

# Analysis of rDNA variation in the genus *Didelphis* (Marsupialia)

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## ABSTRACT

The 18S and 28S rDNA regions from *Didelphis aurita* and *D. albiventris* were analyzed by using restriction endonucleases and hybridizations with 18S and 28S probes. The rDNA regions of *D. aurita* were cloned and analyzed by physical mapping. Mammalian conserved restriction sites were found. Variation between the two species in the restriction sites allocation were described in the intergenic spacer. Intraindividual variation between the repeat units was not found. A *Bam*HI restriction site not described in other mammals was found in the two species.

## INTRODUCTION

The rRNA genes are presented in tandem repeats, with 200 copies per genome in mammals (Britten and Davidson, 1971). Each repeat unit has a intergenic spacer (IGS), a intergenic transcribed spacer, the region that transcribes for 18S rRNA, an internal transcribed spacer (ITS), the 5.8S rRNA region, another ITS, and the 28S rRNA region (Gerbi, 1985). The 18S, 5.8S and 28S regions are highly evolutionary conserved. Nevertheless, the IGS has a great variability. Therefore, the rDNA genes can be used in populational studies (with the IGS), as well as in higher taxa evolutionary studies (with the 18S, 5.8S and 28S regions).

We analyzed the rDNA variations between two South-American marsupials (*Didelphis aurita* and *D. albiventris*).

## MATERIAL AND METHODS

### One specimens

Each of *D. albiventris* collected from Tatua-munha, State of Alagoas and *D. aurita* from Maricá, state of Rio de Janeiro, Brazil, identified by Dr. Rui Cerqueira from the Vertebrate Ecology laboratory at the Federal University of Rio de Janeiro.

### Genomic DNA isolation

DNAs were isolated from liver as described by Berger and Kimmel (1980).

### Lambda EMBL 4 DNA isolation

EMBL 4 DNA (Frishauf *et al.*, 1983) was isolated as described by Sambrook *et al.* (1989).

