HISTORIA NATURAL Y DEMOGRAFÍA DEL MARSUPIAL ARBÓREO *DROMICIOPS GLIROIDES* EN BOSQUES NATIVOS PRIMARIOS Y DEGRADADOS DEL NORTE DE LA ISLA GRANDE DE CHILOÉ

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RESUMEN

En esta tesis se describen aspectos ecológicos y conductuales del marsupial endémico del bosque templado Monito del monte (*Dromiciops gliroides*) en hábitat de bosques y matorrales en un paisaje rural del norte de la Isla de Chiloé (42 S), Chile. Estudiamos los cambios y las consecuencias de la degradación antrópica del bosque sobre la abundancia poblacional, la demografía y rasgos de historia de vida de *D. gliroides* en parches de bosque antiguo, bosques expuestos a tala selectiva y matorrales como elementos más frecuentes de este paisaje rural.

Durante cuatro años (2005–2009), en la época reproductiva (noviembre) y no reproductiva (febrero-marzo), se estimó la densidad poblacional y parámetros demográficos utilizando la metodología de captura-recaptura en dos fragmentos de bosque antiguo y dos fragmentos de bosques con tala selectiva. Junto con ello, durante los dos primeros años, se monitoreó la presencia de individuos en dos hábitat de matorrales. Tanto en bosques antiguos como expuestos a tala selectiva, se caracterizó la vegetación y recursos alimenticios para *D. gliroides*. Además se comparó cambios en las estimaciones del ámbito de hogar, dieta (contenido fecal), tamaño corporal y carga parasitaria. Considerando todos los individuos capturados, se estudiaron aspectos reproductivos mediante análisis de hormonas, y a través del uso de cajas-nidos se describió la conducta de hibernación.

En bosques antiguos, la conducta de sopor grupal fue más frecuente (64.1%), constituyendo el primer registro de sopor comunal, siendo más frecuente en animales juveniles que en adultos. El ámbito de hogar de machos fue significativamente mayor que el de hembras independiente del tipo de manejo del bosque. Su dieta es omnívora, consumiendo principalmente artrópodos, sin embargo también con un alto contenido de frutos en individuos adultos en verano y en bosques antiguos. La proporción de insectos y frutos en el contenido fecal varió en forma proporcional a la disponibilidad en el ambiente. Ni el tipo de manejo del bosque ni el sexo afectaron la prevalencia de ectoparásitos, sin embargo se registró una mayor incidencia de ectoparásitos en individuos juveniles provenientes de bosques expuestos a tala selectiva. La carga parasitaria no afecto el peso ni la sobrevivencia de los individuos.

La tala selectiva modifica la estructura de la vegetación de los bosques. La cobertura del sotobosque y la densidad de troncos y árboles grandes fue mayor en bosques antiguos, mientras que la densidad del bambú nativo o quila (*Chusquea* spp.) y la abundancia artrópodos del sotobosque fue mayor en bosques con tala selectiva.

Se documentó la presencia de individuos de *Dromiciops gliroides* en todos los hábitat estudiados, pero su abundancia en matorrales fue considerablemente menor que en fragmentos de bosque. No se observaron diferencias en la abundancia poblacional entre bosques antiguos y con tala selectiva, pero se observó un patrón con mayores abundancias en el período de verano. Tanto en bosques antiguos como en bosques expuestos a tala selectiva no se encontraros diferencias en la sobrevivencia y reclutamiento poblacional, por ende se registraron tasas de crecimiento poblacional similares. Variaciones interanuales en la sobrevivencia y en el crecimiento poblacional podrían estar asociadas a variaciones en los recursos tróficos y condiciones ambientales. Los resultados permiten concluir que junto con la importancia de mantener remanentes de bosques antiguos, la tala selectiva como manejo tradicional

de bosques a pequeña escala, permite mantener poblaciones viables de *D. gliroides* en un paisaje antropogénico.

ABSTRACT

The ecology and behavior of the endemic marsupial Monito del monte (*Dromiciops gliroides*) was described in remnant temperate rain forest in a rural landscape of northern Chiloé Island (42 S), Chile. *D. gliroides* were studied in two remnant old-growth forest fragments, two selectively-logged forest fragments and two shrublands during four years (2005-2009).

We estimated and compared among habitat types: i) lifetime home range, ii) diet (through fecal content), iii) body size and iv) ectoparasitic load. We also characterized reproductive season, through reproductive hormones, and hibernation behavior using artificial nest boxes.

Dromiciops gliroides presence was found in all studied habitat types, but its abundance in shrublands was significantly lower than in forested habitat. Lifetime home range of males was significantly higher than females regardless of habitat types. *D. gliroides* is omnivorous, consuming mainly arthropods, but also with a high content of fruit in adult individuals in summer and in old-growth forest fragments. The proportion of insects and fruit in diet content varied according to its availability. Neither habitat types nor sex affected ectoparasites prevalence, however, there was a higher incidence in juveniles from selective-logged forest. The parasitic load did not affect animal weight of individuals.

Communal nesting was more frequently (64.1%) than solitary individuals, being the first record of communal hibernation, and being more common in juvenile than adults individuals.

Differences in vegetation structures and food resources as a result of forest degradation was described and its consequences on population density and demography of *D. gliroides* were assessed through capture-recapture procedures for four years (2005-2009), during the reproductive (November) and non-breeding (February-March) season between old growth and selective-logged forest patches as common elements of this rural landscape.

Selective logging altered the structure of forest vegetation. Canopy cover and density of understory and large trees was higher in old forests, while the density of native bamboo or quila (*Chusquea* spp.) and understory arthropod abundance was greater in selective-logged forest.

Population density did not differ between habitats however, there was a trend with higher densities in summer period. Demographic parameters did not differ between both habitats; hence there were similar rates of population growth. Interannual variation in survival and population growth could be linked to changes in trophic resources and environmental conditions. The results strongly suggest that along with the importance of remaining old growth forests, selective logged-forest such as a traditional small-scale logging, allows to maintain viable populations of *D. gliroides* in an anthropogenic landscape of northern Chiloé.

INTRODUCCIÓN

Actualmente, la pérdida mundial de la superficie de bosques, debido principalmente al cambio en el uso del suelo (e.g., expansión de la frontera agrícola y explotación forestal; Sala et al. 2000; FAO 2009), ha implicado una disminución del hábitat para muchas especies (Andren, 1994). El área de los bosques templados del sur de Sudamérica fue originalmente limitada por la presencia de la Cordillera de los Andes y el desierto del norte de Chile, quedando restringidos en gran medida a una estrecha faja en el margen oeste del continente al sur de los 35 grados (Hinojosa & Villagran 1997). La superficie cubierta por bosques templados entre los 35 y 42° S ha disminuido en aproximadamente un 51%, quedando sólo el 38% de la superficie remanente de estas regiones cubierta por bosques nativos y el resto cubierto por plantaciones (Lara & Veblen 1993). Esta reducción, sumado a la ausencia de una adecuada legislación y manejo de los bosques nativos, ha llevado a que junto con la actividad forestal industrial de gran escala, fragmentos remanentes de bosque, sean explotados por los propietarios para extracción local de manera y leña, por lo que el paisaje original se ha transformado en un mosaico de remanentes de bosques antiguos y jóvenes, junto con grandes áreas de plantaciones de especies exóticas, además de extensos matorrales, cultivos y praderas (Willson & Armesto 1996; CONAF–CONAMA–BIRF 1999; Echeverria et al. 2007). Este escenario podría representar una amenaza para muchas poblaciones de especies de vertebrados restringidos a los hábitats de bosques nativos (Acosta-Jamett & Simonetti 2004), lo que se manifiesta en que muchos de ellos han sido incluidos en alguna categoría de amenaza tanto a nivel nacional como internacional (Glade 1993; www.redlist.org).

