Biota Neotropica 18(1): e20170389, 2018
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)

Article

Endozoochory by *Didelphis albiventris* Lund, 1840 (Mammalia, Didelphimorphia) in a Semideciduous Seasonal Forest remnant in the South of Brazil

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JUNGES, S.O., CONSATTI, G., PÉRICO, E., BORDIGNON, S.A.L., FREITAS, E.M., CADEMARTORI, C.V. **Endozoochory by *Didelphis albiventris* Lund, 1840 (Mammalia, Didelphimorphia) in a Semideciduous Seasonal Forest remnant in the South of Brazil**. Biota Neotropica. 18(1): e20170389. http://dx.doi.org/10.1590/1676-0611-BN-2017-0389

Abstract: Seed dispersal is a process that is fundamental to maintenance of forest ecosystems, enabling plants to successfully germinate in sites that are favorable to their growth, minimizing risks of competition, the action of pathogens and predation by herbivores. Intraspecific and seasonal variations in fruit consumption by *Didelphis albiventris,* and its contribution to dispersal and germination of endozoochorous seeds were analyzed in a Semideciduous seasonal forest. The study was conducted at Morro do Coco, which is a hilly area in the municipal district of Viamão, RS, Brazil, bordering the shore of Guaíba lake (30º16'15"S, 51º02'54"W), between June 2013 and May 2014, with a total sampling effort of 2992 trap-nights. A total of 18 individuals were captured and 24 fecal samples were collected. Fruits were identified in 96% of the samples, corresponding to 18 plant species, belonging to 10 families. The most common species were *Ficus cestrifolia* and *Syagrus romanzoffiana*, which occurred in 66% of the samples, followed by *Banara parviflora* and *Cecropia pachystachya*, both with a 25% rate of occurrence. There were no differences between the richness of fruit consumed by males and females (t = 0.083; DF = 32; p = 0.934) and there were no seasonal variations (H = 3.165; p = 0.367). The greatest breadth of dietary niche occurred during the summer, when twice as many fruit species were recorded in the diet than during the autumn, which was the season with the smallest breadth. Both germination percentage and germination velocity of *Ficus cestrifolia* and *Psidium* sp. seeds increased after passage through the animals' digestive tracts (percentage germination increased more than 40% and velocity was up to 7 times highest). *Didelphis albiventris* can be considered a frugivorous-omnivorous species, since fruit are an important item of its diet, and it contributes to dispersal of a large quantity of small endozoochorous seeds, increasing both germination percentage and germination velocity of some species.

Keywords: *white-eared opossum, germination inductor, marsupial, Atlantic Rain Forest, zoochory.*

Endozoocoria por *Didelphis albiventris* Lund, 1840 (Mammalia, Didelphimorphia) em remanescente de Floresta Estacional Semidecidual no sul do Brasil

Resumo: A dispersão de sementes é um processo fundamental à manutenção de ecossistemas florestais, favorecendo o sucesso germinativo de plantas em locais adequados ao seu crescimento, minimizando a competição, a ação de patógenos e a predação por herbívoros. Variações intra-específicas e sazonais no consumo de frutos por *Didelphis albiventris,* bem como sua contribuição à dispersão e germinação de sementes endozoocóricas foram avaliados em Floresta Estacional Semidecidual. O estudo foi realizado no Morro do Coco, localizado no município de Viamão, RS às margens do Lago Guaíba (30º16'15"S, 51º02'54"W), entre junho de 2013 a maio de 2014, resultando em um esforço amostral de 2992 armadilhas-noite. Foram capturados 18 indivíduos e coletadas 24 amostras fecais. Frutos estiveram presentes em 96% das amostras e corresponderam a 18 espécies vegetais, pertencentes a 10 famílias. As espécies mais frequentes foram *Ficus cestrifolia* e *Syagrus romanzoffiana*, que ocorreram em 66% das amostras, seguidas de *Banara parviflora* e *Cecropia pachystachya*, ambas com 25% de ocorrência. Machos e fêmeas não diferiram quanto à riqueza de frutos consumidos (t = 0.083; DF = 32; p = 0.934), o que também não variou sazonalmente (H = 3.165; p = 0.367). A maior amplitude de nicho trófico ocorreu no verão, registrando-se duas vezes mais frutos na dieta do que no outono, estação com a menor amplitude. Tanto a porcentagem quanto a velocidade de germinação das sementes de *Ficus cestrifolia* e *Psidium* sp. aumentaram após a passagem pelo trato digestório do animal (a porcentagem de germinação aumentou mais de 40% e a velocidade foi até 7 vezes maior). *Didelphis albiventris* pode ser considerada uma espécie frugívora-onívora, uma vez que frutos representam um item importante na dieta, e contribui para a dispersão de grande quantidade de sementes endozoocóricas pequenas, aumentando tanto o percentual quanto a velocidade de germinação de algumas espécies.

