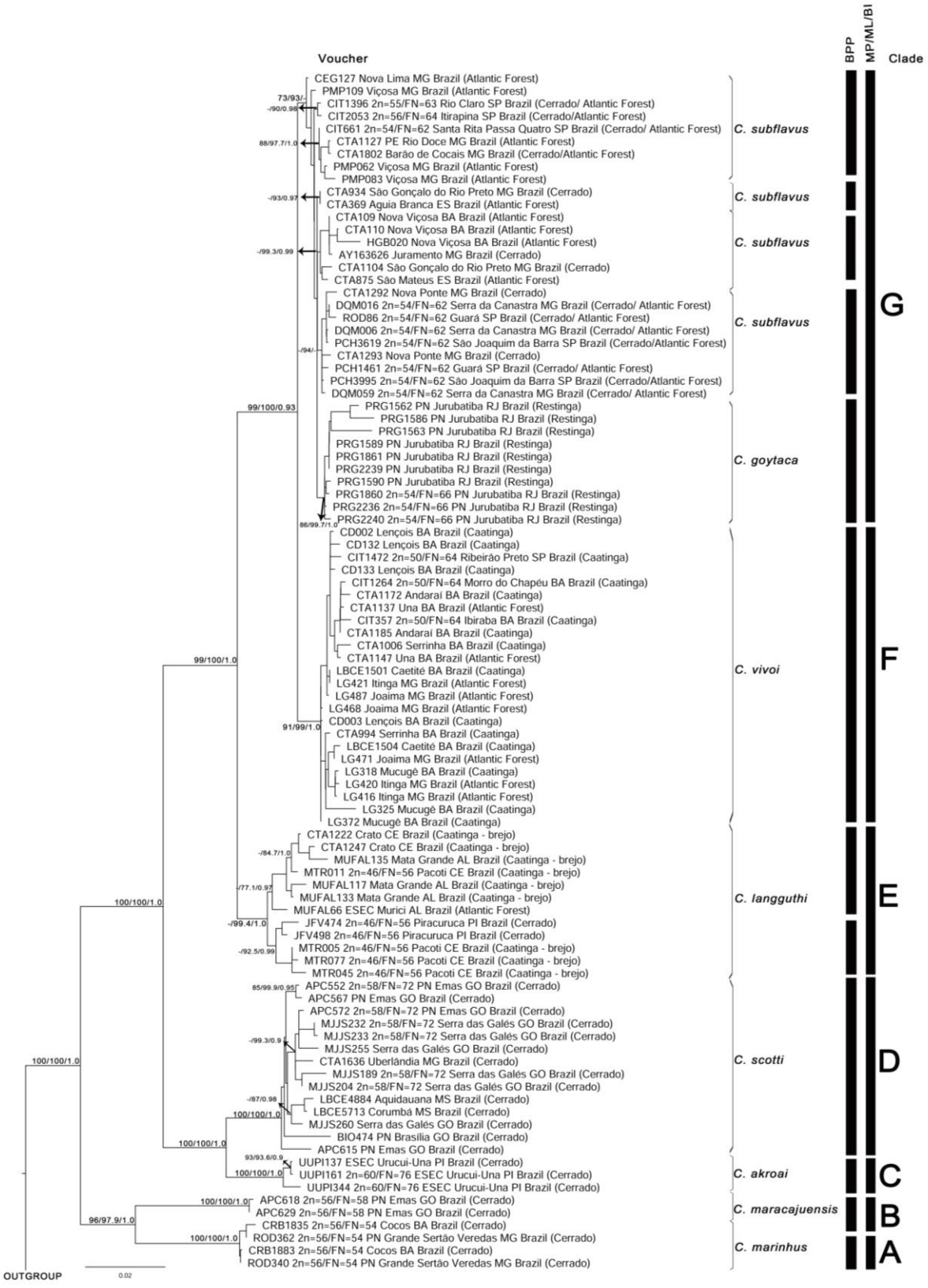

Fig. 2: Maximum Likelihood phylogenetic relationships based on concatenated data set (cyt-*b*, COI, IRBP and i7FBG). Numbers in the nodes indicate MP and ML bootstrap support (above 70) and BI posterior probability (above 0.70), respectively. From the right to the left: first black bar indicate results of multilocus coalescent-based species delimitation (BPP) and second black bars represent clades recovered by MP, ML and BI, respectively.

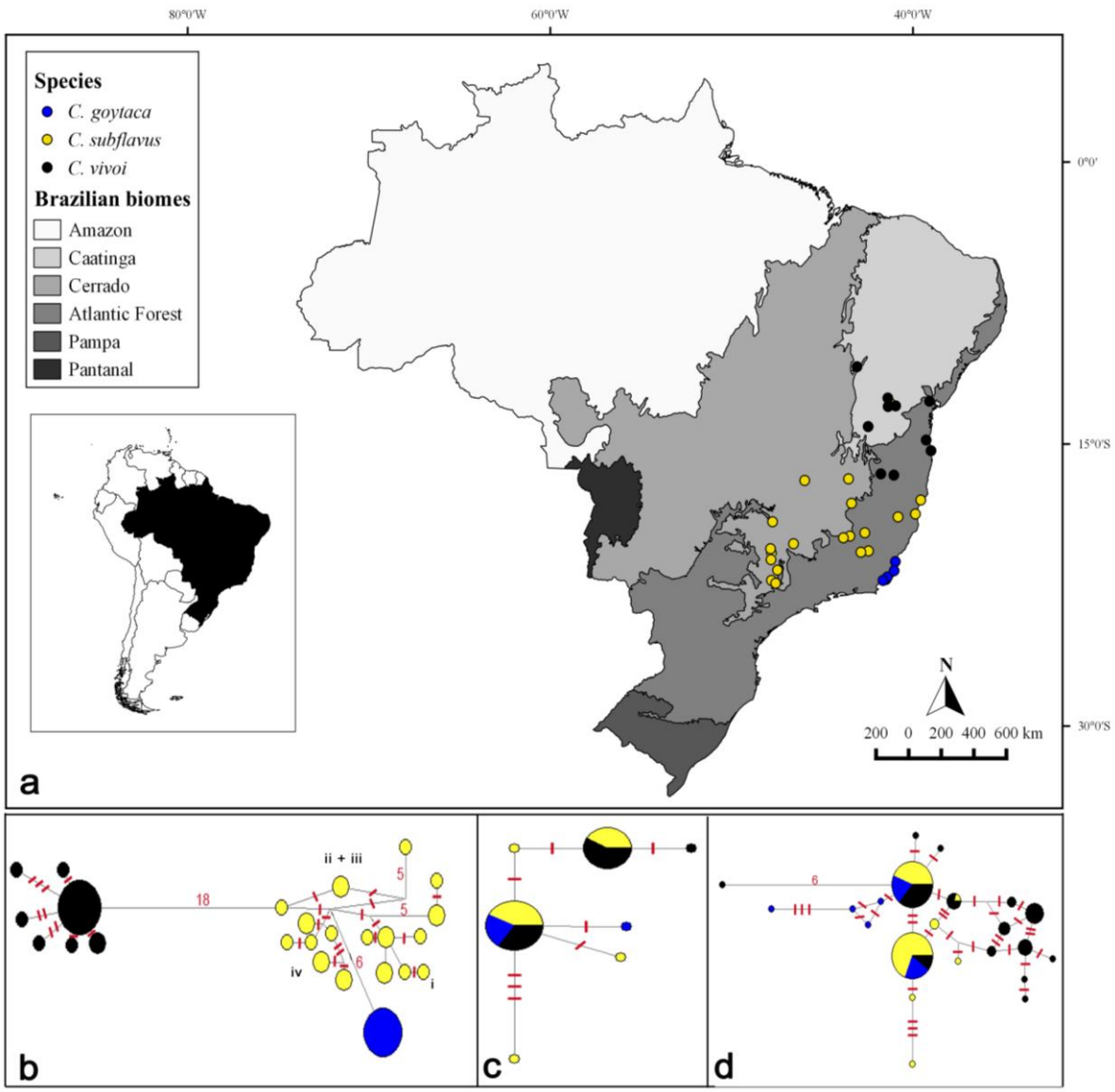

Fig. 3: (a) Distribution of *C. vivoi* (black), *C. subflavus* (yellow) and *C. goytaca* (blue). (b) Haplotype network based on concatenated data set (cyt-*b* and COI). (c, d) Haplotype networks based on nuclear genes IRBP and i7FBG, respectively. Circle size is proportional to the sample size. Each red trace indicates one evolutionary step and five or more steps are indicated by numbers.

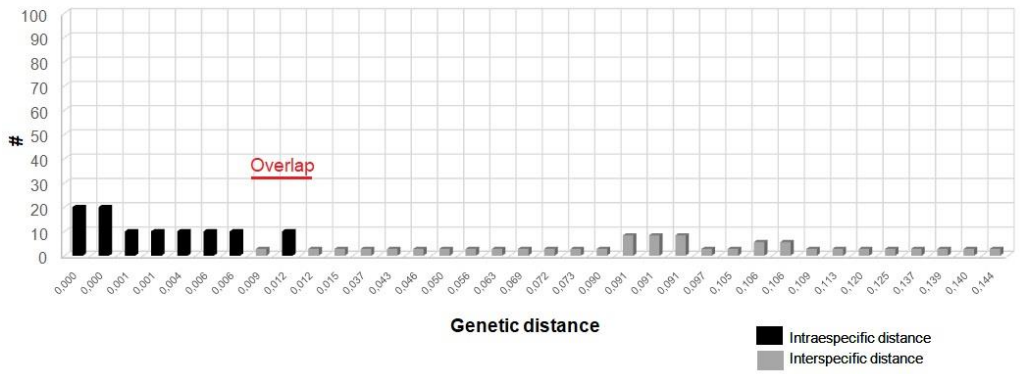

Fig. 4: Distribution of intra and interespecific distance values. Red dash shows region of overlapping.

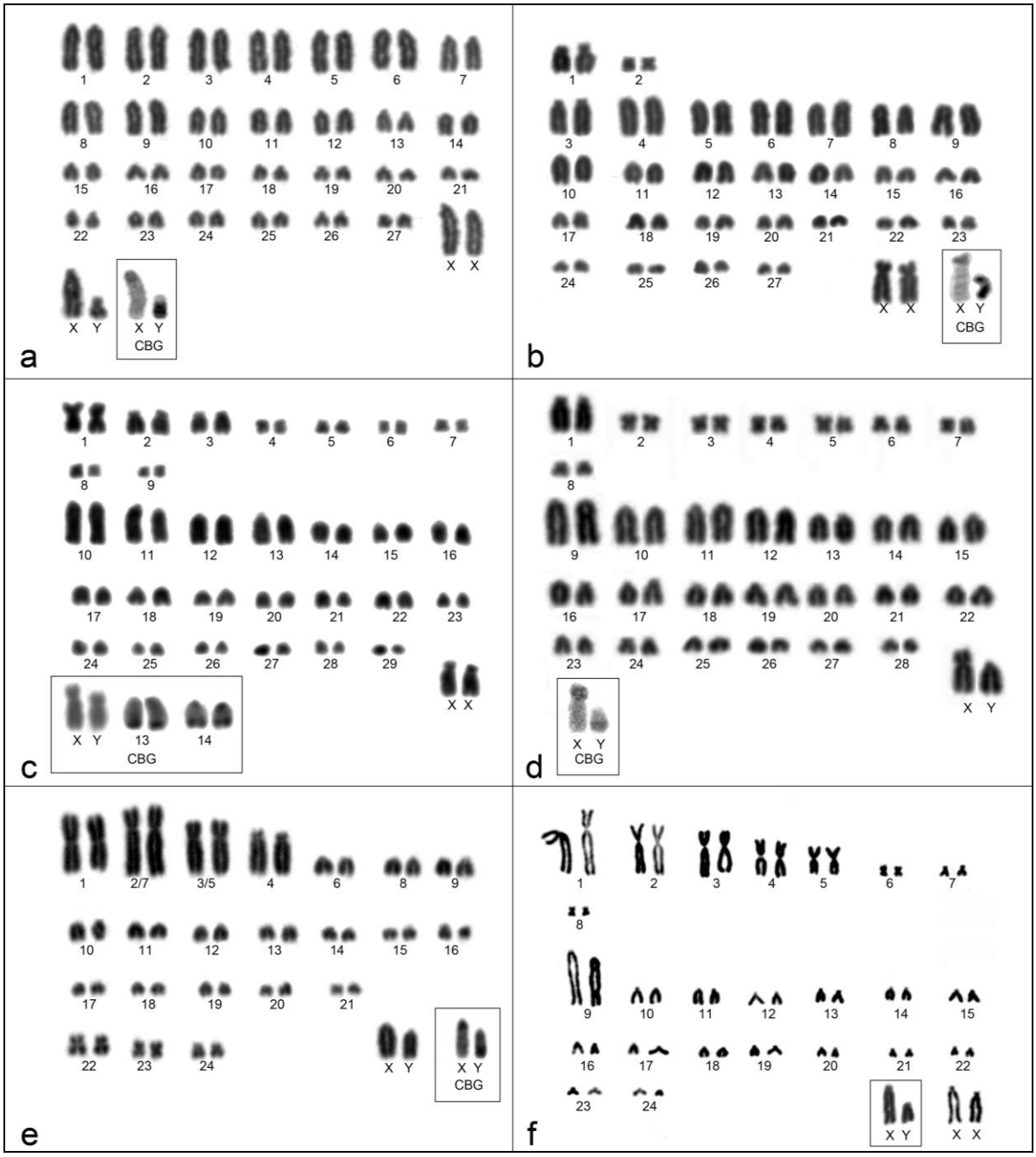

Fig. 5: Karyotypes of *Cerradomys*: (a) ***C. marinhus*** – 2n=56, FN=54 Inset: Sex chromosomes after CBG-banding; (b) ***C. maracajuensis*** – 2n=56, FN=58. Inset: Sex chromosomes after CBG-banding; (c) ***C. akroai*** – 2n=60, FN=76. Inset: Sex chromosomes and pairs 13 and 14 after CBG-banding; (d) ***C. scotti*** – 2n=58, FN=72. Inset: Sex chromosomes after CBG-banding; (e) ***C. langguthi*** – 2n=46, FN=56 Inset: Sex chromosomes after CBG-banding and (f) ***C. vivoi*** – 2n=50, FN=64. Inset: Sex chromosomes of a male.

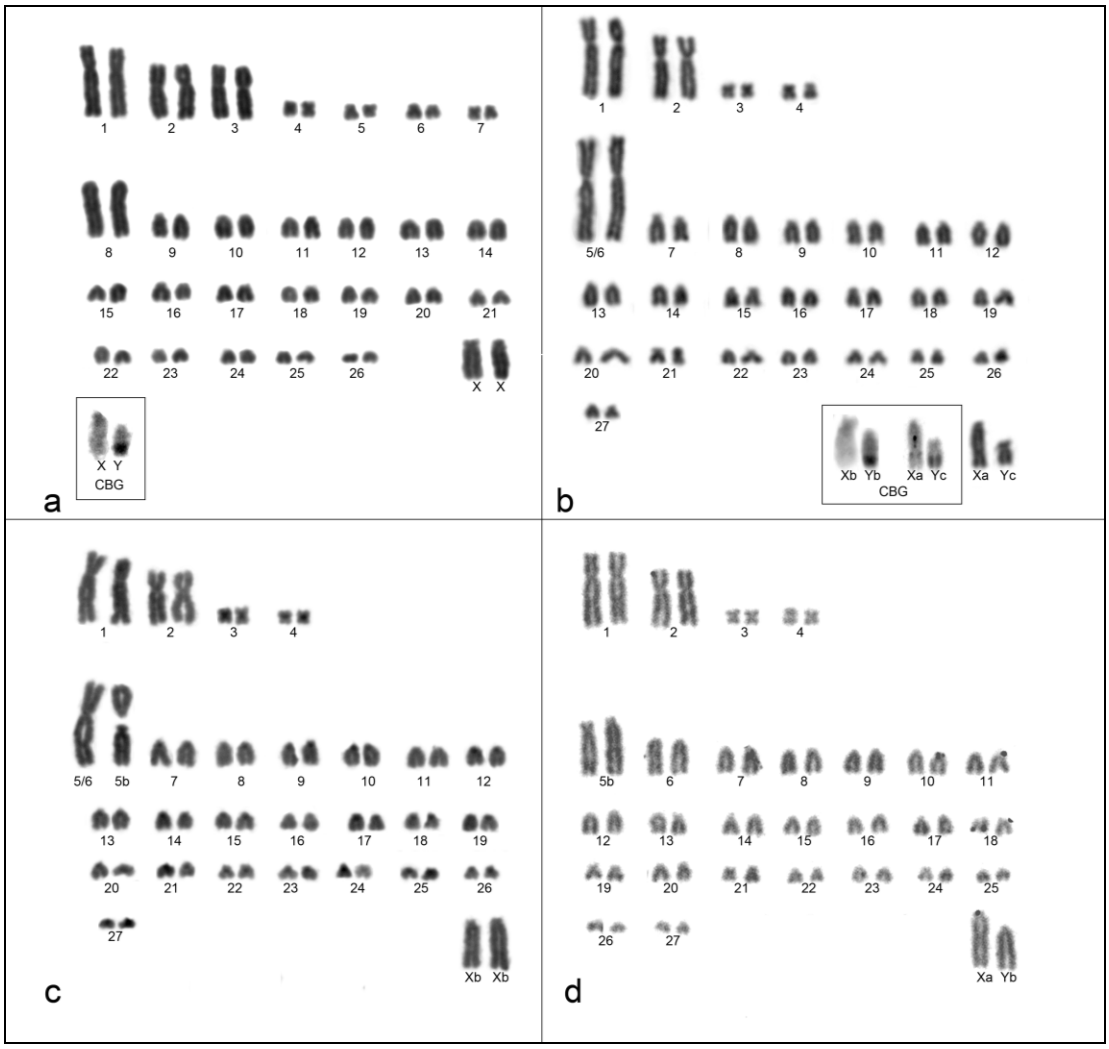

Fig. 6: (a) ***C. goytaca*** – 2n=54, FN=66. Inset: Sex chromosomes after CBG-banding; (b) ***C. subflavus*** with 2n=54, FN=62. Inset: Different types of sex chromosomes after CBG-banding; (c) ***C. subflavus*** with 2n=55, FN=63 and (d) ***C. subflavus*** with 2n=56, FN=64.

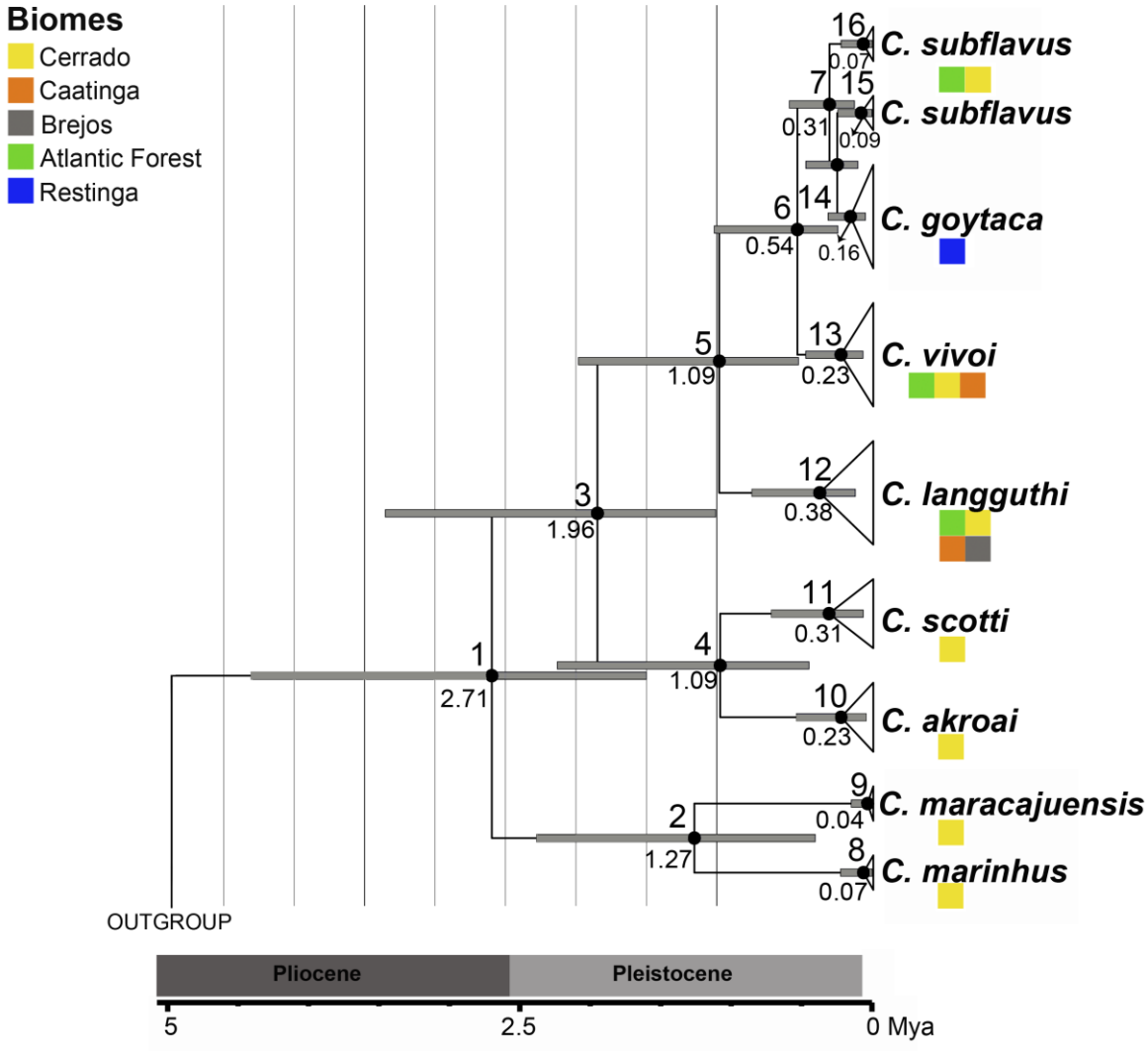

Fig. 7: Divergence times estimation of *Cerradomys* obtained from Bayesian analysis of concatenated data set (cyt-*b*, COI, IRBP and i7FBG). Age are represented below of the main nodes, as mean node height for a maximum clade credibility tree compiled from post-burnin tree topologies after independent runs implemented in BEAST. See Table 9 for 95% highest probability density (HPD) values of the main nodes.

Table 1: Samples analyzed in this work and extracted from GenBank: Species, Field or Lab and Museum number (when available), diploid (2n) and fundamental numbers (FN) when available, mitochondrial genes (cyt-*b* and COI), nuclear genes (IRBP and i7FBG), locality, coordinates, map number and references.

Species	GenBank #	Field #	Museum#	2n	FN	Cyt-*b*	COI	IRBP	i7FBG	Locality	Coordinates	Map	Reference
C. akroai	***to be provided***	**UUPI137**	**MZUSP**	**N/A**	**N/A**	**X**	**X**	**X**	**X**	**ESECUruçuí-**	**08°52'S;44°58'W**	**1**	**This study**
	to be provided	**UUPI161**	**MZUSP**	**60**	**76**	**X**	**X**	**X**	**X**	**Una, PI**			**This study**
	to be provided	**UUPI344**	**MZUSP**	**60**	**76**	**X**	**X**	**X**	**X**				**This study**
	KP122210	N/A	LBCE12016	N/A	N/A	X	N/A	N/A	N/A	Novo Jardim, TO	11º49'S;46º38'W	2	1
	KP122214	N/A	MN80485	N/A	N/A	X	N/A	N/A	N/A	TO			1
	KP122215	N/A	MN80486	N/A	N/A	X	N/A	N/A	N/A				1
	KP122219	N/A	MN80491[a]	N/A	N/A	X	N/A	N/A	N/A				1
C. goytaca	***to be provided***	**PRG2236**	**NPM**	**54**	**66**	**X**	**X**	**X**	**X**	**PARNA**	**22°12'S;41°29'W**	**3**	**This study**
	to be provided	**PRG2240**	**NPM**	**54**	**66**	**X**	**X**	**X**	**X**	**Restinga de**			**This study**
	to be provided	**PRG1860**	**NPM511**	**54**	**66**	**X**	**X**	**X**	**X**	**Jurubatiba, RJ**			**This study**
	to be provided	**PRG1562**	**NPM160**	**N/A**	**N/A**	**X**	**X**	**N/A**	**X**				**This study**
	to be provided	**PRG1563**	**NPM170**	**N/A**	**N/A**	**X**	**X**	**X**	**X**				**This study**
	to be provided	**PRG1586**	**NPM251**	**N/A**	**N/A**	**X**	**X**	**X**	**X**				**This study**
	to be provided	**PRG1589**	**NPM254**	**N/A**	**N/A**	**X**	**X**	**X**	**X**				**This study**
	to be provided	**PRG1590**	**NPM255**	**N/A**	**N/A**	**X**	**X**	**X**	**X**				**This study**
	to be provided	**PRG1861**	**NPM512**	**N/A**	**N/A**	**X**	**X**	**X**	**X**				**This study**
	to be provided	**PRG2239**	**NPM**	**N/A**	**N/A**	**X**	**X**	**X**	**X**				**This study**
	KP122221	LBCE-JCM10	N/A	N/A	N/A	X	N/A	N/A	N/A	Quissamã, RJ	22°04′S;41°24′W	4	1
	KP122222	LBCE-JCM11	N/A	N/A	N/A	X	N/A	N/A	N/A				1
	KR149682	N/A	MN73208	N/A	N/A	X	N/A	N/A	N/A				2
	KR149677	N/A	NPM358	N/A	N/A	X	N/A	N/A	N/A	Presidente	21°15′S;40°58′W	5	2
	KR149678	N/A	NPM364	N/A	N/A	X	N/A	N/A	N/A	Kennedy, ES			2
	KR149679	N/A	MN73261	N/A	N/A	X	N/A	N/A	N/A	São João da Barra, RJ	21°44′S;41°02′W	6	2
	KR149680	N/A	NPM269	N/A	N/A	X	N/A	N/A	N/A	Carapebus, RJ[b]	22°15′S;41°39′W	7	2
C. langguthi	***to be provided***	**MTR005**	**MZUSP**	**46**	**56**	**X**	**X**	**X**	**X**	**Pacoti, CE**	**04º13'S; 38º55'W**	**8**	**This study**
	to be provided	**MTR011**	**MZUSP**	**46**	**56**	**X**	**X**	**X**	**X**				**This study**
	to be provided	**MTR045**	**MZUSP**	**46**	**56**	**X**	**X**	**X**	**X**				**This study**
	to be provided	**MTR077**	**MZUSP**	**46**	**56**	**X**	**X**	**X**	**X**				**This study**

Species	GenBank #	Field #	Museum#	2n	FN	Cyt-*b*	COI	IRBP	i7FBG	Locality	Coordinates	Map	Reference
C. langguthi	***to be provided***	**LPC289**	**CTA1247**	**N/A**	**N/A**	**X**	**X**	**X**	**X**	**Crato, CE**	**07°13'S;39°27'W**	**9**	**This study**
	to be provided	**LPC264**	**CTA1222**	**N/A**	**N/A**	**X**	**X**	**X**	**X**				**This study**
	to be provided	**JFV474**	**MN**	**46**	**56**	**X**	**X**	**X**	**X**	**Piracuruca, PI**	**04°06'S;41°42'W**	**10**	**This study**
	to be provided	**JFV498**	**MN**	**46**	**56**	**X**	**X**	**X**	**X**				**This study**
	to be provided	**N/A**	**MUFAL66**	**N/A**	**N/A**	**X**	**X**	**X**	**X**	**ESEC Murici, AL**	**09º18'S; 35º56'W**	**11**	**This study**
	to be provided	**N/A**	**MUFAL117**	**N/A**	**N/A**	**X**	**X**	**X**	**X**	**Mata Grande,**	**09º07'S;37º43'W**	**12**	**This study**
	to be provided	**N/A**	**MUFAL133**	**N/A**	**N/A**	**X**	**X**	**X**	**X**	**AL**			**This study**
	to be provided	**N/A**	**MUFAL135**	**N/A**	**N/A**	**X**	**X**	**X**	**X**				**This study**
	AF181276	N/A	MN69789[a]	N/A	N/A	X	N/A	N/A	N/A	João Pessoa, PB	07º07'S;34º51'W	13	3
	KP122223	N/A	LBCE15905	N/A	N/A	X	N/A	N/A	N/A	Sousa, PB	06º45'S;38º14W	14	1
	KR149696	PHA282	N/A	N/A	N/A	X	N/A	N/A	N/A	Paudalho, PE	07°52S ;35°09W	15	2
C.maracajuensis	***to be provided***	**APC618**	**MZUSP**	**56**	**58**	**X**	**X**	**N/A**	**X**	**PARNA Emas,**	**18°15'S;52°53'W**	**16**	**This study**
	to be provided	**APC629**	**MZUSP**	**56**	**58**	**X**	**X**	**X**	**X**	**GO**			**This study**
	AF181278	N/A	MN44178[a]	N/A	N/A	X	N/A	N/A	N/A	Maracaju, MS	21°38′S, 55°09′W	17	3
	KP122224	CRB2790	N/A	N/A	N/A	X	N/A	N/A	N/A	São José Xingu, MT	10°48'S; 52°45W	18	1
	KP122226	SVS883	N/A	N/A	N/A	X	N/A	N/A	N/A	Campo Verde, MT	15°29′S;55°09′W	19	1
C. marinhus	***to be provided***	**ROD340**	**MZUSP**	**56**	**54**	**X**	**X**	**X**	**X**	**PARNA**	**15º14'S; 45º37'W**	**20**	**This study**
	to be provided	**ROD362**	**MZUSP**	**56**	**54**	**X**	**X**	**X**	**X**	**Veredas, MG**			**This study**
	to be provided	**CRB1883**	**N/A**	**56**	**54**	**X**	**X**	**X**	**X**	**Cocos, BA**	**14º10'S; 44º32'W**	**21**	**This study**
	to be provided	**CRB1835**	**N/A**	**56**	**54**	**X**	**X**	**X**	**X**				**This study**
	AF181279	N/A	MN63834[c]	N/A	N/A	X	N/A	N/A	N/A	Jaborandi, BA	13º37'S;44º25'W	22	3
	KP122228	N/A	LBCE17294	N/A	N/A	X	N/A	N/A	N/A	Uberlândia, MG	18º55'S;48º17'W	23	1
C. scotti	***to be provided***	**APC552**	**MZUSP**	**58**	**72**	**X**	**X**	**X**	**X**	**PARNA Emas,**	**18°04'S;52°55'W**	**16**	**This study**
	to be provided	**APC567**	**MZUSP**	**N/A**	**N/A**	**X**	**X**	**X**	**X**	**GO**			**This study**
	to be provided	**APC572**	**MZUSP**	**58**	**72**	**X**	**X**	**X**	**X**				**This study**
	to be provided	**APC615**	**MZUSP**	**N/A**	**N/A**	**X**	**X**	**X**	**X**				**This study**
	to be provided	**MJJS189**	**MZUSP**	**58**	**72**	**X**	**N/A**	**X**	**X**	**Serra das Galés,**	**16º59'S;50º37'W**	**24**	**This study**
	to be provided	**MJJS204**	**MZUSP**	**58**	**72**	**X**	**X**	**X**	**X**	**GO**			**This study**
	to be provided	**MJJS232**	**MZUSP**	**58**	**N/A**	**X**	**X**	**X**	**X**				**This study**
	to be provided	**MJJS233**	**MZUSP**	**58**	**N/A**	**X**	**X**	**X**	**X**				**This study**
	to be provided	**MJJS255**	**MZUSP**	**N/A**	**N/A**	**X**	**X**	**X**	**X**				**This study**