El Monito del Monte (*Dromiciops gliroides*), una especie restringida a los bosques templados del sur de Sudamérica (Hershkovitz 1999) es una de las especies más carismáticas y, sin embargo, menos estudiadas en su hábitat natural (Redford & Eisenberg 1992; Kelt 2000). Este marsupial es considerado un fósil viviente, ya que es el único representante vivo del Orden Microbiotheria, un Orden monotípico de una línea filogenéticamente primitiva de los mamíferos a nivel mundial (Reig, 1955; Palma & Spotorno 1999). *Dromiciops gliroides* posee un hábito arborícola, frecuentemente asociado a fragmentos de bosque nativo con denso sotobosque, mayoritariamente constituido por quila o bambú (*Chusquea* spp.) de 2-10 m de alto, y con un dosel ocupado por gran diversidad de elementos epífitos y enredaderas (Mann 1955; Patterson et al. 1990; Hershkovitz 1999). *D. gliroides* nidifica en cavidades de árboles que fueron abandonadas por otras especies, o construye nidos inmersos en el denso sotobosque de quila, para hibernar y reproducirse (Mann 1955). Por esta razón, la presencia de árboles con cavidades y de sotobosque denso podrían ser elementos claves del hábitat para asegurar el éxito de la reproducción y sobrevivencia invernal de esta especie.

Dromiciops gliroides posee un hábito trófico insectívoro-omnívoro, ya que consume una gran variedad de artrópodos, especialmente coleópteros (Mann 1955; Meserve et al. 1988). También consume frutos, por lo que algunos autores lo consideran un importante dispersante de las semillas de la planta hemiparásita *Tristerix corymbosus* (Loranthaceae) y de otras especies epífitas de las familias Gesneriaceae, Philesiaceae y arbustos del genero *Gaultheria* (Ericaceae) (Armesto et al. 1987; Salinas 2008; Rodríguez-Cabal et al. 2007; Amico et al. 2009). Ocasionalmente, depreda huevos y polluelos de *Elaenia albiceps*, *Aphrastura spinicauda* y *Troglodytes musculus* (Jiménez & Rageot 1979; Cornelius 2007).

Estos atributos de la historia de vida de *Dromiciops gliroides*, los que están fuertemente asociados al hábitat de bosques nativos (Mann 1978; Jiménez & Rageot, 1979, Kelt & Martínez 1989), hacen de esta especie un interesante sujeto de estudio para evaluar las consecuencias de la pérdida de cobertura y degradación de bosques por acción antrópica.

En el paisaje rural, sus poblaciones podrían quedar limitadas exclusivamente a los remanentes de vegetación nativa (Patterson et al. 1990; Saavedra & Simonetti 2005). Sin embargo, estos remanentes de bosque son comúnmente degradados a través de la tala selectiva o “floreo”, que modifica la estructura y composición del bosque posiblemente afectando la viabilidad de las poblaciones de *Dromiciops* en los paisajes fragmentados. Además, *Dromiciops gliroides*, está categorizado por la legislación chilena como “inadecuadamente conocido”, debido a la falta de información ecológica y por las actuales tasas de pérdida de su hábitat lo que implica que se considera en la categoría vulnerable o en peligro de extinción, (D.S. N° 151, 2007). A nivel mundial, *D. gliroides* integra la lista de especies con problemas de conservación, clasificada como “casi amenazado” (Díaz & Teta 2008; véase en www.redlist.org).

La degradación de los bosques remanentes, en paisajes rurales del sur de Chile, actúa como un proceso invisible a las estimaciones de cobertura forestal debido a las tradicionales prácticas de tala selectiva, floreo, tala, roce o quemas (Armesto et al. 2009) y la remoción del sotobosque para la protección del ganado (Willson et al. 1994). En el caso de los bosques templados de Chile, se estima que el 62% de la superficie remanente de bosques nativos (entre Maule y la región de Los Lagos, 35 - 42° S) se encuentra en distintos estados de degradación (i.e., en las condiciones denominadas renoval, adulto-renoval y achaparrado; según CONAF–CONAMA–BIRF 1999). Una de las formas de degradación más común es la tala selectiva, la que involucra la remoción selectiva de los árboles maduros, alteración de la estructura de la vegetación por apertura de claros, aumentos de la cobertura de quila, desecación del hábitat y pérdida de diversidad y biomasa de epifitas (Laurance 1996; Lindenmayer et al. 1997; Díaz et al. 2005; Pérez et al. 2009; Díaz et al. in press). Algunos elementos estructurales considerados "críticos" para especies de vertebrados están relacionados con sitios de alimentación, refugio y nidificación (Stenseth 1980; Tews et al. 2004). La pérdida de estas estructuras (e.g., árboles de gran tamaño, troncos muertos, presencia de sotobosque, y elementos epífitos) sumada a la modificación de los recursos tróficos (i.e., flores, frutos e invertebrados) contribuyen a cambiar la calidad del hábitat, modificando así los procesos demográficos y las abundancias poblacionales de muchas especies que dependen de la presencia de estos elementos estructurales y tróficos (Raphael & White 1984; Saunders et al. 1991; Cornelius 2007). Mamíferos arborícolas, tales como *D. gliroides*, pueden ser particularmente vulnerables a la pérdida de estructuras y recursos tróficos del bosque nativo ya que, como propondré, la densidad del sotobosque, la presencia de grandes árboles e indirectamente de cavidades y también la abundancia de frutos carnosos e insectos serían determinantes para la persistencia de esta especie en los fragmentos de bosques remanentes del paisaje rural. En consecuencia, postulo que la degradación de los remanentes de bosque por efectos de la tala selectiva podría afectar negativamente las poblaciones de *Dromiciops gliroides* en estos ambientes.

Se ha documentado que la tala de árboles y la remoción del sotobosque en bosques fragmentados del norte de la Isla de Chiloé han afectado la riqueza y abundancia de aves, alterando la abundancia de sus presas (Reid et al. 2004), y que la presencia de una matriz de matorrales y praderas restringe su capacidad de dispersión y colonización de nuevos hábitats (Willson 2004), confinando los individuos a fragmentos de bosque de reducido tamaño y de menor calidad de hábitat (Willson et al. 1994; Sieving et al. 1996; Willson 2004; Díaz et al. 2005; Cornelius 2007; Castellon & Sieving 2006 a, b). En bosques tropicales de Madagascar, Australia y sudeste de Brasil, la pérdida de árboles de gran tamaño y cambios en cobertura del dosel, lianas y sotobosque a consecuencia de la explotación maderera ha afectado negativamente las abundancias de poblacionales de aves y mamíferos, incidiendo directamente en su densidad y éxito reproductivo (Laurance 1990; Laurance 1996; Aleixo 1999; Gentile & Fernández 1999; Quental et al. 2001; Rendigs et al. 2003; Pardini 2004; Viveiros de Castro & Fernández 2004; Watson et al. 2004; Lambert et al. 2005; Bragagnolo et al. 2007).