Palavras-chave: *gambá-de-orelha-branca, indutor de sementes, marsupial, Mata Atlântica, zoocoria.*

Introduction

Seed dispersal is a process that is fundamental for maintenance of plant species in forest systems, enabling successful germination in sites that are favorable to their growth, minimizing risks of competition, the action of pathogens and predation by insects and other herbivores (Howe & Smallwood 1982, Jordano et al. 2006). It has been estimated that around 50 to 90% of the trees in tropical forests produce fruit that are adapted to dispersal by animals and in these environments the diversity of frugivores is relatively high, primarily comprising reptiles, fish, mammals and birds (Howe & Smallwood 1982, Fleming 1987).

Many authors (e.g. Bocchese et al. 2008, Camargo et al. 2011, Oliveira & Leme 2013) consider that endozoochory is the most important of all of the various different animal-plant dispersal mechanisms. However, this interaction process can have varying results in terms of induction of germination because animals species' habits and behavior differ. Many plants also have features that make germination less likely after consumption by an animal. For example, small seeds can be damaged by gastric juices. As a result, several species may not be considered potential dispersers (Oliveira & Leme 2013).

Among neotropical mammals, marsupials are part of a rich fauna of small mammals and the majority of species are considered omnivorous, although some are more frugivorous. The degree to which didelphids specialize in frugivory is variable, and depends on factors such as the plant's capacity to attract consumers by smell, color and shape of fruit, the position of fruit on the trunk and their availability in the environment (Howe & Smallwood 1982, Cáceres et al. 2009). Marsupials do not fit into a defined dispersal syndrome, but are attracted by fruit that is also dispersed by birds or bats, and for this reason their frugivory is considered eclectic (Cáceres & Lessa 2012). Notwithstanding the fact that they don't have a defined dispersal syndrome, some species are considered more frugivorous than others, such as those that belong to the *Caluromys* genus, with a diet that includes around 45 species of fruit, in addition to nectar and sap (Cáceres 2006).

Didelphis albiventris Lund, 1840 is a generalist and omnivorous marsupial species that eats invertebrates, fruit and small vertebrates and occasionally carrion and parts of plants (Santori & Moraes 2006). Its behavior is also considered opportunist, because it feeds on both vegetable and animal material, depending on the availability in its environment or seasonal variations in the supply of fruit (Lessa & Costa 2010). Cantor et al. (2010) reported that *D. albiventris* ate 37 plant species in a disturbed area in the state of São Paulo, Brazil, including three genera that are considered pioneers in succession of tropical forests (*Piper*, *Cecropia* and *Solanum*), demonstrating the potential importance of these didelphids to recovery of degraded areas. Furthermore, the period of time that a seed may remain within the animal's intestine can be as long as 24 hours, which means it is possible that the seed may be deposited further from the seeding plant, compared to dispersal by other small mammals (Cáceres 2006).

Knowledge about the distinct dietary habits of didelphids is fundamental to understanding the ecological functions that they perform in their respective habitats, as predators of small vertebrates and invertebrates, and as seed dispersers. Although the number of studies investigating dietary habits has increased over recent years, primarily focusing on *Didelphis*, *Micoureus* and *Philander*, further studies of this type are considered indispensable to acquisition of knowledge about relationships between niches and the distribution of resources in the environment (Cáceres 2006, Cantor et al. 2010, Lessa & Cost, 2010; Lessa & Geise 2010). In particular, little is known and has been studied about the contribution made by *D. albiventris* to dispersal of seeds and induction of germination. The few studies that do exist were conducted in Brazil, in the Cerrado, Southeast and South, investigating this marsupial's potential to contribute to regeneration of forest fragments, and the results suggest that it can be an effective disperser, since seeds remain intact even after defecation and it is tolerant of altered environments and of urbanization (Cáceres 2002, Cantor et al. 2010, Oliveira & Leme 2013). In view of this knowledge gap and the fact that this is a common and widely-distributed species, including in degraded habitats, this study was conducted in a remnant of the Atlantic Rain Forest Domain in South Brazil to investigate: 1. intra-species and seasonal variations in fruit consumption by *D. albiventris* and its contribution to endozoochorous seed dispersal, and 2. the viability and speed of germination of seeds after passage through the animal's digestive tract.

Materials and Methods

1. Study area

The study was conducted on the Morro do Coco, which is a hill in the municipal district of Viamão, RS, Brazil, bordering the Guaíba lake (30º16'15"S, 51º02'54"W) (Figure 1). This is an area of Semideciduous seasonal forest of about 140 ha that is part of the Atlantic Rain Forest domain. It has characteristics that are still close to the original characteristics and is made up of mosaics of more preserved areas, in advanced stages of succession, and degraded areas, in initial stages (Backes 2000). The region has a type Cfa climate, according to the Koeppen classification, i.e., subtropical with a cool winter and hot summer, mean temperature greater than 22 ºC in the hottest month, and rainfall well-distributed throughout the year (Livi 1998, Backes 2000).