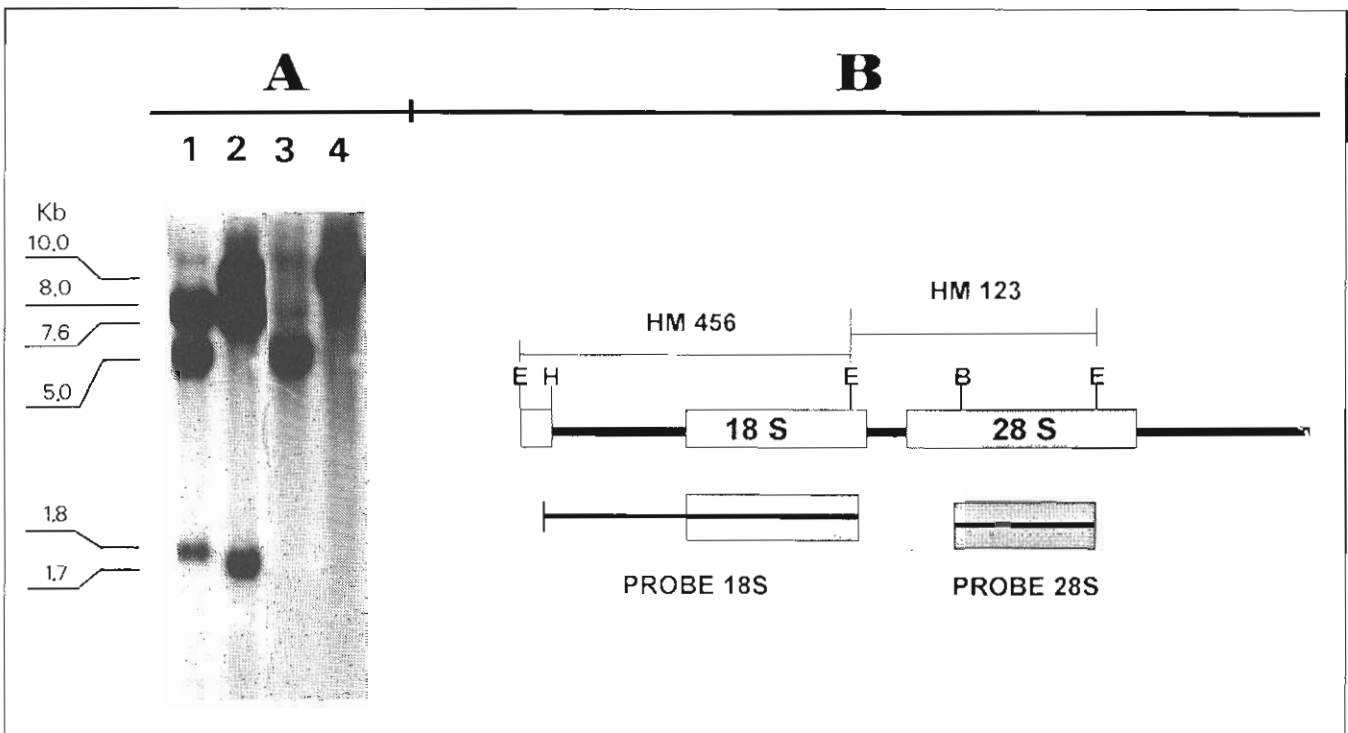
### Plasmid DNA isolation

We used the lysis alkaline method, as described by Sambrook *et al.* (1989).

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**Figure 1** - A: Genomic DNA of *Didelphis albiventris* (1 and 3) and *D. aurita* (2 and 4) were digested with *EcoRI*, electrophoresed in 0.7% agarose gel, transferred to nylon filter. The same filter was hybridized with HM123 + HM456 (1 and 2) and 18S region of HM456 (3 and 4). B: Schematic representation of the probes HM123 and HM456, the black regions were isolated by electroelution and used as probes in some hybridizations to identify the sequences 18S and 28S. The letters in C indicate restriction sites: B - *BamHI*; E - *EcoRI*; H - *HindIII*.

## Probes HM456 and HM123

These two probes (Figure 1b) enclose a whole rDNA repeat unit of *Xenopus laevis* (Meurnier-Rotival et al., 1979). The 28S (between *EcoRI* and *BamHI* sites in HM123) and the 18S (between *EcoRI* and *HindIII* in HM456) regions were isolated by electroelution (Sambrook et al., 1989), and were used as probes in some hybridizations.

## DNA labelling

Probes were labelled by nick-translation using the BRL nick-translation system, and alpha  $^{32}\text{P}$  dATP.

## Cloning and screening

Genomic DNA of *D. aurita* and Lambda EMBL-4 DNA were totally digested with *EcoRI*. Ligation was carried out with three micrograms of vector DNA, 0.6 micrograms of genomic DNA, and one unit of T4 DNA ligase. The reaction was packed *in vitro* with a prepared packaging extract from *E. coli* SMR 10, as described by Rosenberg (1987). Screening was carried out with nylon membranes, using recombinant probes HM456 and HM123 as described by Benton and Davis (1977). Inserts

in positive recombinant phages were sub-cloned in pUC18, at the *EcoRI* site.