Species	GenBank #	Field #	Museum#	2n	FN	Cyt-*b*	COI	IRBP	i7FBG	Locality	Coordinates	Map	Reference
C. scotti	***to be provided***	**MJJS260**	**MZUSP**	**N/A**	**N/A**	**X**	**X**	**X**	**X**	**Serra das Galés, GO**	**16º59'S;50º37'W**	**24**	**This study**
	to be provided	**BIO474**	**N/A**	**N/A**	**N/A**	**X**	**X**	**X**	**N/A**	**PARNA Brasília,GO**	**15º40'S;47º59'W**	**25**	**This study**
	AF181277	N/A	MN50379	N/A	N/A	X	N/A	N/A	N/A	Cavalcante, GO	13º47'S;47º27'W	26	3
	KP122243	N/A	LBCE9446	N/A	N/A	X	N/A	N/A	N/A	Aporé, GO	18º58'S;51º55'W	27	1
	KP122232	N/A	LBCE10907	N/A	N/A	X	N/A	N/A	N/A				
	KP122240	N/A	LBCE5848	N/A	N/A	X	N/A	N/A	N/A				
	KP122242	N/A	LBCE8749	N/A	N/A	X	N/A	N/A	N/A	Campo Alegre de Goiás, GO	17º38'S; 47º47'W	28	1
	KP122241	N/A	LBCE7546	N/A	N/A	X	N/A	N/A	N/A	Luziania, GO	16º15'S;47º57'W	29	1
	to be provided	**LPC688**	**CTA1636**	**N/A**	**N/A**	**X**	**X**	**X**	**X**	**Uberlândia, MG**	**09°05'S;47°56'W**	**23**	**This study**
	KP122234	N/A	LBCE17287	N/A	N/A	X	N/A	N/A	N/A	Campo Florido, MG	19º45'S;48º34'W	30	1
	to be provided	**N/A**	**LBCE4884**	**N/A**	**N/A**	*****	**X**	**X**	**X**	**Aquidauana, MS**	**20°28'S;55°47'W**	**31**	**This study**
	to be provided	**N/A**	**LBCE5713**	**N/A**	**N/A**	*****	**X**	**X**	**X**	**Corumbá, MS**	**19°00'S;57°39'W**	**32**	**This study**
	KP122238	N/A	LBCE5724	N/A	N/A	X	N/A	N/A	N/A				1
	KP122239	N/A	LBCE5725	N/A	N/A	X	N/A	N/A	N/A				1
	KP122233	N/A	LBCE11618	N/A	N/A	X	N/A	N/A	N/A	Sítio D'Abadia, MS	14º48'S;46º15'W	33	1
	KP122244	N/A	MN61672	N/A	N/A	X	N/A	N/A	N/A	Jaborandi, BA	13º37'S;44º25'W	22	1
	KP122231	CRB2731	N/A	N/A	N/A	X	N/A	N/A	N/A	Correntina, BA	13º20'S;44º37'W	34	1
	EU579482	N/A	TK61881	N/A	N/A	X	N/A	X	N/A	Canindeyui, Paraguay	24°08′S;55°22′W	35	4
C. subflavus	***to be provided***	**ROD86**	**MZUSP**	**54**	**62**	**X**	**X**	**N/A**	**X**	**Guará, SP**	**20°48'S;47°48'W**	**36**	**This study**
	to be provided	**PCH1461**	**MZUSP**	**N/A**	**N/A**	**X**	**X**	**X**	**X**				**This study**
	to be provided	**CIT2053**	**MZUSP**	**56**	**64**	**X**	**X**	**X**	**X**	**Itirapina, SP**	**22º15'S;47º49'W**	**37**	**This study**
	to be provided	**CIT1396**	**MZUSP**	**55**	**63**	**X**	**X**	**X**	**X**	**Rio Claro, SP**	**22º24'S; 47º34'W**	**38**	**This study**
	to be provided	**PCH3619**	**MZUSP**	**54**	**62**	**X**	**X**	**X**	**X**	**São Joaquim da**	**20°34'S;47°51'W**	**39**	**This study**
	to be provided	**PCH3995**	**MZUSP**	**54**	**62**	**X**	**X**	**X**	**X**	**Barra, SP**			**This study**
	to be provided	**CIT661**	**MZUSP**	**54**	**62**	**X**	**X**	**N/A**	**X**	**Santa Rita do Passa Quatro, SP**	**21º42'S; 47º28'W**	**40**	**This study**

Species	GenBank #	Field #	Museum#	2n	FN	Cyt-*b*	COI	IRBP	i7FBG	Locality	Coordinates	Map	Reference
C. subflavus	***to be provided***	**DPO22**	**CTA1802**	**N/A**	**N/A**	**X**	**X**	**X**	**X**	**Barão de Cocais, MG**	**19°53'S;43°28'W**	**41**	**This study**
	to be provided	**LPC337**	**CTA1292**	**N/A**	**N/A**	**X**	**X**	**X**	**X**	**Nova Ponte, MG**	**19°07'S;47°44'W**	**42**	**This study**
	to be provided	**LPC338**	**CTA1293**	**N/A**	**N/A**	**X**	**X**	**X**	**X**				**This study**
	to be provided	**CEG127**	**N/A**	**N/A**	**N/A**	*****	**X**	**X**	**X**	**Nova Lima, MG**	**19°59'S;43°50'W**	**43**	**This study**
	to be provided	**LC91**	**CTA1127**	**N/A**	**N/A**	**X**	**X**	**X**	**X**	**PE Rio Doce, MG**	**19º42'S;42º38'W**	**44**	**This study**
	AF181274	CEG42	N/A	N/A	N/A	X	N/A	N/A	N/A				3
	to be provided	**DQM059**	**N/A**	**54**	**62**	**X**	**X**	**X**	**X**	**Serra da Canastra, MG**	**20°18'S;46°35'W**	**45**	**This study**
	to be provided	**DQM006**	**N/A**	**54**	**62**	**X**	**X**	**X**	**X**				**This study**
	to be provided	**DQM016**	**N/A**	**54**	**62**	**X**	**X**	**X**	**X**				**This study**
	to be provided	**YL68**	**CTA934**	**N/A**	**N/A**	**X**	**X**	**X**	**X**	**São Gonçalo do Rio Preto, MG**	**18°08'S;43°22'W**	**46**	**This study**
	to be provided	**LC68**	**CTA1104**	**N/A**	**N/A**	**X**	**X**	**X**	**X**				**This study**
	KR149690	N/A	MCNM1242	N/A	N/A	X	N/A	N/A	N/A	Brasilândia de Minas, MG	16°56′S;45°58′W	47	2
	KR149691	BRG160	N/A	N/A	N/A	X	N/A	N/A	N/A	Araponga, MG	20°41′S;42°27′W	48	2
	KP122247	N/A	LBCE17255	N/A	N/A	X	N/A	N/A	N/A	Uberlandia, MG	18º55'S;48º17'W	23	1
	KP122248	N/A	LBCE17293	N/A	N/A	X	N/A	N/A	N/A				
	to be provided	**PMP062**	**MZUFV2800**	**N/A**	**N/A**	**X**	**X**	**X**	**X**	**Viçosa, MG**	**20°45'S;42°52'W**	**49**	**This study**
	to be provided	**PMP083**	**MZUFV2805**	**N/A**	**N/A**	**X**	**X**	**X**	**X**				**This study**
	to be provided	**PMP109**	**MZUFV2911**	**N/A**	**N/A**	**X**	**X**	**X**	**X**				**This study**
	AY163626	N/A	N/A	N/A	N/A	N/A	N/A	X	N/A	Juramento, MG	16°51'S; 43°33'W	50	5
	to be provided	**SLF102**	**CTA109**	**N/A**	**N/A**	**X**	**X**	**X**	**X**	**Nova Viçosa, BA**	**17°53'S;39°26'W**	**52**	**This study**
	to be provided	**SLF104**	**CTA110**	**N/A**	**N/A**	**X**	**X**	**X**	**X**				**This study**
	to be provided	**HGB020**	**N/A**	**N/A**	**N/A**	*****	**X**	**X**	**X**				**This study**
	to be provided	**YL492**	**CTA369**	**N/A**	**N/A**	**X**	**X**	**X**	**X**	**Águia Branca, ES**	**18°52'S;40°49'W**	**53**	**This study**
	to be provided	**SLF191**	**CTA875**	**N/A**	**N/A**	**X**	**X**	**X**	**X**	**São Mateus, ES**	**18°52'S;39°51'W**	**54**	**This study**
C. vivoi	***to be provided***	**LG0468**	**N/A**	**N/A**	**N/A**	*****	**X**	**X**	**X**	**Joaíma, MG**	**16°39'S;41°03'W**	**55**	**This study**
	to be provided	**LG0471**	**N/A**	**N/A**	**N/A**	**X**	**X**	**X**	**X**				**This study**
	to be provided	**LG0487**	**N/A**	**N/A**	**N/A**	**X**	**X**	**X**	**X**				**This study**
	to be provided	**LG0416**	**N/A**	**N/A**	**N/A**	**X**	**X**	**X**	**X**	**Itinga, MG**	**16°35'S;41°46'W**	**56**	**This study**
	to be provided	**LG0420**	**N/A**	**N/A**	**N/A**	**X**	**X**	**X**	**X**				**This study**
	to be provided	**LG0421**	**N/A**	**N/A**	**N/A**	**X**	**N/A**	**X**	**X**				**This study**

Species	GenBank #	Field #	Museum#	2n	FN	Cyt-*b*	COI	IRBP	i7FBG	Locality	Coordinates	Map	Reference
C. vivoi	KP122249	N/A	MN61666	N/A	N/A	X	N/A	N/A	N/A	Juramento, MG	16°51′S;43°35′W	50	1
	to be provided	**LPC225**	**CTA1185**	**N/A**	**N/A**	**X**	**X**	**X**	**X**	**Andaraí, BA**	**12°48'S;41°15'W**	**51**	**This study**
	to be provided	**LPC212**	**CTA1172**	**N/A**	**N/A**	**X**	**X**	**X**	**X**				**This study**
	to be provided	**N/A**	**LBCE1501**	**N/A**	**N/A**	**X**	**X**	**X**	**X**	**Caetité, BA**	**14°04'S;42°28'W**	**57**	**This study**
	to be provided	**N/A**	**LBCE1504**	**N/A**	**N/A**	**X**	**X**	**X**	**X**				**This study**
	to be provided	**CIT357**	**N/A**	**50**	**64**	**X**	**X**	**X**	**X**	**Ibiraba, BA**	**10º53'S;43º05'W**	**58**	**This study**
	KR149688	LG211	N/A	N/A	N/A	X	N/A	N/A	N/A	Itaetê, BA	12°58′S;40°57′W	59	2
	AF181275	N/A	MN35898[a]	N/A	N/A	X	N/A	N/A	N/A	Itabuna, BA	14º47'S;39º16'W	60	3
	to be provided	**CD002**	**N/A**	**N/A**	**N/A**	**X**	**X**	**X**	**X**	**Lençóis, BA**	**12°33'S;41°23'W**	**61**	**This study**
	to be provided	**CD003**	**N/A**	**N/A**	**N/A**	**X**	**X**	**X**	**X**				**This study**
	to be provided	**CD132**	**N/A**	**N/A**	**N/A**	**X**	**N/A**	**X**	**X**				**This study**
	to be provided	**CD133**	**N/A**	**N/A**	**N/A**	**X**	**X**	**X**	**X**				**This study**
	to be provided	**CIT1264**	**N/A**	**50**	**64**	**X**	**X**	**X**	**X**	**Morro do**	**11º36'S;41º38'W**	**62**	**This study**
	KR149684	N/A	MN75905	N/A	N/A	X	N/A	N/A	N/A	**Chapéu, BA**			2
	to be provided	**LG0372**	**N/A**	**N/A**	**N/A**	**X**	**X**	**X**	**X**	**Mucugê, BA**	**13°00'S;41°22'W**	**63**	**This study**
	to be provided	**LG0318**	**N/A**	**N/A**	**N/A**	**X**	**X**	**X**	**X**				**This study**
	to be provided	**LG0325**	**N/A**	**N/A**	**N/A**	*****	**X**	**X**	**X**				**This study**
	to be provided	**YL208**	**CTA994**	**N/A**	**N/A**	**X**	**X**	**X**	**X**	**Serrinha, BA**	**12°43'S;39°05'W**	**64**	**This study**
	to be provided	**YL220**	**CTA1006**	**N/A**	**N/A**	**X**	**X**	**X**	**X**				**This study**
	to be provided	**LC104**	**CTA1137**	**N/A**	**N/A**	**X**	**X**	**X**	**X**	**Una, BA**	**15°21'S;39°00'W**	**65**	**This study**
	to be provided	**LPC114**	**CTA1147**	**N/A**	**N/A**	**X**	**X**	**X**	**X**				**This study**
	to be provided	**CIT 1472**	**N/A**	**50**	**64**	**X**	**X**	**X**	**X**	**Ribeirão Preto, SP**	**21º08'S;47º49'W**	**66**	**This study**

N/A: not available.* GenBank Sequences: LBCE4884 (KP122236); LBCE5713 (KP122237); LG0325 (KR149687); LG468 (KR149685); CEG127 (KR149693); HGB020 (KR149694); In bold: present study; [1]Bonvicino *et al.* (2014); [2]Tavares *et al*. (2016); [3]Bonvicino and Moreira (2001); [4]Hanson and Bradley (unpublished); [5]Weksler (2003); [a]Holotype; [b]Type locality; [c]Paratype. Acronyms of Brazilian states: Alagoas (AL), Bahia (BA), Ceará (CE), Espírito Santo (ES), Goiás (GO), Mato Grosso (MT), Mato Grosso do Sul (MS), Minas Gerais (MG), Paraíba (PB), Pernambuco (PE), Piaui (PI), Rio de Janeiro (RJ), São Paulo (SP) and Tocantins (TO). Museum and collector acronyms: BIO/CIT (Banco de células do laboratório de Citogenética de Vertebrados, IB/USP), CTA (Coleção de tecidos de animais do departamento de Ciências Biológicas, UFES, Brazil), LBCE (Laboratório de Biologia e Parasitologia de Mamíferos Silvestres Reservatórios), MCNM (Museu de Ciências Natutal da PUC Minas Gerais), MN (Museu Nacional, UFRJ, Brazil), MUFAL (Museu de História Natural, UFAL, Brazil), NPM (Coleção Biológica do NUPEM/UFRJ), PMP (Estação de Pesquisa, treinamento e educação ambiental Mata do Paraíso), ROD (Laboratório de Ecologia e Evolução), UFPB (Universidade Federal da Paraiba, Brazil). APC (Ana Paula Carmignotto), CEG (Carlos Eduardo Grelle), CRB (Cibele Rodrigues Bonvicino), DPO (Daniele Pedrosa Oliveira), DQM (Diego Queirolo), HGB (Helena de Godoy Bergallo), JFV (Júlio Fernandes Vilela), LG (Lena Geise), LPC (Leonora Pires Costa), MJJS (Maria José de Jesus Silva), MTR (Miguel Trefaut Rodrigues), PHA (Paulo Henrique Asfora), PRG (BRG: Pablo Rodrigues Gonçalves), SLF (Simone Lóss de Freitas), YL (Yuri Leite).

Table 2: Patterns of DNA amplification and primers used to amplify mitochondrial DNA (cytochrome *b* – cyt-*b* and cytochrome *c* oxidase subunit I – COI) and nuclear genes (first exon of interphotoreceptor retinoid-binding protein - IRBP and intron 7 of β-fibrinogen – i7FGB).

Gene	**Primers**	**References**	**Initial Denaturation**	**# Cycles**	**Cycles**			**Final Extension**
					Denaturation	**Annealing**	**Extension**	
Cyt-*b*	MVZ05 MVZ16	Irwin *et al.* (1991) Smith and Patton (1993)	94°C/5 min	39	94°C/30sec	48°C/45sec	72°C/45sec	72°C/5min
COI	LCO1490 HCO2198	Folmer *et al.* (1994)	94ºC/5 min	39	94°C/30sec	44ºC/45sec	72ºC/45sec	72ºC/5min
IRBP	A1 F	Stanhope *et al.* (1992)	94ºC/5 min	39	94°C/30sec	62ºC/45sec	72ºC/3min	72ºC/5min
i7FGB	B17-mammL Bfib-mammaU	Matocq *et al.* (2006)	94ºC/5 min	39	94°C/30sec	60ºC/90sec	72°C/60sec	72ºC/5min

Table 3: Samples of the Family Cricetidae analyzed in this work and sequences extracted from GenBank used as outgroups for phylogenetic and molecular dating analyses: Species, mitochondrial genes (cyt-*b* and COI), nuclear genes (IRBP and i7FGB) and references (respectively, for each gene).

Species	**Cyt-*b***	**COI**	**IRBP**	**i7FGB**	**Reference**
Arvicola terrestris	AY275106	N/A	AY277407	N/A	D'Elía (2003)
Eothenomys melanogaster	AM392374	JF444250	AY163583	N/A	Galewski *et al.* (2006); Eger (unpublished); Weksler (2003)
Cricetulus longicaudatus	AJ973386	JX962141	AY326082	N/A	Neumann *et al.* (2006); Ma and Lu (unpublished); Jansa and Weksler (2004)
Cricetus cricetus	AJ490302	KC953838	AY277410	N/A	Smulders (unpublished); Schröder *et al.* (2014); D'Elía (2003)
Mesocricetus auratus	AM904612	JF444325	AY163591	N/A	Neumann (unpublished); Eger (unpublished); Weksler (2003)
Phodopus sungorus	AJ973390	JF444395	AY163631	N/A	Neumann *et al.* (2006); Eger (unpublished); Weksler (2003)
Neotoma albigula	DQ179858	N/A	AY277411	N/A	Matocq *et al.* (2007); D'Elía (2003)
Peromyscus maniculatus	DQ385645	JF457067	AY163630	N/A	Dragoo *et al.* (2006); Eger (unpublished); Weksler (2003)
Rheomys raptor	KJ921706	N/A	AY163635	N/A	Leite *et al.* (2014); D'Elía (2003)
Sigmodon alstoni	AF293397	HQ919654	AY163640	N/A	Peppers *et al.* (2002); Lim *et al.* (unpublished); Weksler (2003)
Sigmodon hispidus	AF425227	JF492732	AY277479	N/A	Carroll and Bradley (unpublished); Engstrom *et al.* (unpublished); D'Elía (2003)
Juscelinomys huanchacae	AY275119	N/A	AY277452	N/A	D'Elía (2003)
Kunsia tomentosus	AY275121	N/A	AY277454	N/A	D'Elía (2003)
Oxymycterus nasutus	AF175286	N/A	AY277468	N/A	Hoffman *et al.* (unpublished); D'Elía (2003)
Scapteromys tumidus	AY275133	N/A	AY163637	N/A	D'Elía (2003); Weksler (2003)
Auliscomys pictus	U03545	N/A	AY277434	N/A	Smith and Patton (1993); D'Elía (2003)
Calomys lepidus	AF159294	N/A	AY163580	N/A	Anderson and Yates (2000); Weksler (2003)
Calomys callosus	DQ447282	N/A	AY277440	N/A	Almeida *et al.* (2007); D'Elía (2003)
Calomys tener	DQ447300	GU938935	JQ434407	N/A	Almeida *et al.* (2007); Muller *et al.* (2013); Salazar-Bravo *et al.* (2013)
Holochilus brasiliensis	*to be provided*	N/A	*to be provided*	*to be provided*	CTA2049 - *Present study*
Oligoryzomys flavescens	*to be provided*	N/A	*to be provided*	N/A	ROD123 - *Present study* (Chapter 4)
Oligoryzomys fulvescens	GU126529	EU095456	AY163611	N/A	Percequillo *et al.* (2011); Borisenko *et al.* (2008); Weksler (2003)
Oligoryzomys nigripes	GU126530	KF815406	AY163612	JQ282856	Percequillo *et al.*(2011); Vilela *et al.* (2014); Weksler (2003); Agrellos *et al.* (2002)
Pseudoryzomys simplex	*to be provided*	*to be provided*	*to be provided*	*to be provided*	ROD286 – *Present study*

Table 4: Fossil records used as calibration points in molecular divergence estimation, extracted from Leite *et al.* (2014) and Tavares *et al.* (2016).

Calibration point	Taxon	Fossil	Stage	Age	Reference
1	Neotominae	*Paronychomys* spp.	Clarendonian	11.6	1-7
2	Cricetinae	*Mesocricetus primitivus*	MN 13	6.0	8
3	Cricetinae	*Cricetus kormosi*, *Cricetus lophidens*	MN 13	6.0	8,9
4	Arvicolinae	*Prosomys* (=*Promimomys*) *mimus*	early Ruscinian (MN 14) late Hemphillian	5.5	2,7,10-13
5	Sigmodontalia	*Prosigmodon oroscoi*	late Hemphillian	4.8	14, 15
6	*Scapteromys*	*Scapteromys hershkovitzi*	Sanandresan	2.2	16
7	*Oxymycterus*	*Oxymycterus* cf. *rufus*	Ensenadan	1.0	17
8	Phyllotini	*Auliscomys* sp.	early Chapadmalalan	4.0	18, 19
9	*Calomys*	*Calomys laucha*	Ensenadan	1.0	17
10	*Oligoryzomys*	*Oligoryzomys cf. flavescens*	late Ensenadan	0.8	17, 20
11	*Holochilus*	*Holochilus brasiliensis*, *Holochilus primigenius*	late Ensenadan	0.8	17, 21

[1]Baskin (1979); [2]Carleton and Musser (1984); [3]Jacobs (1977); [4]Jacobs and Lindsay (1984); [5]Korth (1994); [6]McKenna and Bell (1997); [7]Savage and Russell (1983); [8]de Bruijn *et al*. (1970); [9]Freudenthal *et al.* (1998); [10]Agustí *et al*. (2001); [11]Chaline *et al*. (1999); [12]Martin *et al.* (2002); [13]Repenning (2003); [14]Jacobs and Lindsay (1981); [15]Peláez-Campomanes and Martin (2005); [16]Quintana (2002); [17]Pardiñas *et al.* (2002); [18]Pardiñas and Tonni (1998); [19]Reig (1978); [20]Voglino and Pardiñas (2005); [21]Pardiñas *et al.* (2013).

Table 5: Distance and standard deviation obtained for *Cerradomys* with K2P model of evolution based on cyt-*b* data set. Diagonal in bold represents instraespecific distance.

	C. maracajuensis	*C. marinhus*	*C. langguthi*	*C. vivoi*	*C. subflavus*	*C. goytaca*	*C. akroai*	*C. scotti*
C. maracajuensis	**0.006±0.002**							
C. marinhus	0.105 ± 0.013	**0.004±0.001**						
C. langguthi	0.146 ± 0.016	0.138 ± 0.015	**0.012±0.003**					
C. vivoi	0.14 ± 0.016	0.124 ± 0.015	0.052 ± 0.008	**0.000±0.000**				
C. subflavus	0.148 ± 0.017	0.136 ± 0.015	0.04 ± 0.007	0.023 ± 0.006	**0.006±0.002**			
C. goytaca	0.151 ± 0.017	0.138 ± 0.016	0.041 ± 0.007	0.022 ± 0.006	0.007 ± 0.002	**0.001±0.001**		
C. akroai	0.132 ± 0.015	0.112 ± 0.014	0.094 ± 0.012	0.096 ± 0.012	0.098 ± 0.013	0.099 ± 0.013	**0.005±0.002**	
C. scotti	0.119 ± 0.014	0.113 ± 0.013	0.089 ± 0.012	0.09 ± 0.012	0.088 ± 0.012	0.091 ± 0.012	0.053 ± 0.009	**0.006±0.001**

Table 6: Estimates of genetic diversity and neutrality test for *C. goytaca, C. subflavus* and *C. vivoi* for mitochondrial data set (cyt-*b* and COI), interphotoreceptor retinoid binding protein (IRBP), and intron 7 of β-fibrinogen (i7FBG) genes. Lineage, number of samples (Nº), segregating sites (S), number of haplotypes (h), haplotype diversity (Hd), nucleotide diversity (π), Tajima's D and Fu's Fs test. Statistical significance: (*) P≤ 0,05.

Gene	**Clade**	**Nº**	**S**	**h**	**Hd**	**π**	**Tajima's D**	**Fu's Fs**
Cyt-*b*+COI	*C. vivoi*	19	10	07	0.608	0.00087	-2.12573*	-3.159*
	C. goytaca	10	0	01	0.000	0.00000	N/A	N/A
	C. subflavus	24	35	17	0.975	0.00573	-0.72408	-4.707
IRBP	*C. vivoi*	23	03	03	0.549	0.00157	1.25697	2.280
	C. goytaca	08	01	02	0.233	0.00034	-0.44832	0.083
	C. subflavus	24	06	05	0.611	0.00185	-0.13302	0.550
i7FBG	*C. vivoi*	23	17	14	0.866	0.00590	-0.46531	-2.954
	C. goytaca	10	07	06	0.705	0.00345	-0.491	-0.374
	C. subflavus	25	09	07	0.602	0.00245	-0.89198	-0.923

Table 7: Analysis of molecular variance (AMOVA) among lineage G (*C. subflavus* and *C. goytaca*) for mitochondrial data set.

Source of variation	**d.f.**	**Sum of squares**	**Variance components**	**Percentage of variation**
Among populations within groups	1	42.855	2.84237	51.03
Within populations	32	87.292	2.72786	48.97
Total	33	130.147	5.57023	
Fst=0.51028				

Table 8: Distance and standard deviation obtained for *Cerradomys* with K2P model of evolution based on COI data set. Diagonal in bold represents instraespecific distance.

	C. maracajuensis	*C. marinhus*	*C. langguthi*	*C. vivoi*	*C. subflavus*	*C. goytaca*	*C. akroai*	*C. scotti*
C. maracajuensis	**0**	-	-	-	-	-	-	-
C. marinhus	0.091 ± 0.013	**0.001 ± 0.001**	-	-	-	-	-	-
C. langguthi	0.125 ± 0.018	0.097 ± 0.018	**0.012 ± 0.003**	-	-	-	-	-
C. vivoi	0.144 ± 0.016	0.109 ± 0.018	0.043 ± 0.012	**0.001 ± 0.001**	-	-	-	-
C. subflavus	0.139 ± 0.016	0.106 ± 0.005	0.050 ± 0.021	0.012 ± 0.017	**0.005 ± 0.002**	-	-	-
C. goytaca	0.140 ± 0.020	0.105 ± 0.022	0.056 ± 0.015	0.015 ± 0.017	0.009 ± 0.011	**0**	-	-
C. akroai	0.120 ± 0.011	0.113 ± 0.022	0.037 ± 0.017	0.069 ± 0.014	0.072 ± 0.009	0.073 ± 0.018	**0.004 ± 0.002**	-
C. scotti	0.137 ± 0.022	0.106 ± 0.012	0.063 ± 0.015	0.090 ± 0.019	0.091 ± 0.004	0.091 ± 0.006	0.046 ± 0.017	**0.006 ± 0.002**

Table 9: Divergence dates for *Cerradomys* estimated with concatenated data set (cyt-*b*, COI, IRBP and i7FBG): nodes, species, and node ages (Mya) and 95% highest probability density (HPD).

Node	Node content	Time (Mya)	95%HPD
1	First *Cerradomys* split	2.71	1.39-3.81
2	*C. marinhus* and *C. maracajuensis*	1.27	0.35-2.06
3	Remaining *Cerradomys* species	1.96	0.96-2.98
4	*C. akroai* and *C. scotti*	1.09	0.39-1.93
5	*C. langguthi* and remaining species	1.09	0.45-1.80
6	*C. vivoi* and *C. goytaca/C. subflavus*	0.54	0.21-0.97
7	*C. subflavus* plus *C. goytaca* lineage	0.31	0.11-0.51
8	*C. marinhus* lineage	0.07	0.0074-0.20
9	*C. maracajuensis* lineage	0.04	0.0007-0.13
10	*C. akroai* lineage	0.23	0.04-0.47
11	*C. scotti* lineage	0.31	0.06-0.62
12	*C. langguthi* lineage	0.38	0.11-0.74
13	*C. vivoi* lineage	0.23	0.06-0.41
14	*C. goytaca* lineage	0.16	0.04-0.27
15	*C. subflavus* lineage	0.09	0.008-0.21
16	*C. subflavus* lineage	0.07	0.006-0.19

Chapter 3
Extensive and rapid rates of genomic reshuffling involved the karyotype evolution of the genus Cerradomys (Sigmodontinae: Oryzomyini)

Abstract

Cerradomys is a genus of the Tribe Oryzomyini (Subfamily Sigmodontinae) distributed in open vegetation formations of South American diagonal belts, with some species reaching Atlantic Forest and one endemic to Restingas. Diploid number ranges from 2n=46 to 60. Previous classic cytogenetic studies showed chromosome variability in many species, mainly due to Robertsonian rearrangements, pericentric inversion and sex chromosome polymorphisms. In order to access the karyotype evolution of the genus, we performed classic cytogenetics (CBG and GTG-banding), fluorescence *in situ* hybridization (FISH) with telomeric probes and chromosome painting using *Oligoryzomys moojeni* (OMO) probes (2n=70). Our results were mapped in a phylogenetic framework (obtained in Chapter 2), aiming to infer the hypothetical rearrangements involved in the evolution and diversification of the genus. GTG-banding pattern is inedited in *C. marinhus, C. maracajuensis, C. akroai* and *C. goytaca*. Cross-species hybridization revealed homology with 23 segments of *C. maracajuensis* and *C. marinhus*, 26 segments of *C. scotti*, 31 segments of "*C. langguthi*", 32 in *C. vivoi* and 27 homologous segments of *C. goytaca* and "*C. subflavus*". Associations of probes OMO 4 and 5, OMO 6 and 7, and OMO 16 and 28 were observed. OMO X painted the whole chromosome X and the euchromatic region of the Y. FISH with telomeric probes showed signals exclusively in telomeric positions in species with higher diploid numbers (*C. akroai*, *C. maracajuensis*, *C. marinhus* and *C. scotti*). Insterstitial telomeric signals (ITS) were observed in species with lower diploid numbers ("*C. langguthi*", *C. vivoi*, *C. goytaca* and "*C. subflavus*"). Comparisons of chromosome painting and molecular phylogeny supported the hypothesis that ITS are remnant of ancient telomeres after chromosome fusions. Rearranged chromosomes without ITS were also observed, showing that these sequences may have been lost during karyotype evolution of the genus. Rapid and extensive chromosome reorganization was responsible for karyotypic differences in *Cerradomys.* The major's drivers for genomic reshuffling were *in tandem* and centric fusion, fission, centromere repositioning or pericentric and paracentric inversions. Complex rearrangements corroborate the hypothesis that *C. goytaca* is not junior synonym of "*C. subflavus*". The small population size and restricted distribution of *C. goytaca* in a hostile habitat such as Restinga may have imposed strong selective pressures with fixation of rearrangements in a short period of time.

Keywords: Chromosomal evolution, GTG-banding, ZOO-FISH, Chromosome painting

1. Introduction

Cerradomys is a genus of the tribe Oryzomyini formerly known as *Oryzomys subflavus*. From 1981 to 2002, different karyotypes were described suggesting that this species was not monotypic (Maia and Hulak, 1981; Almeida and Yonenaga-Yassuda, 1985; Svartman and Almeida, 1992; Andrades-Miranda *et al.*, 2002), which was posterior confirmed with molecular and morphological studies (Bonvicino and Moreira, 2001; Langguth and Bonvicino, 2002; Bonvicino, 2003). Then, *Oryzomys subflavus* became *Oryzomys* gr. *subflavus* and, in 2006, this group of species was erected to the genus category (Weksler *et al.*, 2006).

Currently, eight *Cerradomys* species are formally described and cytogenetic information is important to recognize them (Langguth and Bonvicino, 2002; Bonvicino, 2003; Percequillo *et al.*, 2008; Tavares *et al.*, 2011; Bonvicino *et al.*, 2014; Chapter 2).

Besides the importance in cytotaxonomy, cytogenetic showed an outstanding chromosomal variation, turning *Cerradomys* an excellent group for chromosomal evolution studies. For example, four diploid numbers were described for *C. langguthi* due to Robertsonian rearrangements and GTG-banding comparisons allowed the recognition of pairs involved in centric fusion/fission (pairs 2+7 and 3+5) (Maia and Hulak, 1981). Almeida and Yonenaga-Yassuda (1985) found distinct karyotypes for *C. subflavus* as a result of Robertsonian rearrangements in pairs 5 and 6 and pericentric inversion in pair 5. However, an interdisciplinary study revealed that both species are propably polytypic (Chapter 2).

The aim of this work is to investigate chromosomal homologies among *Cerradomys* species or species group and to infer the rearrangements that have occurred during karyotype evolution of the genus. To achieve these goals, we performed classic cytogenetics, *in situ* hybridization with telomeric probes and chromosome painting using *Oligoryzomys moojeni* whole probes (2n=70). *Oligoryzomys* is another genus of the tribe Oryzomyini that belong to a sister clade of *Cerradomys* (Weksler *et al.*, 2006). Both lineages have a shallow and recent split and diverged approximately 5 Mya (Leite *et al.*, 2014). In order to infer the karyotypic evolution, results were plotted on the molecular phylogeny of the genus (Chapter 2).

2. Material and Methods

2.1. Samples

Species were identified based on the integrative taxonomy of Chapter 2. Thus, herein, we consider *C. goytaca* as a valid species and distinct from *C. subflavus*. Besides, since *C. subflavus* and *C. langguthi* may represent polytypic species, we refer samples with 2n=54, 55, 56; FN=62, 63, 64 to "*C. subflavus*" and 2n=46, FN=52 to "*C. langguthi*", since the different lineages recovered as putative species do not have karyotypic differences (Chapter 2).

Samples comprise 10 individuals (also studied using molecular data in Chapter 2) herein referred as: *Cerradomys marinhus* (CMARI - 2n=56, FN=54); *C. maracajuensis* (CMARA - 2n=56, FN=58); *C. akroai* (CAK - 2n=60, FN=76); *C. scotti* (CSC - 2n=58, FN=72); "*C. langutthi*" (CLA - 2n=46, FN=56); *C. vivoi* (CVI - 2n=50, FN=64); *C. goytaca* (CGO - 2n=54, FN=66) and "*C. subflavus*" (CSU - 2n=54, 55, 56, FN=62, 63, 64) (Table 1). Generally, in cytogenetics studies, species are called by the first letter of the genus followed by the first two letters of the species. However, as *C. maracajuensis* and *C. marinhus* begin with the same letters, we used the first four letters of the species name to designate them.

Animals were euthanized according to the protocol of the "Animal experimentation ethics" (Carpenter *et al*., 1996). The skins, skulls and partial skeletons were deposited at the Museu de Zoologia da Universidade de São Paulo (MZUSP), Museu Nacional da Universidade Federal do Rio de Janeiro (MN) and Núcleo de Pesquisa em Ecologia e Desenvolvimento Sócio-Ambiental de Macaé (NUPEM).