Varios autores argumentan que las actuales superficies de áreas silvestres protegidas en Chile y otros países no serían suficientes para asegurar la protección de toda la biodiversidad, fallando especialmente en el caso de los vertebrados (Simonetti & Mella 1997; Tognelli et al. 2008; Ranganathan et al. 2008). Sobre esta base, es interesante conocer la capacidad que tienen los remanentes de vegetación nativa que se encuentran en el paisaje antropogénico, para albergar poblaciones de especies especialistas de hábitat como *Dromiciops gliroides*, y de esta manera complementar los esfuerzos de conservación hoy limitados a las áreas protegidas formales (Armesto et al. 1998).

El tema central de esta tesis es determinar cuál es el efecto de la tala selectiva de los fragmentos de bosque nativo, remanentes en el paisaje rural del norte de la isla de Chiloé, sobre la densidad poblacional y demografía del marsupial arbóreo *Dromiciops gliroides*. Debido al escaso conocimiento ecológico de *D. gliroides*, el primer objetivo de este trabajo se centrará en investigar aspectos no conocidos de la historia natural y ecología de *Dromiciops gliroides* en paisajes de bosques remanentes, para luego analizar las consecuencias de la degradación del bosque por tala selectiva sobre sus poblaciones locales. Específicamente, responderé las siguientes preguntas: ¿cuál es el tamaño de las poblaciones de *Dromiciops* en fragmentos de bosque antiguo (sin tala), bosques degradados por tala selectiva y en matorrales secundarios, como principales ambientes que caracterizan el paisaje rural actual del norte de la Isla de Chiloé? Considerando el hábito estrictamente arborícola de *D. gliroides* y su reconocida afinidad por hábitats de bosques, también me pregunto ¿cuáles serán las consecuencias de la degradación de los fragmentos de bosque para la demografía de la especie y, por ende, el futuro de las poblaciones de este marsupial en los fragmentos de bosque del actual paisaje rural? Debido a que el proceso de degradación del hábitat actuaría en conjunto con la pérdida de superficie de bosques y acentuaría sus efectos, espero que *D. gliroides* muestre una gran sensibilidad a los cambios de estructura de los parches de hábitat, lo cual debería reflejarse en una menor densidad poblacional y éxito reproductivo, limitando su capacidad de persistir en los actuales paisajes rurales de la isla de Chiloé.

Marco teórico. — El conocimiento de los factores que regulan la dinámica de las poblaciones en su ambiente ha sido uno de los temas centrales de la ecología (Andrewartha & Birch 1954). Los modelos de selección de hábitat predicen que hábitats de mejor calidad, serían preferidos por sobre hábitats de inferior calidad resultando en una distribución "libre-ideal" (Fretwell & Lucas 1970). Bajo este escenario, parches de bosques antiguos serian ocupados primeramente por sobre áreas con bosques expuestos a tala selectiva (supuestamente hábitats de "calidad inferior"), hasta que su oferta de recursos se reduzca debido a la alta densidad poblacional, igualando al hábitat sub-óptimo. Sobre la base de este modelo teórico, se esperaría que la densidad poblacional de *Dromiciops gliroides* sea menor en bosques modificados por la tala selectiva en comparación con bosques antiguos no intervenidos. Este modelo de selección de hábitats supone que los individuos se mueven libremente entre los hábitats, pero en paisajes antropogénicos este puede no ser el caso. Si la conectividad entre los hábitats remanentes en el paisaje antropogénico es reducida, a medida que se incrementa la densidad, procesos denso-dependientes pueden aumentar su importancia y reducir la sobrevivencia poblacional (Turchin 1999).

La estructura del hábitat tiene una considerable influencia en la distribución y abundancia de los vertebrados (MacArthur & MacArthur 1961; Rosenzweig 1991). Estudios recientes realizados en marsupiales arborícolas, que respecto a la utilización de recursos tróficos se comportan como generalistas, han documentado un alto grado de especialización en el uso de ciertas estructuras del hábitat (Laurance 1990; Laurance 1996; Lindenmayer et al. 1994; Lindenmayer et al. 1997). Teóricamente, en términos de tiempo y energía es más "fácil" ser especialista de hábitat que especialista trófico (Schoener 1974; Kozakiewicz 1995; Morris 1996; Bowman et al. 2000). Esto sugiriere que la pérdida o modificación de estructuras del hábitat de los bosques, producto de la degradación por tala selectiva o quema, tendría un mayor impacto sobre las poblaciones de vertebrados que la disminución de algunos recursos.

La densidad de una población es resultado de la interacción entre las características de historia de vida de los organismos y la disponibilidad de hábitat y recursos tróficos. En el caso particular del marsupial arbóreo *Dromiciops gliroides* en bosques de Chiloé, la degradación de un parche de bosque puede reducir marcadamente la heterogeneidad vertical (Aravena et al. 2002; Pérez et al. 2009) y aumentar la varianza espacial y temporal en la disponibilidad de recursos (Foley et al. 2007). De acuerdo a este marco teórico, estas diferencias alterarían los procesos demográficos locales, con consecuencias negativas para la densidad poblacional de *D. gliroide*s. Frecuentemente, la densidad poblacional constituye un indicador de calidad del hábitat, ya que un mayor número de individuos en un área respondería a una mayor cantidad de recursos disponibles. Sin embargo, a veces es posible encontrar altas densidades poblacionales en hábitat subóptimos producto efectos de dominancia, territorialidad o estacionalidad (Van Horne 1983; Pulliam 1988; Loeb 1999; Brawn & Robinson 1996; Manning & Edge 2004), o debido a que la densidad responde a la denominada "deuda de extinción" en hábitats fragmentados (Tilman et al. 1994). En consecuencia, parámetros demográficos de la población, tales como las tasas de sobrevivencia y fecundidad son variables más apropiadas para estimar la probabilidad de persistencia de las poblaciones en parches de hábitats remanentes (Loeb 1999; Brawn & Robinson 1996; Manning & Edge 2004).