## Gel electrophoresis, Southern blotting, hybridization and dehybridization

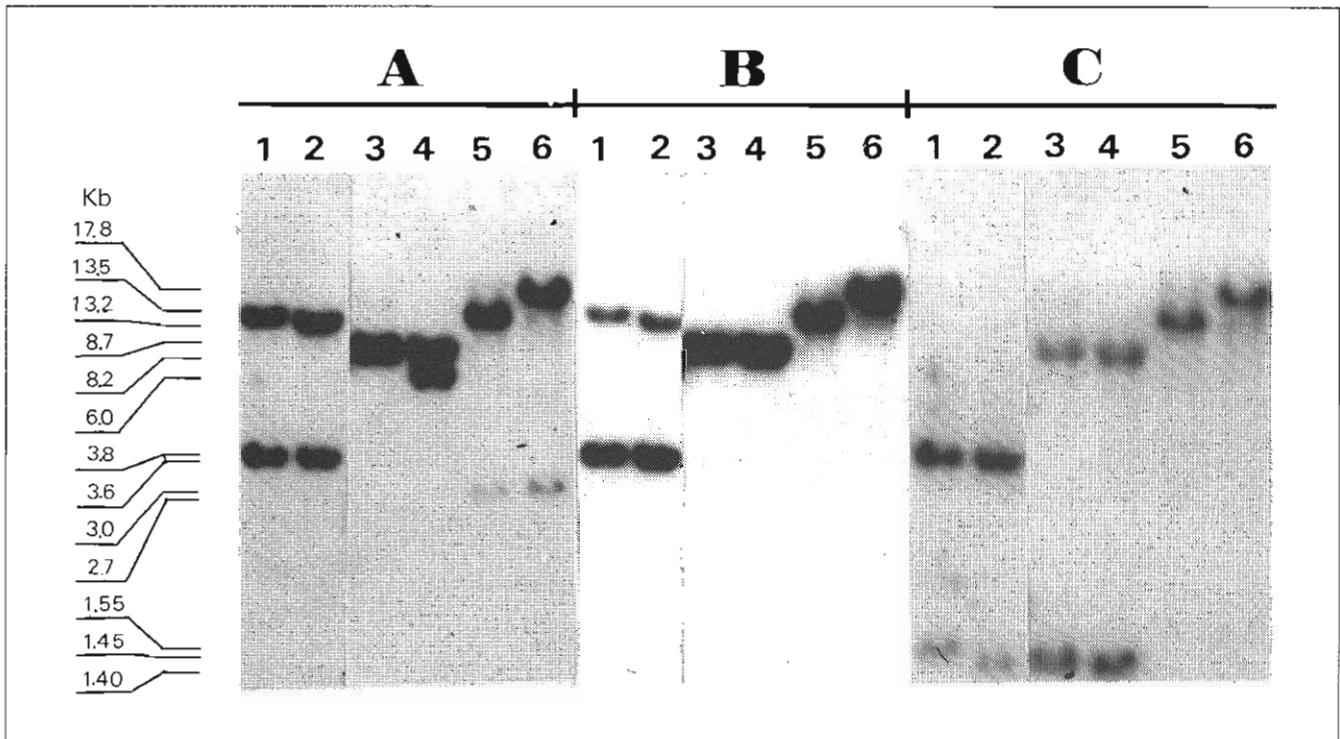
These procedures were carried out as described by Sambrook et al. (1989).

## Restriction enzymes

*BamHI* (Sigma), *BglII* (Sigma), *EcoRI* (FioCruz, Sigma), *HindIII* (Pharmacia, Sigma), *PstI* (Sigma). Reactions were carried out as described by suppliers.

## Physical mapping

Single and double digestions of recombinant plasmids containing rDNA regions of *D. aurita*, were carried out. For allocating restriction sites in *D. albiventris* and for estimating interspecific variations, single, double, and triple digestions of genomic DNA of each species were carried out. Filters were hybridized to probes containing the 28S region in HM123, HM456, and to recombinant plasmid pDa1 (containing the 28S 3' end from *D. aurita*).



**Figure 2** - Genomic DNAs of *Didelphis albiventris* (1, 3, 5) and *D. aurita* (2, 4, 6) were digested with *Bgl*II (1 and 2), *Hind*III (3 and 4), and *Pst*I (5 and 6), electrophoresed in agarose 0.7% and transferred to a nylon filter. The same filter was hybridized to: A - HM123 + HM456; B - 28S region of HM123; C - pDa1 (a recombinant plasmid with the 28S 3'end from *D. aurita*).

