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ORIGINAL ARTICLE

Trajectory assessment of the vulnerable marsupial *Dromiciops gliroides* in the Patagonian temperate forest

Laila D. Kazimierski[1] · Nicolás E. Catalano[2] · Karina Laneri[3] · Agustina Balazote Oliver[4] · Gabriela Calzolari[4] · Julien Joseph[5] · Guillermo C. Amico[4] · Guillermo Abramson[1]

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Abstract

We studied the movement of the marsupial *Dromiciops gliroides* by means of an innovative approach that makes use of two complementary techniques, specifically devised for the monitoring of small animals that live in dense forests. Despite playing a key role in the Southern Temperate Forest of Patagonia, very little is known about the ecology and habitat use of this "almost threatened species" (according to IUCN), the only living representative of an entire order (Microbiotheria). We present here novel results about the movement and explored area of *D. gliroides* at different spatio-temporal scales using complementary approaches: the spool-and-line and a radiotelemetry techniques. Both are complemented in such a way that, while the first one allows to obtain trajectories at small spatial scale with very precise resolution for relatively short periods, the second one provides longer temporal records at larger spatial scales adding temporal resolution. We show in this work very precise nocturnal trajectories unknown so far of 41 individuals of *D. gliroides* using spool and line, analyzing several of their statistical properties. For instance, from the turning angle distribution we find that, after release, the animals followed paths that exhibited little angular deviation between steps. In a complementary way, using radiotelemetry, we were able to study the velocity distribution of their movements, assessing a most probable value of 2.0 ± 0.8 m/min and a median value of 7.2 ± 2.8 m/min. The combination of both approaches brings new opportunities for studying other cryptic and poorly known forest dwellers.

Keywords *Dromiciops gliroides* · Habitat use · Radiotelemetry technique · Spool-and-line technique · Southern Temperate Forest

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✉ Laila D. Kazimierski
lailakazimierski@gmail.com

[1] Consejo Nacional de Investigaciones Científicas y Técnicas, Centro Atómico Bariloche-Comisión Nacional de Energía Atómica (CNEA), Instituto Balseiro - Universidad Nacional de Cuyo - CNEA, R8402AGP Bariloche, Argentina

[2] Centro Atómico Bariloche-Comisión Nacional de Energía Atómica (CNEA), Instituto Balseiro - Universidad Nacional de Cuyo - CNEA, R8402AGP Bariloche, Argentina

[3] Consejo Nacional de Investigaciones Científicas y Técnicas, Centro Atómico Bariloche-Comisión Nacional de Energía Atómica, R8402AGP Bariloche, Argentina

[4] Consejo Nacional de Investigaciones Científicas y Técnicas, INIBIOMA, Universidad Nacional del Comahue, 8400 Bariloche, Argentina

[5] École Normale Supérieure de Lyon, Université Claude Bernard Lyon I, Université de Lyon, 69342 Lyon Cedex 07, France

Introduction

The study of animal movement attempts to analyze the complexity of a behavior that usually arises from the interaction between the individual and the environment (Turchin 1998). In general, movement depends on the animal's internal state such as satiation and reserves, on interactions with others (either of their own or other species), and on previous experiences (Nathan et al. 2008). From an ecological point of view, these movements determine the redistribution of the population over space, and thus have the potential to affect many ecological processes (Kareiva and Wennergren 1995). For the many animals that move to collect food, their trajectories depend strongly on the spatial arrangement of resources. In some cases, these animals play an important role in mutualistic interactions, both as pollinators or seed dispersers of the plants they visit. In the case of keystone species their study becomes very relevant, particularly when human activities such as habitat fragmentation and the introduction of exotic species may change habitat structure, potentially compromising survival or at least altering their behavior (Fontúrbel and Salazar 2016).

The field study of individual movement presents a variety of challenges, in particular when the species of interest inhabits a dense forest where GPS positioning is not reliable because of the attenuation of the signal produced by the vegetation cover. Such is the situation with our species of interest, the arboreal marsupial "monito del monte" (*Dromiciops gliroides*-Microbiotheriidae), the only living species of an entire order, endemic of the Southern Temperate Forest of Patagonia (between 36 °S and 43 °S), see Gurovich et al. (2015). Their nocturnal habits and small size make the field work even more difficult. This animal plays a key role in the forests, since it is able to determine the spatial distribution of at least 16 species of plants, including the mistletoe "quintral" (*Tristerix corymbosus*-Loranthaceae) (Amico and Aizen 2000), which is an important winter resource of the hummingbird (*Sephanoides sephanoides*-Trochilidae), the main pollinator of the forest. The marsupial *D. gliroides* maintains a mutualistic relationship with *T. corymbosus*: they benefit by feeding on the pulp of the fruit, while the plant benefits from the dispersion of its seeds to new places where it can be established (Carlo and Morales 2008; Morales and Carlo 2006; Amico et al. 2017). Hence the relevance of knowing how *D. gliroides* moves in its habitat: their behavior affects the population dynamics of the plants by providing the seed dispersal services (Carlo et al. 2007; Sasal and Morales 2013), largely determining the spatial structure of future generations of plants. Correspondingly, this distribution will affect the movement of many animals feeding on their fruits.

Its phylogenetic uniqueness, as well as the keystone role that *D. gliroides* plays in the forest, makes their preservation a high priority for the conservation of biodiversity (Amico and Aizen 2000; Rodríguez-Cabal et al. 2007). It has been classified as "almost threatened" by the International Union for Conservation of Nature (IUCN 2017[1]). In addition, in Argentina it has been categorized as "vulnerable" (Díaz and Ojeda 2000) and the Argentine Ministry of Environment and Sustainable Development has declared it as "insufficiently known species" (resolution 1030/04 (2005)), and it has the same conservation status in Chile (Fontúrbel et al. 2012). However, little is known about their spatial use of the habitat. Even more, the impact that human activities can have on their population is not fully understood given the scarce data available on its spatial ecology.

In particular, space use at the scale of individuals has not been assessed before in Argentina for *D. gliroides*. To a large extent, this is due to three main facts: they are nocturnal, adult average weight is 30 g and they live in dense forests where GPS signal is screened to the point of making this technique unsuitable to assess a trajectory.

One way to record the movement of small animals is the spool-and-line technique (Miles et al. 1981; Boonstra and Craine 1986), which allows a precise and accurate description of their trajectories. This technique consists in attaching a spool of thread on the back of the individuals and, as they move, the thread unwinds and locks onto the different substrates through which the animal moves. By tracking the thread we can reconstruct the trajectory of each individual. The spool-and-line technique has been used to estimate movement patterns of small mammals (Broughton and Dickman 1991; Onoyama and Saitoh 1991; Hawkins and MacDonald 1992; Mendel and Vieira 2003), including several species of marsupials of Brazil (Loretto and Vieira 2008; Forero-Medina and Vieira 2009; Prevedello et al. 2009, 2010; Prevedello and Vieira 2010), as well as in amphibians (Tozetti and Toledo 2005) and reptiles (Hernández 2010). The method is inexpensive (less than USD 1 per spool) and does not require specialized equipment. It provides detailed information of movement at small scales, which for this particular study is extremely important, and could have a relevant application in conservation-related studies (Steinwald et al. 2006). Furthermore, combined with other observations, it can provide information on the behavior of the animal, including arboreal activity, the use of habitat characteristics (trunks, holes and burrows) and foraging behavior, among others (Glen and Cruz 2009).

