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ASPECTS ON THE BREEDING BIOLOGY OF THE GRACILE MOUSE OPOSSUM *GRACILINANUS MICROTARSUS* IN A SECOND GROWTH FOREST IN SOUTHEASTERN BRAZIL

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ABSTRACT

Nestboxes (n=36) were fixed to trees, 3m above the ground, in a second growth forest fragment in the State of São Paulo. Occupation by the gracile mouse opossum Gracilinanus microtarsus *has been examined for 30 months. This species built nests (n=15) made of leaves (means of 18.68 g and 147.1 leaves per nest) in the boxes. Nests presented a clean aspect, with no feces nor urine. Plant species selection for nest building was not observed. Likely, this species gathers any dry leaves present on the ground around the tree containing the nest; equally, some green leaves are taken from shrubs. Nests were constructed during the early rainy period (September to November). A female with young (8 to 12) attached to the teats was found in every examined nest. This study suggests that* G. microtarsus *is a solitary species and that nests in cavities are built only for breeding, not for shelter. Breeding period is strongly seasonal. Nestboxes can be important tools in studies examining aspects on natural history of mouse opossums.*

KEYWORDS: *Gracilinanus*, mouse opossum, nest, breeding biology, nestbox, second growth forest, Atlantic Forest

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Introduction

Mouse opossums are pouchless, prehensile-tailed marsupials and are among the most abundant, diversified and widespread of New World marsupials (Hunsaker, 1977; Nowak, 1991; Hershkovitz, 1992). They present arboreal or terrestrial habits (Ceballos, 1990; Passamani, 1995; Palma, 1996) and are nocturnal, solitary and omnivorous animals (Enders, 1935; Hunsaker, 1977; Nowak, 1991).

Their behavioral attributes make them difficult to study, placing mouse opossums among the lesser known species of the New World marsupials. Most information results from studies examining habitat utilization (Alho *et al.*, 1986; Bonvicino *et al.*, 1996), microhabitat selection (Nitikman and Mares, 1987; Simonetti, 1989), population ecology (Fleming, 1972; O'Connell, 1989; Lorini *et al.*, 1994; Mares and Ernest, 1995) and general biology (Enders, 1935; Hunsaker and Shupe, 1977; Nowak, 1991).

In relation to reproduction, mouse opossums present seasonal breeding cycles and females generally produce only one single litter per breeding season (Fleming, 1973; O'Connell, 1989; Lorini *et al.*, 1994). They build nests with leaves in cavities of trees and shrubs for shelter or to provide more protection to their young (Enders, 1935; Hunsaker and Shupe, 1977; Ceballos, 1990; Nowak, 1991). Information on nests building has been superficial.

The genus *Gracilinanus* is widely distributed throughout the forested or wooded areas of South America from the Caribbean coast of Colombia and Venezuela in the North, to the Delta del Rio Parana in Argentina (Hershkovitz, 1992). Despite this wide distribution, information on this genus is scarse and concentrates mostly on *Gracilinanus agilis* (Alho *et al.*, 1986; Nitikman and Mares, 1987; Mares and Ernest, 1995; Passamani, 1995). The gracile mouse opossum *Gracilinanus microtarsus* is the only species of this genus which occurs in the Atlantic coastal forests of southeastern Brazil (Hershkovitz, 1992). This is an scansorial species (Palma, 1996) and its biology is still little investigated. Thus, this study aims to research aspects of the natural history, primarily breeding biology, of *G. microtarsus* in a second growth forest fragment in the State of São Paulo.

Material and Methods

Study area

This study was carried out in the domain of the Atlantic Rain Forest, in southeastern Brazil. The region is classified as Cwb under the Köeppen system of climatic classification (Tubelis and Nascimento, 1975). The climate is characterized by low precipitation during the winter, with a dry season from April to August. The mean annual precipitation is approximately 1300mm, with

about 80% of this falling from September to March. February is the month with the most precipitation (mean of 239mm) and August presents the lowest level of precipitation (mean of 26mm). The monthly mean air temperature ranges from 16.3°C (July) to 21.9°C (January).

