Sequence variability of COI in Sigmodontine rodents highlights the taxonomic bottleneck.

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Abstract

Sigmodontinae is a high diverse rodent subfamily with difficult taxonomic identification, which plays a relevant role in environmental impact assessment and public health. We evaluated cytochrome c oxidase subunit I (COX-I) sequence variability in order to investigate the existence of 'barcoding gap' and to assess its phylogenetic potential in evolutionary studies. We generated 130 sequences from 21 species sampled. Also, we added 58 sequences available in Genbank. Preliminary analysis revealed some field misidentifications. Thus, we reclassified them in order to include in the further analysis. The mean distance was 14.7% overall, 15.2% among genera, and 2.4% among species. The 'barcoding gap' was found and an unexpected phylogenetic signal was recovered. Although it proved to be a useful tool that can enhance the discovery of biodiversity, the barcoding approach must be also supported by taxonomy and museum collections.

Keywords: Sigmodontinae, barcoding, COX-1, species identification

Introduction

DNA barcoding is an initiative that proposes fast and accurate species identification through a standardized region in the genome - 648-basepair of a mitochondrial gene (cytochrome c oxidase subunit 1, COX-I) (Hebert et al 2004a). It is especially useful in conservation, because the taxonomic level used in biodiversity assessments and the focus of legislation and conservation programs is the species (Rubinoff 2006). DNA barcodes can serve also as genetic vouchers for ecological surveys (Borisenko et al 2008), offering a more rapid and cheaper way to inventory an area, especially in highly diverse environments (DeSalle 2006, Valentini et al 2009). Several studies have demonstrated the effectiveness of DNA barcoding in different animal groups (Hebert et al 2003a, 2003b, 2004a, 2004b, Ward et al 2005, Hajibabaei et al 2006, Smith et al 2006, Witt et al 2006, Borisenko et al 2008). The success of DNA barcoding as a species identification tool rests upon the disjunction of distance break at the intra and interspecific levels, also known as 'barcoding gap' (Wiemers & Fiedler 2007). Besides the search for disjunctive distribution, researchers have been looking for empirical criteria or distance threshold values to define a species limit. For mammals and birds the limit of interspecific divergence has been gambled at 2% (Hebert et al 2003a). Although this approach is by no means a replacement of taxonomy (Ebach & Holdrege 2005), if applied cautiously among a diverse group, it can provide a reasonable criterion to detect specimens worthy of taxonomic analysis (Stahls & Savolainen 2008). For example, in large scale inventories there are often dominant species, which are abundant, and rare species that require a carefully taxonomic analysis. Ecologists, evolutionary biologists and taxonomists can often be overwhelmed by the amount of work arising from a biodiversity assessment (Valentini et al 2009).

In this study we investigate the barcoding gap approach within the rodent subfamily Sigmodontinae. This is the most diverse family-level mammalian clade in the Neotropical Region (Weksler 2006) with up to 377 species according to Wilson & Reeder (2005). Besides that, these rodents are important vectors and reservoirs of human diseases Hemorrhagic fevers caused by Arenaviruses are transmitted by three species of this subfamily (*Calomys callosus*, *Calomys musculinus,* and *Zygodontomys brevicauda*; Salazar-Bravo et al 2002). Some *Oligoryzomys* species are known to be reservoirs of Hantaviruses, the etiological agents of Hantavirus pulmonary syndromes (Murúa et al 2003, Carbajo & Pardiñas 2007, Porcasi et al 2005) and *Nectomys squamipes* is the host of *Schistosoma mansoni* (Martinez et al 2008). Furthermore,

sigmodontines are an important component of fauna take into account by environmental impact assessment studies. Such aspect is especially relevant since a large number of energetic and infrastructure building sites need fast licensing for the development of Brazilian hydroelectric potential. This step has been pointed out by experts as a major obstacle to development of hydroelectric energy infra-structure.

But, there is a relevant constrain about sigmodontine studies: new species are constantly described (Emmons & Patton 2005, Costa et al 2007, Gonçalves et al 2005, Pardiñas et al 2005, Percequillo et al 2005, 2008) and recognizing taxonomic boundaries has been difficult due to morphological similarities. Molecular markers can offer an alternative to overcome it (Smith & Patton 1999, Weksler 2003, 2006, D'Elía 2003, Almeida et al 2007).

The growth of barcode databases is a potential enhancer to fast species recognition in environmental impact assessment and rapid licensing studies. The ability of using small amount of tissue to obtain barcode DNA sequences enables reliable species identification. In this scenery, the use of barcodes as vouchers could reinforce the urgent need to protect areas as well as well as to control epidemiology issues. However, the extent to which this approach is useful is a matter of empirical evaluation.

In this study we examine the sequence variability of COX-1 in sigmodontine species, especially from southern Brazil, in order to evaluate the existence of a 'barcoding gap' between intra- and interespecific distances. We used such information to infer the barcode utility in species identification by combining this dataset with sequences deposited in Genbank. Also, we emphasize the benefits and pitfalls of this approach both from an economic and taxonomic point of view. Finally, we assess the phylogenetic and phylogeographical potential of COX-I in evolutionary studies.

Material and Methods

Sampling

A total of 130 sigmodontine specimens encompassing 21 species were collected from different localities at Brazil (Table 1), most of them has vouchers and

additional information (diploid number). Tissues obtained from environmental assessments studies, which we received only small piece of ear and no additional information represent 36% of our sampling. The species surveyed are representative of the major tribes: Oryzomyini, Akodontini, Thomasomyini and Phyllotini. Besides that, species with confused allocation in tribal level, such as *Juliomys pictipes, Rhagomys rufescens, Delomys dorsalis* and *D. sublineatus*, were also analyzed. All Sigmodontinae sequences available in GenBank (n = 58, EU095420, EU095443-75, EU095488-93, EU096809-25, EU096953) were added in our analysis.