2.2. Chromosomal preparation and classic cytogenetics

For *C. marinhus*, *C. maracajuensis*, *C. vivoi* and "*C. subflavus*" (2n=54), metaphases were obtained *in vitro* from fibroblast cell culture (Freshney, 1986) and for *C. akroai*, *C. scotti*, *C. goytaca*, "*C. langguthi*", and "*C. subflavus*" (2n=55 and 2n=56), metaphases were obtained *in vivo* from spleen and bone marrow (Ford and Hamerton, 1956). All samples were analyzed by conventional Giemsa staining, CBG-banding and GTG-banding according to Sumner (1972) and Seabright (1971), respectively (Table 1).

2.3. Fluorescence in situ Hybridization (FISH)

Chromosome painting was performed in *C. marinhus*, *C. maracajuensis*, *C. scotti*, "*C. langguthi*", *C. vivoi*, *C. goytaca* and "*C. subflavus*" (2n=54) (Table 1). *Cerradomys akroai* and "*C. subflavus*" (2n=55 and 56) were only analyzed by classic cytogenetics

(CBG and GTG-banding) and FISH with telomeric probes because of the quality of the chromosome preparations.

Twenty specific painting probes of *Oligoryzomys moojeni* 2n=70, FN=72 were used (OMO Xa, 1–8, 11, 16, 17, 25–30, 33, and 34), obtained by fluorescence-activated cell sorter (FACS) (Di-Nizo *et al.* 2015). Probes were made by degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR) and labeled with biotin-16-dUTP (Telenius *et al.*, 1992; Yang *et al.*, 1995). FISH was performed according to Yang *et al.* (1995) and Di-Nizo *et al.* (2015) with probes being detected with avidin-FITC. Metaphases were analyzed with specific filters for DAPI and FITC (emission at 461 and 517 nm, respectively) in a Zeiss Axiophot fluorescence microscope.

Fluorescence *in situ* hybridization (FISH) with telomeric probes $(TTAGGG)_n$ labeled with FITC was carried out in all samples following the recommended protocol (Telomere PNA FISH Kit, Code No. K5325, DAKO). Slides were counterstained with 4′,6-Diamidine-2′-phenylindole dihydrochloride (DAPI) with antifade mounting medium. In some cases, propidium iodide (PI) was added in the fluorescence antifade solution in order to visualize chromosomes.

The hybridization pattern was plotted in the molecular phylogeny obtained with four molecular markers (Chapter 2), in order to infer the chromosome rearrangements directions that have occurred during the karyotype evolution of the genus.

3. Results

3.1. Cerradomys marinhus (CMARI) – 2n=56, FN=54

GTG-banding and allowed the recognition of homologues (Fig. 1a). The hybridization of OMO probes in metaphases of CMARI revealed 23 homologous segments (Table 2). Of the 20 OMO probes, 11 (OMO 2, 3, 8, 11, 25 to 28, 30, 33 and 34) hybridized to whole chromosomes of *C. marinhus* (CMARI 1, 2, 12, 13, 16, 22, 23, 19, 24, 26 and 27, respectively) (not showed). Four paints (OMO 1, 4, 5 and 6) hybridized to more than one pair, and the remaining autosomal probes (OMO 7, 16, 17 and 29) produced a single signal in one chromosomal region of *C. marinhus*. The association of probes OMO 4 and OMO 5 was observed in chromosome CMARI 3 (Fig. 3). OMO Xa painted the whole CMARI X and Yp (not showed).

FISH with telomeric probes showed signals exclusively on telomeric regions (Fig. 4a).

3.2. Cerradomys maracajuensis (CMARA) – 2n=56, FN=58

GTG-banding allowed the recognition of homologues (Fig. 1b). The hybridization of OMO probes in *C. maracajuensis* showed 23 homologous segments (Table 2). Eleven out of the 20 probes (OMO 2, 3, 8, 11, 25-28, 30, 33 and 34) hybridized to whole chromosomes of *C. maracajuensis* (CMARA 1, 3, 14, 17, 18, 19, 22, 23, 24, 25 and 27, respectively) (not showed). Four probes (OMO 7, 16, 17 and 29) hybridized to one chromosomal region. In addition, four probes (OMO 1, 4, 5 and 6) hybridized to more than one chromosome. The chromosome CMARA 5 showed an association of the probes OMO 4 and OMO 5 (Fig. 3). OMO Xa painted the CMARA X and Yp (not showed).

FISH with telomeric probe showed signals on telomeric regions only (Fig. 4b).

3.3. Cerradomys akroai (CAK) – 2n=60, FN=76

GTG-banding was important for the recognition of homologues and sex chromosomes (Fig. 1c). FISH with telomeric probes showed signals on telomeric regions (Fig. 4c).

3.4. Cerradomys scotti (CSC) – 2n=58, FN=72

GTG-banding was important for the recognition of homologues (Fig. 1d). Cross-species hybridization of OMO probes revealed 26 homologous segments (Table 2). Eight probes (OMO 8, 11, 25, 26, 28, 29, 30 and 34) hybridized to whole chromosomes of *C. scotti* (CSC 21, 19, 15, 25, 18, 23, 2 and 28, respectively) (not showed). Four probes (OMO 2, 7, 17 and 27) hybridized to one chromosomal region. Seven paints (OMO 1, 3 to 6, 16 and 33) hybridized to more than one pair. The association OMO 4 and OMO 5 was observed in pair CSC 10 (Fig. 3). OMO Xa painted the CSC X entirely (not showed).

FISH with telomeric probes showed signals on the telomeric regions (Fig. 4d).

3.5. "Cerradomys langguthi" (CLA) – 2n=46, FN=56

GTG-banding allowed the recognition of homologues (Fig. 2a). Hybridizations of OMO paints showed 31 homologous segments. Only five probes (OMO 25, 26, 28, 29 and 30) painted whole chromosomes (CLA 14, 19, 20, 18 and 23, respectively). Four probes (OMO 8, 17, 27 and 34) hybridized to one chromosomal region. Ten probes hybridized to more than one chromosome / region (OMO 1 to 7, 11, 16 and 33). OMO 4

and OMO 5 were associated in CLA 3 (Fig. 3). Six OMO probes hybridized in chromosome CLA 2/7 and five OMO probes hybridized in CLA 3/5 (Fig. 5). OMO Xa showed signals in CLA X entirely and Y proximal (not showed).

FISH with telomeric probes showed signals in the telomeric regions and in interstitial telomeric sites (ITS) in three autosomal pairs. The largest submetacentric pair (2/7) showed multiples ITS and pairs 3/5 and 4 showed signals in the pericentromeric position (Fig. 5 and 6a).

3.6. Cerradomys vivoi (CVI) – 2n=50, FN=64

GTG-banding allowed the recognition of homologues (Fig. 2b). Thirty-two homologous segments were observed after hybridization of OMO probes in *C. vivoi* metaphases. Six out of the 20 probes (OMO 8, 11, 25, 26, 30 and 34) painted whole chromosomes (CVI 12, 19, 13, 18, 6 and 22, respectively). Four probes (OMO 7, 16, 17, 27 and 28) hybridized to one chromosomal region or arm. Eight probes painted more than one pair or region (OMO 1 to 6, 29 and 33). In addition, twelve probes formed the four largest pair of *C. vivoi* (Fig. 7). Two associations were observed: OMO 4 and 5 on CVI 1(OMO 5 painted two regions of the same pair) (Fig. 3) and OMO 16 and 28 on CVI 2 (Fig. 7). OMO Xa painted CVI X entirely and Y proximal (not showed).

FISH showed interstitial telomeric sites (ITS) in the centromeres of pairs 1 and 4, besides the telomeric regions itself in all chromosomes (Fig. 6b and 7).

3.7. Cerradomys goytaca (CGO) – 2n=54, FN=66

GTG-banding allowed homologues to be paired (Fig. 2c). The hybridization of OMO probes in metaphases of *C. goytaca* showed 27 homologous segments. Seven probes (OMO 8, 11, 25 to 27, 30 and 34) painted whole chromosomes (CGO 17, 18, 19, 20, 23, 24 and 26). Four probes (OMO 7, 17, 28 and 29) hybridized to one chromosome region or arm. Eight probes painted more than one chromosome or region (OMO 1 to 6, 16 and 33). The following associations were observed: OMO 4 and 5 (OMO 5 painted two regions of the same pair) (Fig. 3) and OMO 28, 16 and 6 (Fig. 8). Four probes painted CGO 1 and three CGO 2 (Fig. 8). Xa painted CGO X entirely (not showed).

FISH with telomeric probes presented positive signals in all telomeric sites and ITS in the pericentromeric region of pair 1 (Fig. 6c).

3.8. "Cerradomys subflavus" (CSU) – 2n=54, 55 and 56; FN=62, 63 and 64

Three different diploid numbers were observed: (i) 2n=54, FN=62; (ii) 2n=55, FN=63 and 2n=56, FN=64. GTG-banding allowed the recognition of homologues (Fig. 2d). Cross-species chromosome painting with OMO probes was performed in CSU (2n=54). Twenty-seven homologous segments were detected. Seven probes (OMO 8, 11, 25-27, 30 and 34, respectively) painted whole chromosomes (CSU 15, 21, 16, 17, 19, 24 and 26). Four probes (OMO 7, 17, 28 and 29) hybridized to one chromosomal region or arm. Eight probes (OMO 1 to 6, 16 and 33) painted more than one chromosome. Probes OMO 4 and OMO 5 were associated in CSU 1 (OMO 5 painted two regions of the same pair) and OMO 28, 16 and 6 were associated in CSU 2 (Fig. 9). Four probes painted CSU 1 (Fig. 9) and three probes painted CSU 2. OMO Xa showed signal in CSU X and Y proximal (not showed).

After FISH with telomeric probe, all chromosomes presented positive signals in telomeric positions. In addition, samples with 2n=54 and 2n=55 showed ITS in pericentromeric regions of pairs 1 and 5/6 while the sample with 2n=56 showed ITS only in pair 1 (Fig. 6d).

4. Discussion

This is the first work that establishes chromosome homology maps within the genus *Cerradomys* based on classic and molecular cytogenetics. Besides, in order to infer the directions of the chromosome rearrangements that have occurred during the karyotype evolution of the genus, we compared our results of chromosome painting to the molecular phylogeny and times of divergence of *Cerradomys* (Chapter 2) (Fig. 10).

4.1. Chromosome changes revealed by GTG-banding and chromosome painting

GTG-banding pattern is inedited for *C. marinhus*, *C. maracajuensis*, *C. akroai* and *C. goytaca*, reiterating the importance and lack of classic cytogenetic studies in this group. This banding pattern was important for the recognition of homologues and intra/interspecific comparison of the largest pairs.

Chromosome painting using *Oligoryzomys moojeni* probes in metaphases of seven *Cerradomys* species revealed complex rearrangements among very closely related species. Of the 20 OMO probes, only three painted whole chromosomes in all *Cerradomys* species studied, showing that these chromosomes are conserved. In

addition, five OMO probes produced single signals on metaphases of *Cerradomys*, but they are associated to fusion/fission rearrangements since they painted only a region of these chromosomes. The remaining probes showed more than one signal in at least one species, revealing that they are rearranged in the genome of *Cerradomys*.

OMO 1 and OMO 6 painted more than one region in all *Cerradomys* species, showing that a fission event occurred in the ancestral of the genus (Fig. 10).

Cerradomys marinhus and *C. maracajuensis*, two sister species that diverged early in the phylogeny, presented the same diploid number (2n=56), but different fundamental numbers (FN=54 and FN=58, respectively). Comparative chromosome painting revealed a very similar hybridization pattern, corroborating the close relationship between them. In addition, the difference between both FN is due to pericentric inversions in two pairs (CMARI 1/ CMARA 1 and CMARI 25/ CMARA 2) (Fig. 10).

The remaining species clustered in the sister clade of *Cerradomys marinhus* and *C. maracajuensis* (Fig. 10). Comparison between chromosome painting and molecular phylogeny revealed that many rearrangements occurred during karyotype evolution of this clade, such as fission of OMO 3, OMO 16 and OMO 33 and fusion of OMO 27 (Fig. 10).

Internal relationship showed that *C. akroai* and *C. scotti* are closely related (Fig. 10). Although it was not possible to perform chromosome painting in *C. akroai* metaphases, comparative GTG-banding on the largest pairs suggested that the karyotype of *C. akroai* (2n=60, FN=76) and *C. scotti* (2n=58, FN=72) differed by pericentric inversions in two medium pairs (CAK 1 / CSC 1 and CAK 2 / CSC 14) (Fig. 11). In addition, a fusion/fission event plus at least two pericentric inversions or centromere repositioning, which could not be detected by GTG-banding comparison, is necessary to explain karyotypic differences between both species, showing that extensive chromosome change occurred in this clade. Besides, addition/deletion of constitutive heterochromatin occurred since *C. akroai* showed heterochromatic band in two autosome pairs while *C. scotti* showed heterochromatin restricted to centromeric position (Chapter 2).

The other species nested in a clade in which "*C. langguthi*" was the sister of *C. vivoi*, *C. goytaca* and "*C. subflavus*" (Fig. 10). The probe OMO 2 painted more than one region in all of them, revealing fission events. In addition, "*C. langguthi*" was one of the species that suffered the largest number of rearrangements (fission of OMO 2, OMO 6,

OMO 7 and OMO 11 and *in tandem* and centric fusion of OMO 8+16+34+27+3+1, OMO 3+16+6+4+5 and OMO 6+11+6+7+10), leading to the lowest diploid number of the genus.

Chromosome painting showed that a fusion event occurred in OMO 5 and OMO 28 in the clade composed of *C. vivoi*, *C. goytaca* and "*C. subflavus*" (Fig. 10). Many rearrangements were observed in *C. vivoi*, such as fission of OMO 4, OMO 29 and OMO 33 and Robertsonian and *in tandem* fusions of OMO 3+33+29+6+5+4+5, OMO 16+28+6+5, OMO 2+7 and OMO 17+4+1 (Fig. 7 and 10).

Hybridization pattern with OMO probes and GTG-banding comparison in *C. goytaca* and "*C. subflavus*", showed that both are closely related. However, complex rearrangements were observed between them. Comparing *C. goytaca* with "*C. subflavus*" (2n=56), both differed by a centric fusion of pairs CSU 13 and CSU 6 (=CGO 3) or centric fission of pair CGO 3 (Fig. 11) plus a paracentric inversion in one pair (CGO 14, CSU 12) and pericentric inversions in two small pairs. Considering *C. goytaca* and "*C. subflavus*" (2n=54), despite presenting the same diploid number, a complex scenario with a fission of CSU 5/6 followed by a centric fusion of CSU 6 + CSU 13 (= CGO 3) or a centric fission in CGO 3 (leading to CSU 6 and CSU 13) followed by a centric fusion (CSU 5/6) plus a paracentric inversion in one pair (CGO 14 and CSU 12) and pericentric inversions in two small pairs are necessary to explain karyotypic differences between both species (Fig. 12). Thus, our results corroborate previous work that suggested that "*C. subflavus*" with 2n=56 gave rise to 2n=55 and 2n=54 (*C. subflavus*) (Almeida and Yonenaga-Yassuda, 1985) and *C. goytaca*.

Regarding OMO Xa, this probe was conserved and painted the whole X chromosomes of all *Cerradomys* species. In addition, OMO Xa painted the euchromatic region of *C. marinhus*, *C. maracajuensis*, *C. langguthi*, *C. vivoi* and *C. subflavus* Y. This pattern was also observed in *Oligoryzomys* species and *Pseudoryzomys simplex* (using *Holochilus brasiliensis* X probe) and may be associated to the pseudoautosomal region (Moreira *et al.*, 2013; Di-Nizo *et al.*, 2015).

4.2. Probes associations revealed by chromosome painting

The hybridization experiments revealed an association between probes OMO 4 and OMO 5 in all *Cerradomys* species (Fig. 3). This association is plesiomorphic since it was also observed in five *Oligoryzomys* species (Di-Nizo *et al.*, 2015). In addition, these probes painted more than one chromosome region in all species, including regions

of the same chromosome in *C. vivoi*, *C. goytaca* and "*C. subflavus*" (Fig. 3, Table 2), showing that complex rearrangements are involved in the evolution of both chromosomes.

The autapomorphic synteny of OMO 16 and OMO 28 was observed in the clade composed of *C. vivoi*, *C. goytaca* and "*C. subflavus*", with a pericentric inversion or centromere repositioning involving these two probes, since OMO 16 painted the distal region of CVI 2 and the proximal region of CGO and CSU 2.

4.3. Distribution of telomeric repeats

Patterns of distribution of telomeric repeats are being described for the first time for *C. marinhus*, *C. maracajuensis*, *C. akroai* and *C. goytaca*. Herein, the species with the highest diploid numbers of the genus (*Cerradomys marinhus*, *C. maracajuensis*, *C. akroai* and *C. scotti*), presented telomeric signals restricted to telomeric sites (Fig. 4), whereas ITS were observed in the species with lower diploid numbers ("*C. langgutthi*", *C. vivoi*, *C. goytaca* and "*C. subflavus*") (Fig. 6).

"*Cerradomys langguthi*" has the lowest diploid number of the genus and presented multiple ITS at pair 2/7 and centromeric ITS in pairs 3/5 and 4. Comparative analyses of telomeric distribution and chromosome painting revealed that from the five ITS observed in pair 2/7, four coincide with sites of association between two OMO probes and one occurred in the middle of OMO 1. The centromeric ITS observed in pairs 3/5 and 4 also occurred between two OMO probes (Fig. 5 and 6a). These results are in accordance with the ones described by Nagamachi *et al.* (2013), except that the authors called pairs 2/7, 1 and 3/5 by 1, 2 and 3, respectively. Herein, we kept the original nomenclature of chromosomes described by Maia and Hulak (1981).

Cerradomys vivoi presented ITS at pairs 1 and 4, corroborating the pattern observed by Andrades-Miranda *et al.* (2002). *Cerradomys goytaca* and "*C. subflavus*" (2n=56) presented ITS in centromeric position of pair 1 while "*C. subflavus*" with 2n=54 and 2n=55 also presented ITS in the centromeric region of pair 5/6. These centromeric ITS also overlapped associations between two OMO probes (Fig. 7, 8 and 9).

Our results, associated with the molecular phylogeny, suggests that the ITS observed in *Cerradomys* species with the lowest diploid numbers are relicts of telomeres resulted from past fusions (Fig. 10), since they belong to a clade that diverged recently in the phylogeny. In the case of CLA 2/7, multiple intrachromosomal telomeric

sequences resulted from at least five *in tandem* fusions and in the cases of CLA 3/5, CLA 4, CVI 1, CVI 4, CGO 1, CSU 1 and CSU 5/6, ITS resulted from centric fusions.

These observations are in accordance with CBG-banding pattern that showed a weak heterochromatic signal in the pericentromeric region of CLA 3/5, CLA 4, CVI 4, CGO 1,CSU 1 and CSU 5/6, discarding the possibility of the ITS, in these cases, be a consequence of heterochromatin amplification and reiterating that loss of constitutive heterochromatin may be associated to fusion events (Sumner, 2003).

Myriad vertebrate species presented ITS that were related to remnants of telomere after chromosome fusion events (Meyne *et al.*, 1990; Lee *et al.*, 1993; Fagundes *et al.*, 1997b; Pellegrino *et al.*, 1999). However, the presence of ITS not always is related to rearrangements, since these sequences were observed in species that present supposedly conserved karyotypes such as hylid frogs and marsupials (Wiley *et al.*, 1992; Pagnozzi *et al.*, 2000; Metcalfe *et al.*, 2004).

Alternative mechanisms by which non-telomeric repeats are generated were described, such as amplification of $TTAGGG_n$ sequences, components of satellite DNA, exchange, transposition or unequal sister chromatide exchange and introduced by telomerase or by transposons elements (Wiley *et al.*, 1992; Ventura *et al.*, 2006; Ruiz-Herrera *et al.*, 2008).

On the other hand, several species with highly rearranged karyotypes (detected by GTG-banding and chromosome painting), did not present ITS, suggesting that these sequences may have been lost by chromosome breaks (Silva and Yonenaga-Yassuda, 1997; 1999; Di-Nizo *et al.*, 2015). Although ITS were observed in *Cerradomys*, several rearrangements were detected without the presence of ITS (Fig. 3, 5, 7, 8 and 9), showing that this genus underwent both retention and loss of ITS throughout its karyotypic evolution.

Additionally, regarding *C. goytaca* and "*C. subflavus*", because the chromosome CSU 6 is included in a complex rearrangement involving CGO 3 and this pair do not present ITS, it is likely that the non-telomeric repeat observed in CSU 5/6 are an ancient telomere of pair CSU 5 and that the telomere of pair CSU 6 was lost after chromosome breakage to form CGO 3.

4.4. Chromosome evolution in Cerradomys and its role in speciation

Classic and molecular cytogenetics placed in a phylogenetic context were essential to detect patterns of chromosomal evolution of *Cerradomys*. Complex

rearrangements are involved in the karyotype evolution of the genus, such as *in tandem* and centric fusion, fission, centromere repositioning, pericentric and paracentric inversions.

Such rearrangements require inactivation/loss and activation/gain of centromeres and telomeres to keep the chromosomal stability. According to Bolzán (2017), ITS in the majority of cases are probably inactivated, due to the loss of telomere proteins, changes in chromatin or epigenetic mechanisms.

Fusion events produce chromosomes with more than one centromere, which require the extra centromere to be eliminated or inactivated (alternatively, if two centromeres are physically close, they can act as one monocentric structure) (Choo, 1997). Fissions that do not involve centromere require the formation of neocentromere while on the opposite, in centric fission, centromere would be divided in two functional parts (Choo, 1997). Mechanisms by which centromeres are inactivated/activated and neocentromere formation remain unclear, although studies suggest that epigenetic factors and the presence of centromere specific sequences, ectopic to centromere region (latent centromere) are the main causes (Choo, 1997; Ferreri *et al.*, 2005; Marshall *et al.*, 2008)

Different rates of chromosome changes were observed within *Cerradomys*, as the clade composed of *C. marinhus* and *C. maracajuensis* almost did not change karyotypically, contrarily to its sister clade, in which extensive chromosome rearrangements were observed. Chromosome evolution was associated to the increase in diploid number in the clade composed of *C. akroai* and *C. scotti*, followed by decrease in the sister clade ("*C. langguthi*" and *C. vivoi*) and posterior increase in *C. goytaca* and "*C. subflavus*" (Fig. 10).

Chromosome changes have been associated to speciation. Specifically, Robertsonian rearrangements and tandem fusions may form trivalents, quadrivalents or multivalents in meiosis, that can be disjoined, leading to unbalanced gametes, reducing gene flow and posteriorly being a reproductive barrier (Sumner, 2003).

According to Garagna *et al.* (2014), species with different chromosomal variants may represent precursor for new species. This may be the case of "*C. langguthi*" and "*C. subflavus*", since both present polymorphisms associated to Robertsonian rearrangement and pericentric inversion, although exclusive karyotypes could not be observed in structured clades (Chapter 2).

In addition, the non-telomeric sequences observed in the junction of pairs CLA 2/7, CLA 3/5 and CSU 5/6 are probably yielding chromosomal instability since samples with acrocentrics/subtelocentrics pairs CLA 2, CLA 7, CLA 3, CLA 5, CSU 5 and CSU 6 were described in the literature (Maia and Hulak, 1981; Almeida and Yonenaga-Yassuda, 1985). In this case, ITS can be acting as hotspots for chromosome rearrangements, conferring chromosomal plasticity to their holders.

Thus, ITS is congruent with the hypothesis that "*C. langguthi*" and "*C. subflavus*" may be incipient species (Chapter 2) and that what is being called now as polymorphisms, is part of an intermediate process of speciation.

In *Cerradomys,* very close related species have experienced recent and huge genomic reorganization, as corroborated by molecular dating (Chapter 2). The process of occurrence and fixation of rearrangements can be surprisingly fast, as demonstrated by the unexpected chromosomal diversity of the Madeiran house mouse, that occupied this island together with humans, about 500 years ago (Britton-Davidian *et al.*, 2000).

As a matter of fact, house mouse is an extraordinary example of emergence and fixation of Robertsonian rearrangement in wild population. With the standard karyotype of 2n=40 (all chromosomes acrocentrics), more than 90 chromosomal races were described in populations of Western Europe. These karyomorphs were associated to centric fusions, leading to lower diploid numbers variants (Capanna *et al.*, 1977; Searle, 1998; Garagna *et al.*, 2014). In addition, natural hybrids were captured at the contact areas between standard and rearranged karyotypes population (Capanna *et al.*, 1977; Searle, 1998; Garagna *et al.*, 2014), indicating that this species may be in process of speciation.

Similarly, in Neotropical fauna, the Akodontini species *Akodon cursor* is another outstanding example of chromosomal evolution and speciation. Distributed in East Atlantic Forest, this species present a great chromosomal variability due to Robertsonian rearrangement and pericentric inversions (2n=14-16, FN=18-26) (Yonenaga-Yassuda, 1979; Fagundes *et al.*, 1998). Phylogenetic analyses showed two geographical structured North and South clades and, although Geise *et al.* (2001) and Silva *et al.* (2006) recovered samples with 2n=16 separated from 2n=14 and 15, recent studies showed that the subclades are not associated with exclusive karyotypes (Massariol, 2016). Also, sterile hybrids between *A. cursor* and *A. montensis* were detected in wild population (Yonenaga *et al.*, 1975) and experimental crosses involving specimens of *A. cursor* with different diploid numbers do not indicate reproductive

isolation, although reduced fertility was detected in individuals with 2n=15 (Massariol, 2016). Thus, the reciprocal monophyly between North and South clades, in addition to the reduction in fertility of 2n=15 individuals, possibly indicates an incipient process of speciation, with a tendency of 2n=16 be fixed in the North and 2n=14 in the South.

This work corroborates that *C. goytaca* and "*C. subflavus*" are two distinct species as observed in Chapter 2, since complex karyotypic differences between them, compatible with a reproductive isolation, were observed. The complex rearrangements observed between both species are enough to corroborate the hypothesis that *C. goytaca* and "*C. subflavus*" are distinct species because hybrids may present meiotic problems due to mal-segregation and may not be viable.

Cerradomys goytaca has a small effective population size and is geographically isolated from *C. subflavus*, occupying restricted areas of Restinga, a harsh and adverse environment (Tavares *et al.*, 2011; Lemos and Gonçalves, 2015). An accelerate rate of phenotypic evolution was observed for this species due to strong directional selection (Tavares *et al.*, 2016). The Restinga characteristics, small population size, restricted dispersion because of dense forests around Restinga and geographic isolation may have facilitate chromosome fixation in *C. goytaca*, being an indicative of adaptive chromosomal evolution (Árnason, 1972).

4.5.Final considerations

Comparative cytogenetic studies within subfamily Sigmodontinae, including chromosome painting, have increased in the last decade. Nevertheless, this latter approach was only performed in tribes Akodontini and Oryzomyini, and still lacks for other Sigmodontinae groups (Fagundes *et al.*, 1997a; Hass *et al.*, 2008; Ventura *et al.*, 2009; Nagamachi *et al.*, 2013; Di-Nizo *et al.*, 2015; Suárez *et al.* 2015; Pereira *et al*. 2016; Malcher *et al.*, 2017).

Karyotype evolution in Oryzomyini was studied with specific chromosome painting in the genus *Oligoryzomys*, revealing extensive chromosome rearrangements and increase / decrease in diploid numbers in different lineages (Di-Nizo *et al.*, 2015). Comparisons between two Oryzomyini genera *Hylaeamys megacephalus* and *Cerradomys langgutthi* was also performed by chromosome painting and the huge genomic reorganization observed is expected, since both genera are distant (Nagamachi *et al.*, 2013). More recently, *Hylaeamys megacephalus* probes were used to compare tribes Akodontini and Oryzomyini (Suárez *et al*., 2015; Pereira *et al.*, 2016), two

populations of *Oecomys catherinae* (Malcher *et al*., 2017) and three undescribed species of *Neacomys* (tribe Oryzomyini) (da Silva *et al.*, 2017).

This work shed light on the karyotype evolution of *Cerradomys*, showing inedited results of classic and molecular cytogenics. The modern technique of chromosome painting not only corroborates the GTG-banding pattern but also detected more interespecific rearrangements. The use of a phylogeny to guide chromosome painting results is mandatory to infer the directions of rearrangements.

Herein, high rates of chromosome exchange within closely related species were observed and karyotype evolution showed a tendency of reduction in *Cerradomys*.

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Figures and Tables

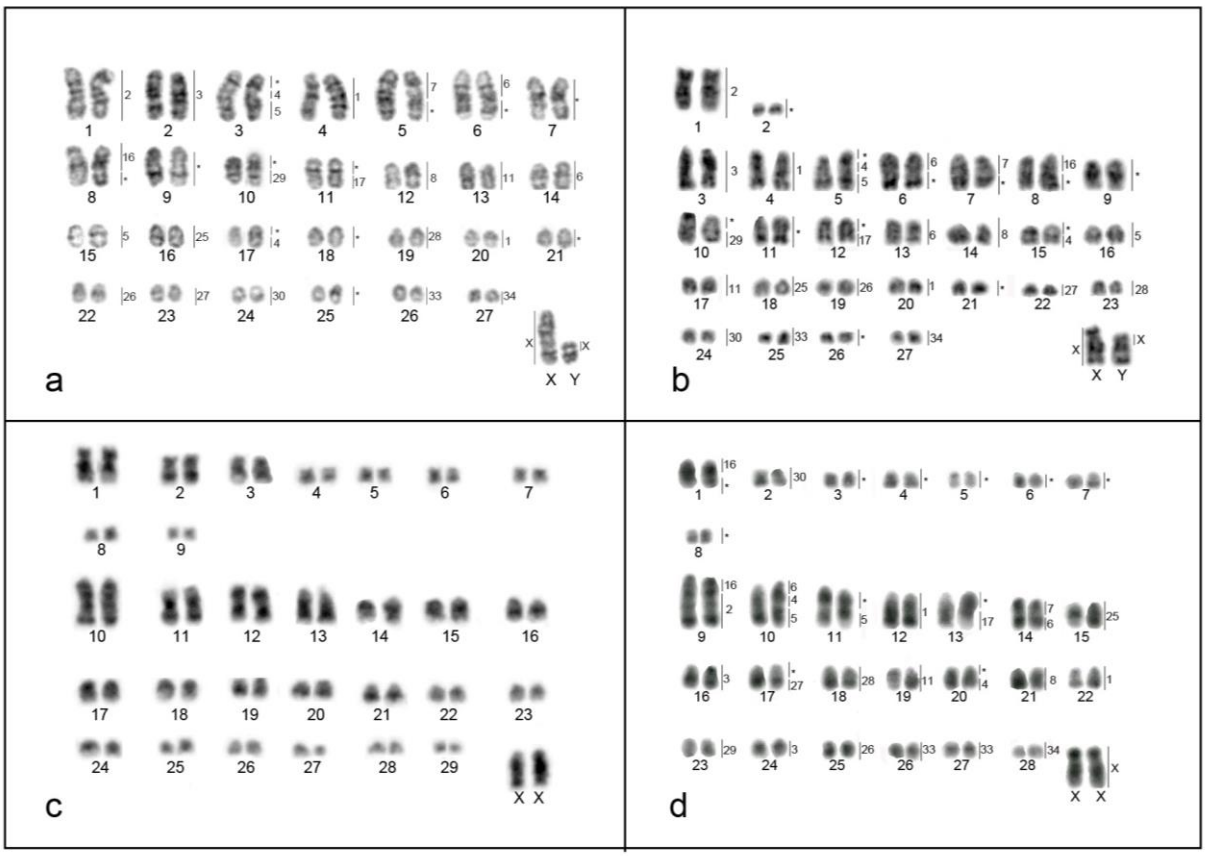

Fig. 1: GTG-banding of *Cerradomys* species: (a) ***C. marinhus*** – 2n=56, FN=54; (b) ***C. maracajuensis*** – 2n=56, FN=58; (c) ***C. akroai*** – 2n=60, FN=76 and (d) ***C. scotti*** – 2n=58, FN=72. Except for *C. akroai*, hybridization pattern of OMO probes are indicated beside the chromosomes.