Field work was done in the "Fazenda Experimental Lageado", an experimental ranch of the Universidade Estadual Paulista, UNESP (campus of Botucatu), located in the State of São Paulo. On this ranch, elevations range from 675 to 775m. The study site (22°50'S, 48°25'W) is a 60 years old second growth forest fragment (50 ha) that is recovering on an abandoned *Eucalyptus* plantation. Now, this semideciduous forest presents an arboreal stratum dominated by *Piptadenia gonocantha* and by *Eucalyptus sp.* Most trees range from 20 to 25m in height. Fruiting trees are scarse and the understory is well developed. A road separate this forest from a mosaic (700 ha) of fragments presenting secondary and primary forests. They are of the same forest type of the study site and present a floristic inventory for arboreal species (Ortega and Engel, 1992).

Methodology

Nest boxes (n=36) were built with sections of giant bamboo, having an entrance hole and an inspection window (Figure 1). They were fixed to trees, at a height of 3m, with the entrance facing North. This height is situated in the range (0.70m to 5.0m) in which several nests of *M. canescens* were found in natural cavities in trees and shrubs (Ceballos, 1990). Enders (1935) installed a nest box about 2.5m above ground and also reported nest construction by *Marmosa*.

The boxes were spaced 70m from each other in a grid (6x6) of about 12.5 ha. Six box types were constructed to increase the probability of nestbox acceptance, not to examine preference for entrance hole. Boxes presented entrances of distinct diameters: 3.3, 4.0, 4.7, 5.0, 5.8 and 6.5cm. Six boxes of each type were built.

Diurnal inspections were made every two weeks from September 1991 to December 1993. During the last two breeding seasons the inspections were weekly. In the first breeding season, the females present in all boxes with nests were collected. I have observed that the manipulation and transport lead the females to abandon their nests when they were taken again to the forest. Thus, in the following breeding seasons, only half of the boxes containing nests were randomly selected and then brought to the laboratory. In December of each year, the material used in nest building was collected. Bird nests and invertebrate species, such as termites, ants, bees and wasps were also taken out of the boxes. For each nest, the number of leaves was counted and the total was weighed when dry.

Results

A total of 15 nests, made of leaves, was built in the nestboxes from September 1991 to December 1993. Examination of nine of them always resulted in the record of a female *Gracilinanus microtarsus* with a variable number of young attached to the mamae (Table 1). The young were of an early development stage, with no fur (Figure 2). One of the nests brought to the laboratory contained an adult with no young attached to the teats. However, two nights later, 12 young were born. This fact suggests that this species builds nests in cavities only for breeding and not for shelter.

All types of nestboxes were used (Table 1), suggesting that this species accepted the range of entrance size offered. However, the preference for entrance diameter was not investigated due to the low number of occupied boxes. The more frequent distance between nests was 140m (N=6). Other four nests (26.7%) were separated by only 70m from each other (Table 1), suggesting that this species may occur in high densities in this forest. In a single breeding season, the greatest number of occupied boxes was 6 (16.7%), resulting in a mean density of 0.5 nest/ha.

Feces and urine were never found in the occupied boxes. The nests presented a clean aspect, containing only leaves variable in form and size (Figure 3). The mean (±SD) number of leaves per nest was 147.1 ± 33.2, ranging from 96 to 188 leaves (Table 1). The mean (±SD) total weight of leaves in the nests was 18.68 ± 5.55g (range: 11.09 to 28.26 g). The leaves were arranged in such a manner that created a central camera used by the female for resting. The lateral and inferior sides of this camera were formed by several layers of leaves arranged side by side. On the other hand, the leaves situated in the highest positions were disordely arranged (Figure 3). They were about 6 to 13cm below the box entrance and covered the female.

It was not possible to identify the plant family of leaves. However, it was possible to separate them into groups. Distinct groups of leaves predominated in each nest, suggesting that this species collects any leaf around the tree, not selecting plant species. Some green leaves, often pieces of composite leaves, were found in the nests. This fact suggests that *G. microtarsus* collects dry leaves on the ground and occasionally gathers some from shrubs around the tree containing the nest.