Estudios previos han documentado grandes diferencias en las densidades de poblaciones de *D. gliroides* en bosques del sur de Chile. Murúa (1996) clasificó a esta especie como escasa, con rangos de densidad entre 10-19 individuos/ha. Por otra parte, densidades mucho menores se registran en hábitats fragmentados (Saavedra & Simonetti 2005; Rodriguez-Cabal et al. 2007), mientras que Patterson et al. (1990) registraron altas densidades en claros y bordes del bosque, lo que atribuyeron a una mayor abundancia de artrópodos asociados al bambú nativo, *Chusquea* sp. Sin embargo, Fortúrbel (2008) registró densidades de hasta 21 individuos/ha en un fragmento de bosque primario en Las Cascadas en Osorno, argumentando que las menores densidades estimadas en estudios previos se debían a problemas de detectabilidad de *D. gliroides* (Kelt & Martínez 1989). En este sentido, metodologías más robustas para estimar la densidad de poblaciones (Parmenter et al. 2003) de *D. gliroides* en hábitats de bosques antiguos y bosques perturbados por tala selectiva nos ayudarían a comprender las consecuencias poblacionales de la degradación antropogénica del hábitat, proceso que ha ido en incremento en las últimas décadas (Echeverria et al. 2008).

Finalmente, los parámetros demográficos están relacionados con atributos de historia de vida de las especies. Por ello, un conocimiento más profundo de estos atributos en el caso de *Dromiciops gliroides*, nos ayudaría a comprender su susceptibilidad a la modificación del hábitat y a explicar o predecir las tendencias demográficas en parches de bosques remanentes del ambiente rural. Por lo tanto, el conocimiento de la historia natural de esta especie, y otras especies amenazadas por la pérdida y degradación de sus hábitats, constituye una herramienta fundamental para estimar la probabilidad de persistencia de las especies en distintos escenarios de paisajes de bosques fragmentados.

Tal como se señaló al principio, el objetivo general de esta tesis es determinar cómo los cambios antropogénicos de la vegetación (bosque antiguo vs. bosque con tala selectiva y matorrales) -- que involucran cambios en cobertura de sotobosque y productividad del hábitat -- afectan la ecología y demografía de las poblaciones locales de *Dromiciops gliroides,* y determinan así el valor del paisaje rural del norte de la Isla de Chiloé como reservorio para las poblaciones de esta especie y complemento del Sistema Nacional de Áreas Protegidas del Estado.

Estructura de la Tesis. —La presentación de esta tesis está dividida en dos capítulos y dos anexos. El primer capitulo, describe los aspectos menos conocidos de historia natural, atributos de historia de vida y ecología de *Dromiciops gliroides* en fragmentos de bosques nativos antiguos, bosques afectados por tala selectiva y matorrales secundarios, como principales tipos de comunidades en el paisaje rural del norte de la

Isla de Chiloé. En este capitulo se estima su densidad poblacional en cada tipo de comunidad y analizan las consecuencias de la degradación de los parches de bosque y cambio potencial del paisaje (relación bosque/matorral) sobre la ecología de las poblaciones.

Los objetivos específicos del capítulo fueron: (i) Estimar la densidad poblacional de *D. gliroides* en hábitats de bosque antiguo, expuestos a tala selectiva y matorrales del paisaje rural del norte de la isla de Chiloé, (ii) estimar el ámbito de hogar y dieta, (iii) describir su ciclo reproductivo y conducta de hibernación y (iv) describir su carga parasitaria, caracterizada con mayor detalle en ambos anexos. Esta información ecológica nos permitiría comprender la respuestas a los cambios del hábitat de *Dromiciops gliroides* a nivel del paisaje.

En el segundo capitulo se caracteriza la demografía de las poblaciones de *D. gliroides* en remanentes de bosques antiguos y bosques sujetos a tala selectiva en el paisaje rural del norte de la Isla de Chiloé. Los objetivos específicos de este capítulo fueron: (i) caracterizar las diferencias en la estructura de la vegetación y recursos alimenticios (e.g., frutos e insectos) entre bosques antiguos y sujetos a tala selectiva, (ii) estimar los tamaños poblacionales en los dos tipos de bosques y (iii) estimar y comparar los parámetros demográficos (sobrevivencia, reclutamiento y tasa de crecimiento poblacional) de *D. gliroides* en bosques antiguos y sujetos a tala selectiva.

Hipótesis de trabajo.

1. La tala selectiva modifica la estructura de la vegetación y los recursos tróficos (e.g., frutos e insectos), a través de: a) la remoción de grandes árboles del dosel e

indirectamente de especies epifitas, b) la disminución del sotobosque, y c) la pérdida de hábitats para las poblaciones de artrópodos que sirven de alimento a *Dromiciops*. Como consecuencia, se espera que la densidad poblacional de *Dromiciops gliroides* en bosques antiguos sea mayor que en los bosques sujetos a tala selectiva.

2. Debido a la remoción de árboles de gran tamaño por tala selectiva, se espera una reducción de la densidad de potenciales sitios de hibernación y nidificación, lo que afectaría negativamente la tasa de crecimiento poblacional de *Dromiciops gliroides* a través de la modificación de parámetros demográficos (i.e., sobrevivencia y reclutamiento) en comparación con los bosques antiguos sin tala.

3. La extracción de grandes árboles del dosel produce claros que conllevan aumentos en la densidad de quila (*Chusquea* spp.) en el sotobosque, la que es un hábitat para una alta densidad de artrópodos (Reid et al. 2004) y, a la vez, constituye un refugio contra los depredadores de *Dromiciops gliroides.* El aumento de la quila podría compensar la pérdida de grandes árboles con cavidades y especies epífitas con frutos carnosos. Por lo tanto, se espera como hipótesis alternativa, que la densidad de *Dromiciops gliroides* en bosques con tala selectiva y con una alta densidad de *Chusquea* spp., sea igual o mayor a la densidad en los bosques antiguos.

CAPITULO I

POPULATION DENSITIES, FOOD HABITS, AND BEHAVIOR OF THE THREATENED ARBOREAL MARSUPIAL *DROMICIOPS GLIROIDES* IN A RURAL MOSAIC OF FORESTS AND SHRUBLANDS IN CHILOE ISLAND, CHILE

ABSTRACT

The ecology and behavior of the endemic and threatened Monito del monte (*Dromiciops gliroides*) were studied in different habitats in a rural area of northern Chiloé Island (42 S), Chile. Individuals were live-trapped for four years (2005–2009) during their breeding (November) and non-breeding (February) season. MNKA was used as an estimation of population density, and home range, diet (through fecal content) and health status (ectoparasite load) were assessed. The breeding season was estimated through reproductive hormones and winter torpor behavior was documented using artificial nest boxes. Population varied seasonally in accordance with breeding, with higher densities recorded during summer. Population densities were similar in old growth than in selective logged forest, but were considerable higher in comparison with shrublands. Social torpor was documented for the first time and was highly frequent (64.1%), especially among juveniles. Home ranges were two times larger for males than for females. *Dromiciops gliroides* is omnivorous, with a diet based mainly on arthropods, and a higher consumption of fleshy fruits in summer. Habitat and age had significant effects on ectoparasite prevalence with higher incidences in juveniles at selective logged forest. Deeper knowledge on the ecology of *D. gliroides* is crucial for the assessment of its conservation status in a changing forest landscape.

Key words: countryside, selective-logged forest, old-growth forest, communal nesting, natural history, marsupial, temperate rainforest.