Table 1. Species surveyed in this study. Specimens with * were reclassified.

Taxa	Sample ID	Locality
Akodon azarae	JR328, JR329	Alegrete, RS, BR
Akodon montensis	JR338	Barracão, RS, BR
	JR407	Erechim, RS, BR
	DG21, DG22, DG23	Passo Fundo, RS, BR
	JR386, JR389	Barracão, RS, BR
	JR197, JR198, JR199, JR203, JR264, JR461, JR535	Terra de Areia, RS, BR
	JR335, JR336	Ronda Alta, RS, BR
	9826, 9595	Blumenau, SC, BR
Akodon paranaensis	JR411	Erechim, RS, BR
	6844, 6963	São Domingos, SC, BR
***Akodon* sp.1**	PCE24	Margarida do Sul, RS, BR
Akodon* sp.*2	A7, A10, A17*, A24, A25, A26, A29, A33, A43	S. F. de Paula, RS, BR
Calomys expulsus	LBCE1547, LBCE1548	Caetité, BA, BR
	CRB2732, CRB2733	Correntina, BA, BR
	LG408	Mucugê, BA, BR
	CRB2374	Mimoso de Goiás, GO, BR
	LG443	Itinga, MG, BR
	CRB2582*	BR
Calomys sp	1787*, 1789*	BR
Calomys sp.nov	LBCE5556	Capitão Andrade, MG, BR
Calomys tener	JR405	Alegrete, RS, BR
	AB6, AB10, AB519	São Paulo, BR
Delomys dorsalis	JR207, JR229, JR230, JR290, JR349, JR527, JR536, JR538	Terra de Areia, RS, BR
	DD2, DD3, DD40	S. F. de Paula, RS, BR
	9954*	Blumenau, SC, BR
	JR224	*Unknown*, BR
Delomys sublineatus	9331, 9950, 9994*	Blumenau, SC, BR
Deltamys kempi	PCE05, PCE06, PCE11, PCE12, PCE13	Rocha, UY
Euryoryzomys russatus	JR194, JR205, JR208, JR281, JR297, JR298, JR299, JR458*, JR459*	Terra de Areia, RS, BR
	9409, 9695, 9773	Blumenau, SC, BR
Juliomys pictipes	9667, 9735	Blumenau, SC, BR
Necromys lasiurus	JR346	Rondinha, RS, BR

	6859	Blumenau, SC, BR
Oligoryzomys flavescens	JR332	Alegrete, RS, BR
	JR202, JR209, JR210, JR258, JR259, JR467	Terra de Areia, RS, BR
Oligoryzomys nigripes	JR339	Barracão, RS, BR
	JR409	Erechim, RS, BR
	DG00, DG01, DG05C, DG06, DG15*	Passo Fundo, RS, BR
	JR337	Ronda Alta, RS, BR
	JR363 JR425, JR426, JR457, JR518	Terra de Areia, RS, BR
	OLN31, ON1, OPN52, OPN55, OPN57, OPN58, OPN59, OPN61, OPN64, OPN87	S. F. de Paula, RS, BR
	9600	Blumenau, SC, BR
	6884	São Domingos, SC, BR
Oxymycterus sp	9792	Blumenau, SC, BR
Rhagomys rufescens	9908, 9921	Blumenau, SC, BR
***Scapteromys* sp**	1007*	S. J. dos Pinhais, PR, BR
Sooretamys angouya	JR196	Terra de Areia, RS, BR
	9793	Blumenau, SC, BR
Thaptomys nigrita	JR372	Derrubadas, RS, BR
	TN3	S. F. de Paula, RS, BR
	6559	Blumenau, SC, BR
Sp sp	9629	*Unknown,* BR

DNA extraction, PCR amplification and sequencing

DNA was extracted from different tissues like heart, liver, muscle, kidney and mainly ear samples (stored at -20ºC in 100% ethanol) using CTAB protocol (Doyle & Doyle 1987) or phenol chloroform protocol (Sambrook et al 1989). The 648-bp target region of COX-I was amplified through polymerase chain reaction (PCR) using primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al 1994). Amplification reaction followed Folmer et al (1994). PCR products were purified with a mix of Shrimp Alkaline Phosfatase and Exonuclease (GE Healthcare, EUA) and sequenced in an ABI 377© (Applied Biosystems Inc.).

Data analysis

Sequences were visually checked in Chromas and aligned in Clustal X software implemented in MEGA 4.2 (Kumar et al 2008). Sequence divergence was calculated using Kimura two-parameter (K2P) base substitution model (Kimura 1980). A distance matrix was processed to calculate divergence averages (standard errors

and ranges) within and among species and also within and among tribes. A Neighbor-joining (NJ) tree based on K2P distances was reconstructed to represent pattern of divergence among taxa using ape library (Paradis et al 2005) in software R (R development core team, 2008).

Distances were analysed among different hierarquical levels. Mean intraspecific, interspecific, intrageneric and intertribal distances were calculated. Distance distributions were evaluated through histograms. The 'barcoding gap' was evaluated by comparing intraspecific and interspecific distance distribution assessed through kernel density estimation.

Model selection of sequence evolution was estimated using MrAIC (Nylander 2004). Phylogenetic reconstruction was carried out using Maximum likelihood (ML) and Bayesian inference (BI). ML trees were obtained with the PHYML v2.4.4 software (Guindon & Gascuel 2003) and BI using MrBayes3.1 (Huelsenbeck & Ronquist 2001) with 5000000 generation number of replicates. *Peromyscus maniculatus* was used as outgroup in all analysis (obtained from Genbank, accession number EF568630).

Results

Barcode divergences between species

We first performed an analysis evaluating intraspecific and intrageneric genetic distances, which showed a few discrepant values. Then we compare it to phylogenetic results and reclassified these specimens according to the more related species in the present study. Here we describe K2P distances values highlighting differences after specimen reclassification (Fig.1).