Despite its benefits, the spool-and-line technique has certain limitations. On the one hand, this technique allows to monitor the movement of the animal only on a relatively small spatial scale, since the length of the thread is limited by the weight that the animal can carry. In the case of *D. gliroides*, for example, it would not be possible to monitor movements of the order of days using this technique. In addition, the spool-and-line technique does not provide temporal information. As a consequence, while we can study the animals' movements and the substrates through which they moved, we do not know when or for how long they moved.

Thus, it is convenient to complement this technique with another one. One technique that can cope with those limitations and provides a tool for monitoring animals that are difficult to observe directly is radiotelemetry (Mennill et al. 2012; Gottwald et al. 2019). Several studies using different implementations of radiotelemetry already showed some success in tracking small animals. Such are the cases of "tuco tuco de Río Negro" (*Ctenomys rionegrensis*-Ctenomyidae) (Tassino et al. 2011) where the use of space is studied, and the study of the home range and daily movement of "Laotian rock rat" (*Laonastes aenigmamus*-Diatomyidae)

[1] https://www.iucnredlist.org/species/6834/22180239.

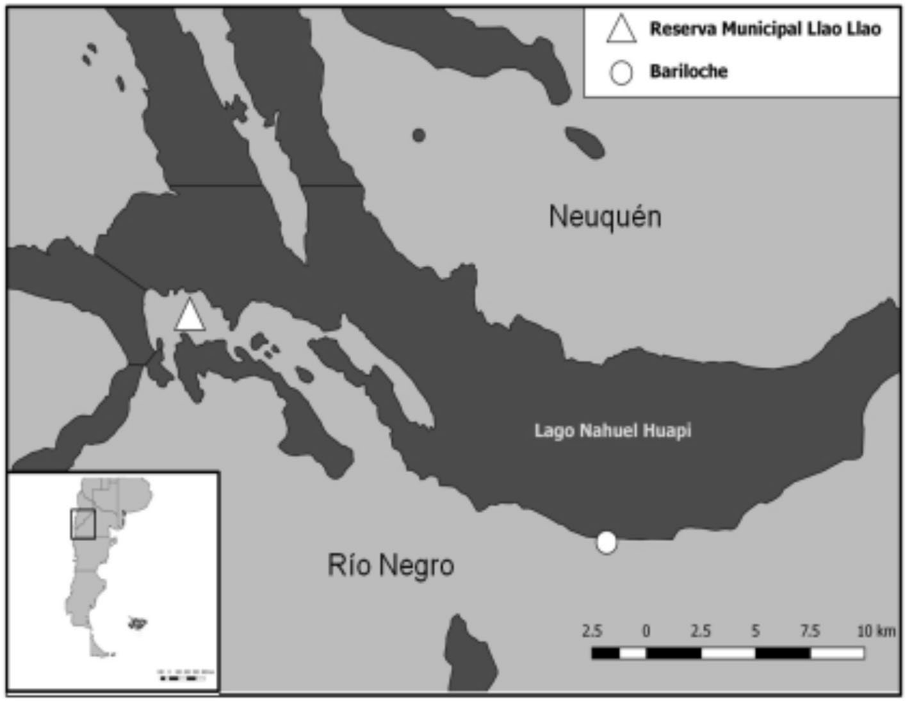

Fig. 1 Our study was carried out in an 1 ha area of old growth native forest of *N. dombeyi*, with the undergrowth dominated by *A. chilensis* and *C. culeou*. The area is close to the Nahuel Huapi lake in west Argentina, South America. Altogether, 43 individuals of *D. gliroides* were captured with Tomahawk traps to study their movement patterns

(Khotpathoom et al. 2020) and of *D. gliroides* in Chile (Fontúrbel and Salazar 2016; Magrach et al. 2015).

An example of radiotelemetry implementation in animal tracking consists of measuring the power of pulses emitted by a radio transmitter attached to the animal. Usually, traditional radio receivers are handheld devices and the tagged animals are monitored primarily while the researchers follow them with an antenna (Amlaner and MacDonald 1980). This method may show to be useful to track animals that can be observed without perturbing them, as can be the case of big animals. However, in the case of small species it is not easy to track them with mobile receivers without modifying their behaviour. Another example of radiotelemetry implementation consists of obtaining the direction of arrival of the emitted pulses by the transmitter attached to the animal at different locations and then triangulate the transmitter position (Rivarola 2009). This method involves moving to different locations and registering the direction of arrival of the signal. Nevertheless, moving at night in a dense forest without disturbing the animal under study presents many difficulties.

Our implementation of radiotelemetry does not require mobile receivers but static receiver stations instead. Three stations measure simultaneously the received power at each one of the pulses emitted by the transmitter attached to the animal. That power is associated with a distance between the transmitter (the animal) and each receiver station, i.e. as the animal with the attached transmitter moves away from a station, the received power at that station decays. Using a calibration method, it is possible to fit a correspondence between received power and distance transmitter-receiver. Finally, the estimated position of the animal at each time is calculated through the trilateration of the radial distances between each receiver station and the transmitter as described with more detail in Materials and methods. While sophisticated and expensive radiotelemetry systems had been developed (Kays et al. 2011; Weiser et al. 2016) we present here a low cost system (under USD 150 per station), that employs off the shelf equipment.

In the present study, we describe the movement of *D. gliroides* at two different scales using these two very different and complementary techniques: we used the spool-and-line technique to record the trajectories with very precise spatial resolution and radiotelemetry to monitor the visited extension on a larger spatial scale with temporal resolution. In this sense, our work complements techniques innovating in the monitoring of small animals that live in dense forests, which allows us to characterize properties of their movement and use of space determining daily home ranges, movement velocity and turning angles.

Materials and methods

Study site

We carried out field work in the Llao Llao Municipal Park located 30 km west from the city of San Carlos de Bariloche, Rio Negro, Argentina (41°8′ S, 71°19′ W) (see Fig. 1). The area is located within the Southern Temperate Forest, inside the biogeographical region of the Sub-Antarctic Province (Mermoz and Martin 1986; Cabrera 1976; Morrone 2015). The climate in this area is cold temperate, with a dry season in spring-summer and a humid season in autumn-winter. On average, only 12% of the annual precipitation (1800 mm) falls during summer (December–February) and snowfalls are common during winter. The annual average temperature is 9°C, and the average temperature of the warmest month (January) is around 15°C, and that of the coldest month (July) is approximately 3°C (Mermoz and Martin 1986; Barros et al. 1983).

Dominant canopy trees are the coihue (*Nothofagus dombeyi*-Nothofagaceae) and the patagonian cypress (*Austrocedrus chilensis*-Cupressaceae). The undergrowth includes 15 woody species but is dominated by the colihue cane (*Chusquea culeou*-Poaceae, 25% of the shrub cover), the maqui (*Aristotelia chilensis*-Elaeocarpaceae, 30% of the cover), the chin-chin (*Azara microphylla*-Salicaceae) and the maitén (*Maytenus boaria*-Celastraceae) (Mermoz and Martin 1986; Amico et al. 2017). Two forest layers are well differentiated with tree canopy reaching up to 40 m in height and the understory reaching up to 5 m in height. Branches in the forest are mostly available within the first 2 m above the ground; they are relatively thin (< 1.5 cm in diameter), and slightly over half of them belonging to *A. chilensis*. The canopy cover is commonly more than 50% (Amico et al. 2017; Mermoz and Martin 1986).

Fig. 2 Tomahawk traps (30 cm×14 cm×14 cm) in the forest used to capture individuals of *D. gliroides*, located at a height of between 1 and 2 m on branches of trees and shrubs, primed with banana and apple

Spool-and-line technique

We used the spool-and-line technique to register the movement of individuals of *D. gliroides* at the scale of the microsite (Johnson 1980). As mentioned, this technique provides information about habitat use and, in addition, it is also possible to recover the animal's path following the thread through the vegetation.