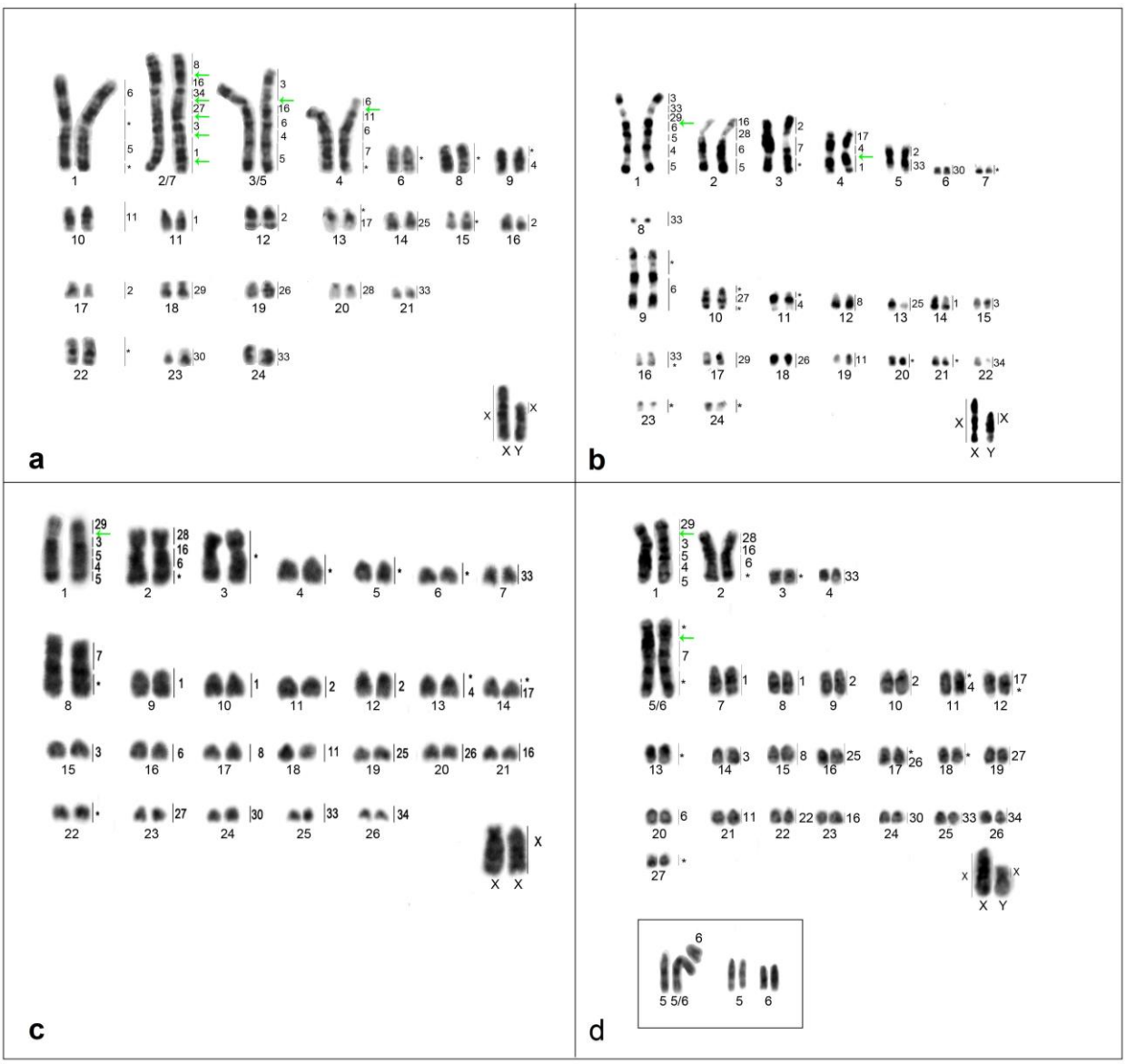

Fig. 2: GTG-banding of *Cerradomys* species: (a) "***C. langguthi*"** – 2n=46, FN=56; (b) ***C. vivoi*** – 2n=50, FN=64; (c) ***C. goytaca*** – 2n=54, FN=66 and (d) "***C. subflavus*"** – 2n=54, FN=62. Inset: pairs 5 and 6 involved in centric fusion in specimens with 2n=55 and 2n=56, respectively. Hybridization pattern of OMO probes are indicated beside the chromosomes. Green arrows indicate ITS.

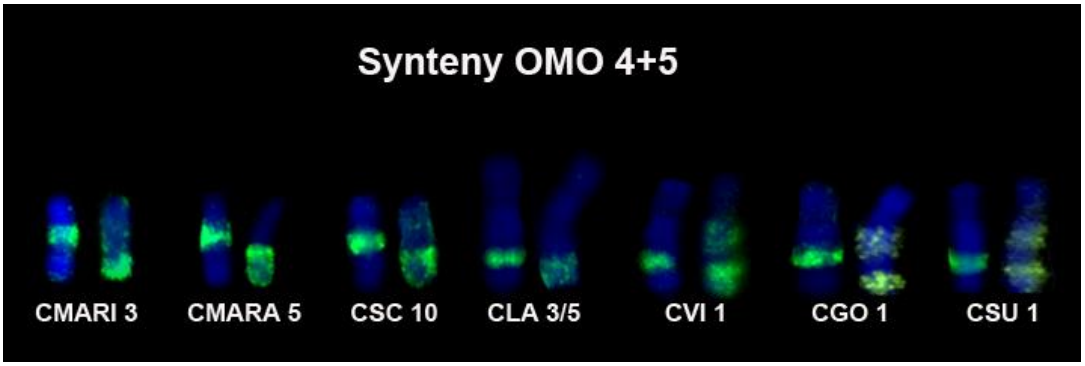

Fig. 3: Associations between OMO probes 4 and 5 detected in all *Cerradomys* species studied.

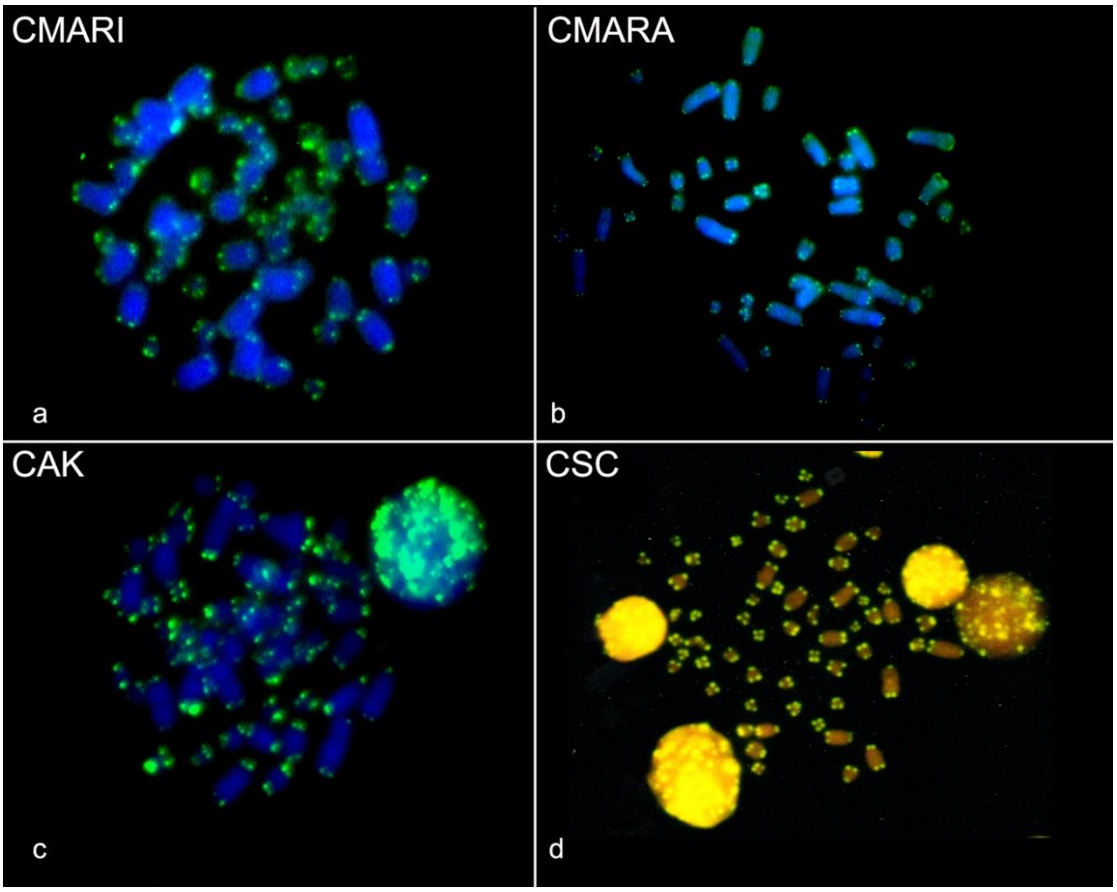

Fig. 4: FISH with telomeric probes in species with highest diploid number (a) ***C. marinhus*** – 2n=56, FN=54; (b) ***C. maracajuensis*** – 2n=56, FN=58; (c) ***C. akroai*** – 2n=60, FN=76 and (d) ***C. scotti*** – 2n=58, FN=72.

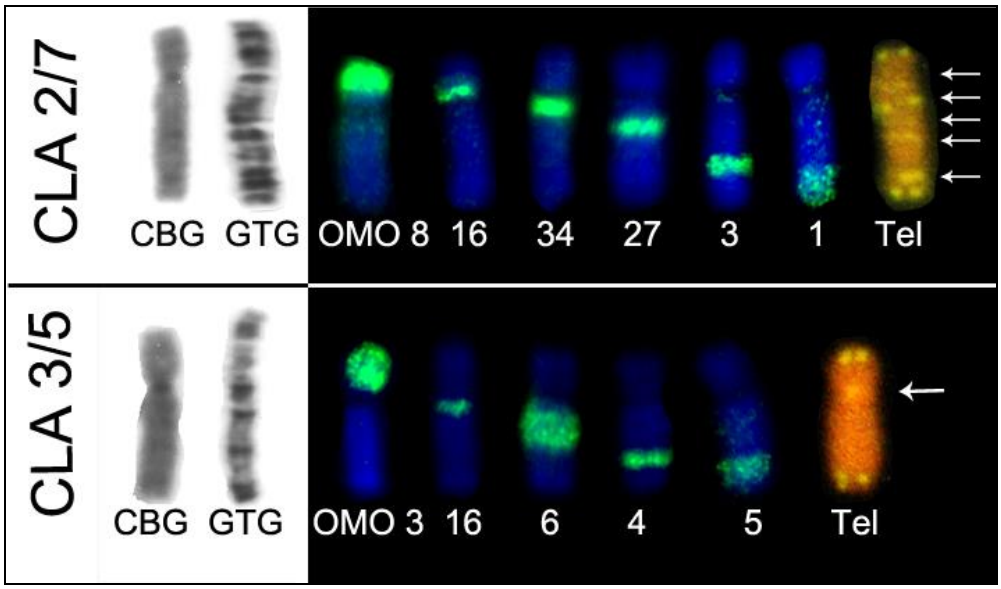

Fig. 5: CBG and GTG-banding pattern and hybridizations with OMO and telomeric probes in chromosomes CLA 2/7 and CLA 3/5 of "*C. langguthi*". Arrows indicate ITS.

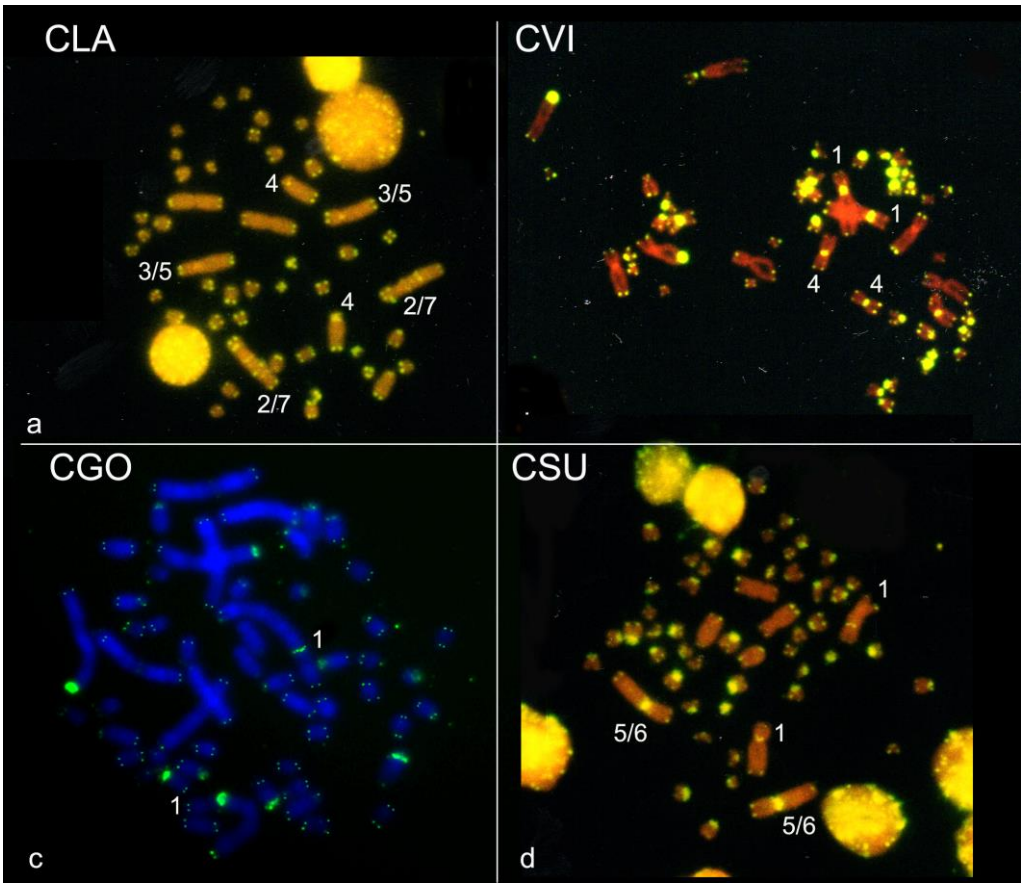

Fig. 6: FISH with telomeric probes in species with lowest diploid number (a) "***C. langguthi***" – 2n=46, FN=56; (b) ***C. vivoi*** – 2n=50, FN=64; (c) "***C. subflavus***" **–** 2n=54, FN=62 and (d) ***C. goytaca*** – 2n=54, FN=66. Chromosomes that bears ITS are indicated.

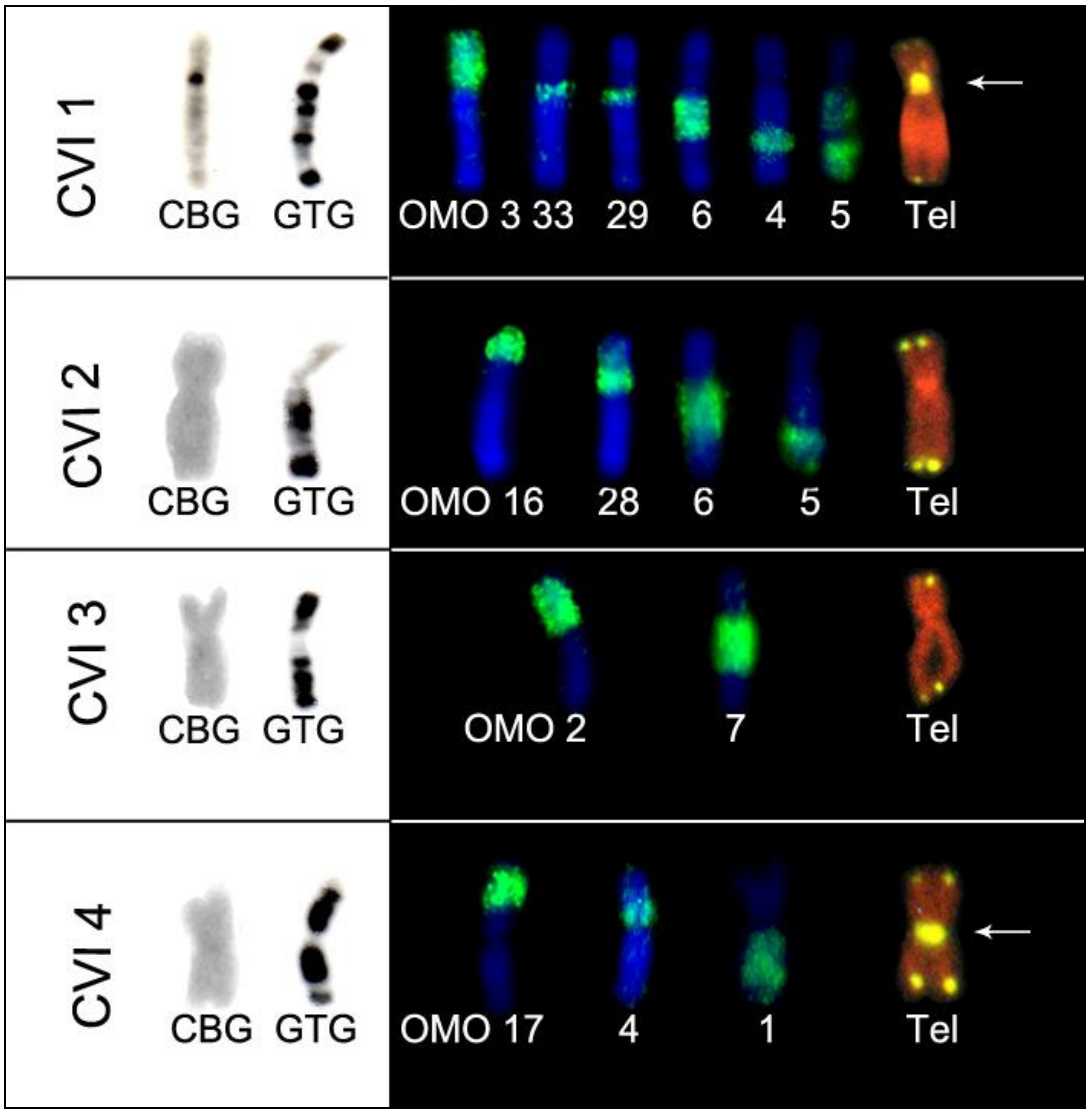

Fig. 7: CBG and GTG-banding pattern and hybridizations with OMO and telomeric probes in four pairs of *C. vivoi* (CVI 1 to 4). Arrows indicate ITS.

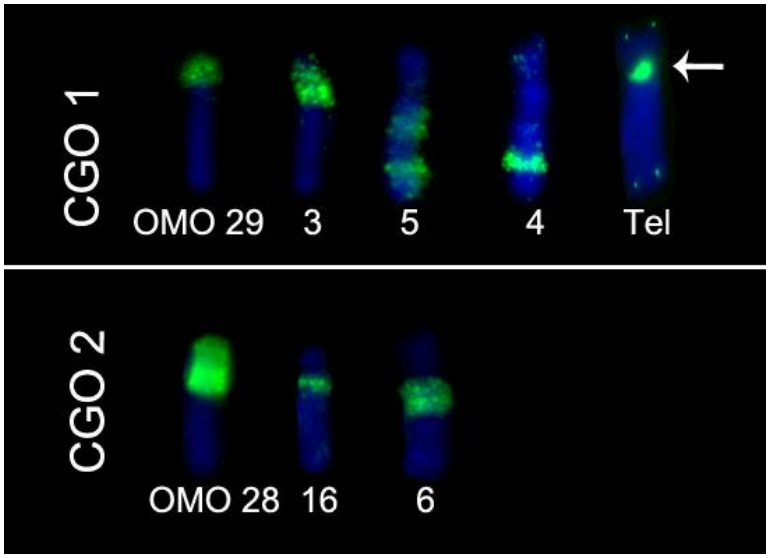

Fig. 8: Hybridizations of OMO and telomeric probes in pairs 1 and 2 of *C. goytaca*. Arrow indicates ITS.

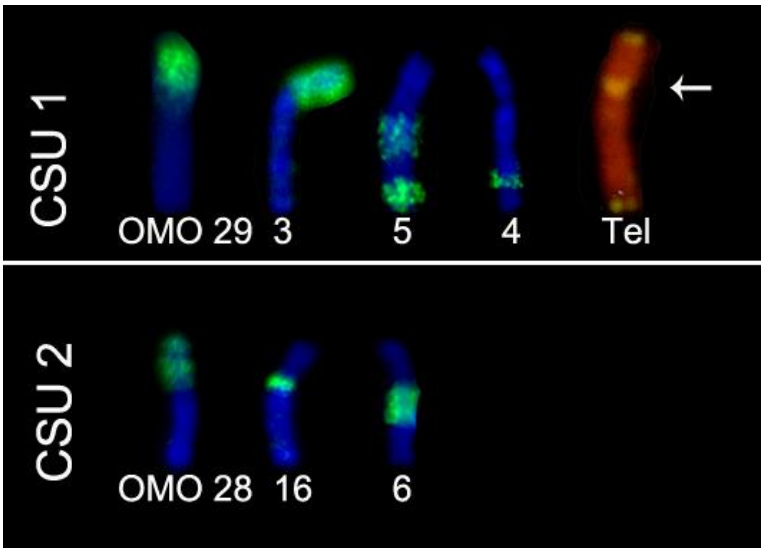

Fig. 9: Hybridizations of OMO and telomeric probes in pairs 1 and 2 of "*C. subflavus*" (CSU 1). Arrow indicates ITS.

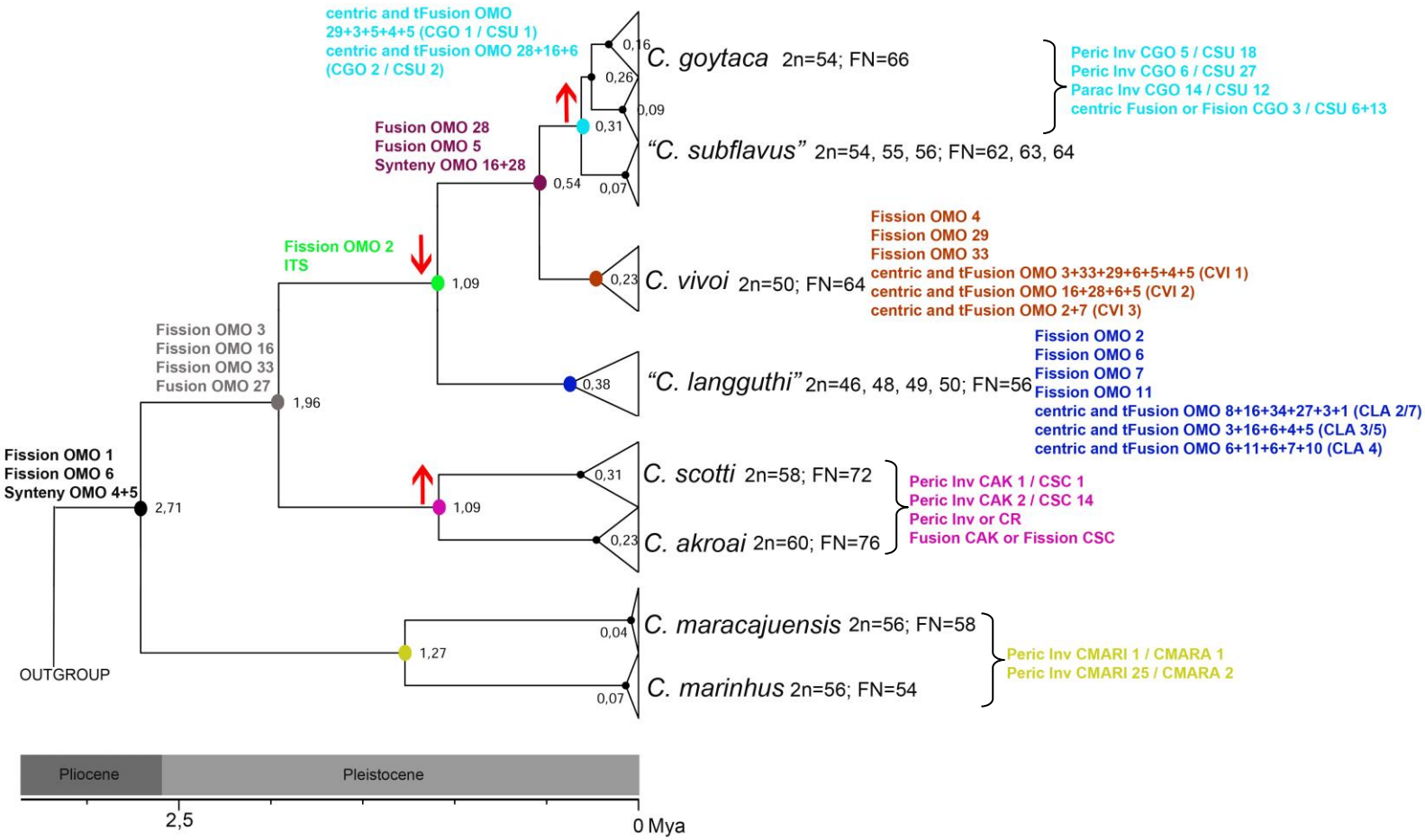

Fig. 10: Phylogenetic relationships of *Cerradomys* based on concatenated matrix (cyt-*b*, COI, i7FBG, IRBP) and diversification times obtained from Bayesian analysis. Values in the nodes represent divergence times. Rearrangements detected by chromosome painting and GTG-banding are plotted. Brackets indicate rearrangements between sister species. Arrows indicate increase or decrease of diploid number.

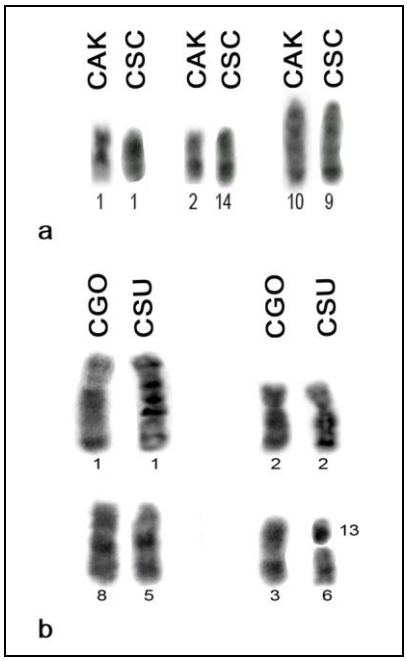

Fig. 11: GTG-banding pattern comparison among largest pairs of *C akroai* (CAK) and *C. scotti* (CSC) (a) and *C goytaca* (CGO) and "*C. subflavus*" (CSU) (b).

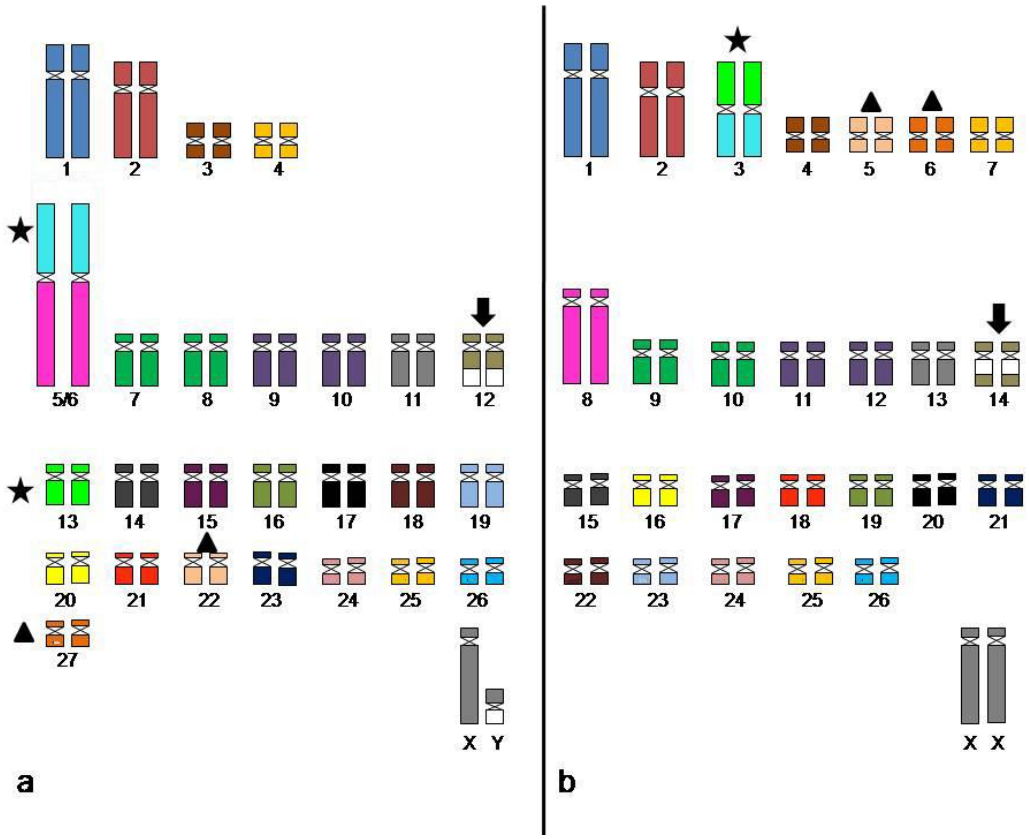

Fig. 12: Homologies between *C. subflavus* - 2n=54, FN=62 (a) and *C. goytaca* – 2n=54, FN=66 (b) detected by chromosome painting with OMO probes and GTG-banding. Symbols represent rearrangements observed between both species. Stars: centric fusion / fission of CSU 13 and 6 (= CGO 3), triangles: pericentric inversions and arrows paracentric inversion.

Table 1: Specimens analyzed in this work. Species or species group, specimen number, diploid number (2n), fundamental number (FN), sex (M = male, F = female) and chromosome painting with *Oligoryzomys moojeni* probes.

Species	**Specimen #**	**2n**	**FN**	**Sex**	**FISH OMO**
C. marinhus (CMARI)	CRB1835	56	54	M	X
C. maracajuensis (CMARA)	APC618	56	58	M	X
C. akroai (CAK)	UUPI161	60	76	M	-
C. scotti (CSC)	MJJS189	58	72	F	X
"*C. langguthi*" (CLA)	JFV474	46	56	M	X
C. vivoi (CVI)	BIO555*	50	64	M	X
C. goytaca (CGO)	PRG2236	54	66	F	X
"*C. subflavus*" (CSU)	DQM059	54	62	M	X
	CIT1396	55	63	F	-
	CIT2053	56	64	M	-

*Sample analyzed in Silva (1994). Collector acronyms: BIO/CIT (Banco de células do laboratório de Citogenética de Vertebrados, IB/USP), APC (Ana Paula Carmignotto), CRB (Cibele Rodrigues Bonvicino), DQM (Diego Queirolo), JFV (Júlio Fernandes Vilela), MJJS (Maria José de Jesus Silva) and PRG (Pablo Rodrigues Gonçalves).

Table 2: Homologous segments detected by chromosome painting with *Oligoryzomys moojeni* (OMO) probes in metaphases of seven *Cerradomys* species: *C. marinhus* (CMARI), *C. maracajuensis* (CMARA), *C. scotti* (CSC), *C. langguthi* (CLA), *C. vivoi* (CVI), *C. subflavus* (CSU) and *C. goytaca* (CGO)

OMO Probes (2n=70)	**CMARI (2n=56,FN=54)**	**CMARA (2n=56,FN=58)**	**CSC (2n=58,FN=72)**	**CLA (2n=46,FN=56)**	**CVI (2n=50,FN=62)**	**CGO (2n=54,FN=66)**	**CSU (2n=54,FN=62)**
OMO 1	4 20	4 20	12 22	2/7 (distal) 11	4q (distal) 14	9 10	7 8
OMO 2	1	1	9 (distal)	12 16 17	3p 5p	11 12	9 10
OMO 3	2	3	16 24	2 (interstitial) 5	1p 15	1p 15	1p 14
OMO 4	3 (interstitial) 17 (distal)	5 (interstitial) 15 (distal)	10 (interstitial) 20 (distal)	3 (interstitial) 9 (distal)	1 (intersticial) 4 (proximal) 11 (distal)	1 (interstititал) 13 (distal)	1 (interstitial) 11 (distal)
OMO 5	3 (distal) 15	5 (distal) 16	10 (distal) 11 (distal)	3 (distal) 1q	1q (two regions) 2q (distal)	1q (two regions)	1q (two regions)
OMO 6	6 (proximal) 14	6 (proximal) 13	10 (proximal) 14 (distal)	1q (proximal) 3 (pericentromeric) 4 (interstitial) 4p	1 (interstitial) 2 (proximal) 9 (distal)	2q (interstitial) 16	2q (interstitial) 20
OMO 7	5 (proximal)	7 (proximal)	14 (proximal)	4 (interstitial)	3q (proximal)	8 (interstititал)	6 (proximal)
OMO 8	12	14	21	7 (distal)	12	17	15
OMO 11	13	17	19	4 (proximal) 10	19	18	21
OMO 16	8 (proximal)	8 (proximal)	1p 9 (proximal)	3 (proximal) 7 (proximal)	2p (distal)	2q (proximal) 21	2q (proximal) 23
OMO 17	11 (distal)	12 (distal)	13 (distal)	13 (distal)	4p	14 (distal)	12 (proximal)
OMO 25	16	18	15	14	13	19	16
OMO 26	22	19	25	19	18	20	17
OMO 27	23	22	17 (distal)	2 (interstitial)	10 (intersticial)	23	19
OMO 28	19	23	18	20	2p (proximal)	2p	2p
OMO 29	10 (distal)	10 (distal)	23	18	1 (intersticial) 17	1q (distal)	1p (distal)
OMO 30	24	24	2	23	6	24	24
OMO 33	26	25	26 27	21 24	1 (intersticial) 5q 8 16 (proximal)	7 25	4 25
OMO 34	27	27	28	2 (proximal)	22	26	26
OMO Xa	X, Yp	X, Yp	X	X, Y (proximal)	X, Y (proximal)	X	X, Y (proximal)
Total	**23**	**23**	**26**	**31**	**32**	**27**	**27**

Chapter 4
Evolutionary complexity, diversification and species limits in Oligoryzomys (Sigmodontinae: Oryzomyini) revealed by molecular phylogeny, phylogeography, cytogenetics and distributional data

Abstract

Molecular phylogenetic inferences were performed in order to recover the evolutionary history and species limits of *Oligoryzomys.* A new phylogenetic hypothesis is proposed on the basis of two molecular markers (mitochondrial cyt-*b* and the nuclear IRBP). *Oligoryzomys* was recovered as monophyletic and internal relationships were poorly resolved, highlighting the possible rapid diversification of the genus. Results of molecular phylogeny integrated to cytogenetic and geographic distribution aids in reveal species limits. Four lineages could not be related to any name (*Oligoryzomys* sp. A-D). *Oligoryzomys flavescens* was found paraphyletic in relation to *O. fornesi*. *Oligoryzomys microtis* and *O. stramineus* showed two structured subclades each. *Oligoryzomys nigripes* also showed two geographically structured major clades: one from northeastern Brazil (with 2n=62, FN=78), and the other from central-south-southeastern Brazil, Argentina, Uruguay and Paraguay (with 2n=62, FN=80-82). Phylogeographic approaches were performed in order to investigate the population structure and patterns of genetic divergence in *O. nigripes*. Results corroborate phylogenetic data, revealing two distinctive phylogroups: a northeast clade (NE) and central-southeast-south clade (CS). The phylogeographic break within these two major phylogroups coincides with Pardo and Jequitinhonha Rivers. CS phylogroup is not well structured and the pattern of differentiation of this population might be related to the past climate shifts. Additionaly, the correlation between phylogenetic, phylogeographic and cytogenetic data suggest that populations from NE and CS might be experiencing a process of speciation. Cytogenetic data were congruent with previous study and could be related to six names: *O. mattogrossae*, *O. moojeni*, *O. chacoensis*, *O. stramineus*, *O. nigripes* and *O. flavescens* although the last two should be taxonomic reviewed. An undescribed karyotype (2n=70, FN=72) for an Amazonian sample here treated as *Oligoryzomys aff. utiaritensi*s is being reported. Moreover, new records were found in Brazil for the following species or species-complex: *O. microtis*, *O. mattogrossae*, *O. moojeni* and *O. flavescens*. 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. These data reinforce the importance of interdisciplinary approaches in uncovering species richness and evolutionary history in speciose genus, such as the rice rat *Oligoryzomys.*

Keywords: Molecular markers, phylogenetic, phylogeography, cytotaxonomy

1. Introduction

Sigmodontinae currently comprises one of the most complex Neotropical mammalian subfamilies. It was subdivided into tribes being Oryzomyini the most diverse of the group. Within this tribe, *Oligoryzomys* is the most speciose genus, distributed throughout the Neotropical region, ranging from northeastern Mexico to Tierra del Fuego, in southern Argentina and Chile, from sea level to 3,500 meters high and occupying different phytophysiognomies (Musser and Carleton, 2005; Weksler and Bonvicino, 2015). Due to the remarkable morphological similarity and few broadly-sampled systematic studies, the taxonomy of the genus is considered provisional (Weksler and Bonvicino, 2015), and the number of species varies according to different authors.