K2P distance between all specimens ranged from 0 and 29.3% (mean(X)=14.7%, variance(σ^2)=0.002) with a bimodal frequency distribution, one,mode centered near 0% and the other around 15% (not shown). Intraspecific variation ranged from 0 to 16.7%. Before reclassification the mean distance was 3.2% (σ^2 =0.002) and after was 2.4% (σ^2 =0.001). Intrageneric mean distance values retrieved more differences.

Before reclassification it varied between 14.2% and 17.6% (X=16.1%, σ^2 =0.0002) and after between 11.2% and 19.7% (X=15.2%, σ^2 =0.0007). Eleven genus (*Deltamys, Handleyomys, Juliomys, Necromys, Nectomys, Nephlomys, Oxymycterus,*

Rhagomys, Scapteromys, Sooretamys, and *Thaptomys*) were not surveyed in this analysis since they are represented by only one species.

Oligoryzomys vegetus and *O. fulvescens* were removed from data set because they clustered together and were not related to others *Oligoryzomys* spp. We also removed *Necromys urichi* due to very discrepant phylogenetic position with regards of other member of its tribe, the Akodontini. Histograms from each species showed a mode around 0%. Intertribal distances calculated among Akodontini, Oryzomyini and *incertae sedis* group varied between 15.4 and 17.2% (X=16.1%, σ^2 =0.0001). However, after reclassification the values ranged from 9.6 to 14.6% (X=12.3%, σ^2 =0.0006). Histograms from each taxa showed a bimodal distribution, all with one small mode centered at 0% and another mode around the mean (not shown).

Reclassifications were based either in distances and phylogenetic position. *Akodon* sp. (PCE24) was classified as *A. montensis* and the sample 9629 as *Oligoryzomys* sp. Specimen DG15 previously identified as *O. nigripes* clustered together with *O. flavescens* indicating a clearly field misidentification. *Scapteromys* sample 1007 from Paraná clustered with *Hylaeamys megacephalus* from Suriname and thus was reclassified. *A. azarae*, *Calomys* sp and *Akodon* sp2 (A17) grouped together, therefore we classified all as *A. azarae*. *Akodon* sp.*2* were thought to be a new *Akodon* species from field notes. Eight of 9 samples previously identified as this *Akodon* sp. clustered together and more related with *Deltamys kempi* than to other *Akodon*. According to K2P distance and phylogenetic position we renamed this species as *Deltamys* sp.nov. *Calomys expulsus* showed a cohesive group, but one specimen (CRB 2582) clustered together with *C. tener* specimens revealing another field misidentification that were reclassified.

Euryoryzomys russatus had more unexpected relationships. Two specimens (JR458 and JR459) clustered together with *Oligoryzomys nigripes* and we believe that this represent and misidentification. This is plausible because *E. russatus* juveniles are morphologically similar to *O. nigripes*. *Delomys dorsalis* (9954) and *D. sublineatus* (9994) were reclassified as *E. russatus* due to both phylogenetic position and genetic distance.

After reclassification genetic distance from *A. montensis*, *E. russatus*, *C. expulsus*, *C. tener*, *D. dorsalis* and *D. sublineatus* were reduced (Fig. 1) improving barcoding efficiency. Twenty three species presented genetic distance <2% (Fig. 1).

The *Juliomys pictipes* specimens are probable two different species. More samples from *Sooretamys angouya* are required to evaluate the position of such species.