INTRODUCTION

Dromiciops gliroides (Microbiotheria: Microbiotheriidae), locally known as "Monito del monte", is the only extant member of the order Microbiotheria (Reig, 1955), with strong phylogenetic affinity with Australasian marsupials (Springer et al., 1998; Palma and Spotorno, 1999; Phillips et al., 2006). It is considered a living fossil or relict in the South American mammal fauna (Springer et al., 1998; Palma and Spotorno, 1999). The species is endemic to temperate rainforests of South America (Mann, 1978; Hershkowitz, 1999) with a distribution that extends along the Chilean Pacific rim from Talca (ca. 35° 30') (Lobos et al., 2005) to Chiloé Island (ca. 44° 00') and adjacent forested habitats of Neuquén and Río Negro provinces in southwestern Argentina (Hershkowitz, 1999).

From late 1800's ownwards, deforestation has been intensive in South American temperate rainforests (Armesto et al., 1998; Echeverría et al., 2006), including northern Chiloé Island (Willson and Armesto, 1996; Echeverría et al., 2007; Jaña-Prado et al., 2006). In addition, fragmentation and degradation of forested habitats through logging, grazing by cattle and fire, alter forest structure and tree species composition affecting the populations of forest vertebrates (Díaz et al., 2005). The current rural landscape of northern Chiloé Island comprises a mosaic of remnant forest fragments (including old growth and secondary forest), wet scrublands, pastures and agricultural fields (Willson and Armesto, 1996; Aravena et al., 2002; Armesto et al., 2009).

The massive loss of forested habitat, together with the limited geographical range of *D. gliroides*, are possibly leading to its decline over recent decades, as shown by its inclusion in the IUCN red list first as "vulnerable," recently changed to "near threatened"

(Díaz and Tetas, 2008). *Dromiciops gliroides* has been recently classified in the Chilean legislation as "inadequately known," which means that it could be either endangered or vulnerable, but it remains to be assessed (Chilean law Supreme Decree N° 151, March, 2007).

Despite its near threatened condition, the natural history, food habits, and behavior of this arboreal marsupial remains poorly known in southern South American forests (Jiménez and Rageot, 1979; Kelt and Martínez, 1989; Redford and Eisenberg, 1992; Muñoz-Pedreros and Palma, 2000) and virtually nothing is known about its habitat use in rural landscape mosaics. *D. gliroides* has been described as an scansorial nocturnal marsupial, most commonly found or seen in evergreen-broadleaved temperate rainforests (Mann, 1978; Jiménez and Rageot, 1979; Hershkowitz, 1999), often dominated by southern beeches (*Nothofagus*: Nothofagaceae), with dense understory cover of native bamboos of the genus *Chusquea* (Mann, 1978; Patterson et al., 1990). During winter *D. gliroides* nests in snag cavities or inside the dense bamboo understory (Mann, 1978; Kelt and Martínez, 1989) and the animals hibernate under cold-temperate winter conditions and/or during food shortages (Mann, 1978; Bozinovic et al., 2004). According to Mann (1978), *D. gliroides* reaches sexual maturity in its second year of life, and it has been reported as a monoestrous, seasonal breeder, producing one litter per year, with births occurring in the early austral spring, October to November (Muñoz-Pedreros et al., 2005). Based on summer analyzes of stomach contents of an Andean population, *D. gliroides* has been classified as an insectivore (Meserve et al., 1988), but recent studies highlight its trophic role as a seed disperser of several fleshy-fruited temperate forest trees and vines (Armesto et al., 1987; Amico and Aizen, 2000; Aizen, 2003; Rodríguez-Cabal et al., 2007; Amico et al., 2009), and also as a nest predator of the forest insectivorous bird *Aphrastura spinicauda* (Cornelius 2007).

Considering that most current knowledge of the species derives from Andean forests, despite its widespread range in lowland and coastal Chilean forests, and that lowland areas are changing rapidly due to human land use (Echeverría et al., 2007), this study seeks to advance our knowledge of the ecology of the relict *D. gliroides* in a rural landscape of northern Chiloé Island and its propensity to become endangered due to the loss of forested habitats.

We concentrate our study in the two main habitats within the rural landscape where this arboreal species could dwell, including forested patches (both old growth and selectively logged patches) and shrublands. We did not sample the other two main rural habitats, pastures and agricultural fields, because previous intensive sampling in northern Chiloé (E. Palma and M. Bustamante, personal communication, and J.L.C. personal observations), and data from mainland sites (Fontúrbel et al., 2009) showed no evidence of presence of *D. gliroides* in these habitats.

Our specific goals were: (1) to assess the population densities of *D. gliroides* in forest patches (i.e., old growth and selectively-logged) and shrublands that are part of the rural landscape mosaic in Chiloé Island; (2) to assess lifetime home range and diet of the species; (3) to establish the seasonality of breeding and torpor behaviors; and (4) to describe its ectoparasites. We use this information to discuss the conservation status of this relict marsupial in the rural mosaic of northern Chiloé Island. Such knowledge is critical for proposing science-based guidelines for the conservation of this unique marsupial, and accompanying biodiversity, especially where old growth forests are becoming scarce (Gutiérrez et al., 2009) and limited land can be allocated to new reserves.

MATERIAL AND METHODS

Study site. — The study was conducted in a rural area of northeastern Chiloé Island, Ancud County, Chiloé province (Fig. 1). Within the northern Chiloé rural landscape, we studied two types of forest habitat (Fig. 2; Aravena et al., 2002: Díaz et al., 2005; Cornelius 2007): two old growth forests (OGF; one within Senda Darwin Biological Station and the other in Caulín) and two selective-logged forests (SLF; named Llanquihue and Quilar), where valuable timber trees had been removed by landowners with no defined silvicultural system (Cornelius 2007). We also looked for *Dromiciops gliroides* in dense shrublands (ESS) surrounding forested areas within Senda Darwin Biological Station (ESS1 and ESS2). OGF habitats had an age of at least 300 years based on tree-ring counts of dominant canopy trees, and had no visible signs of logging or fire (Aravena et al., 2002; Gutiérrez et al., 2004). These OGF patches were characterized by a multi-layered canopy, with large emergent trees of *Nothofagus nitida* (Nothofagaceae), *Drimys winteri* (Winteraceae), *Podocarpus nubigena* and *Saxegothaea conspicua* (both Podocarpaceae), plus few individuals of *Eucryphia cordifolia* (Eucryphiaceae) and *Caldcluvia paniculata* (Cunoniaceae) in the lower canopy. Trees were often covered with vines and epiphytes such as ferns, mosses and bromeliads (Muñoz et al., 2003). Large amounts of dead biomass (snags and logs) are frequent within OG stands. The understory had a high density of tree saplings, few shrubs, and dense thickets of 2-3 m tall native bamboo (*Chusquea* spp.) within tree-fall gaps (Gutiérrez et al., 2004). SL patches are frequent in the rural landscape due to the widespread practice of small-scale logging, and had areas with a closed canopy of dense juvenile trees, 10–20 m tall, predominantly shade-intolerant *D. winteri*, and *N. nitida*. There are also few large trees, legacies from the pre-disturbance forest. In SLFs, open areas with a dense cover of native bamboos were frequent, with a sparse presence of tree seedlings and saplings due to shading (Aravena et al., 2002). Shrublands developed after anthropogenic forest fires, and were dominated by a 1-2 m tall layer of *Baccharis magellanica* (Asteraceae) and a <1 m tall layer of *Blecchnum chilensis* (Dicksoniaceae), with sparse cover of *Berberis darwinii* and *B. microphylla* (both Berberidaceae) (Díaz et al., 2005). These shrublands have sparse regeneration of shade-intolerant tree species such as *D. winteri*, and *N. nitida* (1–3 m tall) forming small patches.