Nest construction was seasonal, been restricted primarily to the period between September and November (Figure 4). In early December 1992, construction had begun on nest 9, but it was not finished. This nest presented only about 40 leaves. Probably, this was an unsuccessful attempt for nesting. It was observed that nest building was associated with the early rainy period

(Figures 4 and 5). This pattern suggests that females of this species breed within a period of 3 months (during the spring).

The relatively long interval between inspections, the presence of several leaves above the female, and the fact that females abandoned nests after manipulation of box made the estimation of the permanence period of the females in the boxes uncertain.

Discussion

No rodent nor marsupial species, other than *G. microtarsus*, was found in the nestboxes. This is the only *Gracilinanus* species that inhabits the Atlantic Forest in southeastern Brazil (Hershkovitz, 1992). Thus, I consider that *G. microtarsus* is responsible for all nests of leaves found in the boxes. The utilization of nestboxes by mammals for reproductive purposes has already been recorded (McComb and Noble, 1981; Rose and Walke, 1988; Morris *et al.*, 1990) and this is not the first record of a mouse opossum breeding in nestboxes. A female *Marmosa robinsoni* with 13 young was found in a nestbox in a Venezuelan forest (O'Connell, 1979). In the Panama Canal zone, *Marmosa isthmica* built nests with leaves in a nestbox fixed to a tree, but no young were recorded with the female (Enders, 1935). Similarly, in southeastern Brazil, nestboxes were used as shelter by *Caluromys philander* (Monteiro-Filho and Marcondes-Machado, 1996).

According to Hunsaker and Shupe (1977) and Nowak (1991), mouse opossums build nests in cavities for shelter and breeding. In this study, every time I verified the presence of *G. microtarsus* in a box, I found a female with young attached to the mamae. Thus, I suggest that *G. microtarsus* does not build nests in cavities for shelter, but only for breeding. In the laboratory, a birth of 12 young was observed. However, the litter sizes of other females were smaller, ranging from 8 to 11. This result is similar to that reported by Fleming (1972) who recorded an average litter size of 10 young (range: 6 to 13) for *Marmosa robinsoni*. O'Connell (1989) reported a mean litter size of 14 young (13 to 15) for *M. robinsoni*.

The greatest density of occupied nestboxes was 0.5/ha. However, the density of this species must be higher due to the presence of males and because natural sites may also have been used for nesting in this forest. Thus, the density of *G. microtarsus* might be near that reported by Fleming (1972) who estimated a density ranging from 0.31 to 2.25/ha for *M. robinsoni*.

The great quantity of leaves found in the nests is an observation similar to those recorded for *M. isthmica* (Enders, 1935) and *Marmosa canescens* (Ceballos,

1990). However, it differs slightly from records obtained by Ceballos (1990), in which dry leaves were lined with grasses and plant fibers, which were not observed in the nests of *G. microtarsus*. On the other hand, in the University of São Paulo Zoological Museum, there is a nest (MZUSP 3171) in which a female *G. microtarsus* with young was recorded. This nest, with a diameter of 20 cm, was built on a branching node and has a bulky basal structure made of fibers. In the superior and central region of this structure there is a chamber (diameter of 7 cm) containing grasses and thin shrub barks. However, the construction of this nest by *G. microtarsus* was not confirmed, as the female could be using an abandoned bird nest. It is likely that fibers found in the nests recorded by Ceballos (1990) provide stability for the arrangement of leaves, which is provided by the walls of the box in the nests described in Enders (1935) and the present study. *G. microtarsus* does not appear to select for plant families when gathering leaves for nest building. It probably collects any dry leaves from the ground and even some green leaves from shrubs around the tree containing the nest. It must, however, select leaves small enough to pass through the box entrance.