The entire hill is covered with arboreal vegetation, with a top stratum that can reach 35 m in height, in which species such as *Syagrus romanzoffiana* (Cham.) Glassmsn (Arecaceae) and *Ficus cestrifolia* Schott ex Spreng. (Moraceae) (Knob 1978) predominate. Backes (1999) recorded 44 arboreal species in the upper stratum, predominantly members of the families Euphorbiaceae, Lauraceae, Moraceae, Mimosaceae and Myrtaceae. In the middle and lower strata, the same study recorded 28 and 19 species respectively, predominantly members of the Myrtaceae family in both strata. The formation also includes many epiphytes, primarily of the Bromeliaceae (14 species) and Orchidaceae (22 species) families, which are more abundant at the margins of Guaíba lake, where the humidity of the air is higher (Backes 1999).

2. Capture methods

Sampling was conducted between June 2013 and May 2014, over 11 expeditions, each lasting four consecutive nights, resulting in a total sampling effort of 2992 trap-nights, according to the Stallings method (1989). In order to minimize the effects of moonlight intensity, dates when the moon was new or in the third quarter were preferred (Auricchio 2002). All four seasons of the year were sampled (winter, from June to August 2013; spring, from September to November 2013; summer, from December 2013 to February 2014; and autumn, from March to May 2014), with the objective of encompassing seasonal variations in supply of fruit and its influence on the diet.

Sixty-five "Tomahawk" type traps were used for captures, 50 with dimensions of 40 cm x 20 cm x 20 cm and 15 with dimensions of 45 cm x 25 cm x 21 cm. The traps were arranged in five parallel lines of 10 capture stations each (spaced equidistantly at 20 m intervals), forming a 100 m x 200 m grid. Three stations in each line (one, five and nine) had both a ground trap and an understorey trap (at 1.60 m from the ground, fixed to a wooden platform). Another three traps were set outside of the grid described above, close to the margins of the Guaíba lake, taking the total number of traps to 68. Trap bottoms were lined with plastic sheeting to facilitate collection of feces and avoid loss of material. Bait was put inside the traps, comprising a mixture of sardines, peanut paste, vanilla essence and banana.

Each individual captured for the first time was identified with a numbered tag (SISBIO Permanent License number 11066-1, granted on 17th November 2010). The following details and biometric data were recorded for each individual: species, sex, weight (g), head and body length (mm), tail length (mm) and hind feet length (mm), and reproductive status for females. Animals that were captured more than once in the same expedition were released without collecting biometric data again after the first capture, to avoid pseudo-replicas. Only animals recaptured during different expeditions were included in the analyses more than once.

3 Collection of fecal content

All fecal collections were conducted manually, from inside the cages and during handling of animals and samples were identified and stored individually. Each sample comprised all stools collected from a single individual. At the Unilasalle Biodiversity Conservation and Management Laboratory, feces were washed under running water, using filter paper bags, and dried at room temperature. Once fully dry, fecal content was sorted and seeds were identified using a stereoscopic microscope and the relevant literature (Lorenzi 2003, 2008, 2009), and compared with seeds from fruit gathered in the study area and with a reference collection maintained by the MCN La Salle museum.

4 Tests of seed germination

Experiments to test seed germination were conducted at the UNIVATES Plant Propagation Laboratory in the town of Lajeado, Rio Grande do Sul. Tests were conducted with the seeds of seven plant species: *Ficus cestrifolia* Schott ex Spreng., *Psidium* spp., *Banara parviflora* (A.Gray) Benth, *Cecropia pachystachya* Trécul, *Passiflora amethystina* J.C. Mikan and one species of Solanaceae. Control groups were also tested, comprising seeds from fruit collected in the study area. The test for *Psidium* sp. used *P. guajava* L. and *P. cattleianum* Sabine as controls, since both occur in the study area. The germination test for *P. amethystina* and the unidentified Solanaceae species were only conducted with seeds from the fecal samples, because no fruit for control groups could be found in the study area.

The seeds were sorted, de-infested and immersed for 20 minutes in commercial hypochlorite at 70% and then in 70% ethanol for 60 seconds, before once more being washed three times using autoclaved water (Maguiri 1962). After disinfection in a laminar flow chamber, the seeds were inoculated onto transparent (glass) Petri dishes containing two sheets of Germitest paper moistened with distilled water. This process was performed with two groups of seeds: control groups (seeds extracted from fruit collected in the study area) and test groups (seeds obtained from fecal samples), as shown in Table 1. Wherever possible, repetitions with 20 seeds were conducted of each treatment (control and test), subject to sufficient numbers of seeds being found in the fecal samples. Dishes were kept in propagators with fluorescent lighting at a photoperiod of 16 h light per day and a temperature of 25 ºC (Maguiri 1962, Camargo et al. 2011).