Karyotypes have been used as a valid taxonomic character for species recognition and diploid number ranges from 44 in *Oligoryzomys* sp. 2 (Silva and Yonenaga-Yassuda, 1997) to 72 in *O. utiaritensis* (Agrellos *et al.*, 2012).

In the last decade, several studies involving molecular data investigating species boundaries in *Oligoryzomys* have been developed (Miranda *et al.*, 2009; González-Ittig *et al.*, 2010; Palma *et al.*, 2010; Agrellos *et al.*, 2012; da Cruz and Weksler, 2017; Weksler *et al.*, 2017). Although the genus was recovered as monophyletic by different characters such as allozime (Dickerman and Yates, 1995) and mitochondrial and nuclear genes (Myers *et al.*, 1995; Weksler, 2003; Miranda *et al.*, 2009; Agrellos *et al.* 2012; da Cruz and Weksler, 2017; Weksler *et al.*, 2017), the relationships among species remain unclear.

Interdisciplinary approaches are important to establish species limits in this genus. For instance, Agrellos *et al.* (2012) demonstrated that *O. utiaritensis*, considered as junior synonym of *O. nigripes* by many authors, is a valid species, based on morphology, karyotype and DNA sequences. In the same study, the authors presented a phylogenetic reconstruction in which *O. microtis* was found as the sister group of all other species, and *O. utiaritensis* and *O. moojeni* were closely related in the most derived clade. Weksler *et al.* (2017), after molecular phylogeny and comparative morphology, showed that *O. mattogrossae* is a valid species being the oldest name from samples previously identified as *O. fornesi* from Brazil and northern Paraguay. In

addition, the phylogenetic topology recovered this species as the first to diverge from other *Oligoryzomys* lineages.

González-Ittig *et al.* (2014) performed a phylogenetic analysis based on sequences of the cytochrome *b* gene, in which 25 clades were recovered, each one corresponding to a different putative species, many of which have not been described yet. More recently, da Cruz and Weksler (2017) performed molecular phylogeny, molecular dating and species delimitation in *Oligoryzomys*, recovering at least eight cryptic lineages, reinforcing that current taxonomy is underestimated.

Overall, an accurated identification of *Oligoryzomys* species is necessary for public health programs, since at least ten species of the genus are Hantavirus reservoirs (Delfraro *et al.*, 2003; Rosa *et al.*, 2005; Richter *et al.*, 2010; Agrellos *et al.*, 2012; Weksler *et al.*, 2017).

In this regard, herein we present a molecular phylogenetic reconstruction of the genus, widely sampled in different localities and phytophysiognomies. Two molecular markers were used to investigate phylogenetic relationships: the fast-evolving mitochondrial cytochrome-*b* (cyt-*b*) and the slower evolving nuclear Interphotoreceptor Retinoid Binding Protein (IRBP). These informations were associated to chromosome data and geographical distribution to aid the species limits of *Oligoryzomys*. In addition, phylogeographic approaches were performed in *O. nigripes*.

2. Material and Methods

2.1. Samples

Our sample is composed of 109 individuals collected in 56 localities from 11 Brazilian states and 1 locality from Northern Argentina (Table 1). Figure 1 exhibits a map containing localities from the present work plus localities from samples extracted from GenBank, totalizing 156 localities and 15 countries from Central and South America.

Trapped were carried out under ICMBio licences (numbers 11603-1 and 24003-4) of Instituto Chico Mendes de Conservação da Biodiversidade. Some specimens were captured by collaborators under their respective licences. Animals were euthanized according to the "Animal experimentation ethics" protocol (Carpenter *et al.*, 1996) and under permission of Instituto Butantan Ethics Committee (1260/14). The skins, skulls and partial skeletons were deposited in the Museu de Zoologia da Universidade de São Paulo (MZUSP), Museu de História Natural da Universidade Federal de Alagoas

(MUFAL) and Coleção de Mamíferos da Universidade Federal do Espírito Santo (UFES) (Table 1).

Specimens were named based on preliminary external morphological data and after integration of phylogenetic and chromosome information with geographic distribution and previous studies.

2.2. DNA extraction, amplifications and sequencing

DNA was extracted from liver or muscle with Chelex100 5% (Bio-Rad) according to Walsh *et al.* (1991) or from fibroblast cell culture using DNeasy Blood and Tissue kit (Qiagen, catalogue number 69506). Amplifications of 696 bp fragment of cyt-*b* and 659 bp of IRBP were performed with PCR in a thermal cycler (Eppendorf Mastercycler) using primers MVZ05 and MVZ16 (Irwin *et al.* 1991; Smith and Patton 1993), and A1 and F (Stanhope *et al.* 1992). The reaction mix consisted of a final volume of 18 μL containing 0.216 μL of Platinum® Taq DNA Polymerase (Invitrogen), 1.8 μL of DNA buffer, 0.72 μL of MgCl2 (50mM), 0.36 μL of dNTP, 0.216 μL of each primer, 0.72 μL of DNA and 13.752 μL autoclaved water.

For mitochondrial cyt-*b* gene, 39 amplification cycles were performed, each consisted of denaturation at 94°C for 30 s, annealing at 48°C for 45 s, polymerization at 72°C for 45 s and extension at 72°C for 5 min. For nuclear IRBP gene, 39 amplification cycles were performed with denaturation at 94°C for 30 s, annealing at 60°C for 60 s, polymerization at 72°C for 180 s and extension at 72°C for 5 min. PCR products were purified with the ExoSAP-IT (Code number US78200, GE Healthcare) following the manufacturer's instructions. DNA sequencing was conducted using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). All sequences were submitted to a comparative similarity search on BLAST (*Basic Local Alignment Search Tool*), before the alignment. For IRBP, we codified double peaks in both strands as ambiguous sites according to IUPAC code. Sequences will be submitted to GenBank after the submission and acceptance of the manuscript.

2.3. Molecular Analyses

Alignments were performed using Muscle (Edgar, 2004) implemented in Geneious 7.1.7 (Kearse *et al*., 2012). Phylogenetic reconstructions were based on Maximum Likelihood (ML) and Bayesian Inference (BI) using: (i) each gene separated

(cyt-*b* and IRBP), (ii) a concatenated dataset with missing data and (iii) a concatenated dataset without missing data. Outgroup was composed according to Weksler *et al.* (2006): *Euryoryzomys russatus, Microryzomys minutus, Neacomys spinosus*, *Oreoryzomys balneator* and *Pseudoryzomys simplex* (Table 1).

The cyt-*b* matrix (696 pb) was composed of 102 sequences obtained herein plus 107 sequences downloaded from GenBank (209 terminal taxa). IRBP matrix (659 pb) was composed of 97 sequences obtained herein plus 18 sequences from GenBank (115 terminal taxa). The concatenate matrix without missing data was composed of 102 sequences and the combined-matrix with missing data was composed of 225 sequences, both with a total of 1355 pb (Table 1).

The partitioning and models of nucleotide substitution were selected using Bayesian Information Criterion (BIC), implemented in PartitionFinder 1.1.1 (Lanfear *et al.*, 2012). ML analyses were performed using GARLI 2.0 (Bazinet *et al.*, 2014). Statistical support for the nodes was estimated by the nonparametric bootstrap (Felsenstein, 1985), with 1000 pseudoreplicates. Bayesian analyses were performed using MrBayes 3.2.6 (Ronquist and Huelsenbeck, 2003). Markov chains were started from a random tree and run for 1.0×10^7 generations, sampling every 1000^{th} generation. Sample points prior to the plateau phase were discarded as burn-in, and the remaining trees were combined to find the maximum a posteriori probability estimated of the phylogeny. Branch supports were estimated by Bayesian posterior probabilities.

Levels of cyt-*b* divergence among and within the monophyletic clades with more than one sample were calculated in MEGA 7.0 (Kumar *et al.*, 2016) with K2P model and 1.000 bootstrap replications (Table 2).

2.4. Chromosome analyses

From the total samples, 45 individuals were cytogenetically studied herein or in other previous studies (Silva and Yonenaga-Yassuda, 1997; Andrades-Miranda *et al*., 2001; Paresque *et al*., 2007; Agrellos *et al.*, 2012; Di-Nizo, 2013 and Di-Nizo *et al*., 2014; 2015) (Table 1). For the remaining individuals, karyotypes were not available.

Chromosome preparations were obtained *in vivo* from bone marrow and spleen (Ford and Hamerton, 1956) or *in vitro* from ear biopsies fibroblast culture in DMEM supplied with 20% fetal calf serum (Freshney, 1986). CBG-banding was produced according to Sumner (1972). At least 20 metaphases per individual were analyzed to define the diploid number (2n).

Fundamental number of autosome arms (FN) was classified according to Levan (1964), after being measured with the program ImageJ version 1.46 (Rasband, 2011). Chromosome preparations were analyzed under a Zeiss Axioskop 40 microscope and karyotypes were set up according to the literature, when available.

2.5. Phylogeographic studies in Oligoryzomys nigripes

Phylogeographic studies in *O. nigripes* were performed with cyt-*b* matrix that is the molecular marker with the largest number of samples. The matrix consisted of 83 representatives from 58 localities, including Brazil, Paraguay, Uruguay and Argentina (Table 1 samples with asterisk, Fig. 2).

Haplotype networks were obtained in Network 4.610 (http://www.fluxus-engineering.com/sharenet.htm), using median-joining (MJ) method (Bandelt *et al*., 1999), to verify the relationships between haplotypes. Analysis of molecular variance (AMOVA, Excoffier *et al*., 1992) was performed in ARLEQUIN 3.5 (Excoffier *et al*., 2005) with 10.000 permutations. In addition, Mantel test was performed to check if there is correlation between genetic and geographic distance with GenAlEX 6.502 (Peakall and Smouse, 2012) with 10.000 permutations.

The nucleotide (π) and haplotype (Hd) diversity were estimated in DnaSP 5.10 (Librado and Rozas, 2009). To test if there is any evidence of demographic expansion, the neutrality test indexes Tajima's D (Tajima, 1989) and Fu's (Fu, 1997) were calculated using also DnaSP 5.10 with 10.000 permutations.

3. Results

3.1 Phylogenetic reconstruction

The best model selected for phylogenetic analyses was GTR+I+G (cyt-*b*) and GTR+G (IRBP) and these models were used for the two concatenated analyses.

All analyses recovered *Oligoryzomys* monophyly: cyt-*b* (89ML/1.0BI) (Fig. 3); IRBP (99ML/1.0BI) (tree not shown); cyt-*b*+IRBP without missing data (99ML/1.0BI) (Fig. 4) and cyt-*b*+IRBP with missing data (99ML/1.0BI) (tree not shown).

For cyt-*b* data set the best ML tree had a –ln likelihood score of -10282.1215. Twenty-two main lineages were recovered in both ML and BI. From this amount, nine were composed of sequences from GenBank exclusively: *O. fulvescens*, *O. vegetus*, *O. brendae*, *O. costaricensis*, *O. destructor*, *O. messorius*, *O. delicatus*, *O. flavescens* West and *O. longicaudatus* + *O. magellanicus*. Nine lineages were recovered with samples

from the present work together with sequences from GenBank, as follows: *O. mattogrossae, O. microtis, O. rupestris, O. moojeni, O. utiaritensis, O. flavescens* + *O. fornesi, O. chacoensis, O. stramineus* and *O. nigripes*. In addition, four unidentified lineages were recovered: *Oligoryzomys* sp. A, *Oligoryzomys* sp. B, *Oligoryzomys* sp. C and *Oligoryzomys* sp. D. (Table 1, Fig. 3).

Monophyly was recovered with high supports for: *O. mattogrossae* (100ML/1.0BI), *O. microtis* (100ML/1.0BI), *O. fulvescens* (100ML/1.0BI), *O. vegetus* (100ML/1.0BI), *O. brendae* (100ML/1.0BI), *O. costaricensis* (100ML/1.0BI), *O. rupestris* (99.7ML/1.0BI), *O. messorius* (100ML/1.0BI), *O. delicatus* (94ML/1.0BI), *O. moojeni* (99.9ML/1.0BI), *O. utiaritensis* (99ML/1.0BI), *O. fornesi* (75ML/1.0BI), *O. chacoensis* (95.7ML/1.0BI), *Oligoryzomys* sp. D (100ML/1.0BI) and *O. stramineus* (86.8ML/0.96BI). Besides, monophyly was recovered with low support for *O. nigripes* and polyphyly was recovered for *O. flavescens, O. longicaudatus* and *O. magellanicus*.

For IRBP matrix, the best ML tree obtained had a –ln likelihood score of -2024.8563. Both ML and BI analyses recovered four monophyletic clades: *O. mattogrossae* (76ML/0.77BI), *O. microtis* (84ML/1.0BI), *O. stramineus* (97ML/1.0BI) and *O. chacoensis* (98ML/1.0BI) (tree not shown).

The concatenated matrix without missing data had a –ln likelihood score of -9285.455267. Nineteen main lineages were recovered in both ML and BI. Monophyly was recovered with high support in ML and BI for: *O. microtis* (100ML/1.0BI), *O. mattogrossae* (100ML/1.0BI), *O. chacoensis* (100ML/1.0BI), *O. flavescens* (100ML/1.0BI), *Oligoryzomys* sp. D (100ML/1.0BI), *O. moojeni* (99ML/1.0BI), *O. delicatus* (100ML/1.0BI), *Oligoryzomys aff. rupestris* (92ML/0.99BI) and *O. stramineus* (100ML/1.0BI). Polyphyly was recovered for *O. nigripes.* In addition, only one specimen was included in the analyses for *Oligoryzomys* sp. A, *Oligoryzomys* sp. B, *Oligoryzomys* sp. C, *O. destructor*, *O. vegetus, O. fulvescens, O. longicaudatus, Oligoryzomys aff. utiaritensis* and no IRBP sequences was available for *O. brendae*, *O. costaricensis* and *O. messorius* (Fig. 4). The combined-matrix with missing data had a –ln likelihood score of -13277.55498 and recovered 22 lineages, as in cyt-*b* topology (tree not shown).

Phylogenetic reconstruction of mitochondrial dataset and concatenated matrices recovered different topologies mainly because interespecific relationships had low support in all analyses. For IRBP, the interespecific relationships were not resolved in

both ML and BI analyses, except for *O. microtis* that was the first lineage to diverge in the phylogenetic reconstruction of Maximum likelihood (data not showed).

Thus, we will focus the results on cyt-*b* topology (which has more samples) and concatenated matrix without missing data (which has a larger number of base pairs).

Cyt-*b* single dataset indicated early divergence of a clade composed by *O. mattogrossae*, that are distributed in Cerrado and transition areas of Atlantic Forest and *O. microtis* (<50ML/<0.7BI). *Oligoryzomys microtis* showed two structured subclades, one composed of samples from western Amazon (99.9ML/1.0BI) and the other with samples from southern Amazon and transitional areas of Cerrado (78ML/0.84BI) (Fig. 3).

Oligoryzomys fulvescens and *O. vegetus*, from North and Central America, were found to be closely related (93ML/0.97BI). Next split involved *O. brendae* (from Yungas biome, north Argentina) followed by *O. costaricensis* (distributed in Panama and Costa Rica) (Fig. 3).

Next clade has low to high support (61.3 ML/0.95BI) and is composed of the following lineages: *O. rupestris*, *O. destructor*, *O. messorius*, *O. delicatus*, *Oligoryzomys* sp. A, *O. moojeni*, *O. utiaritensis* and *Oligoryzomys* sp. B (Fig. 3).

Oligoryzomys rupestris (together with samples herein identified as *Oligoryzomys aff. rupestris*) (from Central Brazil) were related to *O. destructor* (from the Andes) (>50ML/>0.7BI) as well as *O. delicatus* and *O. messorius* (both distributed in northern South America), although with low support values (Fig. 3).

Oligoryzomys sp. A is composed of one sequence from Atlantic Forest and is related to *O. moojeni* (Cerrado) (95ML/1.0BI) and both species are related to *O. utiaritensis* (in addition to a sequence identified as *Oligoryzomys aff. utiaritensis*), from transitional areas of Cerrado and Amazonian (>50ML/>0.7BI). This clade is sister to *Oligoryzomys* sp. B, composed of a single sequence from Cerrado (>50ML/1.0BI) (Fig. 3).

Oligoryzomys flavescens (from Argentina, Uruguay and eastern Brazil) was paraphyletic in relation to *O. fornesi* (Paraguay and Argentina) and strongly related to *O. longicaudatus* (from south Argentina and Chile) (100ML/1.0BI). Also, the single sequence of *O. magellanicus* available in GenBank nested with *O. longicaudatus* (98ML/1.0BI). This clade is related to *O. chacoensis* (from Paraguay and north Argentina) (61.9ML/>0.7BI) and sister group of *Oligoryzomys* sp. C (north Brazil) (Fig. 3).

Oligoryzomys sp. D is composed of two sequences from eastern Brazil and was sister to a clade composed of *O. stramineus* and *O. nigripes* (84ML/0.92BI). *Oligoryzomys stramineus* was recovered in two well structured subclades, one with samples from Cerrado and the other with samples from Caatinga. In addition, *O. nigripes* was also recovered in two well structured clades one with samples from Atlantic Forest and Cerrado of central-southeast-south Brazil, Paraguay, Uruguay and Argentina (83.64ML/1.0BI) and the other with samples from northeast Brazil (98ML/1.0BI).

Regarding the combined-matrix without missing data, internal relationships were not congruent with cyt-*b* analyses. Topological inconsistencies involved the phylogenetic position of *O. chacoensis, O. moojeni, Oligoryzomys* sp. C and D, in addition to the relationships between *O. stramineus, O. nigripes* and *Oligoryzomys aff. nigripes*.

In this data set, after the first split of *O. microtis* and *O. mattogrossae*, *Oligoryzomys* sp. C was recovered as sister to all other *Oligoryzomys* species (98ML/1.0BI) followed by the split of *O. chacoensis* (Fig. 4).

Oligoryzomys flavescens from Brazil was sister to *O. longicaudatus* and *Oligoryzomys* sp. D was sister to the closely related species *O. fulvescens* and *O. vegetus* (>50ML/0.82BI). Although with low support, this clade was sister to a clade composed of *O. moojeni*, *O. delicatus*, *Oligoryzomys* sp. A, *Oligoryzomys* sp. B, *Oligoryzomys aff. utiaritensis, Oligoryzomys aff. rupestris* and *O. destructor*. Next clade recovered *O. nigripes* from central-southeast-south Brazil, Paraguay, Uruguay and Argentina as sister of *O. stramineus* and *O. nigripes* from northeast Brazil (>50ML/>0.7BI) (Fig. 4).

3.2. Pairwise distance

Intraespecific distances varied from 0 (zero) in *Oligoryzomys* sp. C to 3.16% in *O. rupestris*. Interespecific distance varied from 1.34% (*Oligoryzomys* sp. B and *O. moojeni*) to 8.12% (*O. chacoensis* and *O. microtis*) (Table 2).

3.3. Cytogenetic analyses

From the 45 cytogenetically samples studied herein or in previous work (Table 1), nine karyotypes were observed and attributed to the following names: *O. chacoensis* (2n=58, FN=74), *O. flavescens* (2n=64-66, FN=66), *O. mattogrossae* (2n=62, FN=64),

Oligoryzomys aff. microtis (2n=64, FN=64), *O. moojeni* (2n=70, FN=72), *O. stramineus* (2n=52, FN=68), *O. nigripes* (2n=62, FN=80-82), *Oligoryzomys aff. nigripes* (2n=62, FN=78) and *Oligoryzomys aff. utiaritensis* (2n=70, FN=72).

The karyotype attributed to *O. nigripes* (samples from central-southeast-south Brazil, Paraguay, Uruguay and Argentina) presented differences in fundamental number due to polymorphisms in the autosome pair 3, which was homomorphic acrocentric (3A), homomorphic metacentric (3M) or heteromorphic acrocentric/metacentric (3H). *Oligoryzomys aff. nigripes* (composed of samples from northeast Brazil) presented the same karyotype described for *O. nigripes*, except for the morphology of pairs 2 and 3 (which was acrocentric in all samples) (not shown).

One specimen (M968497) from Apiacás, Mato Grosso, Brazil, presented 2n=70, FN=72 (Fig. 5). This karyotype presented heteromorphic pairs 1 and 33: pair 1 is composed of a large subtelocentric chromosome and a large acrocentric, while pair 33 is composed of a medium submetacentric and a medium acrocentric. There are 31 acrocentric pairs (pairs 2 to 32) and one small bi-armed pair (pair 34). The X is a large metacentric and Y, a medium submetacentric. C-banding pattern showed constitutive heterochromatin at the pericentromeric region of all autosomes, in the short arm of X and long arm of Y (Fig. 5). This sample was named *Oligoryzomys aff. utiaritensis* because the molecular phylogeny recovered it in the same clade of *O. utiaritensis* paratype, despite *O. utiaritensis* presented 2n=72, FN=76.

3.4. Phylogeography of O. nigripes

Sixty-nine haplotypes were observed for the total sample (83 individuals) (Table 1). Haplotypes were colored according to the geographical distribution (Fig. 2). The most frequent haplotypes were H1, H35 and H49 (Table 1), occurred in three samples each one, and a total of 58 haplotypes were exclusive.

The haplotype network obtained showed two major haplogroups that correspond to *O. nigripes* from central-southeast-south (CS) and *Oligoryzomys aff. nigripes* from northeastern Brazil (NE) of the phylogeny (Fig. 2).

Oligoryzomys nigripes demonstrated high haplotype (Hd=0.995) and low nucleotide diversity (π=0.01717). The same pattern was observed in NE haplogroup (Hd=0.933 and π=0.01403) and CS haplogroup (Hd=0.995 and π=0.01122) (Table 3).

AMOVA between lineages CS and NE showed that the highest percentage of genetic variation was observed among groups (78,2%). AMOVA showed that genetic

variation among population within groups are low (1,26%) and the fixation index are high (F_{st}=0,79, p<0,001). Besides, the variation within populations was moderate (20%), with high fixation index (F_{ct}=0,78) (Table 4).

Mantel test for all sample revealed a significant correlation between genetic and geographic distance (r=0,697, p=0,001), indicating that the genetic variability is structured in geographic space. When applied only for the population of CS, the correlation between genetic and geographic distance was not statistical significant (r=0,153, p=0,060) (Fig. 6).

In addition, neutrality tests deviations were significant negative for the total population and haplogroup CS and not significant for haplogroup NE (Table 3).

4. Discussion

This work brings a new phylogenetic hypothesis for *Oligoryzomys*, including 225 sequences from 156 localities in Central and South America. From the 23 *Oligoryzomys* species recognized so far (Weksler and Bonvicino, 2015), 19 were used in this work, since sequences of *O. arenalis* and *O. griseolus* are not available, *O. victus* is probably extinct and the only sequence from *O. andinus* is too small to be included in our matrix (401 bp). Phylogenetic results were integrated with cytogenetic data and geographic distribution, using an interdisciplinary approach.

4.1. Interdisciplinarity reveals species limits

Each of the lineages recovered in our phylogenetic analyses (including paratypes) were associated to previous phylogenetic reconstruction, K2P distance, cytogenetic data (when available), geographic distribution and phylogeographic approach (in the case of *O. nigripes*).

From the 22 lineages, we were able to name 15 species: *O. brendae, O. chacoensis, O. costaricensis, O. delicatus, O. destructor, O. fulvescens, O. mattogrossae, O. messorius, O. microtis, O. moojeni, O. nigripes, O. rupestris, O. stramineus, O. utiaritensis* and *O. vegetus*.

The present study recovered the monophyly of *O. mattogrossae* and, together with the cytogenetic information, supported the results obtained by Weksler *et al.* (2017) which demonstrated that this is a valid species. Sequences from Brazil reported as NUMTs by González-Ittig *et al.* (2014) were recovered in *O. mattogrossae* clade with high support. The same result was reported by Weksler *et al.* (2017) and we did not

detected pseudogenes characteristic in these sequences. Recently, Weksler *et al.* (2017) validated *O. mattogrossae*; thus, specimens distributed in the Brazilian Cerrado and Caatinga and in the northeastern Paraguay, with 2n=62, FN=64, were for a long time erroneously associated to *O. fornesi*.

In this work, new records are being presented for this species. Samples were collected in Guará and Luis Antonio, in northern São Paulo state, both transitional regions between Atlantic Forest and Cerrado, representing the southeastern species limit and the first records for São Paulo state (together with Vivo *et al.*, 2011 - cited as *O. fornesi* but without the locality record). In addition, this species was trapped in Vila Rica, Mato Grosso state, in a transitional area between Cerrado and Amazon Rainforest, located 1.000 km northwestern from the closest record in Goiás state, Teresina de Goiás (Weksler and Bonvicino, 2015).

Two well structured subclades were recovered in *O. microtis*, one from western Amazon and the other from southern Amazon and transition areas of Cerrado. K2P distance revealed a relatively high intraespecific value (2.82%). Two sequences (UFES1442 from Pium, Tocantins state and AN69329 from Alto Paraíso, Rondônia state) were reported as *Oligoryzomys* sp. by Rocha *et al.* (2011) and Firth *et al.* (2012), respectively, and clustered together in the southern subclade. These results, in addition to previous species delimitation analyses, in which more than one entity was detected for *O. microtis* (da Cruz and Weksler, 2017), suggest that *O. microtis* may be a species-complex or in process of speciation and needs to be reassessed.

Thus, due to proximity of the type locality (north bank of Solimões river, Amazonas state, Brazil) (Musser and Carleton, 2005), the western Amazon subclade is named herein as *O. microtis* and the southern Amazon subclade as *Oligoryzomys aff. microtis*.

The karyotype associated to *O. microtis* (2n=64, FN=64) by Di-Nizo *et al.* (2015) from samples collected in southern Amazonian (which molecular analyses are presented herein) is similar to the one described by Gardner and Patton (1976), Aniskin and Volobouev (1999) and Patton *et al.* (2000) (2n=64, FN=66), from samples from western Amazonian, except for the pair one, which is described as a large metacentric by the latter authors and is a large acrocentric in Di-Nizo *et al.* (2015).

Although cytogenetic information of samples of the subclade *O. microtis* is not available, it is possible that they possess the same karyotype (2n=64, FN=66) as described by Gardner and Patton (1976), Aniskin and Volobouev (1999) and Patton *et*

al. (2000), due to the proximity of their distribution, whereas the clade *Oligoryzomys aff. microtis* is composed of individuals with 2n=64, FN=64.

The karyotypic difference between *O. microtis* (2n=64, FN=66) and *Oligoryzomys aff. microtis* (2n=64, FN=64) is probably explained by a pericentric inversion or centromere repositioning in pair 1 (the largest pair). Because pericentric inversion may cause reduction of recombination between chromosomes carrying different rearrangements, accumulation of incompatibilities may lead to speciation (Noor *et al.*, 2001; Rieseberg, 2001; Faria and Navarro, 2010), reinforcing the hypothesis that *O. microtis* is polytypic or in process of speciation. Major survey efforts in the Amazonian region should be emphasized in order to investigate if both clades present exclusive karyotypes and if *O. microtis* is an incipient or species-complex.

Although *O. microtis* may be a species-complex, the new record for samples related to this taxon (Juruena) is situated in southwestern Amazon Rainforest, in the extreme northwestern of Mato Grosso state (Weksler and Bonvicino, 2015).

Regarding *O. rupestris*, Agrellos *et al.* (2012) and Weksler and Bonvicino (2015) considered samples with 2n=46 and 2n=44 as a single species and both were recovered related in the present phylogeny. However, previously work described these entities as two distinct species *Oligoryzomys* sp. 1 and *Oligoryzomys* sp. 2 (Silva and Yonenaga-Yassuda, 1997) and intraespecific distance showed 3.16% of divergence. Whether individuals with 2n=44 and 2n=46 represent the same entity must be investigate with morphological approach, although it is likely that the Robertsonian rearrangement that differentiate both entities may cause meiotic problems and lead to reproductive isolation.

Although the low intraespecific K2P distance (1.12%), a sequence from Suriname (CM76908) that was identified as *O. fulvescens* in GenBank, clustered with *O. delicatus* and was designated here as *Oligoryzomys aff. delicatus*.

Furthermore, one individual (referred as *Oligoryzomys aff. utiaritensis*) has a peculiar karyotype that and could only be assigned to *O. utiaritensis* after molecular phylogeny. This individual was captured within Amazon Rainforest and presented the same diploid and fundamental number as that described for *O. moojeni* (2n=70, FN=72), an endemic species from the Brazilian Cerrado (Weksler and Bonvicino, 2015). Considering that this sample was recovered in *O. utiaritensis* clade and although it was not possible to obtain a reasonable G-banding pattern, we could hypothesize that a centric fusion of an acrocentric pair and two pericentric inversions are the most

probable rearrangements that explain the differences observed in this karyotype (2n=70, FN=72) and the karyotype described for *O. utiaritensis* (2n=72, FN=76). Nevertheless, it is likely that complex rearrangements are involved in the differentiation of both karyotypes, which could only be confirmed with chromosome painting. Preliminar morphological analyses of this specimen were inconclusive due to the low number of specimens available (Oliveira, M.V.B., personal communication, 2016).

As stated by Weksler *et al.* (2017), *O. fornesi* is restricted to southern Paraguay and northernmost Argentina and its diploid and fundamental number is unknown. Samples assigned as *O. fornesi* were recovered embedded in sequences of *O. flavescens* (González-Ittig el al. 2010; 2014; da Cruz and Weksler, 2017; Weksler *et al.* 2017; present work).

González-Ittig *et al.* (2014) demonstrated that *O. flavescens* is, in fact, a species complex that includes: *O. fornesi*, *O. flavescens* West (North Argentina and Bolivia), *O. flavescens* East (Uruguay and South Argentina) and *O. flavescens* Brazil. Our analyses recovered *O. flavescens* West more related to *O. longicaudatus* and species from Brazil did not clustered in the same monophyletic clade, reiterating the necessity of taxonomic revision in *O. flavescens*. Recent species demilitation analyses supported *O. flavescens* as species complex (da Cruz and Weksler, 2017).

Although the taxonomic status of *O. flavescens* must be investigated, this is the only species of the genus that bears B chromosomes and its karyotype is easy to be distinguished from the karyotypes of the sympatric species *O. mattogrossae* and *O. nigripes*. In addition, Brum-Zorrilla *et al.* (1988) described metacentrics B chromosomes from population of Uruguay and Sbalqueiro *et al.* (1991) described acrocentrics Bs in samples from Brazil, which is corroborated by the phylogenetic differentiation described herein.

For *O. flavescens* from Brazil, this is the first record for northern São Paulo state (Guará), increasing its known area of occurrence about 300 km to the north of Americana, São Paulo, located in the limits of the southern portion of the Cerrado biome, in a transitional region with the Atlantic Forest.

Oligoryzomys magellanicus nested with *O. longicaudatus* probably because both species are strongly related and there is only a single sequence of *O. megellanicus* available in GenBank. The same result was obtained by da Cruz and Weksler (2017). Despite the validity of both species is supported by morphological, cytogenetic and

allozimes analyses (Gallardo and Patterson, 1985; Gallardo and Palma, 1990; Weksler and Bonvicino, 2015), the taxonomic status of *O. magellanicus* needs to be reassessed.