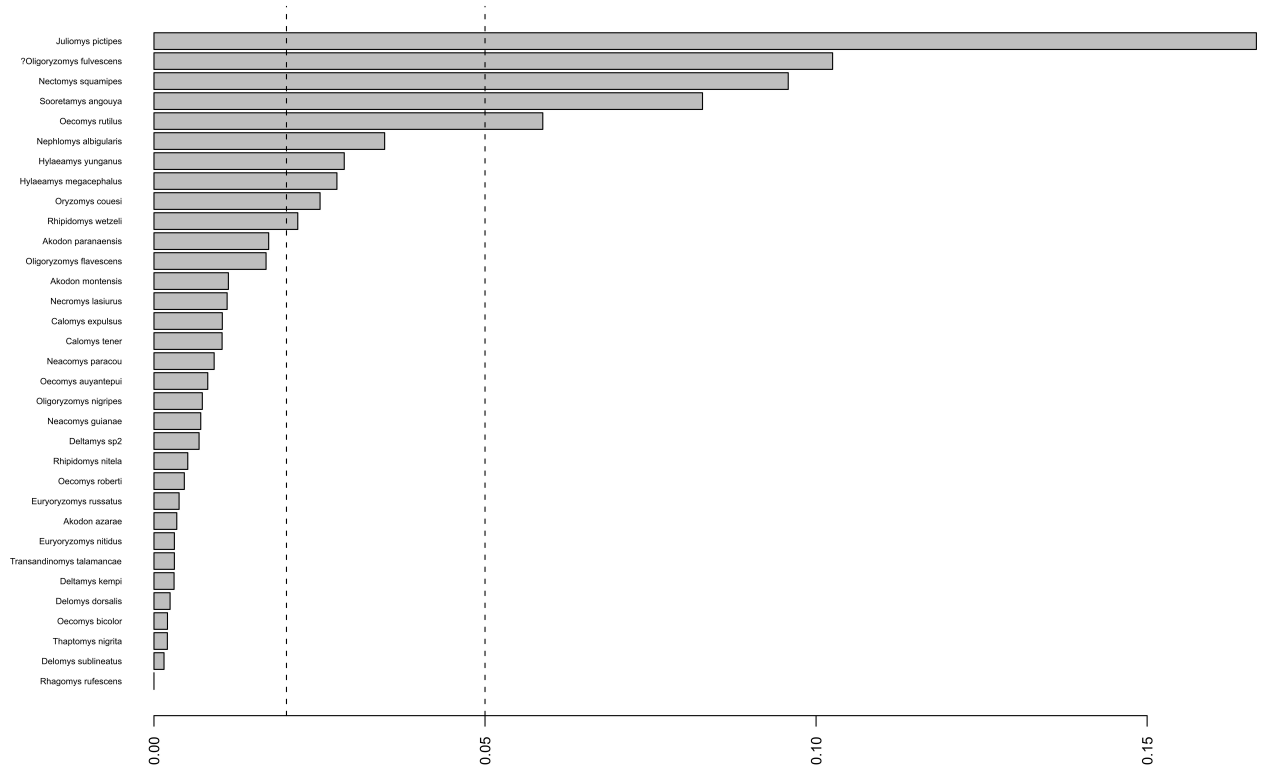

Figure 1. Mean intraspecific K2P pairwise distance after reclassification. First line highlight the suggested 2% limit of intraspecific variation. Twenty-tree species are below this limit and 10 are above.

Model selection and phylogenetic analysis

The model of sequence evolution selected with the Bayesian Information Criteria was HKY. Results of proportion of invariant sites, transition/transversion ratio, each nucleotide frequency, gamma shape parameter and likelihood of ML and Bayesian Inference are compared in Table 2.

Table 2: Comparison between results of Maximum likelihood and Bayesian inference. Mean and variance are represented by X and σ^2, respectively.

Model	**Maximum likelihood** HKY	**Bayesian** HKY
Proportion of invariant	0.338	X=0.359688 $\sigma^{2=}$0.000388
Transition/transversion ratio	4.695	X=6.076899 σ^2=0.104496
"A" Nucleotide frequency	0.28535	X=0.317278 σ^2=0.000152

"C" Nucleotide frequency	0.26002	X=0.279264 σ^2=0.000074
"G" Nucleotide frequency	0.16086	X=0.073416 σ^2=0.000014
"T" Nucleotide frequency	0.29378	X=0.330042 σ^2=0.000090
Likelihood	-14115.49548	-14225.06
Discrete gamma model	YES	YES
Gamma shape parameter	0.712	X=0.521552 σ^2=0.000390

Barcode recovery

NJ, Bayesian and ML tree retrieved the same topology and showed differences mainly in relation to branch support values. Monophyletic groups were recovered for most of the surveyed species, with high probability support. Since the tree is large we present results in four subtrees corresponding mainly to taxonomic groupings; Akondontini (Fig. 2), Phyllotini Thomasomyini and *incertae sedis* (Fig. 3) and Oryzomyini (Fig. 4 and 5). Some specimens grouped within unrelated species [*Oligoryzomys nigripes* DG15, 2 *Euryoryzomys russatus* (JR458, JR459), *Delomys dorsalis* 9954, *D. sublineatus* 9994, 2 *Calomys* sp (1788, 1787), *C. expulsus (CRB2582), Scapteromys sp 1007* and *Sooretamys angouya* 9793], representing a conflict between barcode and field identifications. Two samples of *Juliomys pictipes* specimens did not cluster together. *Akodon* sp PCE24 grouped between *Akodon montensis* sequences revealing his species identification and the sample 9629 could belong to the genus *Oligoryzomys.*

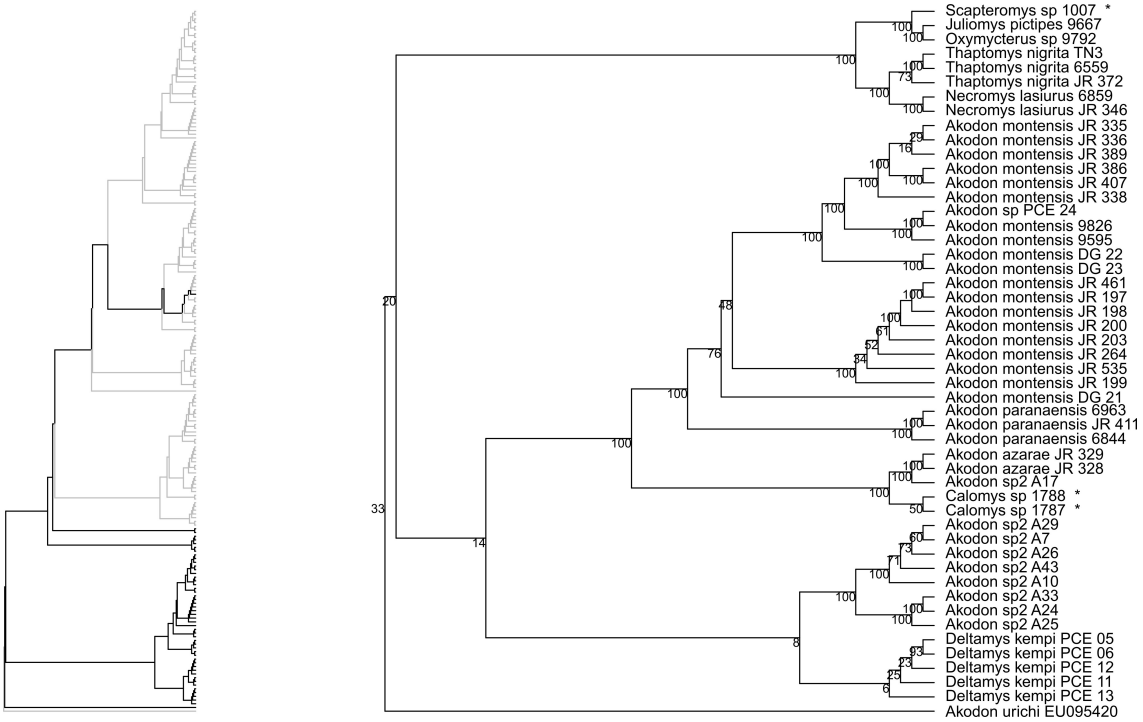

Figure 2: Akodontini tribe subtree, the * represent specimens which are present in more than one tree figure.