Table 1. Number of seeds per plant species and per treatment used to test seed germination in experiments with marsupial *Didelphis albiventris* from a remnant of Semideciduous seasonal forest in South Brazil, RS.

Plant species	Group of seeds	
	Control	Test
Banara parviflora (A.Gray) Benth	133	11
Cereus hildmannianus K.Schum.	8	2
Ficus cestrifolia Schott ex Spreng.	280	134
Psidium sp.	*70	20
Passiflora amethystina J.C.Mikan	-	11
Solanaceae	-	6

* *P. guajava* Linnaeus, Carl von, *P. cattleianum* Sabine

Figure 1. Location of the study area, a hill named Morro do Coco, within the municipal district of Viamão, RS, Brazil (Image from Google Earth, 2014).

The experiment was monitored every 2 days for up to 70 days after inoculation. Sprouting of the primordial radicle was considered evidence of germination.

5 Data analysis

The representativeness of vegetable items identified in the diet was estimated on the basis of the Frequency of Occurrence (FO) of these items in samples, where: FO = number of samples containing the item / total number of samples x 100. Student's *t* test with Welch's correction (for samples with unequal variances) was used to evaluate the significance of differences between sexes in richness of fruit and number of seeds in the fecal samples.

The diversity of fruits in the diet of males and females and per season was measured in terms of breadth of dietary niches, calculated using Levin's Index (B_A), which, according to Krebs (1999), attributes greater weight to fruits that are more abundant in the diet. The Kruskal-Wallis test (H) was used to test whether animals had a preference for any of the fruits in their diet, comparing all of the plant species found in the samples. Seasonal variations in diet were also investigated using the same test. Differences between the numbers of seeds of different species found in fecal samples were tested using ANOVA (F). Dunn's post hoc test for multiple comparisons was used to identify which samples differed from each other.

Analyses of germination were conducted separately for each species, as per Borghetti & Ferreira (2004), by calculating the percentage of germinated seeds (PG) in relation to the number of seeds under experimental conditions, using the formula: $PG = (\sum n_i . N^{-1}) . 100$, where n represents the number of plantlets germinated and N the total number of seeds used in the experiment. The germination velocity index (GVI), in which the number of seeds or plantlets is observed and counted regularly (every 2 days in this study), was obtained as per Borghetti & Ferreira (2004), using the formula: $GVI = G_1/N_1 + G_2/N_2 + ... + G_n/N_n$, where G represents the number of seeds germinated each time they were counted and N is the number of days since seeds were sown. The significance of differences observed between two groups of seeds (control and test) was assessed using Student's *t* test with Welch's correction, Fisher's exact test or the G test (goodness-of-fit), when the number of seeds inoculated was low (six seeds). Each plant was also assessed by calculating odds ratios for the probability of germination after passage through the intestinal tract, in comparison to the control group.

Statistical analyses were conducted using the programs PAST 3.11 and Biostat 5.3 (Ayres et al. 2007). Differences were considered significant when $p \leq 0.05$.

Results and Discussion

During the study, 18 *D. albiventris* individuals (seven females and 11 males) were captured a total of 26 times, producing 24 fecal samples. All individuals were captured on the ground and no animals were captured on the platforms in the understorey.

Among the dietary items found in the fecal samples, fruits were identified in 96% of the samples and corresponded to 18 plant species, belonging to 10 families (Table 2). The most common species were *Ficus cestrifolia* and *Syagrus romanzoffiana*, which occurred in 66% of the samples, followed by *Banara parviflora* and *Cecropia pachystachya*, both with a 25% rate of occurrence.

Cáceres et al. (2009) conducted a 6-month study in Atlantic Rain Forest in South Brazil, using 127 traps and captured 19 *Didelphis aurita* Wiend-Neuwied, 1826, collecting 63 fecal samples and recording consumption of eight plant species. Silva et al. (2014) analyzed 40 fecal samples from *D. albiventris* in two areas in South Brazil and recorded consumption of the fruit of six plant species (accounting for 77.5% of samples), of which *S. romanzoffiana*, *F. cestrifolia* and *Coussapoa microcarpa* (Schott) Rizzini were the most frequent. The *Ficus* genus is considered one of the most important for frugivores in tropical forests, providing a food resource during seasons in which fruit is scarce in the environment (Sugai & Cara 2009). Many frugivorous mammals are attracted by the yellow-green fruit of *F. cestrifolia*, which only become purple during the final phase of maturity (Carauta & Dias 2002).