To capture the individuals we used Tomahawk traps (30 cm×14 cm×14 cm) in the forest, located at a height of between 1 and 2 m on branches of trees and shrubs, primed with banana and apple (see Fig. 2) (Balazote Oliver 2017; Calzolari 2013). Two sets separated by 150 m of 25 traps each were used to capture the individuals. The captures were made during the months of January to April since during those months of summer and early autumn the individuals are most active due to the increase in temperature. We placed 50 traps during 64 nights in those months of the years 2011, 2012, 2014 and 2015 with a sampling effort of 3200 night/traps. Among all the captured individuals during the sampling seasons, we selected 41 individuals of *D. gliroides* to monitor their movement using spools of threat placed on them (Balazote Oliver 2017; Calzolari 2013). The 41 individuals were adults of sufficient size and weight so that the spool did not hinder their movement. All capture and handling methods were performed according to the American Society of Mammalogists rules (Sikes and Gannon 2011) and approved by the Llao Llao Municipal Reserve's authorities and the Province of Río Negro state officials (Municipal Resolution N° 215-DAP-18 and Provincial Resolution N° 1635).

The dimensions of the used spools are 3.5 cm × 1.2 cm, containing 100 m of thread and weighing about 2.5 g (Danfield ®Cotton Cocoon Bobbins) (see Fig. 3, left). These spools are assembled in such a way that the thread unwinds easily from the center and does not require the spool to spin. The animals had their fur trimmed on their back and spools were adhered with cyanoacrylate contact adhesive. The glue residue disappeared a few days later; some of the individuals that had been placed with the spool were recaptured in subsequent campaigns and they presented good health conditions and did not show evidence of the placement of the spools of thread except for the trimmed fur. The animals were released at the same place where they were captured, with the tip of the thread secured to a branch. As the animal moves, the thread is locked at different points of the substrate recording its movement very precisely (Moura et al. 2005). Later, the trajectories of the threads left by the individuals of *D. gliroides* were followed. These trajectories were discretized as vectors of displacement associated with changes of direction. Distances were measured with both a measuring tape and a laser rangefinder (Bosch DLR165) with a resolution of ±1 cm. The changes in direction were measured with a magnetic compass set on the thread and

Fig. 3 Individual of *D. gliroides* with the spool (left) and the transmitter (right) adhered to the back

registering the deviation from magnetic north. Subsequently, the trajectories were plotted using the distance and turning angle distributions for their visualization and mathematical characterization.

Despite that the spool-and-line technique has a very high spatial resolution, it does not provide temporal information and can not be used to track large movement extensions of *D. gliroides*. To overcome these limitations we complemented this technique with radiotelemetry, which is explained below.

Radiotelemetry technique

We implemented radiotelemetry to gauge the movement of two individuals of *D. gliroides* in March 2018 and 2019: we measured the power of a signal emitted by a radio transmitter attached to them. To capture the individuals, we used the same Tomahawk traps already described and the transmitter was glued with cyanoacrylate on the animal's back after trimming its fur (see Fig. 3 right panel). Once the fur grows again, the transmitter detaches and is lost. We used Telenax model TXA-004G and ATS model A2426 transmitters. Both weigh less than 1 g (authorized for species such as *D. gliroides*), measure 1 cm and have flexible antennas of about 15 cm long. These transmitters emit periodic pulses of approximately 20 ms, every 2 or 4 seconds (it depends on the brand and model) at frequencies close to 150 MHz. Once they are activated (connecting two wires in the case of the Telenax or removing a magnet in the case of the ATS ones) the pulses continue for the duration of the battery, which can last up to two months, depending on the model.

To receive the transmitted pulses we developed a low cost reception system consisting of an arrangement of three fixed receiver stations, each one with an omni-directional antenna (Fig. 4, left) tuned to 150 MHz (Stewart et al. 2015), an RTL-SDR receiver (Fig. 4, top right) and a portable computer (see connection diagram in Fig. 4, bottom right). We selected three georeferenced sites for placing the reception stations based on accessibility through the forest and coverage of the studied area, and which were used repeatedly on each measurement session. The three stations receive, display and record the radio pulses using the SDR# software[2]. Notably, this system allows to monitor simultaneously the movement of more than one animal provided their transmitters have different frequencies and lay within the receptor's bandwidth. The measurements were made during the night, since the animal has more activity than during the day (Di Virgilio et al. 2014). It was part of the measurement protocol to arrive at the forest with the last hours of light, in order to set-up the equipment with daylight and start the measurements with darkness. The monitoring times varied between 1 and 2 h each night, depending on the autonomy of the portable computers.

We began measuring from the three stations simultaneously: we received the emitted pulses by the transmitter at each station and recorded those pulses throughout the night until the autonomy of the computers allowed it. Given that the coordination of the beginning of the measurements may not be exact, to provide a common time reference among the stations that would later allow us to align the received pulses, we transmitted a short signal with a portable radio just after beginning the recording. The frequency of this pulse is different from that of the transmitter but close enough to be acquired by the receivers.

[2] Available for free download from: https://airspy.com/download.

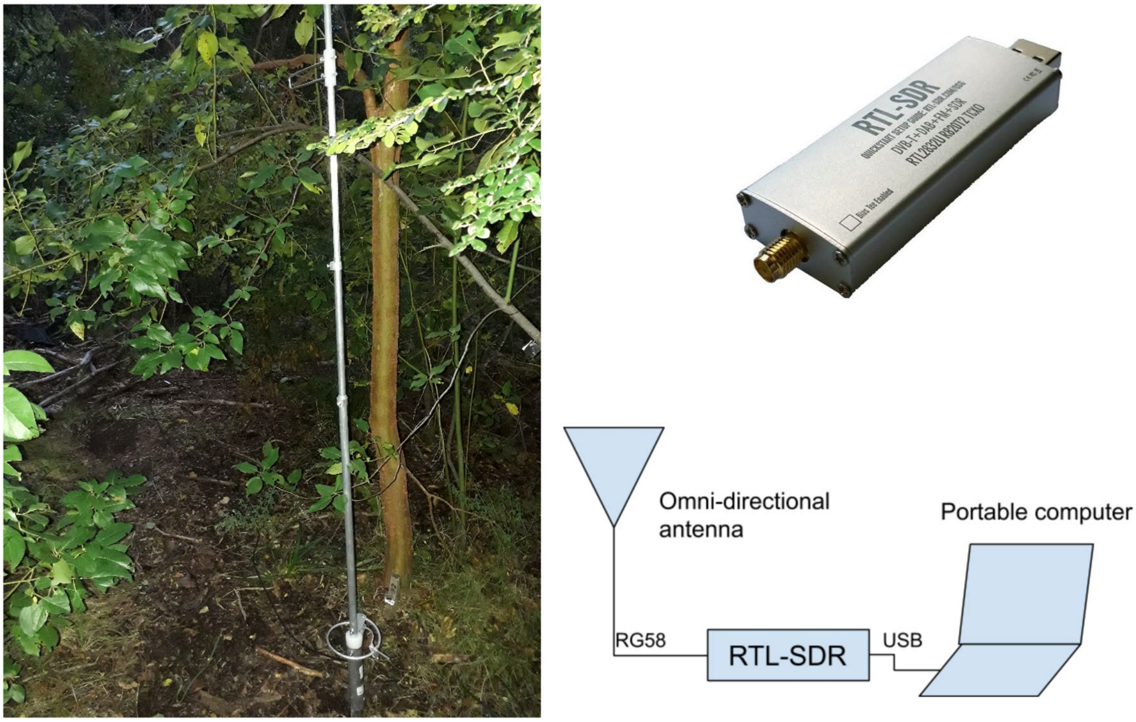

Fig. 4 Equipment of each receiver station: omni-directional antenna located in the forest used to receive 150 MHz radio-frequency signals emitted by the transmitter adhered to the animal (left) and an RTL-SDR receiver (top right). We included a connection diagram of the equipment used at each reception station (bottom right)

The received power at each station depends on the distance between that station and the transmitter. In open space the power received by an antenna decays with distance (r), as r^{-2}. However, in the experiments carried out in the intricate environment of the forest, we observed that this dependence is not strict and that the function that best fits the relation between power and distance is different in this environment. It is possible, nevertheless, to perform a calibration of the functional relation of power with distance. This requires the measurement of pulses emitted from at least two points located at known distances from the antennas (Javaid et al. 2015; Oguejiofor et al. 2013). The calibration process will be described further in this section.