Hunsaker and Shupe (1977) suggested that there are probably all types of annual breeding cycles in mouse opossums. Some studies have reported that female mouse opossums breed only once a year (Enders, 1935), while other works have suggested that it is possible that females may produce more than one litter within a year (Tate, 1933; Fleming, 1973; Lorini *et al.*, 1994). In this study, female *G. microtarsus* were found to have young attached to the teats within a period of 3 months (September to November). Considering that young *M. robinsoni* are fully weaned at some 70 days postconception (Eisenberg, 1981) and assuming a rearing cycle similar to that of *M. robinsoni*, a female *G. microtarsus* could rear one or two litters during one breeding season. However, this was not determined because females were not marked. *G. microtarsus* presented no reproductive activity during the cold and dry season. Females with young were registered in the early rainy period. This seasonal pattern agrees with those of several other studies (O'Connell, 1989; Ceballos, 1990; Lorini *et al.*, 1994; Mares and Ernest, 1995) that reported mouse opossums breeding primarily within wet seasons.

Young were of an early development stage. I did not observe any young with fur in the nests, as was recorded for *M. robinsoni* females by Eisenberg (1981). In this study, females abandoned their nests before the end of the teat-attachment phase, that persists for some 28-30 days after birth (Eisenberg, 1981). Thus, female *G. microtarsus* remained in the nests for a shorter period of time than females *M. robinsoni*, that departed from the nest at about 60 days after giving birth (Eisenberg, 1981). This earlier nest departure by *G. microtarsus* females probably results primarily from the perturbation of inspections, although

other factors might be involved: the increasing difficulty to pass through the entrance hole as a result of the young's development; young should have great difficulty to leave the box by themselves during the 'nest phase' (Eisenberg, 1981) due to the slippery surface of the boxes; and/or avoidance of predators. The risk of nest predation may increase due to the development of the young. It is likely that the increased production of feces and urine by young would increase the possibility of nest detection by predators with high olfactory senses. Snakes, like *Bothrops jararaca* which was recorded once in an empty nestbox, probably encounter more difficulty in finding mouse opossum nests which contain clean leaves, as observed in this study.

In relation to box structure, *G. microtarsus* accepted all kinds of boxes constructed. Despite the difficulty in entering the boxes, due to the slippery surface of the bamboo, the rate of nest construction by *G. microtarsus* was 2.5 times greater than that presented by birds (*pers.observ.*), suggesting that the boxes are suitable for *Gracilinanus* nesting.

Conclusions

During 3 breeding seasons, the gracile mouse opossum, *Gracilinanus microtarsus*, built complete nests in 39% of the nestboxes provided. Only females with young attached to the teats were found in the nests. This fact suggests that *G. microtarsus* is a solitary species and that cavity nests are built only for breeding, and not for shelter. The nest building period was restricted to the period between September and November. Thus, breeding is seasonal, with young being born in the spring, during the early rainy season. It is likely that females produce one or two litters per breeding season. Nests contained only leaves and plant species selection for nest building was not observed. Nests are kept clean, showing no feces nor urine.

In comparison to traditional traps, that often capture only single males and females without young (O'Connell, 1979; Nitikman and Mares, 1987; Mares and Ernest, 1995; Palma, 1996), nestboxes proved to be an efficient methodology to capture females with young. Probably, the rate of nestbox occupation by this marsupial would be greater if the nestboxes were fixed to trees facing the trunk, as in Morris *et al.* (1990), due to an easier access to the nestbox entrance. Also, nestboxes are cheaper than traditional traps and can remain undeteriored in the field for 3 years. Further, nestboxes do not need to be transported several times to the field as occurs with traditional traps. Thus, the use of nestboxes should be considered in studies examining aspects on the natural history, primarily breeding biology, of mouse opossums.

Table 1. Relation of all *Gracilinanus microtarsus* nests built in the nestboxes, with the diameter of box entrance, number of young per female in examined nests, number of leaves per nest, weight of nest and the distance from the next nearest nest built in a box during the same breeding season in southeastern Brazil. The expression "female not caught" represents occupied boxes not brought to the laboratory.