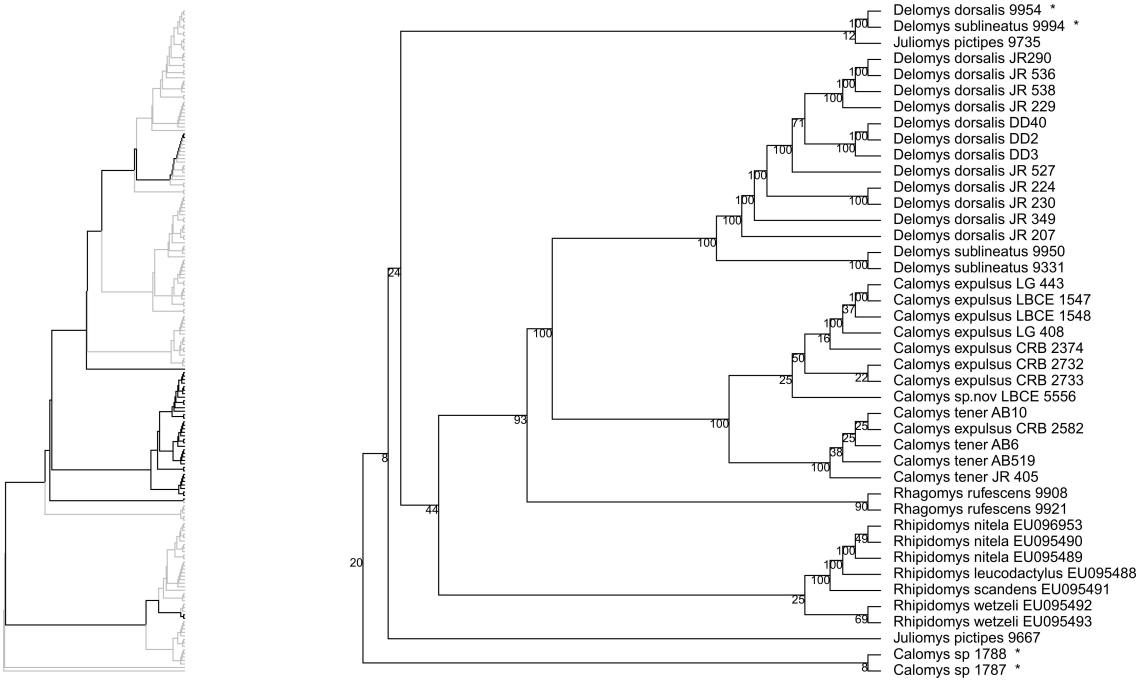

Figure 3: *Incertae sedis* (*Delomys dorsalis, D. sublineatus, Rhagomys rufescens* and *Juliomys pictipes*), Phyllotini (*Calomys spp*) and Thomasomyini (*Rhipidomys spp*) subtree.

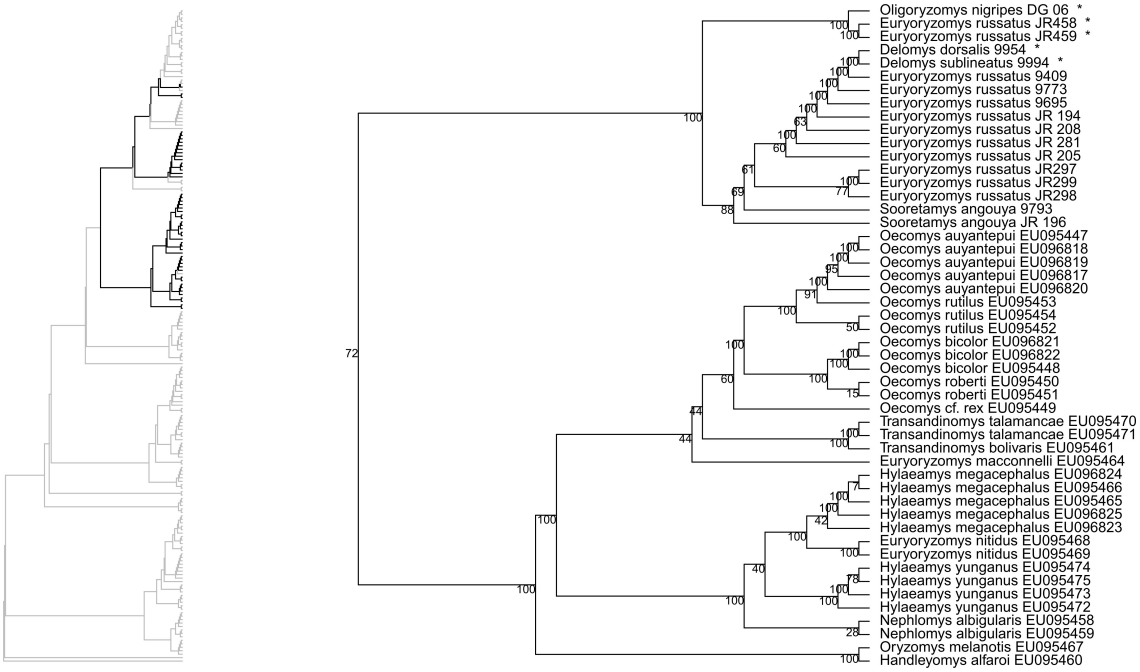

Figure 4: First part of Oryzomyini tribe subtree.