The signal processing was done afterwards in the lab. We synchronized the recordings of the power received by the three stations using the reference signal emitted by the portable radio. In order to reduce the signal-noise ratio we first used a band-pass filter, whose central frequency matches one of the monitored animals. We then implemented an algorithm to identify the transmitted pulses taking advantage of their characteristics: minimum and maximum length, and minimum separation between them. As a result, we obtained a two-column data file with the time of occurrence of each pulse since the beginning of the recording and the detected power. Considering that the pulse is not instantaneous but has a certain duration, we calculated the detected power as the mean value of the squared samples within each pulse.

Due to the dispersion of the received power produced by the heterogeneous attenuation of the signal in the forest, we did not calculate the position of the animal using every recorded pulse of the transmitter. Instead, we considered the maximum value of power obtained within a temporal window of 1 min, considering that *D. gliroides* would move less than our estimated resolution during that period. Subsequently, we converted that maximum power value into distance using the calibration. If at least one of the stations did not have detected pulses for a specific time window, no position was calculated for that window.

The calibration process consisted of recording the received power as a function of the distance between the transmitter and the reception station. To accomplish this, we placed a transmitter at three georeferenced distances from the receiver station recording pulses for 1 min. This temporal window was chosen to be able to identify a power value representative of each distance. Given that the received pulses during the minute of measurement showed some dispersion in power, we chose the pulse with maximum value, assuming that it is the closest to the real one assuming that the influence of the channel conditions such as attenuation of the signal, multi-path fading and shadowing effects, tend to reduce the received power (Javaid et al. 2015). Then, having chosen the maximum value of power

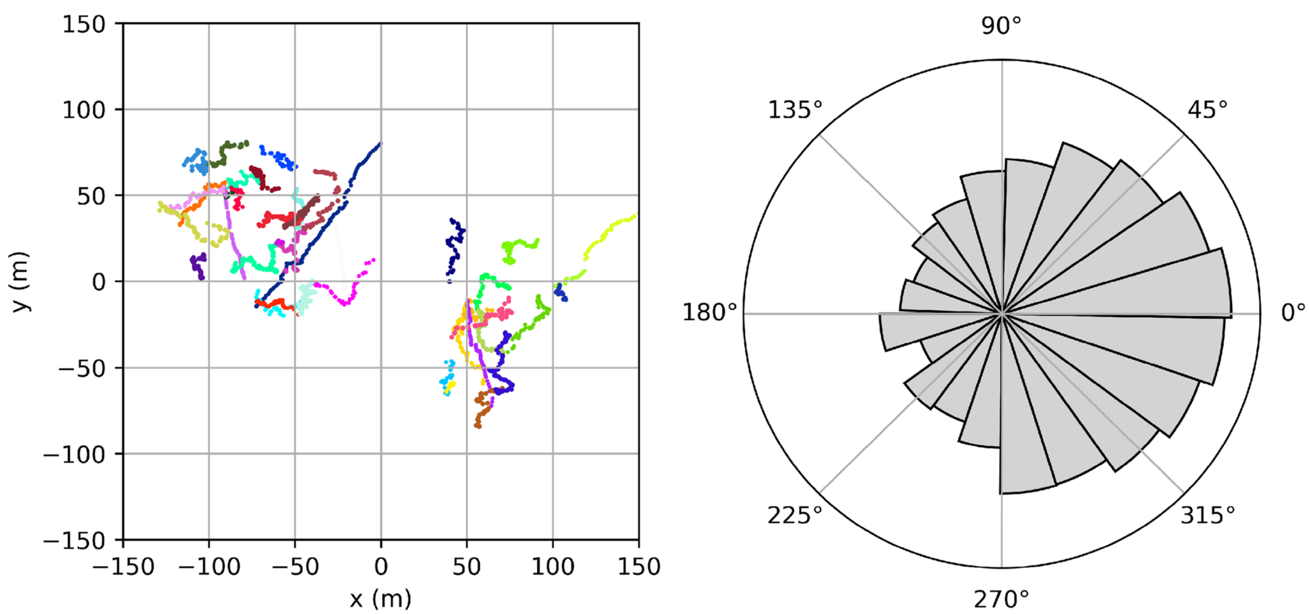

Fig. 5 Left: Trajectories of 41 individuals tracked with the spool-and-line technique. The length of each thread is 100 m. It can be seen that the trajectories appear in two regions of the grid. This is because the individuals were released in the same place where they were captured, and the traps were placed in two sets separated by 150 m. Right: Distribution of the turning angle corresponding to all the trajectories assessed with spool-and-line technique. The radius of the histograms corresponds to 20% of the turning angles measured from all trajectories

received per minute from the three distances at the receiver station, we can associate a distance to each of the antennas.

Since the antennas of each receiver station are omni-directional, each distance measured from a station represents a radius around the corresponding antenna. The position of the individual at each time is calculated by trilateration, i.e. the intersection polygon defined by the three circles (each with its corresponding antenna as its center) (Javaid et al. 2015). The position value is calculated using the method of least squares. By repeating this procedure at each measurement time we obtain an approximate trajectory.

We characterized the error of this technique during the calibration procedure. The procedure described above was repeated 5 times for 3 transmitter positions, including distant sites. The error was considered as the dispersion of the values between these measurements. We took the maximum dispersion obtained as a unique error value, estimated in 7 m radius. It is important to notice that the dispersion of the maximum power is smaller than or equal to the dispersion of all power measurements, but we chose to be conservative by taking the whole dispersion range as measurement error.

Results

Spool-and-line technique

We obtained trajectories of 41 individuals, with a diversity of shape and tortuosity, ranging from about 10 to 50 m on the ground (see their two-dimensional projections in Fig. 5, left). We analyzed the distribution of the turning angles of those trajectories, finding that the animals usually move away from the release point following steps with little angular deviation from the previous one (see Fig. 5, right).

Furthermore, when reconstructing the trajectories we observed that *D. gliroides* uses a great diversity of substrates to transit (see Fig.6). Most of the observations were on the maqui (*A. chilensis*) both on living and dead branches. Other frequently used substrates were the cane (*C. culeou*) and coihues (*N. dombeyi*).

Fig. 6 Segments of threads of spools placed on individuals of *D. gliroides*. We include segments of thread on the substrate *A. chilensis* (left) and *C. culeou* (right). The light colored ovals over the branch of *C. culeou* (right panel) are *T. corymbosus* seeds dispersed by *D. gliroides*. Following the thread in the forest is possible to reconstruct their trajectories

Radiotelemetry technique

We analyzed the trajectories of two individuals of *D. gliroides* monitored for three consecutive nights (Figs. 7 (top and bottom) corresponding to March 2018 and March 2019, respectively).