The high rate of occurrence of the seeds of *F. cestrifolia* in the fecal samples may be related to the species' asynchronous fruit bearing, since its fruit is available in all seasons of the year, which is the reason why members of *Ficus* are considered key species in tropical forests (Carauta & Days 2002, Sugai & Cara 2009). Another relevant factor is the fact that *F. cestrifolia* and *F. luschnathiana* (Miq.) Miq. are common species that are well-distributed in the study area, making their fruits accessible to many frugivores. Although in this study occurrence of *F. luschnathiana* was not identified in the samples analyzed, Silva et al. (2014) have observed seeds of this species in the feces of *D. albiventris* in the same study area.

Syagrus romanzoffiana is also considered a key species in tropical forests, because its fruits are fleshy and rich in nutrients and it provides an

Table 2. List of plant species with life forms (according to Lorenzi 2003, 2008, 2009) and occurrence in feces of marsupial *Didelphis albiventris* collected in a remnant of Semideciduous seasonal forest in Rio Grande do Sul, Brazil, according to APG III (2009).

Family	Taxon	Common name	Life form	Frequency of occurrence (%)
Anacardiaceae	*Lithraea brasiliensis* Marchand	Aroeira-brava	tree	8
Arecaceae	*Syagrus romanzoffiana* (Cham.) Glassm.	Gerivá	tree	66
Cactaceae	*Cereus hildmannianus* K.Schum.	Tuna	tree	8
Moraceae	*Ficus cestrifolia* Schott ex Spreng.	Figueira	tree	66
Myrtaceae	*Campomanesia xanthocarpa* (Mart.)	Guabiroba	tree	4
	Psidium sp. *	Araçá/goiaba	tree	4
Passifloraceae	*Passiflora amethystina* J.C.Mikan	Maracujá-azul	liana	4
Primulaceae	*Myrsine* sp.	Capororoca	tree	4
Salicaceae	*Banara parviflora* (A.Gray) Benth	Farinha-seca	tree	25
Solanaceae	Solanaceae	-	-	4
Urticaceae	*Cecropia pachystachya* Trécul	Embaúba	tree	25
	Coussapoa microcarpa (Schott) Rizzini	Mata-pau	tree	8
Not identified sp.1				17
Not identified 5 spp.				**4

* *P. guajava* Linnaeus, Carl von, *P. cattleianum* Sabine; ** each one

energy source for many tropical frugivores. Additionally, this palm bears fruit throughout the year, with greater intensity from March to August, providing fauna with food in months of seasonal scarcity (Lorenzi 1992, Costantin et al. 2013). Messias & Alves (2009) identified 10 species of mammals feeding on *S. romanzoffiana* in an area of Atlantic Rain Forest in the state of São Paulo, Brazil, one of which was *Didelphis* sp. Larger marsupials, such as *D. albiventris*, are considered occasional dispersers of large seeds, in common with the majority of didelphids, because they only eat the flesh of the fruit, leaving the seeds in the place where they eat (Cáceres 2006). This is the case of *S. romanzoffiana*, according to recent studies (Cáceres et al. 2009, Cantor et al. 2010, Silva et al. 2014). Roman et al. (2010) studied removal of *S. romanzoffiana* fruit, observing that *D. albiventris* removed 13% of the fruit, left 39% intact and masticated 63%, leaving the seeds at the site in a synzoochorous manner. Therefore, because this species does not disperse seeds over long distances, it is not a good disperser, since for palms to germinate successfully the seeds must be a minimum of 4 m from the mother plant.

The size of the most frequent seeds in the samples varied from 0.3 to 3.7 mm (90%), and seeds larger than 3.7 mm were infrequent (10%). Fecal samples differed significantly in terms of the quantity of seeds ($F = 31.134$; Degrees of freedom [DF] = 17; $p = 0.019$), and *F. cestrifolia* was the species with the largest number of seeds in the samples. The quantity of seeds found in the samples varied from one to 1480 seeds (Mean = 150.125; SD = 311.15) in a single sample, but the majority of samples contained large numbers of seeds. As such, small seeds predominated in the samples, and the seeds of *F. cestrifolia* and *B. parviflora* were the smallest seeds found. Cantor et al. (2010) also found large numbers of seeds in samples from *D. albiventris,* ranging from one to 566 seeds per sample. Cáceres (2006) suggests that animals' body sizes can have an influence on the quantity of seeds dispersed and that small seeds (< 5 mm) may be dispersed by both small and large marsupials. In view of this, the observation of a single *S. romanzoffiana* seed (around 17 mm in size) in a fecal sample from a reproductive female with young is considered atypical, since it was the only such record. This does, however, provide evidence that an animal with the body size of *D. albiventris* can occasionally disperse the fruit of palms by endozoochory. Cantor et al. (2010) found a larger amplitude of seed sizes in stools, ranging from 0.5 to 13.3 mm, but still with a higher frequency of small seeds, and also observed that larger seeds were damaged. Lessa et al. (2013) reported similar results, finding small seeds in 68% of samples from seven species of marsupials, including *D. albiventris*. Seeds larger than 6 mm were damaged.