## RESULTS

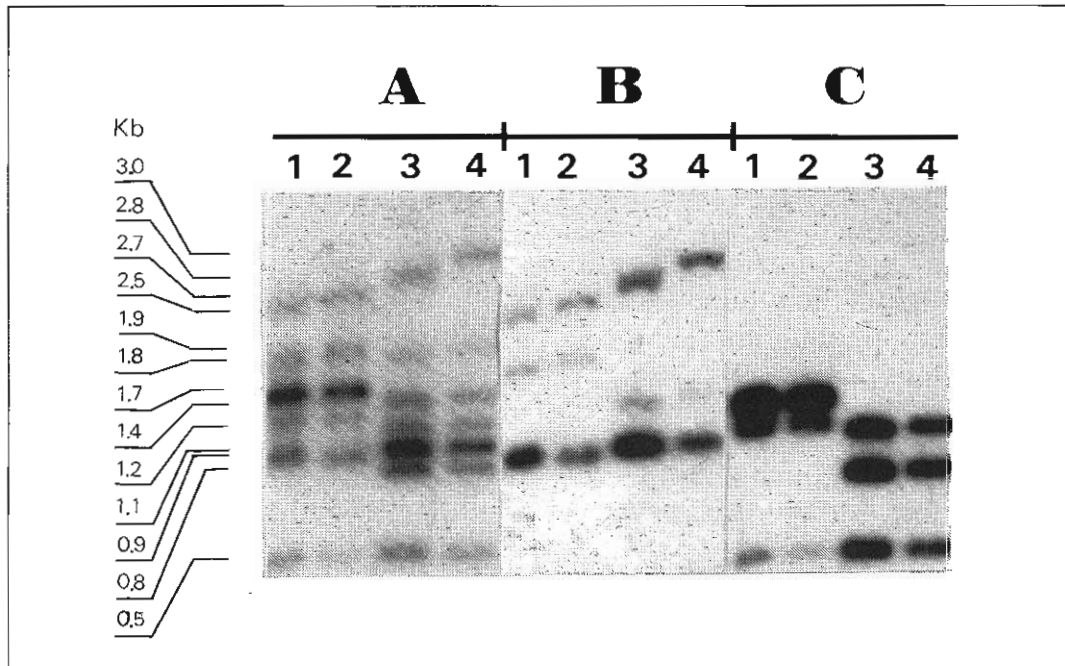
Southern blots of genomic DNAs from *D. aurita* and *D. albiventris*, digested with *Eco*RI, were hybridized to probes HM456 + HM123, and to the 18S region in HM456 (Figure 1a). The first hybridization showed different *Eco*RI restriction patterns: bands around 1.8, 5.0, 8.0 kb for *D. albiventris*; and 1.7, 7.6, and 10 kb for *D. aurita*. Hybridization with 18S probe revealed that the 5.0 kb fragment of *D. albiventris* and 10 kb of *D. aurita* contained the 18S region. The interspecific variation is due to the localization of *Eco*RI sites in the IGS. *D. aurita* *Eco*RI fragments were selected for cloning in EMBL4.

Positive rDNA fragments isolated from recombinant phages were subcloned at the pUC18 *Eco*RI site. Hybridizations to HM456 and HM123 probes showed that pDa1 (1.7 kb) contained the 3'end of 28S region, pDa2 (7.6 kb) contained the 3'end of 18S region, the internal transcribed spacer, and a major part of the 28S region, while pDa3 (10.4 kb) contained a major part of the 18S region and part of the IGS. Physical maps for the three recombinant plasmids were constructed with *Bam*HI, *Bgl*II, *Eco*RI, *Hind*III, and *Pst*I. Conserved restriction sites common to all vertebrates were allocated in the 18S and 28S regions (Figure 4).

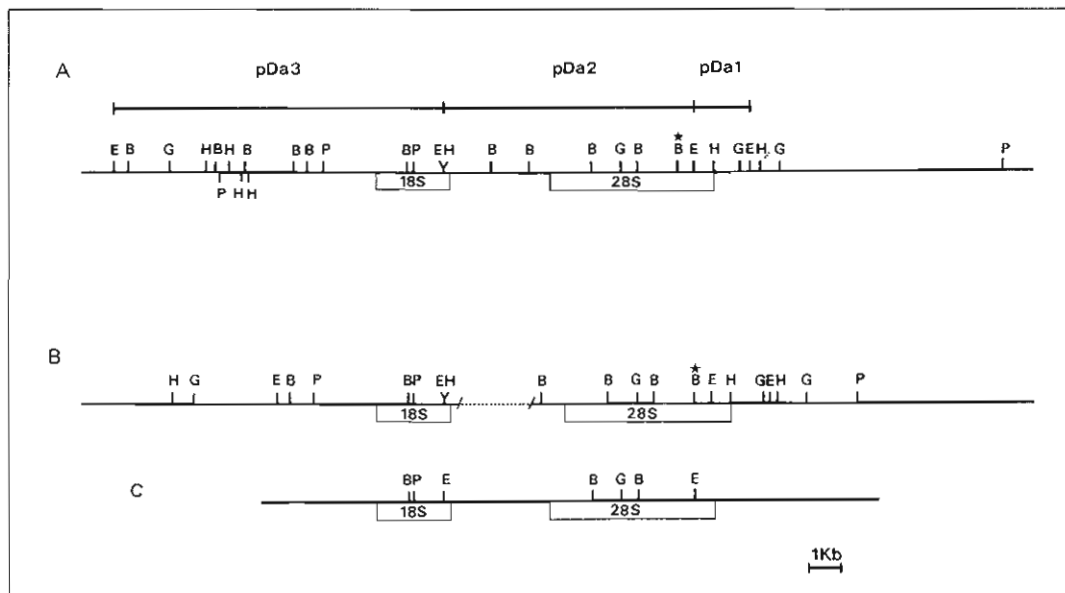
For genomic DNA analysis, hybridizations of blots containing *Bgl*II, *Hind*III, and *Pst*I digests were carried out to detect interspecific differences between *D. aurita* and *D. albiventris* at the IGS region. Hybridizations with HM456 + HM123 and the 28S probe from HM123 detected IGS differences at the 5' of the 18S region (Figures 2a and 2b). With pDa1, IGS variation was found at the 3' direction of the 28S region (Figure 2c).