Even if the points are connected to show the temporal evolution, it should be kept in mind that there may be intervals in which we do not consider any pulse as useful. Using the dispersion of the data during the preliminary calibrations and measurements (see Materials and methods section), and considering that we know the release position exactly (C3), we estimate the error of each point at 7 m in radius (shown in the figures as opacity shades of the corresponding Gaussians). Just as a visualization, Figs. 7 (top right and bottom right) show one of these trajectories (during 15 min of measurement on the night of March 19 of 2018 and during 1 h 52 min on the night of March 10 of 2019, respectively).

The radiotelemetry technique, at variance with the spool-and-line one, provides temporal information, which can be used to derive a velocity of the animals. We analyzed the distribution of the modulus of velocities taking into account only the trajectory points separated by, at most, 5 min (see Fig. 8). Fitting a log-normal function to the distribution of velocities we get a mode of 2.0 ± 0.8 m/min (the most probable value of the velocity) and a median of 7.2 ± 2.8 m/min. Such a skewed distribution is to be expected if the animal tends to avoid spending much time still, to evade predators for example. Besides the fitted log-normal, it is interesting to notice the existence of three local maxima of the velocity, at approximately 2 m/min, 6 m/min and 11 m/min. These values could correspond to different displacement regimes, related to markedly different behaviors, e.g. feeding, exploration and escape or other fast transit. The feature is robust against the change of the binning of the histogram as well as the criterion to filter the trajectory points.

We also analyzed the convex hulls corresponding to all the trajectories (see Fig. 9). These hulls are, in our case, the smallest convex polygons enclosing all the points of the trajectories and were determined using the Python function ConvexHull of the library *scipy.spatial*. The averaged area visited was estimated in 82 ± 32 m^2. Comparing the areas and the corresponding time spent, it is interesting to notice that the marsupial seems to explore the same area during consecutive nights (see Fig. 9). For instance in 2019, the animal spent 2 h exploring the same region every night (see Fig. 9 light grey region). A similar behavior was observed in the trajectories of the individual monitored during 2018 (see Fig. 9 dark grey region).

Conclusions and discussion

We presented two complementary techniques which allow to monitor the movement of small animals living in a dense forest where GPS tracking is of low reliability: spool-and-line and radiotelemetry techniques. The spool-and-line technique allows to record the movement quite precisely, as well as to assess exactly on which vegetation stratum the animal moved (Cunha and Vieira 2002). We applied this technique to 41 individuals of *D. gliroides*, a species of great ecological relevance in the Patagonian South Temperate Forest. We observed that most of the trajectories of *D. gliroides* were on the maqui (*A. chilensis*), and other frequently used substrates were the cane (*C. culeou*) and coihues (*N. dombeyi*).

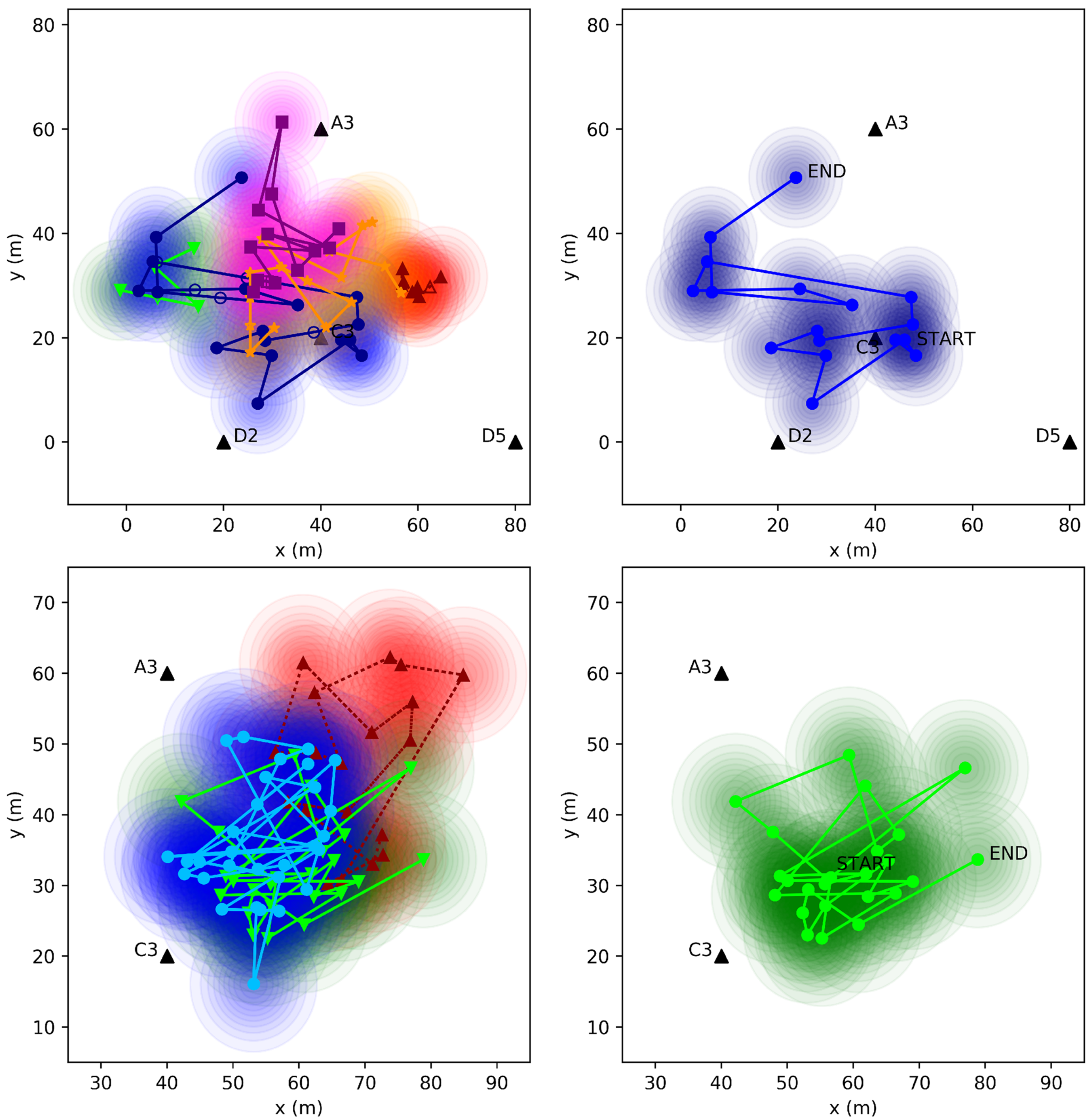

Fig. 7 Top. Left: All the trajectories recorded in 2018 assessed by radiotelemetry. The average duration of the trajectories was of 15 min around midnight of March 18, 19 and 20, 2018. Each color corresponds to a different trajectory, and shadows represent an error with a standard deviation of 7 m around each position. D2, D5 and A3 are the georeferenced points where the reception stations were placed and C3 is the place where the animals were released. Right: One of the trajectories shown at left, recorded on March 19. Bottom. Left: All trajectories recorded in 2019 assessed with radiotelemetry technique. The total average time was of 3 h around midnight of March 9, 10 and 11. Each color corresponds to a different trajectory, and shadows represent an error with a standard deviation of 7 m around each position. D2, D5 and A3 are the georeferenced points where the reception stations were placed and C3 is the place where the animals were released. In this case D2 and D5 are out of the plot area. Right: One of the trajectories, corresponding to a register made on the night of March 10, 2019, from 21:25 to 23:17. In this case D2 and D5 are out of the plot area