The richness of plant species found in samples from males and females did not differ significantly ($t = 0.083$; DF = 32; $p = 0.934$), and there was no significant seasonal variation in fruit richness either ($H = 3.165$; $p = 0.367$), as illustrated in Figure 2. However, the greatest dietary niche breadth was observed in the summer ($B_A = 0.3176$), when twice as many fruits were recorded in the diet than in the autumn, which was the season with the narrowest dietary niche breadth ($B_A = 0.1513$). This difference may be related to availability of fruit in the environment, since 47 arboreal individuals bearing fruit were recorded in the summer and just 16 individuals in the autumn in the same sampling grid (Luana S. Guimarães, personal communication, 2014, data not yet published). Cantor et al. (2010) also failed to detect seasonal differences in fruit richness for *D. albiventris* and attributed this result to continuous fruit bearing by zoochorous plants in the area studied.

Males had a dietary niche breadth ($B_A = 0.3771$) that was 1.3 times larger than that of females ($B_A = 0.2834$). These results are similar to findings reported by Silva et al. (2014) for the same study area. *Didelphis aurita* also exhibited a similar pattern in fragments of Atlantic Rain Forest (Carvalho et al. 2005, Cáceres et al. 2009). Both the increase in size of males' living areas during the reproductive period and the report that young males are more nomadic and don't have a fixed living area (Cáceres & Monteiro-Filho 2006, Graipel & Filho 2006) may explain why it was observed that males' trophic niches were wider in this study.

The quantity of seeds was also not significantly different between the sexes ($t = 1.547$; DF = 11; $p = 0.150$), confirming that the frugivorous-omnivorous diet is independent of sex. So, although males have larger living areas and young males are more nomadic and arboreal (Cáceres & Monteiro-Filho 2006, Graipel & Filho 2006, Vieira 2006), it seems that this ecological differences between the sexes don't influence the consumption of fruits. Cáceres et al. (2009) analyzed fecal samples from *D. aurita* in South Brazil and also failed to detect differences between sexes in terms of the quantity of seeds, which confirms the results obtained in this study.

In relation to the life forms of the plant species that were found in the samples, 91% are tree forms and just one is a liana. All of the species recorded are native, with the exception of the *Psidium* sp., which may have been *P. guajava*, which occurs in the study area as well as other exotic species of plants.

The germination tests of *Ficus cestrifolia* seeds demonstrated that differences observed were not the result of chance. For the first repetition, there was a significant difference in percentage germination ($t = 3.793$; DF = 5; $p = 0.013$) between the control group (PG = 6.6%) and

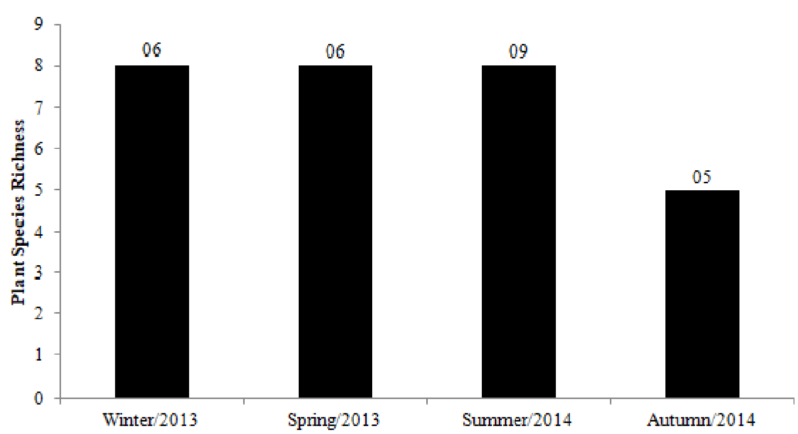

Figure 2. Richness of fruit in fecal samples and number of marsupial *Didelphis albiventris* captures from June 2013 to May 2014 in a remnant of Semideciduous seasonal forest in South Brazil, RS.

test group (PG = 39%). Germination velocity also differed significantly (t = 3.455; DF = 5; p = 0.018) between control (GVI = 0.06) and test groups (GVI = 0.44); velocity in the test group was 7.33 times greater than in the control group (Figure 3B). The second repetition also exhibited significant differences in percentage germination (G = 24.670; DF = 1; p < 0.0001) between the control (PG = 12.72%) and test groups (PG = 57%). Germination velocity was 1.53 times faster in the test group (GVI = 0.29) than in the control group (GVI = 0.19).