The prevailing climate in northern Chiloé is wet-temperate with a strong oceanic influence (Di Castri and Hajek 1976). Climatic records (i.e., air temperature and precipitation) to characterize the studied area were obtained from Senda Darwin Biological Station (45° 53' S, 73° 40' W) (see appendix 1).

Animal trapping and field data. —Live captures of *D. gliroides* were carried out in forest habitats from spring 2005 to spring 2009 and only until summer of 2007 for both shrubland sites. Trapping was conducted over 10 consecutive nights, twice each year: (1) in mid-spring (November-December), after torpor and before the onset of the breeding season, and (2) late austral summer (February-March), after the breeding season had ended. We did not trap animals in autumn or winter because the harsh environmental conditions increases mortality risk and animals are largely inactive. We used modified Tomahawk-live traps (baited with banana) because Sherman traps were not effective (see Kelt and Martínez, 1989; Patterson et al., 1989; Fontúrbel and Jiménez, 2009). Traps were placed on tree branches 1-2 m high, because of dense understory and structural complexity of both forest fragments and shrublands, and arranged spatially in a web design, which consisted of 12 radial lines of 100 m each. Each trapping line had 12 stations; the first four were at 5 m intervals from the center (5, 10, 15, and 20 m), and the following eight were at 10 m intervals (30 m up to 100 m). Thus, 144 traps were set up in each site per trapping night (Parmenter et al., 2003). The area covered by each web of traps was 3.46 ha. Each trap was checked daily between 0600-0800 h.

For all captured animals we recorded sex, age, breeding status (based on scrotum size, pouch morphology and mammary gland development), and body mass, using a Pesola ® (Baar, Switzerland) spring balance, 0.1 g precision. Animals were marked with numerated 2 mm long aluminum ear tags (Tag no. 1005-1, National Band and Tag Co., Newport, KY). Each individual was released at the capture site. To avoid pseudo replication in statistical analyses, we considered only new unmarked individuals. ESS had to be excluded due to low sample sizes and low capture probabilities. Capture procedures followed guidelines approved by the American Society of Mammalogists (Gannon et al., 2007) and complied with current Chilean laws (SAG permit No. 2605).

The effect of habitat (OG forest, SL forest and shrubland), season and sex on adult body mass were analyzed using three-way ANOVA (STATISTICA; Statsoft Inc. 2001), with log-transformed mass as the dependent variable and habitat, season and sex as a grouping factor.

Population abundance. —The minimum number of individuals known alive (MNKA) was used as an index of population abundance for all habitat types and sites (Lima et al., 2001; Rodríguez-Cabal et al., 2007). Population abundances of *D. gliroides* from each forest fragment were associated with monthly temperature and precipitation obtained from Senda Darwin Biological Station using a nonparametric Spearman rank correlation (STATISTICA; Statsoft Inc. 2001).

Home range. —Lifetime home ranges (I.e., four years) were estimated in both forested habitat for marked-recaptured individuals from the entire study period (2005 to 2009), using capture positions (XY-coordinates from trap data) and analyzed using Animal Movement SA (v2.04 beta -ArcView GIS 3.2). Despite some limitations of mark-recapture data, this method still is widely used to estimate home range for small mammals (Ribble et al., 2002). For adult males and females trapped more than three times, the 95% minimum convex polygon (MCP) was used as an estimate of home range size (Agüero and Simonetti, 1988). The MCP method is more robust than other home-range estimators.(e.g. radio-tracking), when the number of fixes is low (Agüero and Simonetti, 1988).

The effect of habitat and sex on home range areas were analyzed using two-way ANOVA (STATISTICA; Statsoft Inc. 2001), with log transformed MCP areas as the dependent variable and habitat and sex as a grouping factor. In addition, we assessed the relationship between home-range area and adult body mass (for both sexes) using a nonparametric Spearman rank correlation (STATISTICA; Statsoft Inc. 2001).

Diet analysis. —The diet of *D. gliroides* in all sites was studied by examining prey and vegetal remains in fecal samples during the first three years of trapping periods. Feces were collected in the field from inside or under traps in both forest fragments. Feces were placed in labeled 1.5-ml Eppendorf vials with 70% alcohol (Pinheiro et al., 2002) and dried in the laboratory. They were later teased apart under a 40X binocular dissecting scope and remains of arthropods were determined to at least ordinal level with keys for exoskeleton fragments (Pinheiro et al., 2002). Seeds were identified using reference samples for plant species in the forest and surrounding areas. Frequencies of occurrence (FO%) were estimated by counting the number of each prey or seed type in feces and then dividing by the total number of prey or seeds identified without correction for differential digestibility (Jiménez, 2007).

We compared variation in the proportions of items in the diet of *D. gliroides* among both forested habitats (i.e., OGF and SLF) and seasons (i.e., spring and summer) using the-log likelihood ratio (G Test: Zar 1999). For this comparison, we pooled data from juveniles and adults individuals, due to inadequate sample size (n < 30) at each category for a simultaneous analysis. In addition, we explored differences in prey content between juveniles and adults individuals. This comparison was done only for summer, due to the larger number of juveniles. Finally, we described the overall composition of the diet of *D. gliroides* in terms of items in fecal content using all samples.

For graphical comparisons, dietary results by habitat and season were simplified further by combining categories into three broad classes (following Meserve et al., 1988): arthropods and vertebrates, vegetative plant material and fungi, and fruits and seeds. We used De Finetti diagrams following procedures of Meserve et al. (1988) for illustration purposes, where the trophic position of a group is represented as a single point whose distance to each endpoint of an equilateral triangle is proportional to the dietary composition along three axes categories.

Ectoparasites and health status. — Ectoparasites were collected from unmarked individuals captured by live trapping from spring 2005 until summer 2007. Ectoparasites were collected after visual examination following Marín-Vial et al. (2007). Samples were placed in labeled 1.5 ml Eppendorf vials with 70% alcohol and identified taxonomically in the laboratory.

Ectoparasites were identified using descriptive keys, and their prevalence and abundance were measured according to definitions given by Bush et al. (1997). To analyze the effect of habitat, season, sex, and age of *D. gliroides* in ectoparasite prevalence, a GLIM model selection with binomial distribution was conducted in R project software (version 2.7.1, 2008). Mass of parasitized adults *versus* non-parasitized individuals was analyzed using one-way ANOVA (STATISTICA; Statsoft Inc., 2001). Proportions of recaptured individual, at least once, as a proxy of survival, were compared between infected and non-infected individuals using a proportion test in R project software (version 2.7.1 2008).