One sequence (MSB80489), named here as *Oligoryzomys aff. chacoensis*, from Fortin Toledo, Boquerón, Paraguay grouped in *O. chacoensis* clade, but showed divergence and high cyt-*b* distance (3%) and should be analyzed by morphology to confirm its identification. In addition, *Oligoryzomys chacoensis* has the same karyotype described for *O. brendae*, thus karyotype alone can not be used for cytotaxonomic identification since both species occur in sympatry in Argentina.

Within *O. stramineus*, two structured subclades with high support were recovered, one with samples from Caatinga and the other with samples from Cerrado, including the paratype. Both subclades presented 2n=52, FN=68 and genetic distance within this species was low (1.33%). Although this subestructured may be due to lack of sample in intermediate distribution, taxonomic revision and phylogeographic studies is recommended.

Our molecular analyses with mitochondrial and nuclear markers separated specimens previously identified as *O. nigripes* in two clades: one with samples from northeastern Brazil (*Oligoryzomys aff. nigripes* with 2n=62, FN=78) and other with samples from central, southeastern and south of Brazil, Argentina, Uruguay and Paraguay (*O. nigripes stricto sensu* with 2n=62, FN=80-82). Additionally, distance value between both taxa is 3.4%.

Oligoryzomys nigripes has been considered a polymorphic species that showed variations in fundamental number due to pericentric inversions in pairs 2, 3, 4 and 8 (Almeida and Yonenaga-Yassuda, 1991; Paresque *et al.*, 2007). Pericentric inversion in pair 2 is restricted in samples from northeastern Brazil while pericentric inversions in pairs 3, 4 and 8 are widespread distributed in the population of south-southeast Brazil (Paresque *et al.*, 2007). The same cytogenetic results was observed in this work; however, as molecular phylogeny, K2P distance and phylogeography strongly support that population from northeast is different from population from central-southeast-south Brazil, Uruguay and Paraguay, we designated 2n=62, FN=78 as *Oligoryzomys aff. nigripes* and 2n=62, FN=80-81 as *O. nigripes*, since neotype of *O. nigripes* is from Ybyaú National Park, Department of Paraguaí, Paraguay. In addition, as in the case of *Oligoryzomys aff. microtis*, pericentric inversion may have played a role in speciation due to crossing-over supression in heterozygous (Faria and Navarro, 2010). Thus, we suggested that both lineages may represent distinct species or in process of speciation.

Based on our results and previous morphological and phylogenetic studies, we found misidentification in GenBank that should be corrected in order to avoid future errors. *Oligoryzomys brendae* (OMNH34497) is identified as *O. destructor* and one sequence of *O. flavescens* West (NK11547) is misidentified as *O. andinus*. Samples MSB93659 and MVZ183088 are identified as *O. eliurus* and *O. delticola*, respectively and both species were considered junior synonym of *O. nigripes* (Francés and D'Elía, 2006; Bonvicino and Weksler, 1998).

According to Weksler and Bonvicino (2015), *Oligoryzomys* species are found frequently in sympatry, with the occurrence of both large body sized and small body sized species in the same locality. Herein, this was the case for (i) *O. nigripes* and *O. flavescens* that were collected in the same localities in the Atlantic Forest (Caieiras and Pedreira) and transitional areas of Atlantic Forest and Cerrado (Flona Ipanema) of São Paulo state and in Atlantic Forest and Pampa of Rio Grande do Sul state (Osório and Charqueada), (ii) *O. moojeni* and *O. mattogrossae* that were recorded on the northern portion of Cerrado, in southern Tocantins state (Peixe) and in Goiás state (Minaçu), (iii) *O. mattogrossae* and *O. microtis* collected in Pium, an ecotonal region between Cerrado and Amazonia of Tocantins state (Rocha *et al.* 2011), (iv) *O. mattogrossae* and *O. stramineus* collected in Cerrado of Goias state, (v) *O. fornesi* and *O. flavescens* East in Chaco, Argentina, (vi) *O. flavescens* East and *O. nigripes* in Buenos Aires, Argentina and for (vii) *O. vegetus* and *O. costaricensis* in Cartago, Costa Rica.

Three species were collected in the same trapline in three localities: (i) *O. flavescens, O. mattogrossae* and *O. nigripes*, in a transitional region between Atlantic Forest and Cerrado in northern São Paulo state (Guará). Weksler and Bonvicino (2015) cited that *O. mattogrossae* possibly can be found in sympatry with *O. flavescens*, but this is the first record; (ii) *O. moojeni, O. mattogrossae* and *Oligoryzomys* sp. B were captured simpatrically in Cerrado of Piaui state (Uruçuí-Una) and (iii) *O. flavescens* West, *O. brendae* and *O. chacoensis* (Salta, Argentina, Yungas biome).

4.2. Putative new species

Four clades (A-D), were recovered as independent lineages and could not be related to any species.

"*Oligoryzomys* sp. A" corresponds to the sample (UNIBAN0160) and was collected in the Atlantic Forest of São Paulo State, Brazil. This sample is related to *O.*

moojeni, *O. utiaritensis* and *Oligoryzomys* sp. B, species from Cerrado and transitional areas of Cerrado and Amazonian Rainforest.

"*Oligoryzomys* sp. B" (CIT1549) was collected in ESEC Uruçuí-Una, Piaui State, Brazil, in which two other species were found: *O. moojeni* and *O. mattogrossae*.

"*Oligoryzomys* sp. C" is represented by a single specimen from Surumu, Roraima State, Brazil, region of both Savanna and Rainforest. This individual was referred as *Oligoryzomys cf. messorius* by Andrades-Miranda *et al.* (2001) and Miranda *et al.* (2009) but based on morphological, karyotypic and phylogenetic studies, Weksler and Bonvicino (2015) stated that this sample is different from *O. messorius*. Cytogenetics (2n=56, FN=58) corroborates the hypothesis that it represents a new species (Andrades-Miranda *et al.*, 2001). In addition, da Cruz and Weksler (2017) based on molecular phylogeny also recovered this sample as a new species.

"*Oligoryzomys* sp. D" is represented by two samples from Una, Bahia state, Northeast Brazil. It is possible that they represent an undescribed species since Una belongs to a region known as one of the endemism centre and refugial area of Atlantic Forest (Vivo, 1997; Carnaval *et al.*, 2009).

In addition, we reiterate that taxonomic revision should be performed in *O. microtis*, *O. rupestris*, and *O. nigripes* in addition to all samples related to *O. flavescens* in order to evaluate if they represent new or incipient species.

4.3. Generic monophyly and phylogenetic relationships

Phylogenetic analyses with two molecular markers corroborate the previously observed monophyly of the genus (Dickerman and Yates, 1995; Myers *et al.*, 1995; Miranda *et al.*, 2009; Gonzalez-Ittig *et al.*, 2014; Weksler, 2006; da Cruz and Weksler, 2017; Weksler *et al.*, 2017). As expected, the single mitochondrial dataset showed more monophyletic lineages due to the higher number of sequences used.

The combination of two markers with different evolutionary rates (mitochondrial cyt-*b* and nuclear exon IRBP) was not enough to achieve internal phylogenetic relationships. Weksler *et al.* (2017) also did not observe strongly supported relationships within *Oligoryzomys* using combined mitochondrial cyt-*b* and nuclear intron 7 of β-fibrinogen. In contrast, da Cruz and Weksler (2017) reported that the nuclear intron 7 of β-fibrinogen was able to recover the monophyletism of species, but it is still not informative since few species have this gene sequenced.

Although with low support, *O. mattogrossae* and *O. microtis* were found together in the ancestral clade within *Oligoryzomys* genus. Inconsistencies in the position of both species have been reported, some authors recovered *O. microtis* as the ancestral species within the genus (Miranda *et al.*, 2009; Agrellos *et al.*, 2012; Teta *et al.*, 2013; da Cruz and Weksler, 2017; IRBP and concatenated analyses with gaps of the present work – not showed) while Weksler *et al.* (2017) reported *O. mattogrossae* as sister to *O. microtis.*

Interespecific relationships were poorly resolved, except for four clades recovered herein and also in previous phylogenies: (i) *O. vegetus* and *O. fulvescens* (Teta *et al.*, 2013; González-Ittig *et al.*, 2014; da Cruz and Weksler, 2017; Weksler *et al.*, 2017); (ii) *O. stramineus* and *O. nigripes* (Miranda *et al.*, 2009; Agrellos *et al.*, 2012; Teta *et al.*, 2013; González-Ittig *et al.*, 2014; Weksler *et al.*, 2017); (iii) *O. longicaudatus, O. magellanicus, O. fornesi* and *O. flavescens* (Miranda *et al.*, 2009; Agrellos *et al.*, 2012; González-Ittig *et al.*, 2014; Weksler *et al.*, 2017) and (iv) *O. utiaritensis, Oligoryzomys* sp. A and *Oligoryzomys* sp. B (present paper).

4.4. Patterns of diversification

Phylogenetic analyses failed to recovered relationships between the majority of species probably because of some reasons, such as (i) the wide distribution of the genus studied and lack of species; (ii) few molecular markers were used until now in phylogenetic approaches and those markers were not enough informative or (iii) cladonegenic events may have occurred in a short period of time. The latter is corroborated by molecular dating that suggest that *Oligoryzomys* diversified during Pliocene (Parada *et al.*, 2013; Leite *et al.*, 2014; Vilela *et al.*, 2014) or late Pliocene/ early Pleistocene (da Cruz and Weksler, 2017).

Then, the origin of the *Oligoryzomys* still remains speculative, since the first clade that diverged in the phylogeny was composed of *O. microtis* and *O. mattogrossae*, it is not clear if the origin of the genus occurred in Amazonian Rainforest, as suggested by some authors (Miranda *et al.*, 2009; Palma *et al.*, 2010).

Our analyses did not reveal a north-to-south geographical pattern as observed by Miranda *et al.* (2009). Instead, a much more complex scenario was found once *Oligoryzomys* species have radiated across multiple biomes.

Taking into account previous hypothesis of Reig (1984) in which *Oligoryzomys* is autochthonous of South America, molecular dating (Parada *et al.*, 2013; Leite *et al.*,

2014; Vilela *et al.*, 2014; da Cruz and Weksler, 2017) and our phylogenetic results, it is possible to suggest that this genus originated in central South America and dispersed throught this continent reaching Central and North America through Panama isthmus. In this sense, many events have shaped the diversity of the group, such as rivers formation and glacial cycles of Plio and Pleistocene.

Although internal phylogenetic relationships were poorly recovered, it was possible to observe that species endemic to different phytophysiognomies were closely related. The close relationship between *O. rupestris* from Cerrado and *O. destructor* from the Andes support the hypothesis that this area may have been connected during the Last Glacial Maximum as suggested for other vertebrates (Werneck *et al.*, 2012).

Costa (2003), studying small non-volant mammals, found similarities between samples from the Atlantic Forest and Amazon or central Brazilian forests than within each of the two Rainforest domains, reinforcing that one single hypothesis for speciation can not explain all the diversity found in Neotropics.

Besides, as we will stated below, population genetics in *O. nigripes* and *Oligoryzomys aff. nigripes* revealed the role of riverine barriers and Refugia hypothesis for the diversification of this group. As a matter os fact, climatic shifts may have influenced genetic pattern of all *Oligoryzomys* species since splits occurred during Pleistocene (Leite *et al.*, 2014; da Cruz and Weksler, 2017).

Thereby, the high diversity of *Oligoryzomys* could not be explained by a single model of diversification.

4.5. Phylogeographic pattern in O. nigripes and Oligoryzomys aff. nigripes

Population genetics analyses in *O. nigripes* from Atlantic Forest and transitional areas corroborate the phylogenetic results that grouped samples in two main clades, strongly associated with geographical regions. A separation of northern and southern phylogroups was observed for several taxa, however this discontinuity was found in distinct areas of the Atlantic Forest (Costa, 2003; Carnaval and Moritz, 2008; Costa and Leite, 2012).

Although it should be taking in account the lack of samples in the region between *O. nigripes* and *Oligoryzomys aff. nigripes*, Mantel test was significant, revealing that both lineages are isolated by distance. This isolation coincides with Pardo and Jequitinhonha rivers, suggesting that either one or both of these rivers may be acting as

a vicariant barrier for gene flow between NE and CS lineages. The presence of monophyletic groups on each side of the rivers corroborates this hypothesis.

Regarding *Oligoryzomys aff. nigripes*, two subclades were observed within this lineage, one composed of samples from Bahia (H55) and the other from Alagoas (H56 and H57) and Ceará (H53 and H54). Despite the small sample size of this lineage, as the split of the subclades coincides with São Franscisco River, we can not discard the possibility that this river is acting as a barrier. Further analyses with samples from intermediate locations may elucidate this hypothesis.

Pellegrino *et al.* (2005) observed a correlation between genetic structure of clades and the presence of river systems of Atlantic Forest, including Jequitinhonha River, for the lizards of the ancient *Gymnodactylus darwinii* complex. As in the present study, authors described two chromosome races concordant with the two major clades. Several studies in Atlantic Forest recovered phylogeographic breaks coincident with drenage systems in different vertebrate's species, such as lizards (Pellegrino *et al.*, 2005; Rodrigues *et al*., 2014), frogs (Amaro *et al.*, 2012), birds (Silva *et al.*, 2004; Lacerda *et al.*, 2007) and rodents (Ventura *et al.*, 2012).

Nevertheless, as times of divergence can vary among species, the role of river as primary barrier is being questioned by many authors (Cabanne *et al*., 2007; Colombi *et al,*. 2010). For CS phylogroup, rivers from south and southeast Brazil do not seem to influence patterns of genetic differentiation (see Fig. 2).

Besides the strong population genetics structure between samples from NE and CS and within NE population, absence of structure was observed within CS (*O. nigripes*) lineage. Gonçalves *et al.* (2009) also observed lack of genetic structure in *O. nigripes* from south Brazil sampled in altitudinal gradients in different types of Atlantic Forest habitats, and authors did not find a correlation between altitude and habitats.

Herein, some haplotypes were distinguished by having a greater number of mutations (see Fig. 2). H6 is composed of samples from Argentinean Pampas and since other samples were captured in Pampas from Brazil and Uruguay, this distinct haplotype may be due to lack of samples in intermediate localitions between La Balandra, Argentina (H6) and the other localities. In addition, the highly differentiated haplotypes H15 and H17 are composed of samples from Flona Ipanema and Ribeirão Preto, São Paulo state, both regions of transition between Atlantic Forest and Cerrado. The high divergence of these haplotypes could not be explained by the biome they occupy since samples from Cerrado (*e.g.* Caldas Novas, Goiás state, H5) and other

transitional areas of Atlantic Forest and Cerrado (*e.g.:* Guará, São Paulo, H19) did not show differentiated haplotypes. Finally, three other diversified haplotypes, H18, H26 and H64 are composed of samples from high altitutes and *Araucarias* type vegetation (Wenceslau Bráz and Ortigueira, Paraná state, and Caxias do Sul, Rio Grande do Sul state) but these characteristics are not enough to explain this difference since samples from the same localities exhibit low divergent haplotypes.

The high haplotypic diversity (Hd) and low nucleotide diversity (π) observed in populations from CS in addition to the shared haplotypes from different regions and altitudes, revealed habits of species dispersal. Neutrality tests deviations corroborates that the population is in demographic expansion. In addition, Mantel test performed exclusively in lineage CS showed results not statically significant, since this population is not isolated. This pattern is consistent with Pleistocene refugia (Carnaval *et al.*, 2009). If the ancestral population has not been totally isolated in the retraction periods of Atlantic Forest, when these fragments came into contact again, population expanded.

Carnaval *et al.* (2009), using frogs of the genus *Hypsiboas*, showed low genetic diversity in the southernmost Atlantic Forest, revealing that this region was climatically unstable relative to the central region during late Pleistocene. Nevertheless, recent studies found historical stable forests during Pleistocene, challenging the Refugia hypothesis and proposing alternative explanation for the great Atlantic Forest biodiversity, such as the emergence of continental shelf (Leite *et al.*, 2016) and barriers associated to neotectonic activities (Thomé *et al.*, 2010).

Although the reduced sample (in NE region) and the use of a single locus, these inedited phylogeographic results with strong genetic population structure is consistent with incipient species and reiterates the phylogenetic, cytogenetic and cyt-*b* distance results, pointing out the need of a taxonomic revision in order to clarify the split of *O. nigripes* and *Oligoryzomys aff. nigripes*.

This work also reiterates the complexity of this widespread species and, despite it is not exclusively distributed in Atlantic Forest, our results reiterate the intricacy of the biogeographic history of this biome.

4.6. Final Considerations

This work reiterates that species number and diversity of *Oligoryzomys* is underestimated since at least four new putative species are suggested (one of them also recovered by da Cruz and Weksler, 2017) and new cytogenetic information and

geographic distribution were detected. In addition, we pointed out species that should be taxonomic reviewed.

Cytogenetic is a useful tool for species recognition in the group and can also point the identification of new species, as in the case of *Oligoryzomys* sp. C, that has a distinct karyotype: 2n=56, FN=58 (Andrades-Miranda *et al.*, 2001). *Oligoryzomys* represent one of the most polymorphic genus (Di-Nizo *et al.,* 2015) since several chromosome rearrangements were described (Gallardo and González, 1977; Almeida and Yonenaga-Yassuda, 1991; Sbalqueiro *et al.*, 1991; Silva and Yonenaga-Yassuda, 1997; Aniskin and Volobouev, 1999; Paresque *et al.*, 2007).

Karyotypic evolution of *Oligoryzomys* was studied using chromosome painting and pericentric inversions, fissions, tandem and Robertsonian fusions, centromeric loss/ inactivation, gain/activation or repositioning have occurred during karyotype differentiation of the genus. In addition, it was observed increase and decrease in diploid number in different branches of *Oligoryzomys* evolution (Di-Nizo *et al.*, 2015).

In fact, for a long time chromosome changes were related to speciation because gene flow is reduced in heterozygous due to segregation problems and origin of unbalanced gametes. Recent models suggest that the reduction of recombination between chromosomes carrying different rearrangements is determinant for speciation giving opportunity for the accumulation of incompatibilities (Noor *et al.*, 2001; Rieseberg, 2001; Faria and Navarro, 2010).

Chromosome rearrangements could have had a role in speciation in the case of *O. microtis* (2n=64, FN= 66) and *Oligoryzomys aff. microtis* (2n=64, FN=64), *O. nigripes* (2n=62, FN=80-82) and *Oligoryzomys aff. nigripes* (2n=62, FN=78) and also in *Oligoryzomys aff. rupestris* (2n=46, FN=52 and 2n=44, FN=52), if these species are confirmed to be valid.

As each character has a different rate of evolution, the combination of interdisciplinary approaches such as molecular phylogeny, cytogenetics, morphology, phylogeography and geographic distribution is paramount to better understand and delimit species of this complex group due to its heterogeneity.

This work showed the evolutionary complexity of the genus and phylogeny reiterates the very rapid and possibly adaptative radiation of *Oligoryzomys*, corroborated by the chromosomal evolution data that showed that closely related species have experienced recent autosomal rearrangement (Di-Nizo *et al.*, 2015).

We reiterate also the importance of faunal surveys to increase the knowledge of the species and description of new taxa, as new collection efforts often result in new distributional records (Percequillo *et al.*, 2017) and descriptions of new species or even genera (Percequillo *et al.*, 2011; Pardiñas *et al.*, 2014).

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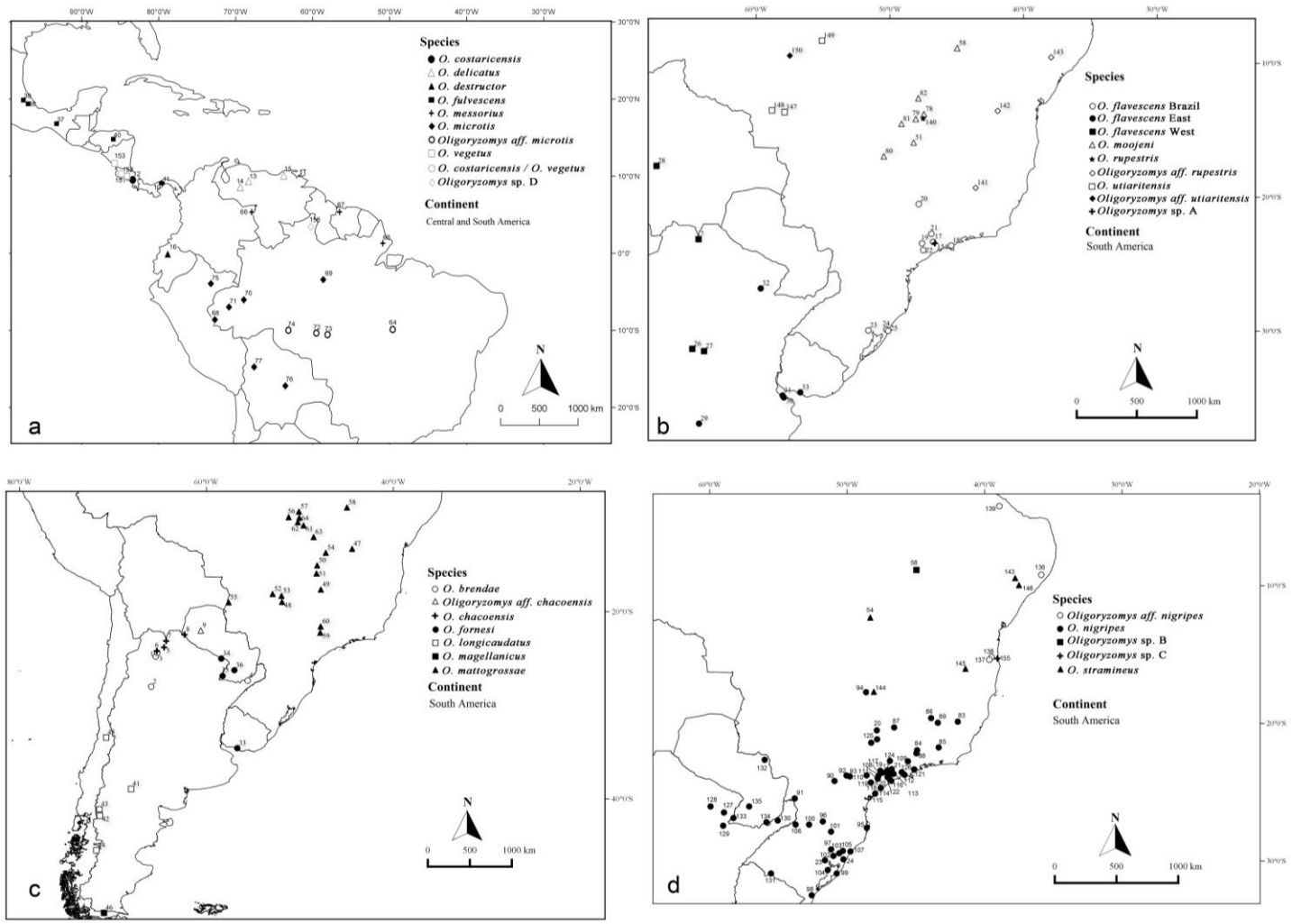

Fig. 1: Geographic distribution of *Oligoryzomys* sampled in this work plus localities of sequences extracted from GenBank. Numbers correspond to localities of Table 1.

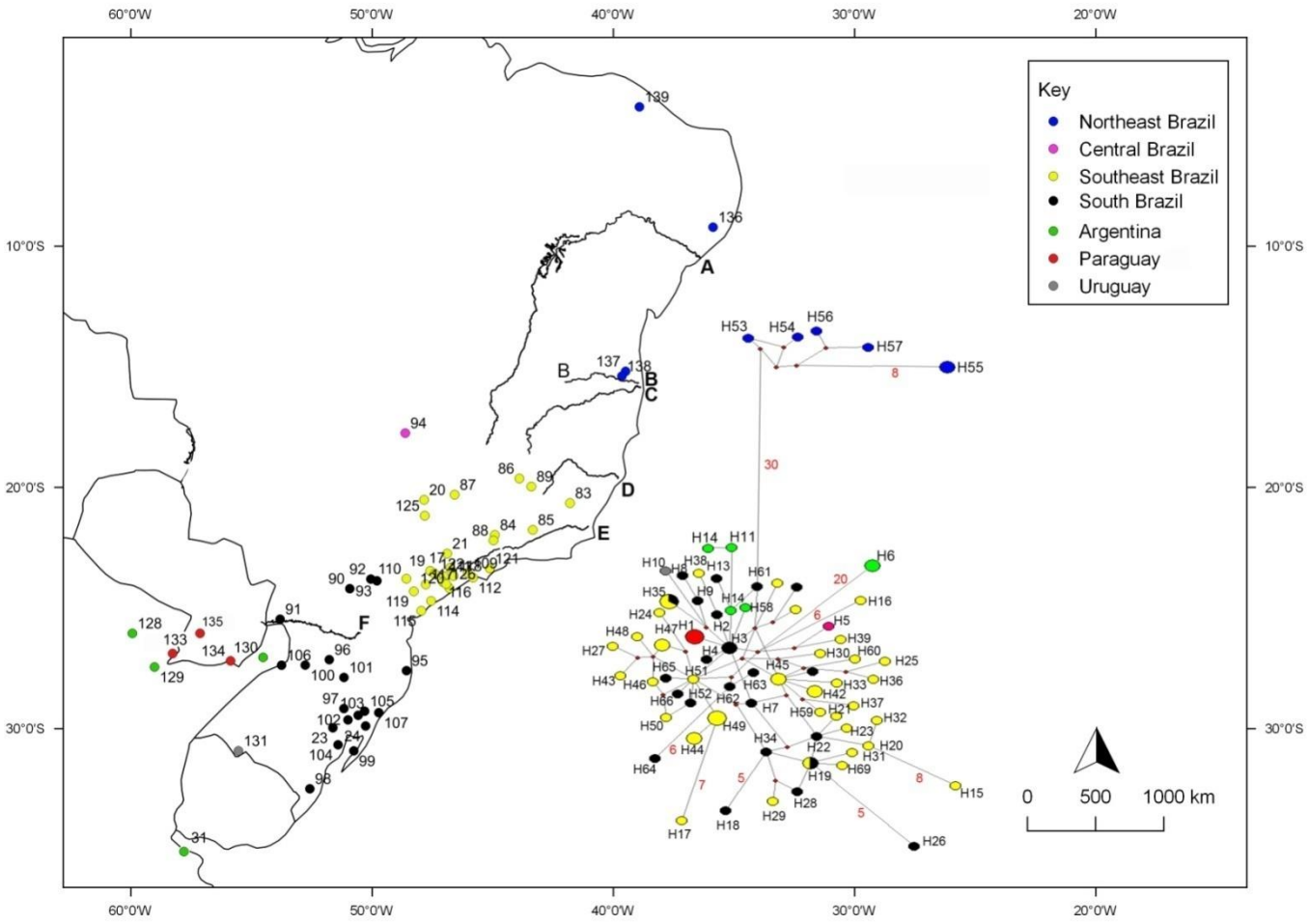

Fig. 2: Map of partial South America with geographic location of *O. nigripes lato sensu* studied in this work and haplotype networks on the right side. Circle size is proportional to the sample size. Five or more evolutionary steps are indicated by numbers in red. Numbers of locations are indicated in Table 1. Rivers: São Francisco (A), Pardo (B), Jequitinhonha (C), Doce (D), Paraíba do Sul (E) and Iguaçu (F).

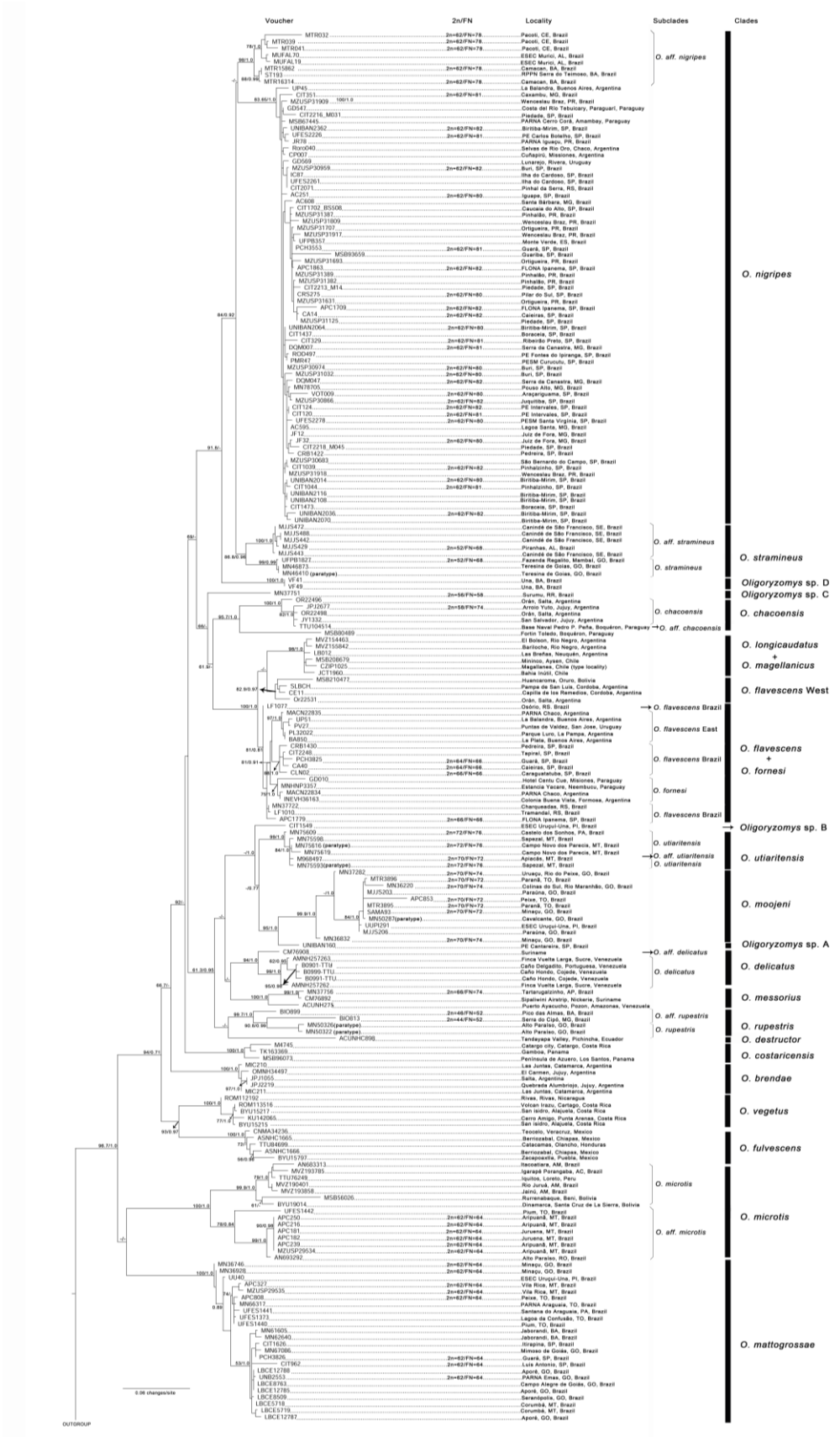

Fig. 3: Maximum likelihood phylogenetic hypothesis based on mitochondrial gene cyt-*b*. Numbers in the nodes indicate bootstrap support (above 50) and BI posterior probability (above 0.70), respectively.

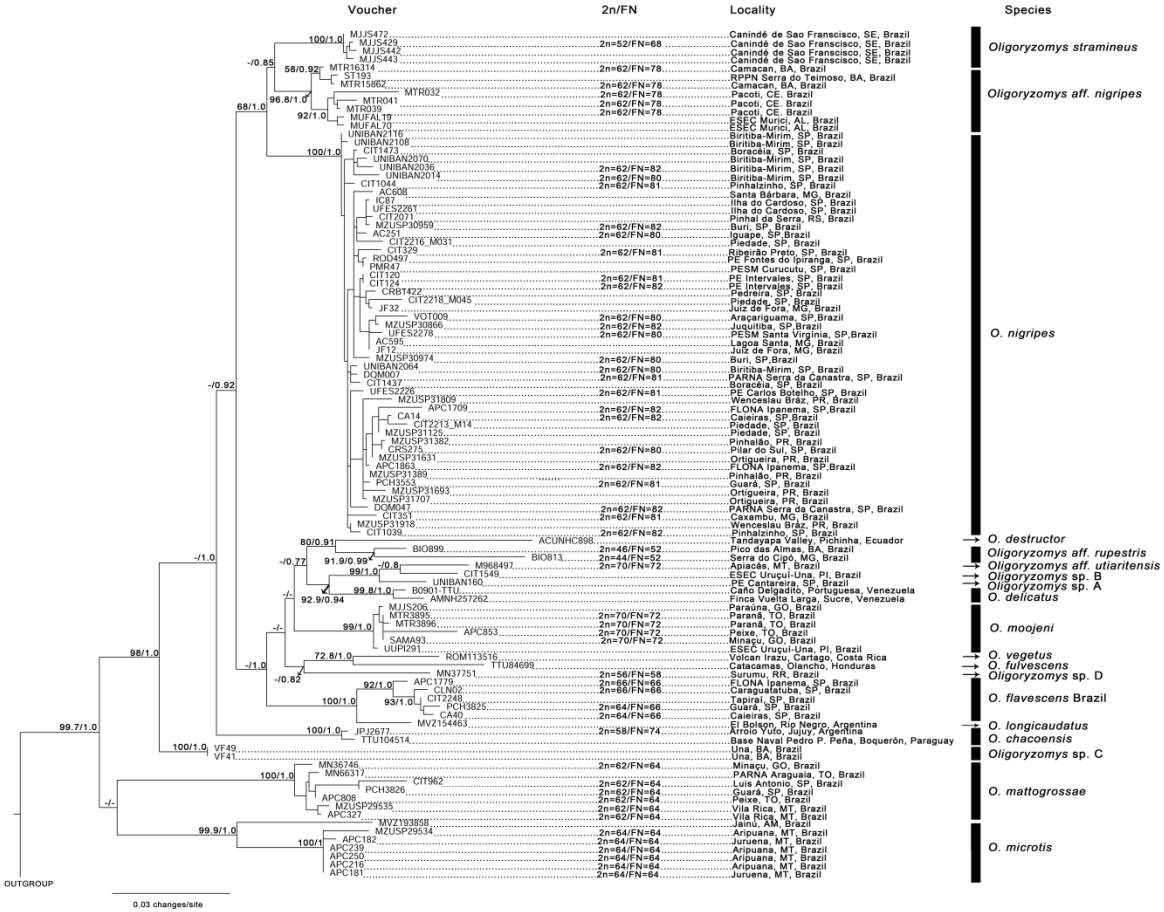

Fig. 4: Bayesian Inference phylogenetic hypothesis based on concatenated matrix dataset (cyt-*b* and IRBP) without missing data. Numbers in the nodes indicate bootstrap support (above 50) and BI posterior probability (above 0.70), respectively.