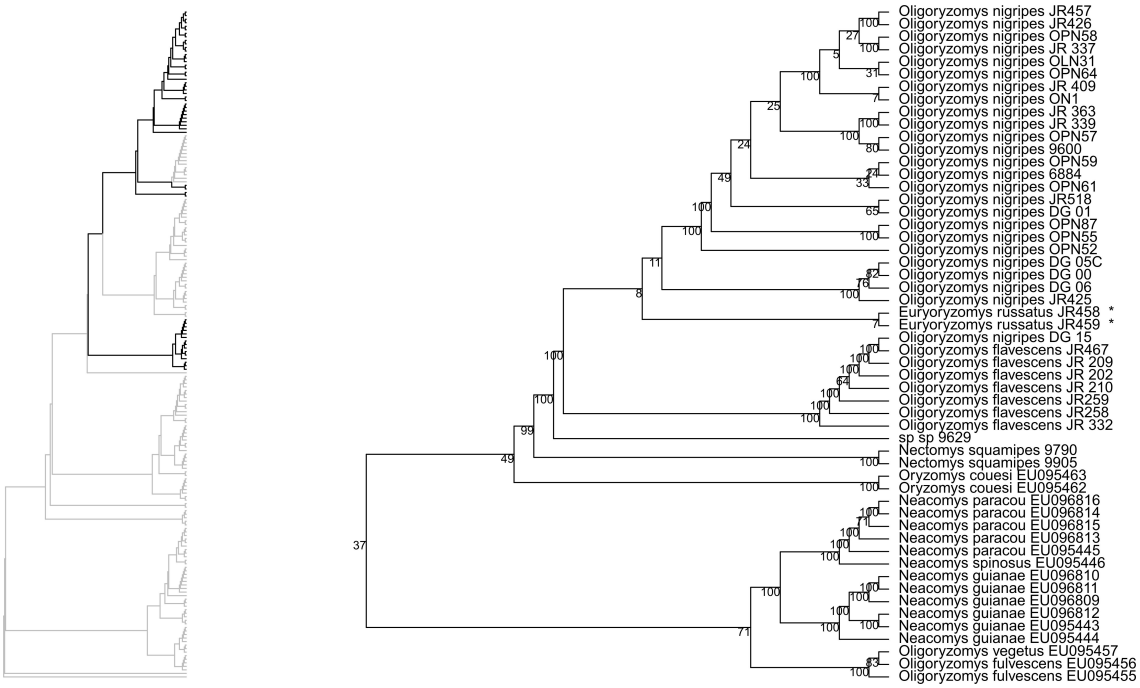

Figure 5: Second part of Oryzomyini tribe subtree.

Sigmodontine species are arranged into tribes which represent groups of monophyletic genera. COX-I showed a phylogenetic signal at the tribal level. All Oryzomyini species were monophyletic. Considering misidentifications, all species are monophyletic except *Sooretamys angouya*, *Oligoryzomys fulvescens* and *O. vegetus*. Genera *Oecomys, Transandinomys* and *Neacomys* are also monophlyletic, however *Oligoryzomys, Euryoryzomys, Oryzomys* and *Hylaeamys* showed paraphyletic. Thomasomyini and Phyllotini tribes were each represented by only one monophyletic genus, *Rhiphidomys* and *Calomys* respectively. *Incertae sedis* species *Delomys dorsalis* and *Delomys sublineatus* clustered and are closely related to Phyllotini, as well other *incertae sedis Rhagomys rufescens*. Akodontini species formed two clusters, one containing most of its species (all of them monophyletic) and the other with *Necromys lasiurus* and *Thaptomys nigrita*. *Akodon* spp. clustered together except *Akodon* sp2, which may be a new *Deltamys sp*.

Discussion

COX-1 sequence divergence

Few studies investigated COX-1 sequence divergence in rodents, especially regarding the barcoding approach. Borisenko et al (2008) studied small mammals (opossums, bats and rodents) from Guiana and Suriname and suggested the effectiveness of such target region in species identification. Although it was the first preliminary survey of COX-I sequence divergence in rodents such study did not sample adequately the most diverse subfamily sigmodontine.

Our results indicate that barcoding approach is a very useful tool for identification of sigmodontine species since taxonomic units were recovered in almost all cases and. We found that interspecific genetic variation exceeds intraspecific variation to such an extent that a clear gap exists (Fig.6), which enables the assignment of unidentified individuals to their species with a negligible error rate.