The germination tests with *Psidium* sp. also revealed significant differences. Percentage germination for *Psidium* sp. and *P. guajava* (control) differed significantly (G = 4.935, DF = 1, p = 0.026) between control (PG = 31.21%) and test groups (PG = 80%) (Figure 3A). Germination velocity was also around 1.2 times greater in the test group (GVI = 0.72) than in the control group (GVI = 0.61) (Figure 3B). However, *Psidium* sp. and *P. cattleianum* (control) did not differ significantly (G = 0.112, DF = 1, p = 0.738), although percentage germination was higher in the control group (PG = 100%) than in the test group (PG = 80%). However, germination velocity was 4.1 times greater in the test group (GVI = 0.726) than in the control group (GVI = 0.177). In turn, *Cereus hildmannianus*, did not exhibit significant differences in percentage germination (*n* = 10, p = 1.0) between the control and test groups. However, the small sample size should be taken into consideration in relation to this result. In contrast, percentage germination of *Banara parviflora* did differ significantly (*n* = 181, p = < 0.0001) between control and test groups, with higher percentage germination in the first group (40%) than in the second (2%).

Passiflora amethystina and Solanaceae seed samples did not germinate during the experimental period (70 days), which may be related to the size of the seeds, the plants' life forms, climatic variations, and/or the length of time the seeds spent in the animals' intestines (Cantor et al. 2010).

Overall, the germination tests indicated that both germination percentage (Figure 3A) and germination velocity (Figure 3B) of *Ficus cestrifolia* and *Psidium* sp. seeds increased after passage through the animals' digestive tracts. Cáceres (2006) states that the majority of seeds that pass through marsupials' digestive tracts remain viable for germination, because their masticatory behavior does not damage seeds, particularly not smaller ones. The action of gastric acids is also insufficient to damage seeds, according to the same author. Notwithstanding, since *B. parviflora* seeds exhibited a higher germination percentage in the control group, it appears that passage through the intestinal tract of *D. albiventris* may compromise their viability. Cantor et al. (2010) investigated the success of germination of five plant species after passage through the digestive tract of *D. albiventris*, observing that the viability of seeds varied across different species. For three species, the germination percentage in the control group was medium to high (*Cecropia pachystachya*, *Psidium guajava* and *Morus nigra*), whereas *Piper amalago* L. did not exhibit significant differences between groups. Only *Passiflora edulis* Sims exhibited a higher germination percentage in the test group.