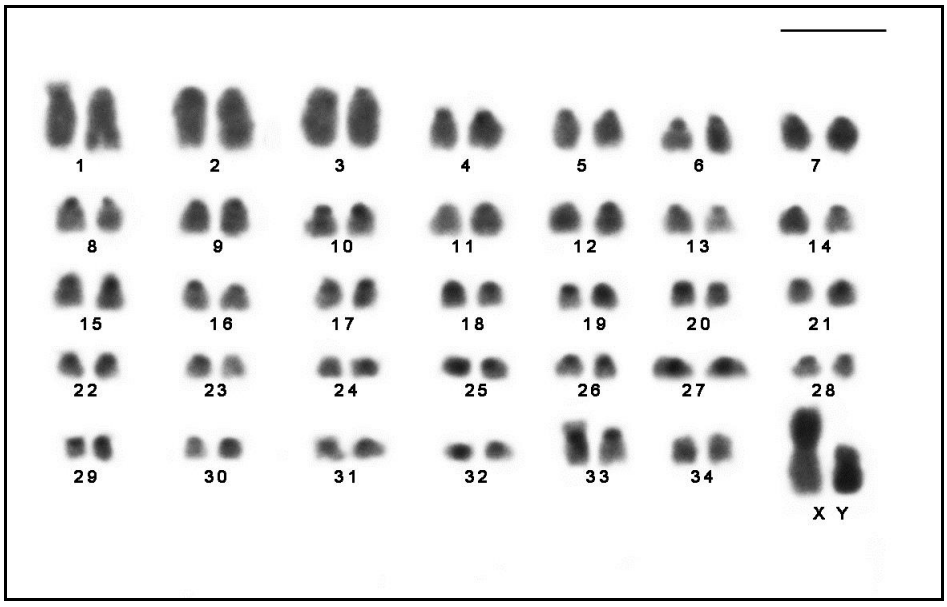

Fig. 5: CBG-banding karyotype of *Oligoryzomys aff. utiaritensis* (2n=70, FN=72, male) from Apiacás, MT, Brazil (extracted from Di-Nizo, 2013). Bar = 10 µm.

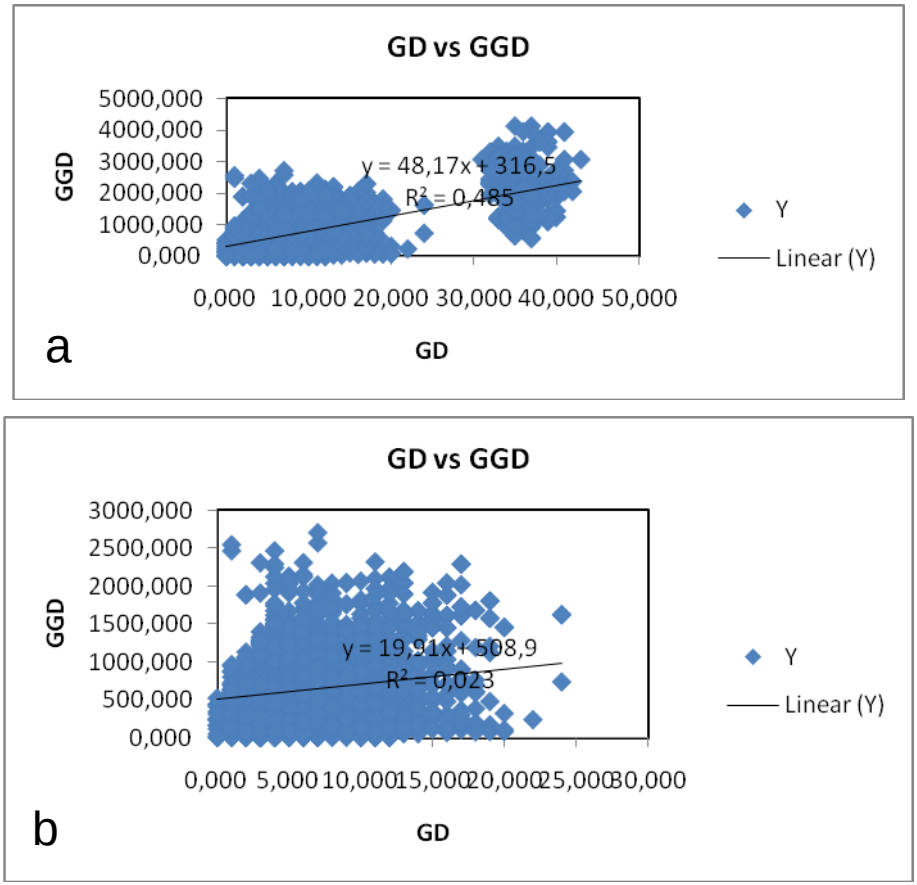

Fig. 6: Mantel test between genetic distance (GD, pb) and geographical distance (GGD, km) matrices. (a) Test among lineages NE and CS, showing significant correlation coefficient (r=0.697; p=0.001). (b) Test with lineage CS, showing no significant correlation coefficient (r=0.153; p=0.060).

Table 1: Specimens used in this study: species, GenBank number, field/lab number, voucher numbers (when available), genes amplified, karyotype (when available), locality with state or province, country (C), coordinates (Coord), locality number (Nº) in the map (Fig. 1), haplotypes (Hap) for phylogeographic studies of *O. nigripes* and references.

Species	GenBank Acession	Field Number	Museum number	Cyt-*b*	IRBP	Karyotype	Locality, State or Province	C	Coord	Map	Hap	Reference
O. brendae	JX154130	JPJ1055	IER- LIEY	X	-	N/A	15 km W Escoipe, Salta	Arg	25º10'S;65º49'W	1	-	Teta *et al.* (2013)
	EU192168	MIC211	-	X	-	N/A	Las Juntas,	Arg	28°06'S;65°55'W	2	-	Palma *et al.* (2010)
	EU192167	MIC210	-	X	-	N/A	Catamarca				-	Palma *et al.* (2010)
	KC841389	-	OMNH34497	X	-	N/A	El Carmen, Jujuy	Arg	24º23'S; 65º15'W	3	-	Coyner *et al.* (2013)
	JX154134	JPJ2219	IER- LIEY	X	-	N/A	Quebrada Alumbriojo, Jujuy	Arg	23º19'S;64º55'W	4	-	Teta *et al.* (2013)
O. chacoensis	***to be provided***	**JPJ2677**	**IER- LIEY**	**X**	**X**	**2n=58,FN=74**	**Arroio Yuto, Jujuy**	**Arg**	**23°38'S;64°32'W**	**5**	**-**	**This study**
	GU185902	JY1332	INEVH	X	-	N/A	San Salvador, Jujuy	Arg	24°12'S;65°19'W	6	-	Gonzalez-Ittig *et al.* (2010)
	GU185903	OR22496	INEVH	X	-	N/A	Orán, Salta	Arg	23°08'S;64°19'W	7	-	Gonzalez-Ittig *et al.* (2010)
	GU185904	OR22498	INEVH	X	-	N/A					-	Gonzalez-Ittig *et al.* (2010)
	EU258543 / EU649059	TK62932	TTU104514	X	X	N/A	Base Naval Pedro P. Peña, Boquéron	Parag	22°27'S;62°21'W	8	-	Hanson *et al.* (2011); Hanson and Bradley (unpubl.)
O. aff. chacoensis	EU192173	NK72388	MSB80489	X	-	N/A	Fortin Toledo, Boquerón	Parag	22°01'S;60°36'W	9	-	Palma *et al.* (2010)
O. costaricensis	GU393988	TK163369	TTU	X	-	N/A	Gamboa	Panamá	09°07'N; 79°42'W	10	-	Hanson *et al.*(2011)
	EU192164	NK101588	MSB96073	X	-	N/A	Península de Azuero, Los Santos	Panamá	07°46'N; 80°17'W	11	-	Palma *et al.* (2010)
	EU258539	M4745	MVZ155316	X	-	N/A	Catargo city, Catargo	C. Rica	09°52'N; 83°54'W	12	-	Hanson *et al.* (2011)
O. delicatus	GU393994	TK138248	B0991-TTU	X	-	N/A	Caño Hondo,	Ven	09º26'N; 68º17'W	13	-	Hanson *et al.*(2011)
	GU393995	TK138247	B0999-TTU	X	-	N/A	Cojedes				-	Hanson *et al.*(2011)
	DQ227457 / EU649060	FHV4083-TK138080	B0901-TTU	X	X	N/A	Caño Delgadito, Portuguesa	Ven	08°47'N; 69°26'W	14	-	Hanson *et al.*(2011)

Species	GenBank Acession	Field Number	Museum number	Cyt-*b*	IRBP	Karyotype	Locality, State or Province	C	Coord	Map	Hap	Reference
O. delicatus	GU393997	-	AMNH257263	X	-	N/A	Finca Vuelta Larga,	Ven	10°24'N; 63°46'W	15	-	Hanson *et al.* (2011)
	GU126529 / AY163611	-	AMNH257262	X	X	N/A	Sucre				-	Weksler (2003)
O. aff. delicatus	GU393993	TK21065	CM76908	X	-	N/A	N/A	Suriname	N/A	-	-	Hanson *et al.* (2011)
O. destructor	EU258544/EU 649061	TEL1479	ACUNHC898	X	X	N/A	Tandayapa Valley, Pichincha	Ecuador	0º00'39"N;78º40'W	16	-	Rogers *et al.* (2009); Hanson and Bradley (unpubl.)
	GU393991	TEL1481	ACUNHC	X	-	N/A					-	Hanson *et al.* (2011)
*O. flavescens*Brazil	***to be provided***	**ROD87 - CA-40**	**MZUSP**	**X**	**X**	**2n=64,FN=66[7]**	**Caieiras, SP**	**Brazil**	**23°20'S; 46°46'W**	**17**	**-**	**This study**
	to be provided	**ROD123-CLN02**	**MZUSP**	**X**	**X**	**2n=66,FN=66[7]**	**Caraguatatuba, SP**	**Brazil**	**23°37'S; 45°25'W**	**18**	**-**	**This study**
	to be provided	**APC1779**	**UFSCAR**	**X**	**X**	**2n=66,FN=66[1]**	**Flona Ipanema, SP**	**Brazil**	**23°25'S; 47°35'W**	**19**	**-**	**This study**
	to be provided	**PCH3825**	**MZUSP**	**X**	**X**	**2n=64,FN=66[7]**	**Guará, SP**	**Brazil**	**20º30'S; 47º51'W**	**20**	**-**	**This study**
	GU126528	CRB1430	-	X	-	N/A	Pedreira, SP	Brazil	22º44'S; 46º54'W	21	-	Percequillo *et al*. (2011)
	AY163609	CRB1405	-	-	X	N/A					-	Weksler (2003)
	to be provided	**CIT2248**	**MZUSP**	**X**	**X**	**N/A**	**Tapiraí, SP**	**Brazil**	**23º57'S; 47º30'W**	**22**	**-**	**This study**
	DQ826014	-	MN37722	X	-	N/A	Charqueadas, RS	Brazil	29°57'S; 51°37'W	23	-	Miranda *et al.* (2009)
	DQ826030	-	-	-	X	N/A					-	Miranda *et al.* (2009)
	DQ826011	LF1077	-	X	-	N/A	Osório, RS	Brazil	29º54'S; 50º16'W	24	-	Miranda *et al.* (2009)
	DQ826010	LF1010	-	X	-	N/A	Tramandaí, RS	Brazil	29º59'S; 50º08'W	25	-	Miranda *et al.* (2009)
O flavescens West	GU185916	SLBCH	MZ-UNC	X	-	N/A	Pampa de San Luis, Córdoba	Arg	31°20'S; 64°47'W	26	-	Gonzalez-Ittig *et al.* (2010)
	GU185915	CE11	MZ-UNC	X	-	N/A	Capilla de los Remedios, Córdoba	Arg	31°30'S; 63°54'W	27	-	Gonzalez-Ittig *et al.* (2010)
	GU185913	Or22531	INEVH	X	-	N/A	Orán, Salta	Arg	23°08'S; 64°19'W	7	-	Gonzalez-Ittig *et al.* (2010)
	AY452200	NK11547	MSB210477	X	-	N/A	Huancaroma, Oruro	Bolivia	17°40'S; 67°28'W	28	-	Palma *et al* . (2010)
O flavescens East	GU185923	PL32022	INEVH	X	-	N/A	Pq Luro, La Pampa	Arg	36°55'S; 64°17'W	29	-	Gonzalez-Ittig *et al.* (2010)

Species	GenBank Acession	Field Number	Museum number	Cyt-*b*	IRBP	Karyotype	Locality, State or Province	C	Coord	Map	Hap	Reference
O flavescens East	GU185925	BA850	IMBICE	X	-	N/A	La Plata, Buenos Aires	Arg	34°58'S; 57°56'W	30	-	Gonzalez-Ittig *et al.* (2010)
	GU185924	UP51	CENPAT	X	-	N/A	La Balandra, B. Aires	Arg	34°49'S; 58°02'W	31	-	Gonzalez-Ittig *et al.* (2010)
	GU185919	-	MACN22835	X	-	N/A	PARNA Chaco, Chaco	Arg	26°49'S; 59°40'W	32	-	Gonzalez-Ittig *et al.* (2010)
	GU185921	PV27	INEVH	X	-	N/A	Puntas de Valdez, San José	Urug	34°35'S; 56°42'W	33	-	Gonzalez-Ittig *et al.* (2010)
O fornesi	HQ890936	-	INEVH36163	X	-	N/A	Colonia Buena Vista, Formosa	Arg	25°01'S; 58°25'W	34	-	Gonzalez-Ittig *et al.* (2014)
	GU185917	-	MACN22834	X	-	N/A	PARNA Chaco, Chaco	Arg	26°49'S; 59°40'W	32	-	Gonzalez-Ittig *et al.* (2010)
	EU258542	TK64399	MNHNP3357	X	-	N/A	Estancia Yacare, Neembucu	Parag	26º52'S; 58º16'W	35	-	Rogers *et al.* (2009)
	AY452199	GD010	-	X	-	N/A	Hotel Centu Cue, Misiones	Parag	26°15'S; 57°01'W	36	-	Palma *et al.* (2005)
O. fulvescens	EU294233	-	ASNHC1665	X	-	N/A	12 km N Berriozabal,	Mex	16º48'N; 93º16'W	37	-	Rogers *et al.* (2009)
	EU294234	-	ASNHC1666	X	-	N/A	Chiapas				-	Rogers *et al.* (2009)
	EU294248	-	CNMA34236	X	-	N/A	18 km NE of Teocelo, Veracruz	Mex	19°23'N; 96°58'W	38	-	Rogers *et al.* (2009)
	EU294235	-	BYU15797	X	-	N/A	10 km N of Zacapoaxtla, Puebla	Mex	19°52'N; 97°35'W	39	-	Rogers *et al.* (2009)
	EU258547 / EU649063	TK102042	TTU84699	X	X	N/A	Catacamas, Olancho	Hond	14°48'N; 85°54'W	40	-	Hanson *et al.* (2011); Hanson and Bradley (unpubl.)
O. longicaudatus	AY275702	LB012	CENPAT	X	-	N/A	Las Breñas, Neuquén	Arg	39º23'S; 71º12'W	41	-	Palma *et al.* (2005)
	GU393998 / EU649064	-	MVZ154463	X	X	N/A	El Bolson, Rio Negro	Arg	41°48'S; 71°25'W	42	-	Hanson *et al.* (2011); Hanson and Bradley (unpubl.)
	U03535 / KC953426	-	MVZ155842	X	-	N/A	Bariloche, Rio Negro	Arg	41°07'S; 71°27'W	43	-	Smith and Patton (1999); Schenk *et al.* (2013)
	AY275697	NK95268	MSB208679	X	-	N/A	Mininco, Aysén	Chile	45°30'S; 71°47'W	44	-	Palma *et al*. (2005)
	EU192160	JCT1960	MNHNCL	X	-	N/A	Bahía Inútil, Antarctica Chilena	Chile	54º59'S; 68º13'W	45	-	Palma *et al.* (2010)

Species	GenBank Acession	Field Number	Museum number	Cyt-*b*	IRBP	Karyotype	Locality, State or Province	C	Coord	Map	Hap	Reference
O. magellanicus	AY275705	-	CZIP1025	X	-	N/A	Río Penitente, Magallanes	Chile	52°06'S; 71°32'W	46	-	Palma *et al.* (2005)
O. mattogrossae	KY952261	-	MN62640	X	-	N/A	Jaborandi, BA	Brazil	13º17'S; 44º25'W	47	-	Weksler *et al.* (2017)
	KY952260	-	MN61605	X	-	N/A					-	Weksler *et al.* (2017)
	KY952254	-	LBCE12787	X	-	N/A	Aporé, GO	Brazil	18º57'S; 51º55'W	48	-	Weksler *et al.* (2017)
	KY952255	-	LBCE12788	X	-	N/A					-	Weksler *et al.* (2017)
	KY952253	-	LBCE12785	X	-	N/A					-	Weksler *et al.* (2017)
	KY952259	-	LBCE8763	X	-	N/A	Campo Alegre de Goiás, GO	Brazil	17º38'S; 47º46'W	49	-	Weksler *et al.* (2017)
	KY952262	-	MN67086	X	-	N/A	Mimoso de Goiás, GO	Brazil	15º03'S; 48º09'W	50	-	Weksler *et al.* (2017)
	DQ826022/ DQ826033	-	MN36746	X	X	N/A	Minaçu, Rio Tocantinzinho, GO	Brazil	13º31'S; 48º13'W	51	-	Miranda *et al.* (2009)
	DQ826023	-	MN36928	X	-	N/A					-	Miranda *et al.* (2009)
	to be provided	**APC565**	**UNB2553**	**X**	**-**	**2n=62,FN=64[7]**	**PARNA Emas, GO**	**Brazil**	**18°06'S; 52°55'W**	**52**	**-**	**This study**
	KY952258	-	LBCE8509	X	-	N/A	Serranópolis, GO	Brazil	18º18'S; 51º58'W	53	-	Weksler *et al.* (2017)
	AY163610	CRB747	-	-	X	N/A	Teresina de Goias, GO	Brazil	13º46'S; 47º15'W	54	-	Weksler (2003)
	KY952256	-	LBCE5718	X	-	N/A	Corumbá, MS	Brazil	19º00'S; 57º38'W	55	-	Weksler *et al.* (2017)
	KY952257	-	LBCE5719	X	-	N/A					-	Weksler *et al.* (2017)
	to be provided	**APC319**	**MZUSP29535**	**X**	**X**	**2n=62,FN=64[7]**	**Vila Rica, MT**	**Brazil**	**09°54'S; 51°12'W**	**56**	**-**	**This study**
	to be provided	**APC327**	**MZUSP**	**X**	**X**	**2n=62,FN=64[7]**					**-**	**This study**
	HM594623	-	UFES1441	X	-	N/A	Santana do Araguaia, PA	Brazil	09°18'S; 50°06'W	57	-	Rocha *et al.* (2011)
	to be provided	**CIT1554-UU40**	**MZUSP**	**X**	**-**	**N/A**	**ESEC Uruçuí-Una,PI**	**Brazil**	**08°52'S; 44°58'W**	**58**	**-**	**This study**
	to be provided	**PCH3826**	**MZUSP**	**X**	**X**	**2n=62,FN=64[7]**	**Guará, SP**	**Brazil**	**20º29'S; 47º51'W**	**20**	**-**	**This study**
	to be provided	**CIT1626**	**MZUSP**	**X**	**-**	**N/A**	**Itirapina, SP**	**Brazil**	**22°15'S; 47°49'W**	**59**	**-**	**This study**

Species	GenBank Acession	Field Number	Museum number	Cyt-*b*	IRBP	Karyotype	Locality, State or Province	C	Coord	Map	Hap	Reference
O. mattogrossae	***to be provided***	**CIT962**	**MZUSP**	**X**	**X**	**2n=62,FN=64[7]**	**Luis Antonio, SP**	**Brazil**	**21°33'S; 47°42'W**	**60**	**-**	**This study**
	HM594620	-	UFES1373	X	-	N/A	Lagoa Confusão, TO	Brazil	10°47'S; 49°37'W	61	-	Rocha *et al.* (2011)
	to be provided	**ARB120**	**MN66317**	**X**	**X**	**N/A**	**PARNAAraguaia, TO**	**Brazil**	**10°27'S, 50°29'W**	**62**	**-**	**This study**
	to be provided	**APC808**	**MZUSP**	**X**	**X**	**2n=62,FN=64[7]**	**Peixe, TO**	**Brazil**	**12º01'S; 48º32'W**	**63**	**-**	**This study**
	HM594622	-	UFES1440	X	-	N/A	Pium, TO	Brazil	09°58'S; 50°04'W	64	-	Rocha *et al.* (2011)
O. messorius	DQ826025	-	MN37756	X	-	2n=66, FN=74[2]	Tartarugalzinho, AP	Brazil	01º30'N; 50º54'W	65	-	Miranda *et al.* (2009)
	EU258537	-	ACUNH275	X	-	N/A	Puerto Ayacucho, Pozon, Amazonas	Ven	05°39'N; 67°37'W	66	-	Hanson *et al*. (2011)
	EU258546	TK17858	CM76892	X	-	N/A	Sipaliwini Airstrip, Nickerie	Suriname	05°46'N; 56°52'W	67	-	Hanson *et al*. (2011)
O. microtis	HM594624	MNFS1321	MVZ193785	X	-	N/A	Igarapé Porangaba, AC	Brazil	08°40'S; 72°47'W	68	-	Patton and da Silva (1995)
	JX443647	AN683313	-	X	-	N/A	Itacoatiara, AM	Brazil	03°08'S; 58°26'W	69	-	Firth *et al.* (2012)
	EU258549/ EU649066	MNFS790	MVZ193858	X	X	N/A	Jainu, AM	Brazil	06°28'S; 68°46'W	70	-	Hanson *et al.* (2011)
	HM594624	-	MVZ190401	X	-	N/A	Rio Juruá, AM	Brazil	06°45'S; 70°51'W	71	-	Rocha *et al.* (2011)
	AY439000	-	BYU19014	X	-	N/A	Dinamarca, Santa Cruz	Bolivia	17°03'S; 63°24'W;	76	-	Carrol *et al*. (2005)
	EU192172	NK13425	MSB56026	X	-	N/A	Rurrenabaque, Beni	Bolivia	14°30'S; 67°34'W	77	-	Palma *et al.* (2010)
	FJ374766	-	TTU76249	X	-	N/A	Iquitos, Loreto	Peru	03°45'S; 73°11'W	75	-	Richter *et al.* (2010)
O. aff. microtis	***to be provided***	**APC216**	**MZUSP**	**X**	**X**	**2n=64,FN=64[7]**	**Aripuanã, MT**	**Brazil**	**10°10'S; 59°27''W**	**72**	**-**	**This study**
	to be provided	**APC239**	**MZUSP**	**X**	**X**	**2n=64,FN=64[7]**					**-**	**This study**
	to be provided	**APC241**	**MZUSP29534**	**X**	**X**	**2n=64,FN=64[1]**					**-**	**This study**
	to be provided	**APC250**	**MZUSP**	**X**	**X**	**2n=64,FN=64[7]**					**-**	**This study**
	to be provided	**APC181**	**MZUSP**	**X**	**X**	**2n=64,FN=64**	**Juruena, MT**	**Brazil**	**10°19'S; 58°29'W**	**73**	**-**	**This study**
	to be provided	**APC182**	**MZUSP**	**X**	**X**	**2n=64,FN=64**					**-**	**This study**

Species	GenBank Acession	Field Number	Museum number	Cyt-*b*	IRBP	Karyotype	Locality, State or Province	C	Coord	Map	Hap	Reference
O. aff. microtis	JX443663	AN693292	-	X	-	N/A	Alto Paraíso, RO	Brazil	09°44'S; 63°17'W	74	-	Firth *et al.* (2012)
	HM594618	-	UFES1442	X	-	N/A	Pium, TO	Brazil	09°58'S; 50°04'W	64	-	Rocha *et al.* (2011)
O. moojeni	JQ013772	-	MN50287 (paratype)	X	-	N/A	Cavalcante, GO	Brazil	14º04'S; 47º45'W	78	-	Agrellos *et al.* (2012)
	DQ826016	-	MN36220	X	-	2n=70, FN=74[2]	Colinas do Sul, GO	Brazil	14°09'S; 48°04'W	79	-	Miranda *et al.* (2009)
	DQ826031	-	-	-	X	N/A					-	Miranda *et al.* (2009)
	to be provided	**SAMA93**	**-**	**X**	**X**	**2n=70,FN=72[1]**	**Minaçu, GO**	**Brazil**	**13º31'S; 48º13'W**	**51**	**-**	**This study**
	DQ826019	-	MN36832	X	-	2n=70, FN=74[2]					-	Miranda *et al.* (2009)
	to be provided	**MJJS203**	**MZUSP**	**X**	**-**	**N/A**	**Paraúna, GO**	**Brazil**	**16°59'S; 50°37''W**	**80**	**-**	**This study**
	to be provided	**MJJS206**	**MZUSP**	**X**	**X**	**N/A**					**-**	**This study**
	DQ826020	-	MN37282	X	-	2n=70, FN=74[2]	Uruaçu, GO	Brazil	14°31'S; 49°08'W	81	-	Miranda *et al.* (2009)
	to be provided	**UUPI291**	**MZUSP**	**X**	**X**	**N/A**	**ESEC Uruçuí-Una,PI**	**Brazil**	**08°52'S; 44°58'W**	**58**	**-**	**This study**
	to be provided	**MTR3895**	**MZUSP**	**X**	**X**	**2n=70,FN=72**	**Paranã, TO**	**Brazil**	**12º36'S; 47º52'W**	**82**	**-**	**This study**
	to be provided	**MTR3896**	**MZUSP**	**X**	**X**	**2n=70,FN=72**					**-**	**This study**
	to be provided	**APC853**	**MZUSP**	**X**	**X**	**2n=70,FN=72[7]**	**Peixe, TO**	**Brazil**	**12º01'S; 48º32'W**	**63**	**-**	**This study**
O. nigripes	DQ826004*	-	UFPB357	X	-	N/A	Monte Verde, ES	Brazil	19°53'S; 41°57'W	83	69	Miranda *et al.* (2009)
	to be provided	**AC530**	**-**	**-**	**X**	**2n=62,FN=82[3]**	**Caxambu, MG**	**Brazil**	**21°58'S; 44°55'W**	**84**	**-**	**This study**
	to be provided	**AC581**	**-**	**-**	**X**	**2n=62,FN=82[3]**					**-**	**This study**
	to be provided*	**CIT351**	**-**	**X**	**X**	**2n=62,FN=81[3]**					**16**	**This study**
	to be provided*	**JF12**	**-**	**X**	**X**	**N/A**	**Juiz de Fora, MG**	**Brazil**	**21°45'S; 43°21'W**	**85**	**45**	**This study**
	to be provided*	**JF32**	**-**	**X**	**X**	**2n=62,FN=80**					**36**	**This study**
	to be provided*	**AC595**	**-**	**X**	**X**	**N/A**	**Lagoa Santa, MG**	**Brazil**	**19º37'S; 43º53'W**	**86**	**45**	**This study**
	to be provided*	**DQM007**	**-**	**X**	**X**	**2n=62,FN=81[7]**	**PARNA Serra da**	**Brazil**	**20°20'S; 46°38'W**	**87**	**49**	**This study**

Species	GenBank Acession	Field Number	Museum number	Cyt-*b*	IRBP	Karyotype	Locality, State or Province	C	Coord	Map	Hap	Reference
O. nigripes	***to be provided***	**DQM047**	**-**	**X**	**X**	**2n=62,FN=82[3]**	**Canastra, MG**				**-**	**This study**
	KF815443* /KF815419	-	MN78705	X	-	N/A	Pouso Alto, MG	Brazil	22°11'S; 44°58'W	88	59	Vilela *et al.* (2014)
	to be provided*	**AC608**	**-**	**X**	**X**	**N/A**	**Santa Barbara, MG**	**Brazil**	**19º57'S; 43º24'W**	**89**	**24**	**This study**
	to be provided*	**IIM170**	**MZUSP31693**	**X**	**X**	**N/A**	**Ortigueira, PR**	**Brazil**	**24º12'S; 50º57'W**	**90**	**26**	**This study**
	to be provided*	**IIM185**	**MZUSP31707**	**X**	**X**	**N/A**					**28**	**This study**
	to be provided*	**IIM087**	**MZUSP31631**	**X**	**X**	**N/A**					**22**	**This study**
	DQ825988*	JR78	-	X	-	N/A	PARNA Iguaçu, PR	Brazil	25º32'S; 54º35'W	91	68	Miranda *et al.* (2009)
	to be provided	**IIM031**	**MZUSP31382**	**X**	**X**	**N/A**	**Pinhalão, PR**	**Brazil**	**23º46'S; 50º02'W**	**92**	**-**	**This study**
	to be provided*	**IIM040**	**MZUSP31387**	**X**	**-**	**N/A**					**34**	**This study**
	to be provided*	**IIM055**	**MZUSP31389**	**X**	**X**	**N/A**					**19**	**This study**
	to be provided*	**IIM231**	**MZUSP31809**	**X**	**X**	**N/A**	**Wenceslau Braz, PR**	**Brazil**	**23º52'S; 49º48'W**	**93**	**18**	**This study**
	to be provided	**IIM295**	**MZUSP31909**	**X**	**-**	**N/A**					**-**	**This study**
	to be provided	**IIM339**	**MZUSP31917**	**X**	**-**	**N/A**					**-**	**This study**
	to be provided*	**IIM340**	**MZUSP31918**	**X**	**X**	**N/A**					**52**	**This study**
	DQ825993*			X		N/A	Caldas Novas, GO	Brazil	17º44'S; 48º37'W	94	05	Miranda *et al.* (2009)
	DQ826001*	-	-	X	-	N/A	Florianópolis, SC	Brazil	27º35'S; 48º34'W	95	03	Miranda *et al.* (2009)
	JQ013778*	-	-	X	-	N/A	Jaborá, SC	Brazil	27º08'S; 51º46'W	96	62	Agrellos *et al.* (2012)
	DQ825995*	-	-	X	-	N/A	Caxias do Sul, RS	Brazil	29º10'S; 51º11'W	97	64	Miranda *et al.* (2009)
	DQ825992*	-	-	X	-	N/A	Charqueadas, RS	Brazil	29º57'S; 51º37'W	23	13	Miranda *et al.* (2009)
	DQ825990*	-	-	X	-	N/A	ESEC Taim, RS	Brazil	32º29'S; 52º34'W	98	63	Miranda *et al.* (2009)
	DQ826000*	-	-	X	-	N/A	Mostardas, RS	Brazil	31º10'S; 51º31'W	99	67	Miranda *et al.* (2009)
	DQ826003*	-	-	X	-	N/A	Nonoai, RS	Brazil	27º21'S; 52º47'W	100	09	Miranda *et al.* (2009)
	DQ825991*	-	-	X	-	N/A	Osório, RS	Brazil	29º54'S; 50º16'W	24	07	Miranda *et al.* (2009)
	to be provided*	**CIT2071**	**-**	**X**	**X**	**N/A**	**Pinhal da Serra, RS**	**Brazil**	**27º52'S; 51º10'W**	**101**	**35**	**This study**