Nest	Diameter (cm)	Young per female	Number of leaves	Weight of nest (g)	Distance (m)	Observation
1	4.7	10	-	-	140	Leaves were lost
2	5.8	10	-	-	140	Leaves were lost
3	3.3	9	147	13.93	350	
4	3.3	8	174	16.34	200	
5	5.8	-	153	21.84	70	Female not caught
6	4.7	12	136	13.75	200	Born in laboratory
7	5.0	9	111	15.24	140	
8	4.7	-	96	16.85	70	Female not caught
9	6.5	-	-	-	-	Incomplete building
10	5.8	11	167	18.15	140	
11	3.3	-	-	-	70	Female not caught
12	4.7	-	188	25.55	70	Female not caught
13	4.0	9	96	11.09	140	
14	6.5	9	172	28.26	140	
15	5.0	-	178	24.52	280	Female not caught

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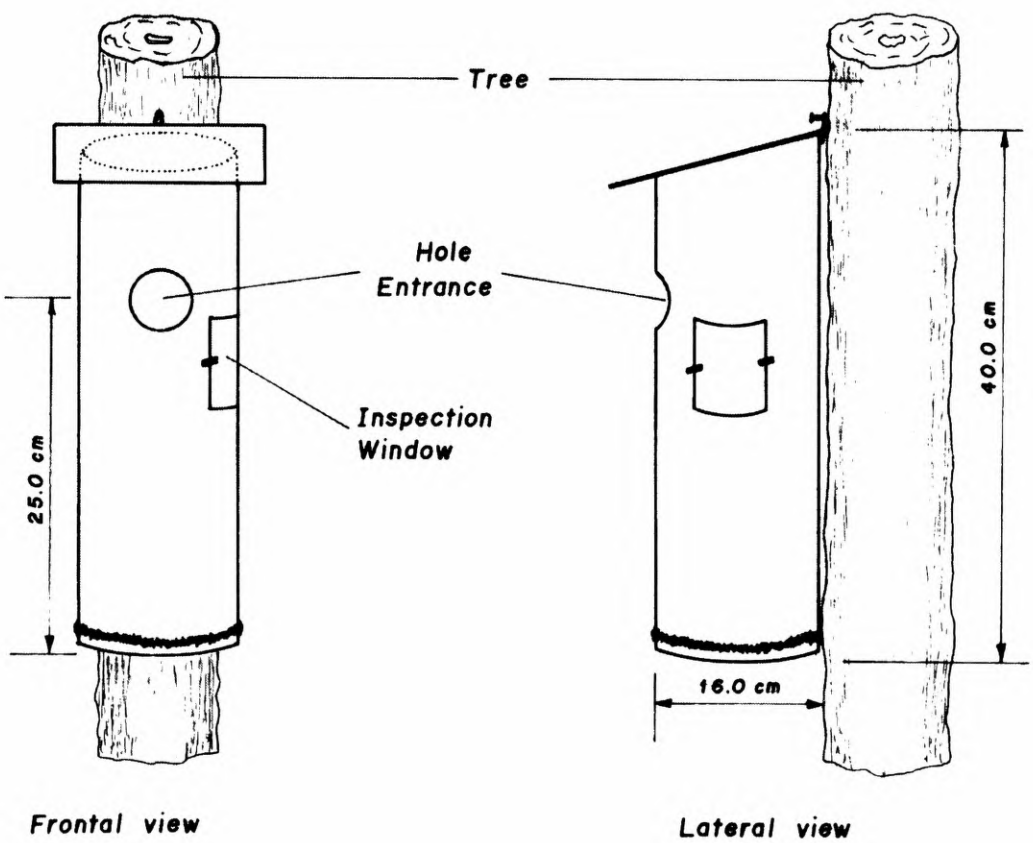

Figure 1. Nestbox constructed with sections of giant bamboo in which *Gracilinanus microtarsus* built nests for breeding.

Figure 2. Female *Gracilinanus microtarsus* with young attached to the teats found in the nestboxes.

Figure 3. An open nestbox, showing the arrangement of leaves of a nest built by the gracile mouse opossum *Gracilinanus microtarsus*.