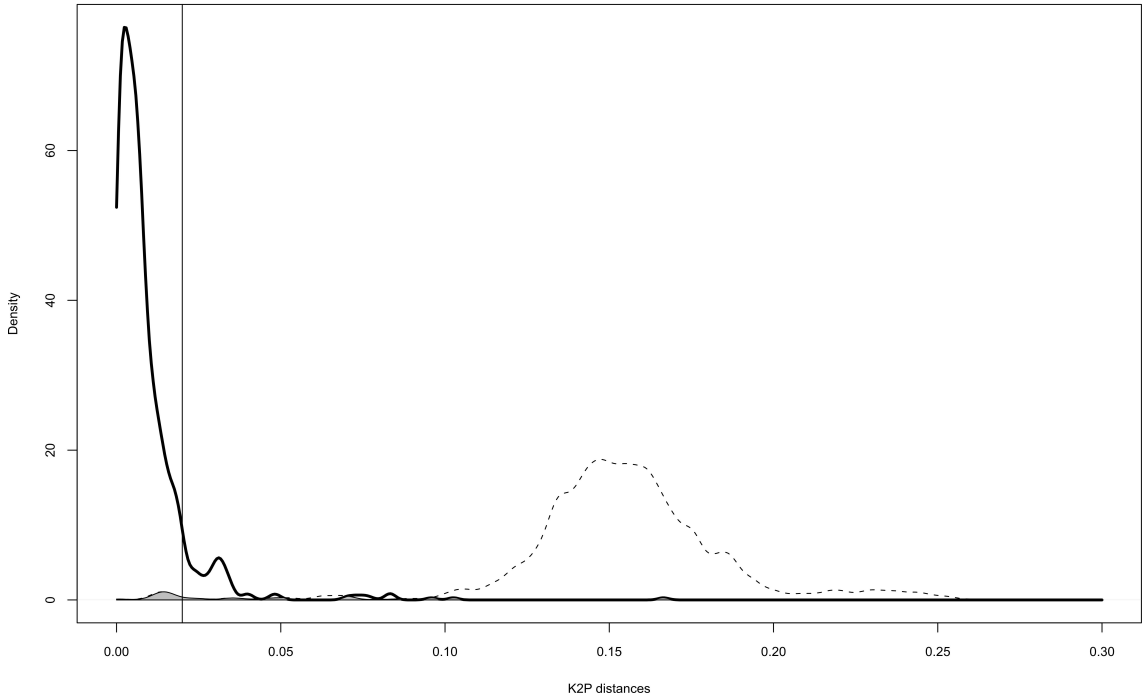

Figure 6: Intra (continuous line) and interspecific (stippled line) distances. Proposed limit of 2% was highlighted and the gap if above this value. Overlap between the distances are represented in grey.

Our results confirmed similarity among COX-1 gene and cytochrome b. Within species cytochrome b sequence divergence is 0.18-0.53 (same population) and 0.26-11.37 (different populations), different species in the same genus present 1.23-14.72 and different genera 9.26-24.34 (D´Elia 2003). Baker and Bradley (2006) compiled

cytochrome b sequence distances obtained by several authors and then calculated the mean for rodents, the divergence intraspecific was 1.5 (0.0-4.7) and intergeneric was 10.9 (4.9-16.9).

Some specimens clustered within unrelated specimens and their genetic distances were higher than intraspecific values expected, then we consider that it represented field misidentifications, instead of incomplete lineage sorting, introgression or current gene flow. Two *Euryoryzomys russatus* sequences more related with *Oligoryzomys nigripes* were obtained from ear tissue and there are no vouchers are available to confirm identification. We consider that these specimens identified in the field and only by external morphology are in fact *O. nigripes* since *E. russatus* juveniles are externally similar to *O. nigripes*. *Calomys* sp. clustered with

Akodon azarae, which is a signal that it might not be a new species, or that it is a case of introgression. *Akodon* sp.2 sample A17 is clearly an *Akodon azarae*.

Specimens of *Juliomys pictipes* showed large sequence divergence in relation to each other, even if it had been collected in the same locality, suggesting the existence of two different taxonomic units. *Juliomys* was recently described by González (2000); and was first considered within the genus *Wilfredomys*. Such genus encompasses three species *J. pictipes*, *J. rimofrons* and *J. ossitenuis* (González 2000, Oliveira and Bonvicino 2002, Costa et al 2007). Paresque et al (2009) recently described a new karyotype for the genus, which may represent a new species from São Francisco de Paula (Rio Grande do Sul, Brazil). In the light of the growing diversity within genus *Juliomys* and recent data we showed that the employment of new molecular markers, such as COX-1 sequences, may reveal cryptic species.

A total of 10 samples of *Euryoryzomys russatus* clustered with three unexpected sequences: *Delomys dorsalis* 9954, *Delomys sublineatus* 9994 and *Sooretamys angouya* 9793. Two *Delomys* sequences collected in Blumenau, Santa Catarina state are more related to three *E. russatus* collected in the same locality. Diploid number analysed for *D. dorsalis*, *D. sublineatus* and *E. russatus* specimens were similar (2n=82, 72 and 80, respectively). However, these karyotypes are not available for comparison. Other 12 *D. dorsalis* specimens collected in Rio Grande do Sul state formed a monophyletic group, as well other 2 *D. sublineatus* sequences, and

both clusters are sister groups. *S. angouya* were collected in the same locality from others and were considered genus *Oryzomys*, as well *Euryoryzomys*, until Weksler et al (2006) divided them. The assumption of misidentification is most probable, even that these species presented clear external differentiation, because some samples can be changed. When a researcher is in the field he can capture many individuals and classified all of them can be difficult, leading to misidentification. Also, after field work, the information can be changed when it is deposited in databases.

Differentiation between *Calomys spp* are not obvious and its taxonomy is complex (Haag et al 2007). Misidentification between *C. expulsus* and *C. tener* is not surprisingly, both species seems similar without refined analyzes. *Calomys expulsus* and *C. tener* have the same diploid number, but they can be separated by karyotype because they differ in fundamental number and in the abundant and distribution of constitutive heterochromatin, they can also be recognized through morphometric analysis mainly in size (Bonvicino & Almeida 2000). According to Wilson & Reeder (2005) the distribution of *C. tener* is Atlantic Forest region and habitat bordering the Cerrado (Southeast Brazil) and in Northeast Argentina and East Bolivia, however they point that these limits need refinement. Haag et al (2007) collected one specimen in Quintão (29°40'S, 50°12'W), coast of Rio Grande do Sul, expanding the knowledge of its distribution, but yet in Atlantic Forest *sensu lato*. Our specimen was captured in the same state, however at a new locality, Alegrete, a grassland area.

Sampling of different populations is necessary to be more precise in determining variability of a specific gene. *Oligoryzomys nigripes*, *Akodon montensis* and *Thaptomys nigrita* were the species with major geographical area sampled, likely representing different populations. All of them presented monophyletic groups with low genetic distance and mean below 2%.