We were able to conclude that, after release, the animals moved away from the release point following paths with steps with little angular deviation from the previous one (see Fig. 5, right). Some of our trajectories are similar to those found for other marsupial movement studied with the spool-and-line technique (Ríos-Uzeda et al. 2019), but we can't

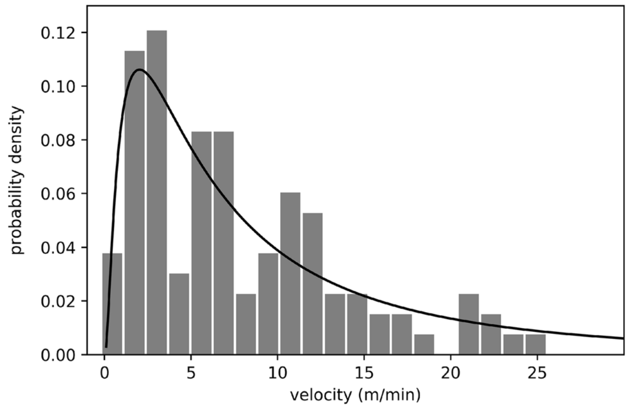

Fig. 8 Probability density of the absolute value of the velocity, calculated from all the trajectories recorded in 2018 and 2019 assessed by radiotelemetry. The black line corresponds to a log-normal fit to the histogram, $P(v) = (v\sigma\sqrt{2\pi})^{-1}\exp(-(\ln v - \mu)^2/(2\sigma^2))$, with parameters $\mu = 1.98 \pm 0.24$, $\sigma = 1.13 \pm 0.14$, corresponding to a mode of 2.0 ± 0.8 m/min, a median of 7.2 ± 1.7 m/min

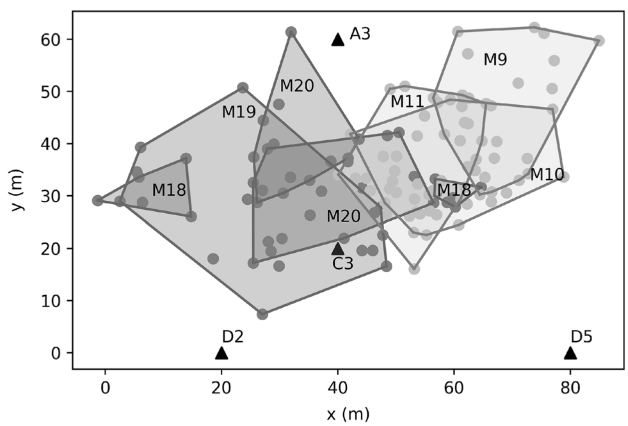

Fig. 9 Convex hulls for all trajectories assessed by radiotelemetry in 2018 (dark grey areas) for one individual and 2019 (light grey areas) for another individual. Areas were visited during different periods, i.e., for year 2018: March 18 (M18 right: 7 min and M18 left: 4 min), March 19 (M19: 21 min) and March 20 (M20 up: 12 min, M20 down: 14 min); for year 2019: March 9 (M9: 2 h 3 min), March 10 (M10: 1 h 52 min), March 11 (M11: 3 h 28 min). D2, D5 and A3 are the georeferenced points where the reception stations were placed and C3 is the place where the animals were released

conclude that there is an absolute predominant direction for the whole trajectory (see Fig. 5, left).

The patterns of rectilinear movements are usually repeated in many mammals at the time of release (Ramos-Fernandez et al. 2004; Razafindratsima et al. 2014). The less tortuous and more linear routes suggest that the individual is only crossing an area, while the more tortuous routes indicate that the individual is using it more intensively, for example, foraging (Nams and Bourgeois 2004). At the time of release, linear movements predominate as individuals may have an escape behavior. It is possible that the animals are trying to find their bearings in the forest. Given that the length of thread is limited by the weight of the animal, in our case this technique can only register short trajectories, insufficient to determine the home range of *D. gliroides*. It also lacks a temporal scale of the deployment of the thread, so it does not provide an estimate of the time during which *D. gliroides* explore its environment. The trajectories were originally registered in three dimensions, but they are effectively quasi-two-dimensional because *D. gliroides* moves preferentially at heights between 1 and 2 m (Balazote Oliver 2017; Calzolari 2013). We presented here their two-dimensional projections at ground level in order to confront them with the radiotelemetry measurements.

The radiotelemetry technique, on the other hand, can provide complementary information. The aforementioned techniques are not only complementary but also compatible with their simultaneous implementation provided that the animal weight allows it. The radio measurements allow to determine the position of the animal at precise times. Besides, the recording can continue for days or weeks, depending on the lifetime of the battery of the transmitter, allowing to measure the movement of the animals during several nights (long after their manipulation) as well as to identify their refuges during daytime. This kind of observations can be used to compute values such as the home range size, the habitat use on a large spatial scale and the speed of movement.

We found that *D. gliroides* moves with a most probable speed of 2.0 ± 0.8 m/min and a median of 7.2 ± 2.8 m/min. These values are in agreement with those previously reported in this same area, determined with infrared cameras (Di Virgilio et al. 2014). We can estimate that in a few hours *D. gliroides* can explore almost all its home range. We can suppose that, as a result of such exploration, *D. gliroides* has to know very well its environment. Indeed, it is not infrequent to recapture the same individuals after releasing them far from their initial capture place. The multimodal structure of the velocity histogram suggests different regimes of movement, something that should be further analyzed with additional data in the future.

We found that *D. gliroides* remains within a Daily Home Range (DHR) of 82 ± 32 m^2. This value is smaller than the one measured by (Fontúrbel et al. 2009) for this species in Chile as well as for other marsupial species of a weight similar to *D. gliroides* (30 g). For instance, (Leiner and Silva 2007), using the spool-and-line technique, estimated *Marmosops paulensis* DHR (weight 39 g) in 4000 m^2. Besides, for the rodent *Rhabdomys pumilio* (weight 40 g) the DHR is approximately 1500 m^2 (Schradin and Pillay 2005). The small DHR found for *D. gliroides* in this work could be related to the fact that the study was done in the season with more availability of resources. However, the reduction of DHR could also be related to an increasing predation pressure exerted by domestic animals. It is important to notice that even if the study was done within a protected area, it is surrounded by roads and houses at approximately 200 m, and domestic cats were seen within the DHR of *D. gliroides*. It is also worth noting that *D. gliroides*' DHR is embedded in a touristic area that might be producing some disturbance, therefore reducing the area visited by the animals. Further investigation during several seasons will allow us to test these hypotheses.

Let us also make a few considerations regarding the role of *D. gliroides* as a seed disperser. The time of digestion, the home range size, the speed and the movements of *D. gliroides* are key factors in the dispersion of seeds, in particular those of *T. corymbosus*, which relies on their ingestion to germinate and be deposited on a suitable host (Amico et al. 2017). Given that the time of the seed passing through the digestive tract is half an hour (Balazote Oliver 2017), and the results shown in this work with respect to the home range, we conclude that the average dispersion distance would be consistent with that reported by (Morales et al. 2012).

As an arboreal animal, *D. gliroides* needs the dense forest to move and browse for its resources, an activity that translates into seed dispersion. A change in the traits induced by human activity could significantly affect the dispersion modality and threaten the plant subsistence. For these reasons, the conservation status of *D. gliroides* should not only be based on their own population decrease, but also on the change of their habits and their impact on the potential survival of many species of plants.