Southern blots of triple digested genomic DNA (with *Bam*HI/*Eco*RI/*Bgl*II and *Bam*HI/*Eco*RI/*Pst*I), and hybridizations with HM456 + HM123, HM456, and the 28S region in HM123 (Figure 3 and Table I), showed homologous cleavage sites between *D. aurita* and *D. albiventris* and conserved sites in vertebrates. The probe containing part of the 28S region and HM456 did not show homology with the 1.9 kb fragment appearing in blot hybridization with HM123 + HM456. This fragment was found between the *Bam*HI site at the ITS and the next *Bam*HI site in the 28S region. Another *Bam*HI site, not present in other vertebrates, was also observed (Figure 4).

Data from clone and genomic DNA analyses are synthesized in Figure 4. A segment of the ITS from *D. albiventris* is not shown in Figure 4, because the unequivocal allocation of restriction sites in this region, as well in the IGS, would require the cloning of an rDNA repeat unit of this species.



**Figure 3** - Genomic DNAs from *Didelphis aurita* (1 and 3) and *D. albiventris* (2 and 4), were triple digested with *Bam*HI/*Eco*RI/*Pst*I (1 and 2), and *Bam*HI/*Eco*RI/*Bgl*II (3 and 4), electrophoresed in agarose 1.2% and transferred to a nylon filter. The filter was hybridized with: A - HM123 + HM456; B - HM456; C - 28S region of HM123.



**Figure 4** - Representation of the physical maps of the rDNA sequences from *Didelphis aurita* (A), *D. albiventris* (B), and the conserved sites in mammals (C) (Arnheim et al., 1980; Tanhauser et al., 1986). The data from *D. aurita* came from the clones and genomic DNA analysis, from *D. albiventris* from genomic DNA analysis. The cloned regions of *D. aurita* (pDa1, pDa2, and pDa3) are presented in A. The letters indicate the position of restriction sites: B - *Bam*HI; E - *Eco*RI; G - *Bgl*II; H - *Hind*III; P - *Pst*I. The dotted line in *D. albiventris* (B) indicates a region not mapped. \* indicates a *Bam*HI site not present in other vertebrates.

## DISCUSSION

Taxonomic arrangements of the genus *Didelphis* either consider *D. marsupialis* and *D. albiventris* as different species or *D. aurita* as a subspecies of *D.*

*marsupialis* (Kirsch et al., 1993). Cerqueira (1985) considered *D. aurita* as a different species from *D. marsupialis*, with separate ecological domains.

Location of nucleolar organizer regions (NORs) in *Didelphis* chromosomes ( $2n=22$ ) has shown from two to nine NORs per cell, per specimen (Yonenaga-Yas-

**Table I** - Fragments (in kilobases) from hybridizations of triple digestions of the genomic DNA shown in Figure 3. *Didelphis aurita*: *Da*; *D. albiventris*: *Dal*.