Nest box occupancy and torpor. — In winter 2002 and 2004, artificial nest boxes were installed in two OG forests (i.e., Senda Darwin and Caulín) as part of a parallel bird survey (see Moreno et al., 2005). All nest boxes (N=194) were placed more than 500 m away from the trapping area and were fastened to tree trunks or branches ca. 150 cm above the ground. Nest box dimensions were described in Moreno et al. (2005). To describe torpor behavior of *D. gliroides* we monitored the nest boxes in the austral winter period (July-August) during 2005 and 2006, when birds were not using them. All animals found in boxes were measured and marked as those captured with web live trapping method.

To assess the effect of age and sex on the probability of communal *versus* solitary nests found in the occupied boxes, data were analyzed using GLIM model selection with binomial distribution at R project software (version 2.7.1, 2008).

Reproduction. — At all sites sexual maturity and breeding season were assessed by measuring the levels of plasma testosterone in males and progesterone in females from blood samples, as signs of reproductive activity (i.e., follicular development and corpora lutea). Blood samples (100µl) were obtained from adults or juveniles that were anesthetized in a closed chamber for 20 – 30 sec with isofluran in 0.5 – 1 ml volume and blood extracted from the infraorbital sinus with heparinized hematocrit capillary tubes (Van Herck et al., 2000). Samples were centrifuged and the plasma analyzed. All individuals were released after recovery from anesthesia at the site of capture. Due to the small amount of plasma, individuals captured at different sites but with similar ages were pooled. Plasma testosterone and serum progesterone concentrations were analyzed with solid phase I^{125} radioimmunoassay, using a total testosterone or progesterone Siemens kit.

The relationship between reproductive hormones and secondary sexual traits, such as testicular size, was assessed using a nonparametric Spearman rank correlation (STATISTICA; Statsoft Inc., 2001).

We quantified the proportion of reproductive females and counted in-pouch young for all females at all sites throughout study period. To confirm reproductive data, which suggest that *D. gliroides* becomes sexually active during the second year of life (Mann, 1978), we recorded the age at maturity based on morphological changes (i.e., testicular size, and pouch development and coloration). For this analysis, we considered only reproductive individuals with previously recorded body mass information at the juvenile stage.

RESULTS

Dromiciops *population description.* — From spring 2005 to summer 2009, we live-trapped 502 different individuals of *D. gliroides* with 1065 captures in all habitats and sites. A total of 119 animals were trapped at Senda Darwin, 111 at Caulín, 181 at Llanquihue, 83 at Quilar, 4 in ESS1 and 6 in ESS2 (Table 1). Only two juveniles captured in shrubland (i.e., at ESS2) were trapped again as adults in the Senda Darwin OG forest patch. On average, each animal was recaptured 2.12 times (3.4 times for recaptured individuals). Accidental trapping death was 1.8% (19 out of 1065 captures), and was marginally significantly higher during the spring months (13) than in summer (6, $\chi^2 = 3.31$, *d.f.* = 1, $P = 0.06$; Table 1). On two occasions we observed owls (*Strix rufipes* and *Glaucidium nanum*) killing trapped individuals at SL patches.

Population varied seasonally in all habitats (Fig. 3). In both forested habitats (i.e., OGF and SLF), we observed a trend towards higher abundances in the austral summer (February), which corresponded to the juvenile recruitment period, than in spring (November), when mating and breeding takes place (Fig. 3). In contrast, even though abundances were extremely low, we observed an inverse trend in shrublands (Fig. 3). Abundances were significantly correlated with mean temperature, but only for Senda Darwin OG forest (Spearman rank correlation $R = 0.87$, $n = 8$, $P = 0.004$). Population abundances were higher than 45 individuals/ha, and occasionally lower than 6 individuals/ha in spring or in shrublands. Over all seasons, years, and sites, OGF had a mean of 4.91 individuals/ha (SE = 0.7); SLF a mean of 5.36 individuals/ha (SE = 0.8) and shrublands a mean of 0.40 individuals/ha (SE = 0.14) (Table 1).

We found no significant differences in body mass of *Dromiciops* adults captured between habitats, OG forest, SL forest and shrublands ($F_{1, 268} = 3.75$, $P > 0.06$; Table 2). However, adult mass differed significantly between seasons within a year ($F_{1, 268} = 28.71$, $P < 0.01$; Table 2) and between sexes ($F_{1, 268} = 5.43$, $P = 0.02$; Table 2), with lower body mass during spring when individuals were recovering from torpor (n = 197, mean ± SE = 26.87 g ± 0.4), and higher body mass at the end of summer period (n = 79, 30.54 g ± 0.56). On the other hand, females (n = 95, mean ± SE = 29.5 g ± 0.5) were heavier than males (n = 181, mean ± SE = 27.9 g ± 0.46). In addition, the interaction between seasons and sex of the animals was statistically significant ($F_{1, 268} = 5.52$, $P < 0.02$), because summer body mass of females was higher than females in spring and for males in both seasons.

Home range. — A total of 80 adults out of 287 individuals were captured three or more times during the study period (Table 3), enabling a reliable estimation of lifetime home ranges. Because no individuals were captured more than three times in ESS, we did not estimate home range in this habitat. Estimated home range sizes of the 80 individuals from forest habitats ranged from 0.12 to 0.35 ha (Table 3). Home range was not statistically different between OG and SL forest habitats, but it was significantly larger for males than for females, with considerable spatial overlap between sexes (Table 3 and Fig. 4). Body mass and home range area were uncorrelated ($r_s = 0.12$, $P = 0.29$, n = 80).

Diet. —A total of 146 fecal samples of *D. gliroides* were obtained from forested habitats (i.e., OGF and SLF) in two seasons of the year (i.e., spring and summer) from 2005 to 2008. Only one fecal sample was obtained from a shrubland animal, and hence we excluded this habitat form further analyses. Arthropods were the most frequent item in *D. gliroides* feces, over 40% in all habitats and seasons. However, significant differences were found in the proportion of food items between forest habitats and seasons (G = 13.95, *d.f.* = 6, *P* < 0.03, Fig. 5). The proportion of arthropods was higher in SLD than in OGF in both spring and summer (Fig. 5). On the contrary, the proportion of seeds in feces was higher in OGF than in SLF, and this differences increased in summer (Fig. 5). No differences were found between age classes in the proportion of food items from summer samples (G = 4.74, *d.f.* = 2, *P* < 0.09). In particular, adults from OGF showed a higher proportion of seeds in summer, when most of shrubs and trees were in fruiting (Smith-Ramírez and Armesto, 1994), in comparison with spring. Hence, in summer samples, fruits were more frequent in feces than were arthropods.