We believe that the movement behavior of *D. gliroides* deserves deeper investigation. On the one hand, we need a better characterization of the statistical properties of the trajectories, including a better estimation of the home range, the recurrence times and the role of vertical displacements. These properties could give us clues on the mechanisms behind search strategies or other behaviors. For this, it would be helpful the use of dynamical models of movement, a step on which we are working now. On the other hand, a more extensive study in time would be necessary, in order to probe the eventual spatial structure of seed dispersal, beyond what we can derive from our current data. For instance, *D. gliroides* acts as a seed disperser for numerous species of plants within the Southern Temperate Forest (Amico et al. 2009) and this role is essential for the conservation of the biodiversity of Patagonia's forests (Rodríguez-Cabal et al. 2007). By combining data of the movement of *D. gliroides* with the seed dispersal distances and with a map of the forest structure we will be able to study the spatial dynamics of the Southern Temperate Forest in an integral way. The integration of mathematical modeling and simulations with the spatial distribution of the resource can shed light on factors determining seed dispersion and the successful persistence of *T. corymbosus*. Besides, future research will allow to study little known aspects of the ecology of this species, such as the location of their nests and the social structure of their communities.

As implemented during the field campaigns of 2018 and 2019, our system is still affected by instrumental issues with an impact on the errors in the determination of the individuals' position. These shortcomings can nevertheless be improved by upgrading the material at our disposal (mainly the antennas and receivers), and streamlining the measurement protocol. One of the limitations presented by monopole antennas such as the ones we used is that the received power depends on the polarization of the transmitted signal. This can be overcome to a good extent by better antenna designs. We have already built and tested cloverleaf antennas, optimized to have an axial ratio close to 1 at the frequency of the transmitters. Future work will implement their use in the field.

Further analysis of the data collected during 2019 is also still underway, and will be reported elsewhere. Future studies should also attempt to evaluate the role of potential intraspecific interaction between individuals on their distribution and use of space, and to test hypotheses about the resource dynamics and other behavioral mechanisms. Future work will also include the design of dynamical models of movement incorporating all characteristics found, to better understand the behavior of this marsupial. Their strong dependence on densely vegetated habitats makes them particularly sensitive to human disruptions of the habitat, such as fragmentation through deforestation and other activities. In this regard we believe, as (Fontúrbel et al. 2009), that the key role played by *D. gliroides* makes them a very relevant species for the conservation of the Patagonian forest. Additional knowledge of this ecosystem will certainly strengthen the conservation efforts.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Amico G, Aizen MA (2000) Mistletoe seed dispersal by a marsupial. Nature 408:929–930

Amico GC, Rodríguez-Cabal MA, Aizen MA (2009) The potential key seed-dispersing role of the arboreal marsupial *Dromiciops gliroides*. Acta Oecol 35:8–13

Amico G, Sasal Y, Vidal-Russell R, Aizen M, Morales J (2017) Consequences of disperser behaviour for seedling establishment of a mistletoe species. Austral Ecol 42:900–907

Amlaner CJ, MacDonald DW (1980) Handbook on biotelemetry and radio tracking. Pergamon Press, Oxford

Balazote Oliver A (2017) Efectos del comportamiento de dispersores en la dinámica de plantas: Selección de hábitat y patrones de movimiento del monito del monte (*Dromiciops gliroides*) y sus efectos en la dinámica poblacional de la planta parásita aérea *Tristerix corymbosus*. Ph. D. thesis, Universidad Nacional del Comahue

Barros VR, Cordón V, Moyano C, Méndez R, Forquera J, Pizzio O (1983) Cartas de precipitación de la zona oeste de las provincias de Río Negro y Neuquén

Boonstra R, Craine ITM (1986) Natal nest location and small mammal tracking with a spool and line technique. Can J Zool 64:1034–1036

Broughton SK, Dickman CR (1991) The effect of supplementary food on home range of the southern brown bandicoot, *Isoodon obesulus* (Marsupialia: Peramelidae). Aust J Ecol 16:71–78

Cabrera AL (1976) Enciclopedia argentina de agricultura y jardinería: Regiones fitogeográficas argentinas. ACME, Buenos Aires

Calzolari G (2013) Uso de micro-hábitats por el monito del monte (*Dromiciops gliroides*) y posibles consecuencias para la dinámica del quintral (*Tristerix corymbosus*). Biology degree thesis, Universidad Nacional del Comahue

Carlo TA, Morales JM (2008) Inequalities in frugivory and seed dispersal: consequences of bird behaviour, neighbourhood density and landscape aggregation. J Ecol 96:609–618

Carlo T, Aukema J, Morales JM (2007) Plant-frugivore interactions as spatially explicit networks: Integrating frugivore foraging with fruiting plant spatial patterns. Seed dispersal: theory and its application in a changing world, CABI, Wallingford, UK, chap 6:369–390

Cunha AA, Vieira MV (2002) Support diameter, incline, and vertical movements of four didelphid marsupials in the Atlantic Forest of Brazil. J Zool 258:419–426

Di Virgilio A, Amico GC, Morales JM (2014) Behavioral traits of the arboreal marsupial *Dromiciops gliroides* during *Tristerix corymbosus* fruiting season. J Mammal 95:1189–1198

Díaz GB, Ojeda RA (2000) Libro Rojo de los mamíferos amenazados de la Argentina. Sociedad Argentina para el Estudio de los Mamíferos, SAREM, Mendoza, Argentina

Fontúrbel FE, Salazar DA (2016) Beyond habitat structure: landscape heterogeneity explains the monito del monte (*Dromiciops gliroides*) occurrence and behavior at habitats dominated by exotic trees. Integr Zool 11:413–421

Fontúrbel FE, Silva-Rodriguez EA, Cárdenas NH, Jiménez JE (2009) Spatial ecology of monito del monte (*Dromiciops gliroides*) in a fragmented landscape of southern Chile. Mamm Biol 75:1–9

Fontúrbel F, Franco M, Rodríguez-Cabal MA, Rivarola MD, Amico GC (2012) Ecological consistency across space: a synthesis of the ecological aspects of *Dromiciops gliroides* in Argentina and Chile. Naturwissenschaften 99:873–881

Forero-Medina G, Vieira M (2009) Perception of a fragmented landscape by neotropical marsupials: effects of body mass and environmental variables. J Trop Ecol 25:53–62

Glen DRA, Cruz J (2009) An improved method of microhabitat assessment relevant to predation risk. Ecol Res 25:311–314

Gottwald J, Zeidler R, Friess N, Ludwig M, Reudenbach C, Nauss T (2019) Introduction of an automatic and open-source radiotracking system for small animals. Methods Ecol Evol 1:1–10

Gurovich Y, Stannard HJ, Old JM (2015) The presence of the marsupial *Dromiciops gliroides* in Parque Nacional Los Alerces, Chubut, Southern Argentina, after the synchronous maturation and flowering of native bamboo and subsequent rodent irruption. Revista Chilena de Historia Nat 88:17

Hawkins CE, MacDonald DW (1992) A spool-and-line method for investigating the movements of badgers. Mammalia 56:322–325

Hernández I (2010) Desplazamientos de la serpiente de cascabel *Crotalus catalinensis* (Viperidae) a través de su ciclo anual, en la isla Santa Catalina, Golfo de California, México. Bachelor's thesis

Javaid R, Qureshi R, Enam RN (2015) RSSI based node localization using trilateration in wireless sensor network. Bahr Univ J Inf Commun Technol 8:2