Probes:	Hybridized fragments (kb)					
	HM123+HM456		HM456		28S	
	<i>Da</i>	<i>Dal</i>	<i>Da</i>	<i>Dal</i>	<i>Da</i>	<i>Dal</i>
<i>Bam</i> HI/ <i>Eco</i> RI/ <i>Pst</i> I	2.5	2.8	2.5	2.8		
	1.9 <sup>a</sup>	1.9 <sup>a</sup>				
	1.7	1.8	1.7	1.8		
	1.4	1.4			1.4	1.4
	1.2	1.2			1.2	1.2
	0.9	0.9	0.9	0.9		
	0.5	0.5			0.5	0.5
<i>Bam</i> HI/ <i>Eco</i> RI/ <i>Pst</i> I	2.7	3.0	2.7	3.0		
	1.9 <sup>a</sup>	1.9 <sup>a</sup>				
	1.4	1.4	1.4	1.4		
	1.2	1.2			1.2	1.2
	1.1	1.1	1.1	1.1		
	0.8	0.8			0.8	0.8
	0.5*	0.5*			0.5*	0.5*

<sup>a</sup>These fragments do not shown homology with probes HM456 and 28S region from HM123.

\*Two fragments of the same size.

suda *et al.*, 1982), though this extensive variation was not observed in other didelphid genera (Souza *et al.*, 1990).

At the individual level, *D. aurita* and *D. albiventris*, showed homogeneity in their genomic restriction patterns, without detectable variations among intraindividual repeat units. The absence of intraindividual variation in *Didelphis* may be due to a fast homogenization process between different repeat units in at last six chromosomes of the chromosome complement (see Arnhein *et al.*, 1980; Dover, 1982; Wilson *et al.*, 1984; Dover and Tautz, 1986; Hilles *et al.*, 1991).

Interspecific differences were observed in the IGS region. The IGS region, at the 5' proximal region to the 18S unit did not show conserved restriction site when genomic DNA was analyzed. At the 28S 3' end, the IGS region showed limited variation (ca. 100bp) between *Eco*RI, *Bgl*III, and *Hind*III sites. Hybridization of a DNA fragment, spanning between a *Hind*III site at the 28S 3' end and the next 3' *Hind*III site in the IGS of *D. albiventris*, with pDa1 (Figure 2c) indicated sequence homology between the two species. Other interspecific variations were related with the distances (ca. 0.5 kb) between the *Eco*RI sites in the 18S and 28S regions, and *Hind*III sites in 18S and 28S regions (Figures 1a and 2b).

These are probably related to variations in the internal transcribed spacer.

There were no interspecific differences between 18S and 28S regions, and conserved sites common to mammals were observed (see Figure 4). However, data from the clones and genomic DNA analyses disagreed with Tanhauser *et al.* (1986). These authors postulated the existence of two *Bam*HI sites in the 28S region of *D. marsupialis*. Our data indicated a different interpretation. Tanhauser *et al.* (1986), in order to locate conserved sites in vertebrates, made a triple digestion with *Bam*HI/*Eco*RI/*Bgl*III and detected a 0.5 kb band in the 28S region of *D. marsupialis*. Examination of Figure 3 of this report showed that the 0.5 kb band of *D. marsupialis* has the highest intensity when compared to other mammals. We propose that this could be due to the presence of a double band, actually comprising two fragments hybridizing to the 28S probe. Data from *D. aurita* and *D. albiventris* actually showed the existence of three *Bam*HI sites (Figures 3 and 4). The additional *Bam*HI site herein reported is 5' located, approximately 0.5 kb from the 28S *Eco*RI site. The fragment between these two sites has the same length as the fragment spanning between the single *Bgl*III site and the middle *Bam*HI site of the 28S region. This may

be particular to the genus *Didelphis* and other marsupials, however in the report of Tanhauser *et al.* (1986), in a specimen reported as *D. marsupialis*, there are no specifications on the taxonomic criterion used for species identification. The possibility exists that this specimen might actually be *D. aurita* rather than *D. marsupialis*; in which case, our hypothesis could not be extended to the entire genus.

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## RESUMO

As regiões do rDNA 18S e 28S de *Didelphis aurita* e *D. albiventris* foram analisadas através de digestões do DNA genômico com enzimas de restrição e hibridações contra sondas de rDNA. Regiões do rDNA de *D. aurita* foram clonadas e mapeadas fisicamente. Sítios de restrição conservados em mamíferos foram encontrados. São descritas variações nos sítios de restrição no espaçador intergênico das duas espécies. Não foram observadas variações entre as unidades de repetição de um mesmo indivíduo. Um sítio de restrição para *Bam*HI na região 28S, encontrado nas duas espécies, não é descrito em outros mamíferos. Este sítio provavelmente é característico do gênero *Didelphis*.

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