Species	GenBank Acession	Field Number	Museum number	Cyt-*b*	IRBP	Karyotype	Locality, State or Province	C	Coord	Map	Hap	Reference
O. nigripes	DQ825997*	-	-	X	-	N/A	Sapiranga, RS	Brazil	29º38'S; 51º00'W	102	65	Miranda *et al.* (2009)
	DQ825996*	-	-	X	-	N/A	São Francisco de Paula, RS	Brazil	29º27'S; 50º35'W	103	61	Miranda *et al.* (2009)
	DQ825999*	-	-	X	-	N/A	Tapes, RS	Brazil	30º40'S; 51º23'W	104	04	Miranda *et al.* (2009)
	DQ826005*	-	-	X	-	N/A	Tainhas, RS	Brazil	29º16'S; 50º18'W	105	66	Miranda *et al.* (2009)
	DQ825994*	-	-	X	-	N/A	Tenente Portela, RS	Brazil	27º22'S; 53º45'W	106	08	Miranda *et al.* (2009)
	DQ826029	-	-	-	X	N/A	Torres, RS	Brazil	29°19'S; 49°46'W	107	-	Miranda *et al.* (2009)
	DQ825987*	-	-	X	-	N/A					03	Miranda *et al.* (2009)
	DQ825998*	-	-	X	-	N/A					02	
	to be provided	**CIT355 VOT009**	**MZUSP**	**X**	**X**	**2n=62,FN=80[3]**	**Araçariguama, SP**	**Brazil**	**23°26'S; 47°03'W**	**108**	**-**	**This study**
	to be provided*	**UNIBAN 2014**	**MZUSP**	**X**	**X**	**2n=62,FN=80[7]**	**Biritiba-Mirim, SP**	**Brazil**	**23°34'S; 46°02'W**	**109**	**46**	**This study**
	to be provided*	**UNIBAN 2036**	**MZUSP**	**X**	**X**	**2n=62,FN=82[7]**					**27**	**This study**
	to be provided*	**UNIBAN 2362**	**MZUSP**	**X**	**-**	**2n=62,FN=82[7]**					**41**	**This study**
	to be provided*	**UNIBAN 2064**	**MZUSP**	**X**	**X**	**2n=62,FN=80[7]**					**49**	**This study**
	to be provided*	**UNIBAN 2070**	**MZUSP**	**X**	**X**	**N/A**					**43**	**This study**
	to be provided*	**UNIBAN 2116**	**MZUSP**	**X**	**X**	**N/A**					**47**	**This study**
	to be provided*	**UNIBAN 2108**	**MZUSP**	**X**	**X**	**N/A**					**47**	**This study**
	to be provided*	**ITM0459**	**MZUSP30959**	**X**	**X**	**2n=62,FN=82[7]**	**Buri, SP**	**Brazil**	**23°48'S; 48°36'W**	**110**	**39**	**This study**
	to be provided*	**ITM0474**	**MZUSP30974**	**X**	**X**	**2n=62,FN=80[7]**					**51**	**This study**
	to be provided*		**MZUSP30991**	**X**		**2n=62,FN=82[7]**					**35**	**This study**
	to be provided*	**ITM0556**	**MZUSP31032**	**X**	**-**	**2n=62,FN=81[7]**					**30**	**This study**
	to be provided*	**ROD27 CA-14**	**MZUSP**	**X**	**X**	**2n=62,FN=82[7]**	**Caieiras, SP**	**Brazil**	**23°21'S; 46°44'W**	**17**	**32**	**This study**

Species	GenBank Acession	Field Number	Museum number	Cyt-*b*	IRBP	Karyotype	Locality, State or Province	C	Coord	Map	Hap	Reference
O. nigripes	***to be provided****	**CIT1702 - BS508**	**MZUSP**	**X**	**-**	**N/A**	**Caucaia do Alto, SP**	**Brazil**	**23°40'S; 47°01'W**	**111**	**29**	**This study**
	to be provided*	**CIT1437**	**MZUSP**	**X**	**X**	**N/A**	**Estação Biológica de Boracéia, SP**	**Brazil**	**23°38'S; 45°52'W**	**112**	**49**	**This study**
	to be provided*	**CIT1473**	**MZUSP**	**X**	**X**	**N/A**					**48**	**This study**
	to be provided*	**APC1709**	**UFSCAR**	**X**	**X**	**2n=62,FN=82[7]**	**Flona Ipanema, SP**	**Brazil**	**23°25'S; 47°35'W**	**19**	**15**	**This study**
	to be provided*	**APC1863**	**UFSCAR**	**X**	**X**	**2n=62,FN=82[7]**					**31**	**This study**
	to be provided*	**PCH3553**	**MZUSP**	**X**	**X**	**2n=62,FN=81[7]**	**Guará, SP**	**Brazil**	**20º29'S; 47º51'W**	**20**	**19**	**This study**
	EU192163	NK42266	MSB93659	X	-	N/A	Guariba, SP	Brazil	21°25'S; 48°15'W	113	-	Palma *et al.* (2010)
	to be provided*	**AC251**	**-**	**X**	**X**	**2n=62,FN=80[3]**	**Iguape, SP**	**Brazil**	**24°42'S; 47°33'W**	**114**	**38**	**This study**
	to be provided	**-**	**UFES2261**	**X**	**X**	**N/A**	**Ilha do Cardoso, SP**	**Brazil**	**25°08'S; 48°00'W**	**115**	**-**	**This study**
	to be provided*	**ROD304 - IC87**	**-**	**X**	**X**	**N/A**					**35**	**This study**
	to be provided*	**ITM0387**	**MZUSP30866**	**X**	**X**	**2n=62,FN=82[7]**	**Juquitiba, SP**	**Brazil**	**23°56'S; 47°04'W**	**116**	**37**	**This study**
	to be provided	**ROD160**	**UFES2227**	**-**	**X**	**N/A**	**PE Carlos Botelho, SP**	**Brazil**	**24°08'S; 47°58'W**	**117**	**-**	**This study**
	to be provided*	**ROD164**	**UFES2226**	**X**	**X**	**2n=62,FN=81[7]**					**40**	**This study**
	to be provided*	**ROD497**	**MZUSP**	**X**	**X**	**N/A**	**PE Fontes do Ipiranga, SP**	**Brazil**	**23°38'S; 46°37''W**	**118**	**44**	**This study**
	to be provided*	**CIT120**	**MZUSP**	**X**	**X**	**2n=62,FN=81[3]**	**PE Intervales, SP**	**Brazil**	**24°16'S; 48°24'W**	**119**	**33**	**This study**
	to be provided*	**CIT124**	**MZUSP**	**X**	**X**	**2n=62,FN=82[3]**					**33**	**This study**
	EU649062	-	MVZ183088	-	X	N/A					-	Hanson and Bradley (unpubl.)
	GU126530 */ AY163612	CRB1422	-	X	X	N/A	Pedreira, SP	Brazil	22º44'S; 46º54'W	21	60	Agrellos *et al.* (2012); Weksler (2003)
	to be provided*	**PMR47**	**MZUSP**	**X**	**X**	**N/A**	**PESM Curucutu, SP**	**Brazil**	**23º59'S; 46º44'W**	**120**	**44**	**This study**
	to be provided*	**-**	**UFES2278**	**X**	**X**	**2n=62,FN=80[4]**	**PESM Santa Virgínia, SP**	**Brazil**	**23°26'S; 45°14'W**	**121**	**42**	**This study**

Species	GenBank Acession	Field Number	Museum number	Cyt-*b*	IRBP	Karyotype	Locality, State or Province	C	Coord	Map	Hap	Reference
O. nigripes	***to be provided****	**ITM0236**	**MZUSP31125**	**X**	**X**	**N/A**	**Piedade, SP**	**Brazil**	**23º55'S; 47º04'W**	**122**	**20**	**This study**
	to be provided*	**CIT2213 - M014**	**MZUSP**	**X**	**X**	**N/A**					**23**	**This study**
	to be provided*	**CIT2216 - M031**	**MZUSP**	**X**	**X**	**N/A**					**21**	**This study**
	to be provided*	**CIT2218_ M045**	**MZUSP**	**X**	**X**	**N/A**					**25**	**This study**
	to be provided	**CIT1279 - CRS275**	**MZUSP**	**X**	**X**	**2n=62,FN=80**	**Pilar do Sul, SP**	**Brazil**	**23°48'S; 47°42'W**	**123**	**-**	**This study**
	to be provided	**CIT1039**	**MZUSP**	**X**	**X**	**2n=62,FN=82[7]**	**Pinhalzinho, SP**	**Brazil**	**22º46'S; 46º35'W**	**124**	**-**	**This study**
	to be provided	**CIT1044**	**MZUSP**	**X**	**X**	**2n=62,FN=81[7]**					**-**	**This study**
	to be provided*	**CIT329**	**MZUSP**	**X**	**X**	**2n=62,FN=81[3]**	**Ribeirão Preto, SP**	**Brazil**	**21°10'S; 47°48'W**	**125**	**17**	**This study**
	to be provided*	**ITM0068**	**MZUSP30683**	**X**	**-**	**2n=62,FN=80[7]**	**São Bernardo do Campo, SP**	**Brazil**	**23º48'S; 46º25'W**	**126**	**50**	**This study**
	GU185908*	UP45	CENPAT	X	-	N/A	La Balandra, Buenos Aires	Arg	34°49'S; 58°02'W	31	06	González-Ittig *et al.* (2010)
	GU185909*	UP46	CENPAT	X	-	N/A					06	
	GU185905	Roro040	CENPAT	X	-	N/A	Selvas Río Oro, Chaco	Arg	26°29'S; 58°58'W	127	-	González-Ittig *et al*. (2010)
	KF207850*	Roe_293	-	X	-	N/A	Pampa Del Indio	Arg	26°02'S; 59°56'W	128	11	Orozco *et al.* (2014)
	GU185910*	LIF122	CML	X	-	N/A	Caminito a la isla cerrito, Misiones	Arg	27°19'S; 58°46'W	129	12	González-Ittig *et al*. (2010)
	GU185906*	CP007	CENPAT	X	-	N/A	Cuñapirú, Misiones	Arg	27°04'S; 55°03'W	130	14	González-Ittig *et al.* (2010)
	GU185907*	Ol105	MACN22262	X	-						58	
	EU192162*	GD569	UC	X	-	N/A	Lunarejo, Rivera	Urug	31º06'S; 55º58'W	131	10	Palma *et al.* (2010)
	AY041196	NK22527	MSB67445	X	-	N/A	PARNA Cerro Corá, Amambay	Parag	22°39'S; 56°00'W	132	-	Rinehart *et al.* (unpubl.)
	EU258551*	TK61708	-	X	-	N/A	Ñeembucú	Parag	26°52'S; 58°16'W	133	01	Hanson (unpubl.)
	EU258550*	TK65938	-	X	-	N/A	Itapua	Parag	27°11'S; 55°52'W	134	01	Hanson (unpubl.)
	EU192161*	GD547	UC	X	-	N/A	Costa del Río Tebuicary, Paraguarí	Parag	26º30'S; 57º14'W	135	01	Palma *et al.* (2010)

Species	GenBank Acession	Field Number	Museum number	Cyt-*b*	IRBP	Karyotype	Locality, State or Province	C	Coord	Map	Hap	Reference
O. aff. nigripes	***to be provided****	-	**MUFAL19**	**X**	**X**	**N/A**	**ESEC Murici, AL**	**Brazil**	**09º12'S; 35º51'W**	**136**	**57**	**This study**
	to be provided*	-	**MUFAL70**	**X**	**X**	**N/A**					**56**	**This study**
	to be provided*	**ST193**	**MZUSP**	**X**	**X**	**N/A**	**RPPN Serra do Teimoso, Jussari, BA**	**Brazil**	**15º09'S; 39º32'W**	**137**	**55**	**This study**
	to be provided*	**MTR15862**	**MZUSP**	**X**	**X**	**2n=62,FN=78[7]**	**Serra Bonita, Camacan, BA**	**Brazil**	**15°23'S; 39°33'W**	**138**	**55**	**This study**
	to be provided	**MTR16314**	**MZUSP**	**X**	**X**	**2n=62,FN=78[7]**					**-**	**This study**
	to be provided	**MTR032**	**MZUSP**	**X**	**X**	**2n=62,FN=78[3]**	**Pacoti, CE**	**Brazil**	**04°13'S; 38°55'W**	**139**	**-**	**This study**
	to be provided*	**MTR039**	**MZUSP**	**X**	**X**	**2n=62,FN=78[3]**					**54**	**This study**
	to be provided*	**MTR041**	**-**	**X**	**X**	**2n=62,FN=78[3]**					**53**	**This study**
O. rupestris	JQ013763	-	MN50322 (paratype)	X	-	N/A	Alto Paraíso, GO	Brazil	14°01'S; 47°32'W	140	-	Agrellos *et al.* (2012)
	JQ013764	-	MN50326 (paratype)	X	-	N/A					-	
O. aff. rupestris	***to be provided***	**BIO813**	**MZUSP29013**	**X**	**X**	**2n=44,FN=52[5]**	**Serra do Cipó, MG**	**Brazil**	**19°18'S, 43°35'W**	**141**	**-**	**This study**
	to be provided	**BIO899**	**MZUSP29016**	**X**	**X**	**2n=46,FN=52[5]**	**Pico das Almas, BA**	**Brazil**	**13°33'S; 41°56'W**	**142**	**-**	**This study**
O. stramineus	DQ826027	-	UFPB1827	X	-	2n=52, FN=68[2]	Fazenda Regalito, Mambaí, GO	Brazil	14º29'S; 46º06'W	144	-	Miranda *et al.* (2009)
	JQ013747	-	MN46410 (paratype)	X	-	N/A	Teresina de Goias, GO	Brazil	13º46'S; 47º15'W	54	-	Agrellos *et al.* (2012)
	AY163613	-	MN46406	-	X	N/A					-	Weksler (2003)
	GU126531	-	MN46873	X	-	N/A					-	Percequillo *et al* . (2011)
	to be provided	**MJJS429**	**MUFAL0254**	**X**	**X**	**2n=52,FN=68**	**Piranhas, AL**	**Brazil**	**09º33'S; 37º56'W**	**143**	**-**	**This study**
	to be provided	**MTR 17413**	**-**	**-**	**X**	**2n=52,FN=68[7]**	**Jequitinhonha, MG**	**Brazil**	**16°26'S; 41°00'W**	**145**	**-**	**This study**
	to be provided	**MJJS442**	**MUFAL0260**	**X**	**X**	**2n=52,FN=68**	**Canindé de São Francisco, SE**	**Brazil**	**09º33'S; 37º56'W**	**146**	**-**	**This study**
	to be provided	**MJJS443**	**MUFAL0259**	**X**	**X**	**N/A**					**-**	**This study**

Species	GenBank Acession	Field Number	Museum number	Cyt-*b*	IRBP	Karyotype	Locality, State or Province	C	Coord	Map	Hap	Reference
O. stramineus	***to be provided***	**MJJS462**	**MUFAL0268**	**-**	**X**	**N/A**					**-**	**This study**
	to be provided	**MJJS470**	**MUFAL0274**	**-**	**X**	**2n=52,FN=68**					**-**	**This study**
	to be provided	**MJJS472**	**MUFAL0276**	**X**	**X**	**N/A**					**-**	**This study**
	to be provided	**MJJS488**	**MUFAL0285**	**X**	**-**	**2n=52,FN=68**					**-**	**This study**
O. utiaritensis	JQ013754	-	MN75616 (paratype)	X	-	2n=72, FN=76[6]	Campo Novo do Parecis, MT	Brazil	13º40'S; 57º53'W	147	-	Agrellos *et al.* (2012)
	JQ013749	-	MN75619	X	-	N/A					-	Agrellos *et al.* (2012)
	JQ013760	-	MN75598	X	-	N/A	Sapezal, MT	Brazil	13º20'S; 55º36'W	148	-	Agrellos *et al.* (2012)
	JQ013758	-	MN75593 (paratype)	X	-	2n=72, FN=76[6]					-	Agrellos *et al.* (2012)
	JQ013762	-	MN75609	X	-	2n=72, FN=76[6]	Castelo dos Sonhos, PA	Brazil	08º18'S; 55º05'W	149	-	Agrellos *et al.* (2012)
O.aff. utiaritensis	***to be provided***	**M968497**	**MZUSP**	**X**	**X**	**2n=70,FN=72[7]**	**Apiacás, MT**	**Brazil**	**09°34'S; 57°23'W**	**150**	**-**	**This study**
O. vegetus	EU294251	-	BYU15217	X	-	N/A	San Isidro, Alajuela	C. Rica	10º05'N; 84º12'W	151	-	Rogers *et al.* (2009)
	EU294252	-	BYU15215	X	-	N/A					-	Rogers *et al.* (2009)
	EU192165	-	KU142065	X	-	N/A	Cerro Amigo, Punta Arenas	C. Rica	10°18'N; 84°46'W	152	-	Palma *et al.* (2010)
	EU258541 / EU649067	-	ROM113516	X	X	N/A	Volcan Irazu, Cartago	C. Rica	09°58'N; 83°50'W	12	-	Hanson *et al.* (2011); Hanson and Bradley (unpubl.)
	EU258538	-	ROM112192	X	-	N/A	Rivas, Rivas	Nic	11°26'N; 85°50'W	153	-	Rogers *et al.* (2009)
Oligoryzomys sp.A	***to be provided***	**UNIBAN 160**	**MZUSP**	**X**	**X**	**N/A**	**PE Cantareira, SP**	**Brazil**	**23°26'S; 46°37'W**	**154**	**-**	**This study**
Oligoryzomys sp. B	***to be provided***	**CIT1549**	**MZUSP**	**X**	**X**	**N/A**	**ESEC Uruçuí-Una,PI**	**Brazil**	**08°52'S; 44°58'W**	**58**	**-**	**This study**
Oligoryzomys sp. C	***to be provided***	**CIT830-VF41**	**-**	**X**	**X**	**N/A**	**Una, BA**	**Brazil**	**15°17'S; 39°03'W**	**155**	**-**	**This study**
	to be provided	**CIT838-VF49**	**-**	**X**	**X**	**N/A**					**-**	**This study**
Oligoryzomys sp.D	DQ826024 / DQ826032	-	MN37751	X	X	2n=56, FN=58[2]	Surumu, RR	Brazil	04º11'N; 60º47'W	156	-	Miranda *et al.* (2009)

Species	GenBank Acession	Field Number	Museum number	Cyt-*b*	IRBP	Karyotype	Locality, State or Province	C	Coord	Map	Hap	Reference
Outgroups						-	-	-	-	-	-	
E. russatus	GU126542	-	MN50230	X	-	-	-	-	-	-	-	Weksler (2003)
	AY163625	ORG67	-	-	X	-	-	-	-	-	-	Weksler (2003)
M. minutus	AF108698	-	MVZ173975	X	-	-	-	-	-	-	-	Smith and Patton (1999)
N. spinosus	EU579504/ AY163597	-	MVZ155014	X	X	-	-	-	-	-	-	Weksler (2003)
O. balneator	EU579510/ AY163617	-	AMNH268144	X	X	-	-	-	-	-	-	Weksler (2003)
P. simplex	EU579517/ AY163633	GD065	-	X	X	-	-	-	-	-	-	Weksler (2003

N/A: not available. In bold: sample which karyotype and sequences were obtained in the present study. *Specimen in which phylogeographic studies were performed. Superscript numbers in the column karyotype referred to 2n and NF described by: [1]Di-Nizo *et al.* (2015); [2]Andrades-Miranda *et al.* (2002); [3]Paresque *et al.* (2007); [4]Di-Nizo *et al.* (2014); [5] Silva and Yonenaga-Yassuda (1997); [6]Agrellos *et al.* (2012); [7]Di-Nizo (2013). Acronyms of Brazilian states: Acre (AC), Alagoas (AL), Amapá (AP), Amazonas (AM), Bahia (BA), Ceará (CE), Espírito Santo (ES), Goiás (GO), Mato Grosso (MT), Mato Grosso do Sul (MS), Minas Gerais (MG), Pará (PA), Paraná (PR), Piaui (PI), Rio Grande do Sul (RS), Rondônia (RO), Roraima (RR), São Paulo (SP), Sergipe (SE) and Tocantins (TO). Countries: Arg (Argentina), C. Rica (Costa Rica), Hond (Honduras), Mex (Mexico), Nic (Nicaragua), Parag (Paraguay), Uruguay (Urug), Ven (Venezuela). Museum and lab acronyms: ACUNHC (Abilene Christian University of Natural History Collection), AMNH (American Museum of Natural History), ASNHC (Angelo State National History Collection), BIO/CIT (Laboratório de Citogenética de Vertebrados, Instituto de Biociências, Universidade de São Paulo), BYU (Monte L. Bean Museum, Brigham Young University), CENPAT (Centro Nacional Patagónico), CM (Carnegie Museum of Natural History), CNMA (Colección Nacional de Mamíferos, Universidad Nacional Autonôma de México), IER-LIEY (Instituto de Ecologia Regional, Laboratorio de Investigaciones Ecológicas de las Yungas, Universidad Nacional de Tucuman), IMBICE (Instituto Multidisciplinario De Biologia Celular, La Plata, Argentina), INEVH (Instituto Nacional de Enfermedades Virales Humanas 'Dr. Julio I. Maiztegui'), LBCE (Laboratório de Biologia e Parasitologia de Mamíferos Silvestres Reservatórios), MACN (Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia'), NK (Museum of Southwestern Biology, University of New Mexico, Albuquerque), MN (Museu Nacional, UFRJ, Brazil), MNHNCL (Museu Nacional de Historia Natual Chile), MNHNP (Museo Nacional de Historia Natural de Paraguay), MSB (Museum of Southwestern Biology), MUFAL (Museu de História Natural, UFAL, Brazil), MVZ (Museum of Vertebrate Zoology, University of California, USA), MZUNC (Museo de Zoologia Universidad Nacional de Córdoba), MZUSP (Museu de Zoologia, USP, Brazil), OMNH (Sam Noble Museum, University of Oklahoma, Norman), ROD (Laboratório de Ecologia e Evolução, Instituto Butantan), TTU (Texas Tech University), UFPB (Universidade Federal da Paraiba, Brazil), UFES (Coleção de Mamíferos, UFES, Brazil), UFSCAR (Universidade Federal de São Carlos), UNB (mammal collection, Universidade de Brasília). Collector acronyms: AC (Alexandre Christoff), APC (Ana Paula Carmignotto), ARB (Alexandra Bezerra), CRB (Cibele Rodrigues Bonvicino), DQM (Diego Queirolo), GD (Guillermo D'Elía), JPJ (Pablo Jayat), MIC (Maria Inés Carma), MJJS (Maria José de Jesus Silva), MNFS (Maria Nazaré F. da Silva), MTR (Miguel Trefaut Rodrigues), PMR (Priscila Roswell) and VF (Valéria Fagundes). Localities abbreviations: ESEC (Estação Ecológica), FLONA (Floresta Nacional), PE (Parque Estadual), PESM (Parque Estadual da Serra do Mar), PARNA (Parque Nacional) and RPPN (Reserva Particular do Patrimônio Nacional).

Table 2: Pairwise nucleotide divergence of cyt-*b* using K2P model (in percentage) among species. Diagonal values represent intraespecific distance (in cases of more than one sample per species). Number of individuals is informed in parenthesis.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	***O. brendae* (N=5)**	0.24																					
2	***O. chacoensis* (N=6)**	6.44	3.00																				
3	***O. costaricensis* (N=3)**	5.81	7.07	2.26																			
4	***O. delicatus* (N=6)**	4.78	6.17	4.00	1.12																		
5	***O. destructor* (N=1)**	6.73	7.66	5.68	6.21	0.00																	
6	***O. flavescens+O. fornesi* (N=22)**	5.77	5.17	5.20	4.07	6.38	1.17																
7	***O. fulvescens* (N=5)**	5.94	7.07	4.93	4.28	6.23	4.98	0.98															
8	***O. longicaudatus* + *O. magellanicus* (N=6)**	6.31	6.32	4.97	3.91	6.97	2.24	4.72	0.41														
9	***O. mattogrossae* (N= 23)**	7.18	7.82	6.16	5.17	5.91	6.05	5.06	6.19	1.10													
10	***O. messorius* (N=3)**	5.73	6.00	5.69	3.54	6.68	4.97	4.83	5.15	5.45	2.07												
11	***O. microtis* (N=15)**	6.44	8.12	7.24	4.81	7.40	6.17	6.42	6.34	7.05	7.10	2.82											
12	***O. moojeni* (N=11)**	3.84	4.66	4.04	2.40	5.42	2.62	4.84	2.48	5.59	3.80	4.97	0.21										
13	***O. nigripes* (N=66)**	6.03	5.70	5.54	4.17	6.54	4.17	5.44	3.90	6.47	5.07	6.40	2.86	0.73									
14	***O. aff. nigripes* (N=8)**	6.68	5.21	6.00	4.76	7.13	4.13	6.00	4.12	6.54	5.44	6.82	2.94	3.46	1.60								
15	***O. rupestris* (N=4)**	5.22	7.16	5.81	4.27	5.63	4.87	5.35	5.29	7.33	5.86	5.78	3.51	5.55	5.66	3.16							
16	***O. stramineus* (N=8)**	5.98	4.76	4.84	4.40	5.50	3.38	4.49	3.35	5.69	4.85	6.03	2.39	2.64	2.65	5.05	1.33						
17	***O. utiaritensis* (N=6)**	5.45	6.81	6.13	3.60	5.99	5.11	5.63	4.78	6.00	4.21	5.98	2.84	5.70	5.64	5.09	5.03	0.51					
18	***O. vegetus* (N=5)**	6.78	7.10	5.81	5.24	6.83	6.13	4.68	6.31	6.38	6.38	6.87	4.54	5.75	5.62	6.10	4.58	6.16	1.13				
19	***Oligoryzomys* sp. A (N=1)**	4.41	5.09	5.60	2.84	5.72	3.32	5.26	3.12	5.62	3.73	5.99	1.85	4.01	4.03	4.88	3.35	2.31	5.11	0.00			
20	***Oligoryzomys* sp. B (N=1)**	3.75	5.09	4.60	2.31	4.72	3.82	4.07	3.76	4.94	3.51	5.30	1.34	4.03	4.03	3.42	3.59	1.58	4.45	1.85	0.00		
21	***Oligoryzomys* sp. C (N=2)**	6.40	5.55	5.48	3.58	6.73	3.93	5.98	3.86	6.92	5.68	5.02	3.22	2.93	3.51	5.19	3.12	4.89	6.10	3.75	3.75	0.00	
22	***Oligoryzomys* sp. D (N=1)**	5.48	4.27	5.24	3.79	5.36	3.07	4.53	4.48	5.63	3.93	5.85	2.83	4.96	4.48	4.86	4.04	4.75	5.43	3.73	3.41	5.35	0.00

Table 3: Estimates of genetic diversity and neutrality test for *Oligoryzomys aff. nigripes* and *O. nigripes* for cytochrome *b* gene. Group, number of samples (Nº), segregating sites (S), number of haplotypes, haplotype diversity (Hd), nucleotide diversity (π), Tajima's D test and p-value (P), Fu's FS test and p-value (P). Statistical significance: (*) P≤ 0,05 and (**) P≤ 0,01.

Lineages	**Nº**	**S**	**h**	**Hd**	**π**	**Tajima's D**	**Fu's Fs**
Total	83	118	69	0.995	0.01717	-1.89531*	-2.43106*
NE	06	19	05	0.933	0.01403	0.60846	0.84484
CS	77	95	64	0.995	0.01121	-2.18153**	-3.78083**

Table 4: Analysis of molecular variance (AMOVA) among *Oligoryzomys aff. nigripes* and *O. nigripes* for cytochrome *b* gene.

Source of variation	**d.f.**	**Sum of squares**	**Variance components**	**Percentage of variation**	**Fixation index**	**P-value**
Among groups	1	158.210	13.72244	78.22	F_{sc}=0.05780	0.00
Among populations within groups	1	12.089	0.22084	1.26	F_{st}=0.79481	0.00
Within populations	80	287.978	3.59972	20.52	F_{ct}=0.78222	0.34213+-0.01289
Total	82	458.277	17.54300			

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.

Appendix

Appendix 1

COMISSÃO DE ÉTICA NO USO DE ANIMAIS
INSTITUTO BUTANTAN
Av. Dr. Vital Brazil, 1500, CEP 05503-900, São Paulo, SP, Brazil
Telefone: (55) (011) 2627-9585 - Fax: (55) (011) 2627-9505
ceuaib@butantan.gov.br

CERTIFICADO

Certificamos que o projeto intitulado "Estudos cromossômicos e de filogenia molecular em roedores da tribo Oryzomyini (Rodentia, Cricetidae, Sigmodontinae), com ênfase no gênero *Cerradomys*", **protocolo nº 1151/13**, sob a responsabilidade de Maria José de Jesus Silva e Camilla Bruno Di Nizo – que envolve a criação e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica – está de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto 6.899, de 15 de julho de 2009 e de normas complementares, bem como está de acordo com os Princípios Éticos na Experimentação Animal adotado pelo Colégio Brasileiro de Experimentação Animal (COBEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS DO INSTITUTO BUTANTAN (CEUAIB) em reunião de 19/03/2014.

Vigência do Projeto: 03/2014 - 03/2018	**N° de animais/espécie**
Laboratório Especial de Ecologia e Evolução	100 animais de espécies silvestres brasileiras 10-150g (M,F) (roedores da Tribo *Oryzomyini*)

São Paulo, 24 de março de 2014

Dr. Marcelo L. Santoro
Coordenador da CEUAIB

Appendix 2

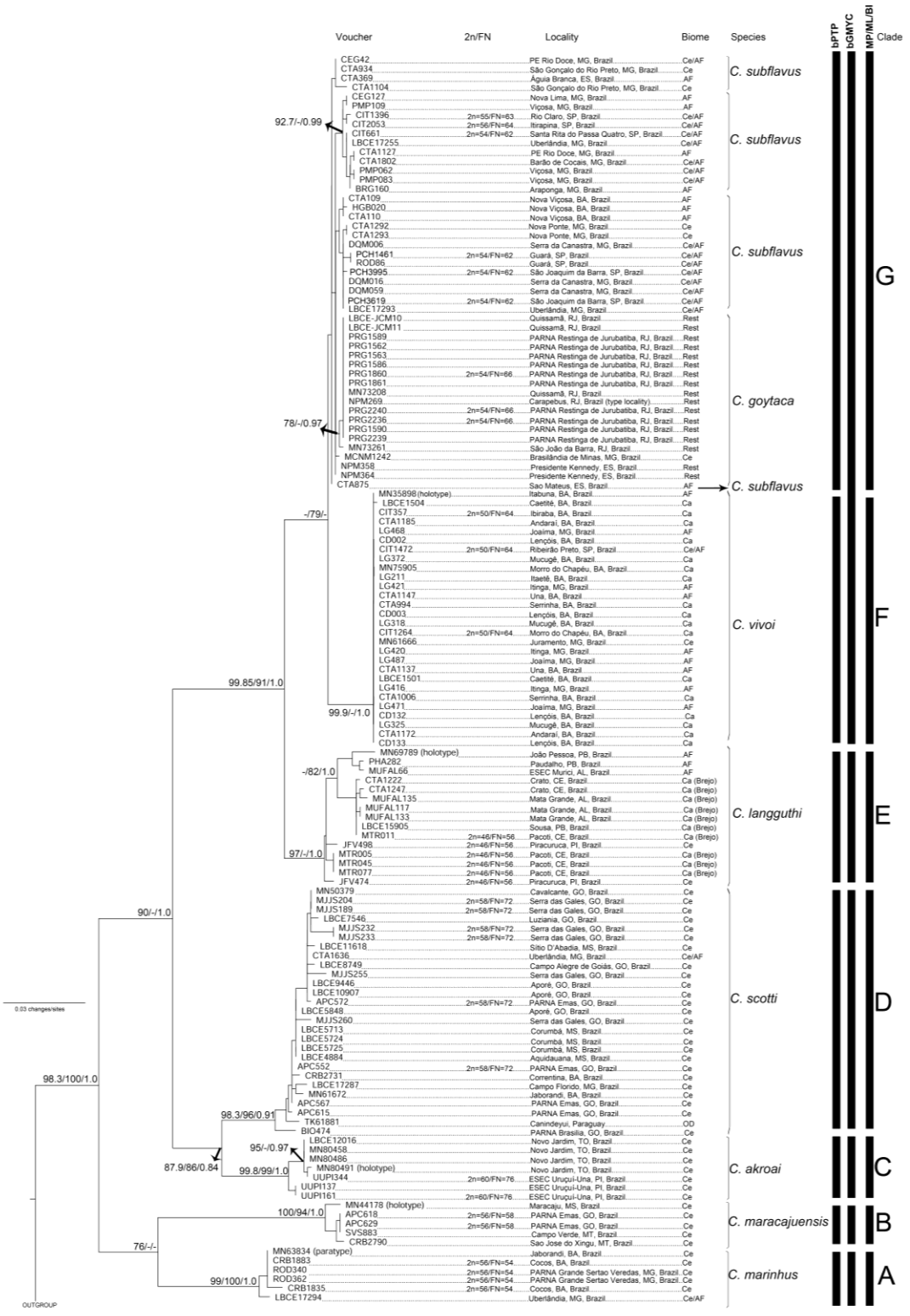

Fig. 2: Maximum Likelihood phylogenetic hypothesis based on mitochondrial gene cyt-*b*. Numbers in the nodes indicate MP and ML bootstrap support (above 70) and BI posterior probability (above 0.70), respectively. From the right to the left: black bars indicate results of single-locus coalescent-based species delimitation (bPTP and bGMYC) and clades recovered by MP, ML and BI, respectively. Biomes: Ce (Cerrado), AF (Atlantic Forest), Rest (Restinga), Ca (Caatinga) and OD (open diagonal).

Biografia

Formação Acadêmica

Graduação
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