Johnson DH (1980) The comparison of usage and availability measurements for evaluating resource preference. Ecology 61:65–71

Kareiva P, Wennergren U (1995) Connecting landscape patterns to ecosystem and population processes. Nature 373:299–302

Kays R, Tilak S, Crofoot M, Fountain T, Obando D, Ortega A, Kuemmeth F, Mandel J, Swenson G, Lambert T, Hirsch B, Wikelski M (2011) Tracking animal location and activity with an automated radio telemetry system in a tropical rainforest. Comput J 54:1931–1948

Khotpathoom T, Vu TT, Bhumpakphan N, Sukmasuang R, Bumrungsri S (2020) Using radiotelemetry to identify the home range and daily movement of a living fossil: the Laotian rock rat (*Laonastes aenigmamus*). Mamm Biol 100:377–384

Leiner NO, Silva WR (2007) Seasonal variation in the diet of the Brazilian slender opossum (*Marmosops paulensis*) in a montane Atlantic forest area, southeastern Brazil. J Mammal 88(1):158–164

Loretto D, Vieira M (2008) Use of space by the marsupial *Marmosops incanus* (Didelphimorphia, Didelphidae) in the Atlantic Forest. Mammalian Biology - Zeitschrift für Säugetierkunde 73(4):255–261

Magrach A, Rodríguez-Pérez J, Piazzon M, Santamaría L (2015) Divergent effects of forest edges on host distribution and seed disperser activity influence mistletoe distribution and recruitment. J Ecol 103(6):1475–1486. https://doi.org/10.1111/1365-2745.12472

Mendel SM, Vieira MV (2003) Movement distances and density estimation of small mammals using the spool-and-line technique. Acta Theriol 48(3):289–300

Mennill DJ, Doucet SM, Ward KAA, Maynard DF, Otis B, Burt JM (2012) A novel digital telemetry system for tracking wild animals: a field test for studying mate choice in a lekking tropical bird. Methods Ecol Evol 3(4):663–672. https://doi.org/10.1111/j.2041-210X.2012.00206.x

Mermoz M, Martin C (1986) Mapa de vegetación del Parque y la Reserva Nacional Nahuel Huapi, vol 22. Secretaría de Ciencias y Técnica de la Nación

Miles MA, de Souza AA, Póvoa MM (1981) Mammal tracking and nest location in Brazilian forest with an improved spool-and-line device. J Zool 195(3):331–347

Morales JM, Carlo TA (2006) The effects of plant distribution and frugivore density on the scale and shape of dispersal kernels. Ecology 87:1489–1496

Morales J, Rivarola MD, Amico GC, Carlo TA (2012) Neighborhood effects on seed dispersal by frugivores: testing theory with a mistletote marsupial system in Patagonia. Ecology 93:741–748

Morrone JJ (2015) Biogeographical regionalisation of the Andean region. Zootaxa 3936:207–236

Moura MC, Caparelli AC, Freitas SR, Vieira MV (2005) Scale-dependent habitat selection in three didelphid marsupials using the spool-and-line technique in the Atlantic forest of Brazil. J Trop Ecol 21:337–342

Nams VO, Bourgeois M (2004) Fractal analysis measures habitat use at different spatial scales: an example with American marten. Can J Zool 82(11):1738–1747

Nathan R, Getz WM, Revilla E, Holyoak M, Kadmon R, Saltz D, Smouse PE (2008) A movement ecology paradigm for unifying organismal movement research. Proceedings of the National Academy of Sciences 105(49):19052–9. https://doi.org/10.1073/pnas.0800375105, https://www.pnas.org/content/105/49/19052

Oguejiofor OS, Okorogu VN, Adewale A, Osuesu BO (2013) Outdoor localization system using RSSI measurement of wireless sensor network. Int J Innovat Technol Explor Eng (IJITEE) 2:2

Onoyama K, Saitoh T (1991) Spool-and-line tracking of the vole *Clethrionomys rufocanus* bedfordiae, with special reference to its home range. Honyurui Kagaku 30:131–142

Prevedello JA, Vieira MV (2010) Plantation rows as dispersal routes: A test with didelphid marsupials in the Atlantic Forest. Biol Conserv 143:131–135

Prevedello JA, Rodrigues RG, Monteiro ELA (2009) Vertical use of space by the marsupial *Micoureus paraguayanus* (Didelphimorphia, Didelphidae) in the Atlantic Forest of Brazil. Acta Theriol 54:259–266

Prevedello JA, Rodrigues RG, Monteiro ELA (2010) Habitat selection by two species of small mammals in the Atlantic Forest Brazil: comparing results from live trapping and spool-and-line tracking. Mammal Biol 75:106–114

Ramos-Fernandez G, Mateos JL, Miramontes O, Cocho G, Larralde H, Ayala-Orozco B (2004) Lévy walk patterns in the foraging movements of spider monkeys (*Ateles geoffroyi*). Behav Ecol Sociobiol 55:223–230

Razafindratsima OH, Jones TA, Dunham AE (2014) Patterns of movement and seed dispersal by three lemur species. Am J Primatol 76:84–96

Rodríguez-Cabal MA, Aizen MA, Novaro AJ (2007) Habitat fragmentation disrupts a plant-disperser mutualism in the temperate forest of South America. Biol Conserv 139:195–202

Ríos-Uzeda B, Brigatti E, Vieira M (2019) Lévy like patterns in the small-scale movements of marsupials in an unfamiliar and risky environment. Sci Rep 9:2737

Rivarola MD (2009) Interacción entre un muérdago y un marsupial: estructura poblacional y área de acción de *Dromiciops gliroides*, y caracterización de la remoción de frutos de *Tristerix corymbosus*. Bachelor's Thesis, Universidad Nacional del Comahue, Bariloche, Río Negro, Argentina

Sasal Y, Morales JM (2013) Linking frugivore behavior to plant population dynamics. Oikos 122:95–103

Schradin C, Pillay N (2005) Demography of the striped mouse (*Rhabdomys pumilio*) in the succulent karoo. Mammal Biol 70(2):84–92

Sikes RS, Gannon WL (2011) Guidelines of the American Society of Mammalogists for the use of wild mammals in research. J Mammal 92:235–253

Steinwald MC, Swanson BJ, Waser PM (2006) Effects of spool-and-line tracking on small desert mammals. Southwestern Nat 51:71–78

Stewart R, Crockett L, Atkinson D, Barlee K, Crawford D, Chalmers I, McLernon M, Sozer E (2015) A low-cost desktop software defined radio design environment using MATLAB, Simulink, and the RTL-SDR. IEEE Commun Mag 53:64–71

Tassino B, Estevan I, Pereira-Garbero R, Altesor P, Lacey E (2011) Space use by Río Negro tuco-tucos (*Ctenomys rionegrensis*): excursions and spatial overlap. Mammal Biol 76:143–147

Tozetti AM, Toledo LF (2005) Short-term movement and retreat sites of *Leptodactylus labyrinthicus* (Anura: Leptodactylidae) during the breeding season: A spool-and-line tracking study. J Herpetol 39(4):640–644

Turchin P (1998) Quantitative analysis of movement: Measuring and modeling population redistribution in animals and plants. Sinauer Associates, Sunderland, Massachusetts, USA

Weiser AW, Orchan Y, Nathan R, Charter M, Weiss AJ, Toledo S (2016) Characterizing the accuracy of a self-synchronized reverse-GPS wildlife localization system. In: 2016 15th ACM/IEEE International Conference on Information Processing in Sensor Networks (IPSN), pp 1–12