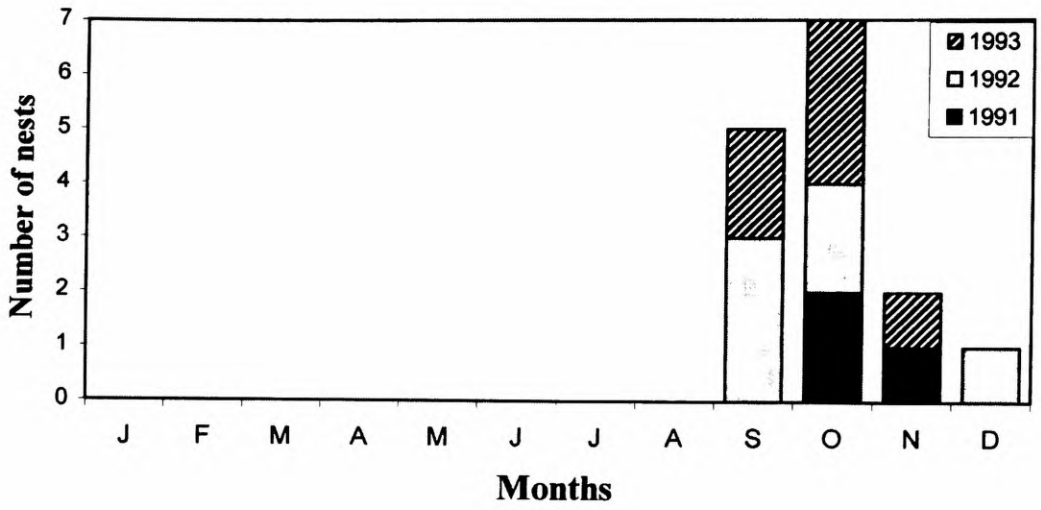

Figure 4. Nest building period of the gracile mouse opossum *G. microtarsus* in the study site, during 3 consecutive years. Construction of the nest made in December was incomplete.

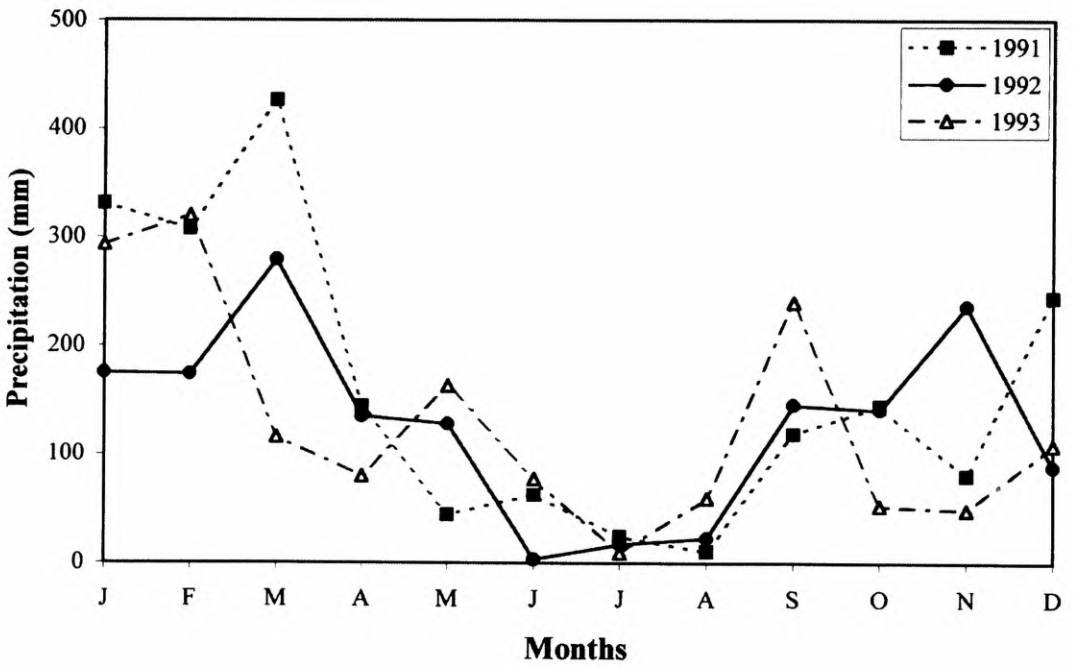

Figure 5. Monthly rainfall during 3 consecutive years in the study site.

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