New *Akodon* species, previously assigned as *Akodon* sp.2, is the sister group of *Deltamys kempi*, with high posterior probability (0.96) support. All other *Akodon* species grouped together in a cohesive cluster (Fig.3). Thun we reclassified *Akodon* sp.2 as *Deltamys* sp. nov. Furthermore, genetic distance data supports such relation. Distance between *Akodon* spp. is less than 8%, between *Deltamys kempi* and *Deltamys* sp.nov. is 10-13%, between *Deltamys* sp.nov and *Akodon* species is 12-16% and between *D. kempi* and *D.* sp.nov is 10-11% (Fig.7). Therefore, it suggests that the new species do not belong to *Akodon* genus, and is more related to *Deltamys*. Also, we cannot refuse belong to a genus not yet described. The karyotype of the new

species is 2n = 34, the same found in *D. kempi*. *Akodon* and *Deltamys* belong to the same tribe and can be differentiated by karyotype comparison: a singular small chromosome pair is present in all *Akodon*, which is absent in *D. kempi*. D´Elia (2003) using nuclear and mitochondrial genes found *D. kempi* as the sister group of *Akodon* spp.

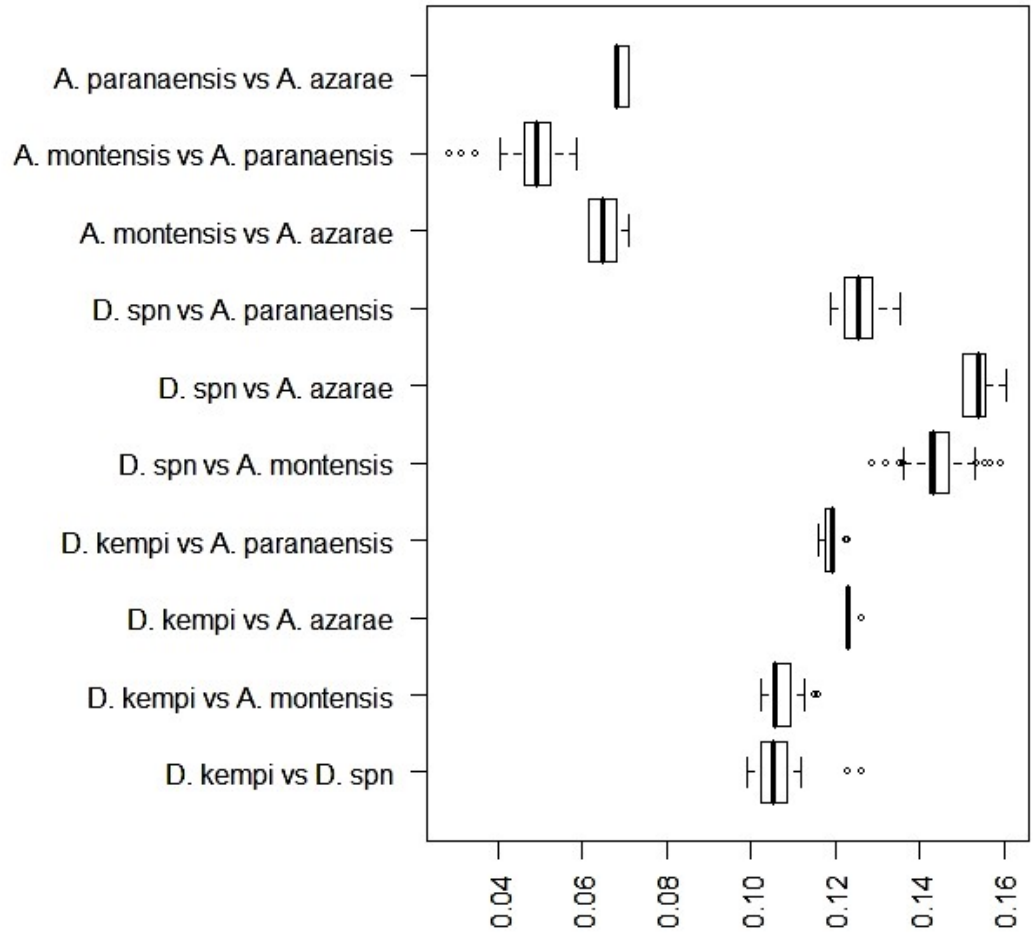

Figure 7: Comparison among K2P distances of *Deltamys kempi, Deltamys* sp. (D.spn) and *Akodon* species.

Phylogenetic relationships

An unexpected phylogenetic signal was recovered by COX-1 sequences. Individuals from Oryzomyini and Phyllotini tribes grouped together, and Akodontini splits in two groups. However, internal evolutionary relationships remains unclear compared with previous works based on others molecular markers and morphological characters (D´Elia 2003, Weksler 2003, D´Elia et al 2006, Weksler et al 2006). Within the group species of Akodontini the relations were similar as evidenced by nuclear and mitochondrial sequences (D´Elía 2003), [[A.montensis+A.azarae] D. kempi].

On the other hand, relationships inside tribe Oryzomyini does not agree with results obtained by Weksler et al (2006) based on IRBP sequences and 99 morphological characters. The genera *Oryzomys, Oligoryzomys, Hylaeamys* and *Euryoryzomys* are not monophyletic according to COX-1 sequences.

Using DNA barcoding in sigmodontine diagnostics

Sigmodontine represents a challenge in environment consulting studies because many field identifications needs to be confirmed by special preparation such as skulls and karyotype, retain of voucher specimens are critical to reliable assessments. Due to difficulty the present study give an alternative for fast preliminary identifications. As proposed by Borisenko et al (2008) collecting a small tissue sample – like ear tips – enables the validations of field identifications when vouchers are not retained.

Our results based on COX-I sequences showed that most species were well identified in field. In general, to consider a misidentification of a given specimen it is necessary information like karyotype, external morphology and collection site, since data showing the extent of intra-and interspecific and intra-intergenera distance were not available. This study showed such extent of variability in sigmodontine species, which allows inferring an identification of taxa based on COX-I sequences. But, to describe putative new species a taxonomist is still required, as well as reference collections. Thus, the barcoding approach must be supported in taxonomy and museum collections (unfortunately there is a few in Brazil). Even so, it is still a reasonable tool that can enhance the discovery of biodiversity.

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