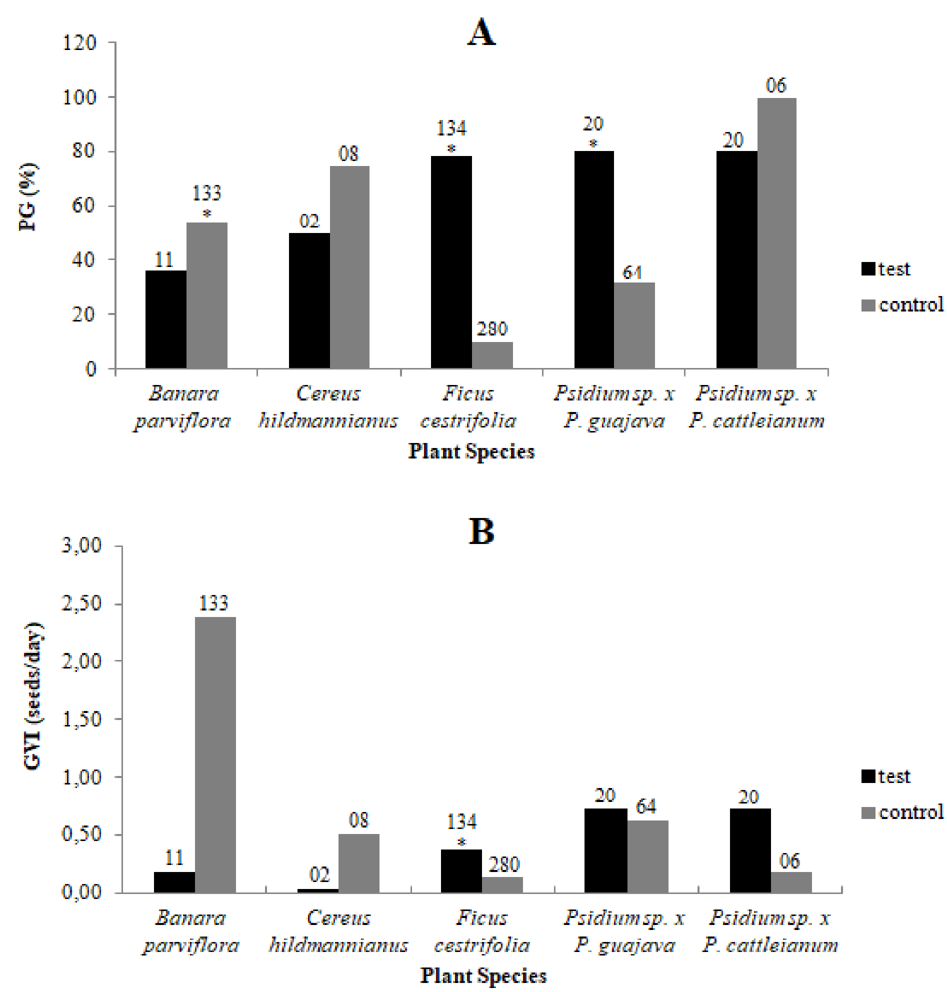

Figure 3. (A) Percentage germination (PG) and (B) germination velocity index (GVI) for seeds in test groups and control groups (above vertical bars are the numbers of tested seeds) in experiments with marsupial *Didelphis albiventris* from a remnant of Semideciduous seasonal forest in South Brazil, RS. (*) indicates significant differences (p<0.05).

In turn, Oliveira and Leme (2013) tested the germination potential of *Rapanea ferruginea* (Ruiz et Pav.) after passage through the digestive tract of *D. albiventris* and reported that both germination percentage and germination velocity were higher than for other treatments and a control group. As a result, they concluded that the animal is a potential disperser of *R. ferruginea* in conserved areas and in anthropized areas. Camargo et al. (2011) tested the viability of seeds of three species of the genus *Miconia* found in fecal content from *Gracilinanus agilis* (Burmeister,1854) and in addition to not detecting significant differences between the groups tested, they found that percentage germination was higher in the control group for *Miconia cuspidata*. These authors suggested that the seeds had been damaged by the animals' gastric acids, because of their small size, contradicting Lessa & Costa (2010), who claim that small seeds (< 1 mm) remain intact when eaten by small marsupials. Cáceres & Monteiro-Filho (2007) tested germination of 14 plant species and the majority of seeds did not exhibit significant differences between control and test groups, with the exception of *Rubus rosifolius* Stokes (the viability of which has been confirmed previously after passage through the intestines of bats and primates). The authors highlighted the influence of the dormancy period of each species' seeds, which is essential to understanding the potential of an effective disperser. In contrast, Cáceres (2006) argues that the length of dormancy does not impact on dispersal, since a seed from a pioneer plant deposited in a site that is inappropriate for germination (such as the forest interior), may spend a long period of time in the soil seed bank until favorable conditions appear, such as a clearing opening, for example. Notwithstanding, Lessa et al. (2013) point out that even if germination after passage through the intestines of these animals is irrelevant, the impact is still positive because of the increased distance of dispersal and maintenance of the soil seed bank.

Conclusions

Didelphis albiventris can be considered a frugivore-omnivore, since fruit is an important item in the diet of both males and females, and was found in all fecal samples and all seasons of the year.

Didelphis albiventris can disperse large quantities of small endozoochorous seeds, increasing both germination percentage and germination velocity of some species, such as *Ficus cestrifolia* and *Psidium* sp. Many small seeds pass through the digestive tracts of these didelphids without being damaged, retaining germination viability and, consequently, resulting in dispersal of plant species between forest fragments. They therefore contribute to regeneration of forests and to maintaining the soil seed bank, both in conserved and in degraded areas, where specialized frugivores are often absent as a result of localized extinctions.

Since not enough is yet known about the mutualistic relationships between marsupials and plants, further studies are needed to better understand the germination dynamics of the many different plant species whose fruit attract this group of mammals. The role that these animals play in the process of seed dispersal, whether they act as occasional or effective dispersers, whether or not they damage the seeds and the part didelphids play in regeneration of damaged environments are just some of the subjects that merit investigation.

Acknowledgements

We are grateful to the research funding agency Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) for a bursary; to the Universidade La Salle, for providing the opportunity and infrastructure needed to carry out this research; and to everybody who helped, directly or indirectly, in carrying out this study.

Author Contributions

Susana de Oliveira Junges and Cristina Vargas Cademartori: substantial contribution to the concept and design of the study; contribution to data collection, data analysis and interpretation; contribution to manuscript preparation and critical revision.

Eduardo Périco: substantial contribution to data analysis and interpretation; contribution to manuscript critical revision.

Elisete Maria de Freitas and Sérgio Augusto de Loreto Bordignon: contribution to data collection, data analysis and interpretation; contribution to manuscript critical revision.

Guilherme Consatti: substantial contribution to data collection and data analysis.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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Received: 07/06/2017
Revised: 20/09/2017
Accepted: 24/11/2017
Published online: 07/12/2017