Among arthropods, Coleoptera and Orthoptera were the most frequent prey item in feces of this marsupial, with a frequency of occurrence FO% = 42.9 and 24.6, respectively (Table 4). Lepidoptera (mostly larvae and pupae), Hymenoptera and Hemiptera were also found at lower frequencies (Table 4). Two fecal samples contained bird bones and feathers (only from spring samples). Seeds of twelve plant species were identified, including forest epiphytes, vines and shrubs. *Tristerix corymbosus*, *Gaultheria insana* and *Luzuriaga polyphylla* were the most frequent seeds found in feces, representing 45, 19 and 8% of the total frequency of occurrence (Table 5); however, considering total seed numbers, *Tristerix corymbosus*, *Myrteola numularia* and *Gaultheria sp.* were more abundant in *D. gliroides* feces (Table 5).

Ectoparasites and health status. — Four different taxonomic groups of ectoparasites were identified infesting *D. gliroides*: ticks, fleas, mites and leaches. Ectoparasites were identified to species level for ticks and fleas. For ticks, we detected *Ixodes neuquenensis*, the only tick previously reported from *D. gliroides* (recently recorded in Chiloé; Marín-Vial et al., 2007) and for fleas, we detected *Chiliopsylla allophyla* and *Plocopsylla diana*, both species shared with other temperate rainforest rodents (Alarcón, 2003).

A total of 102 (55%) of 185 individuals *D. gliroides* were infested with some or all of these ectoparasites in all sites studied (Table 6). Excluding shrublands, due to small sample size (n = 7), a GLIM model including forest habitat, season (spring *versus* summer) and animal age, had the best adjustment to explain the prevalence of ectoparasites (AIC = 236.91, res. dev. = 220.91). Prevalence was statistically different between forest habitats (GLM W = 5.33, $P < 0.039$), with higher values in SL forest (62.73%) than in OG forest (44.78%), and was higher in juveniles (62.1%) than in adults (50.9%). Flea prevalence had the same pattern (GLM W = 5.33, $P < 0.02$), been higher in juveniles (52.2%) than in adults (31.5%). In addition, tick prevalence was statistically different between summer and spring (GLM W = 5.33, $P < 0.02$), with higher prevalence in spring (33.3%) than in summer (15.7%). No significant differences were found between sexes (GLM W = 5.33, $P > 0.05$), unlike the findings of Guglielmone et al. (2004).

Body mass of infested adults was not significantly different from that of non-infested individuals (ANOVA $F_{1, 115} = 2.24$, $P = 0.14$). Moreover, the proportion of recapture rate of infested adults was not significantly different from that of non-infested individuals ($\chi^2 = 0.04$, *d.f.* = 1, $P = 0.8$).

Nest box occupancy and torpor. — In winter 2005, 28 out of 194 nest boxes were used by *D. gliroides* as den sites in two OG forests. Twenty-three (15.4%) nest boxes were occupied in Senda Darwin and five (11.1%) in Caulín. Similar level of occupancy was observed in winter 2006 with twenty-six (17.5%) and six (13.3%) boxes used by *Dromiciops* in Senda Darwin and Caulín respectively. Nests inside boxes were spherical, with a single entrance, built with materials from epiphytes, vines, and understory vegetation (*Chusquea* leaves, *Hymenophyllum* ferns), and lined with epiphytes, ferns, or mosses.

During winter 2005 and 2006, 77 and 62 torpid animals, respectively, were found nesting in these boxes. Overall nest occupancy rate was 16.5 % (n = 388 boxes). Mean number of animals in nests was 2.3 ± 0.2 SE (range = 1-9). Forty-one of 64 nest boxes (i.e., 64.1%) were communal nests, significantly greater than expected by random ($\chi^2 = 9.03$, *d.f.* = 1, $P = 0.002$). Animal age had a significant effect on communal nest behavior (Fisher's exact test, *d.f.* = 1, $P = 0.05$; Table 7); juveniles were found solitary in 17.4% and communally in 38.3% of the nests, whereas adults were solitary in all the remaining nests, 82.6%. Contrary to expectations, neither sex (Fisher's exact test, *d.f.* = 1, $P = 0.34$) nor body mass (three way ANOVA, $F_{1,\ 139} = 0.0049$, $P = 0.94$), had a significant effect on communal nesting.

Reproductive parameters. — A total of 44 blood samples were obtained from adult males captured throughout the study period. Plasma testosterone levels had a single annual peak during austral spring (November), followed by a steady decline from spring to winter (July), when males entered torpor (Fig. 6). Levels of plasmatic testosterone were significantly correlated with testicular volume (Heideman et al., 2000; $r_s = 0.12$, n = 36, $P < 0.001$), and related to mating season. Interestingly, like other marsupials (Scott, 1979), we observed that most adult males individuals had different scrotal colorations.

A total of 21 blood samples were obtained from 19 adult and 2 subadult females, captured in all sites. Plasma progesterone concentration in adult females showed an increase during spring (November) lasting until summer (February), followed by a reduction in winter (August), when females entered torpor (Fig. 6). During spring, subadult females had lower progesterone concentrations than mature females (22.3 ng/ml *versus* 73.0 ng/ml). Overall, considering all forest sites and years, we recorded 27 adult females with their young still attached to the marsupium, with a mean litter size of 2.26 ± 0.17 (1 SE). Two of these females were also trapped pregnant in the reproductive season (i.e., November) of a previous year. In addition, in the summer (i.e., post reproduction) of 2009, six females were trapped with young still attached to the marsupium (mean ± SE: 2.17 ± 0.17), from which three were found breeding in the previous (i.e., reproductive) trapping session, suggesting a second reproductive event. Anecdotally, in spring of 2005, we observed parturition and migration of two immature embryos into the pouch. Three days later, we trapped the same female bearing young inside the pouch.

Based on hormone profiles and/or primary sexual characters, field observations suggest that subadult individuals become sexually mature when they reach a body mass of 25.2 g ± 0.47 (n = 21) for males and 27.0 g ± 0.98 (n = 8) for females from birth to the next reproductive season (i.e., 10 month approximately)

DISCUSSION

In all remnant forest patches in the rural landscape of northern Chiloé Island, regardless of whether some logging of large trees had taken place, we recorded abundant presence of the arboreal marsupial *D. gliroides*. Population densities were similar in OG and SL forest, and there were no differences in adult body mass between habitats. Previous knowledge suggested that *D. gliroides* populations were strongly associated with old-growth forest patches, because of its arboreal habit and nesting behavior, occupying tree holes (Mann, 1978; Jiménez and Rageot, 1979, Kelt and Martínez, 1989; Patterson et al., 1989; Hershkovitz, 1999). Other studies have found similar densities of *D. gliroides* in habitats that differed in successional stage such as old growth and secondary forest patches (e.g., Fundo San Martín Experimental Forest Reserve in Valdivia, and Las Cascadas in Osorno), which are characterized by dense bamboo cover in the forest understory (Patterson et al., 1990; Fontúrbel et al., 2009).

Dromiciops gliroides abundances in forested habitats remaining in northern Chiloé Island ranged from 5 to 48 individuals/ha, a density comparable to estimates available for continuous forests in the Andes (Rodríguez-Cabal et al., 2007) and slightly higher than estimates for an arboreal rodent of southern temperate rainforests, *Irenomys tarsalis* (see Murúa 1996).

Considering an annual forest loss rate of 1.1 % (Echeverría et al., 2007), and an increase in the proportion of SL in remnant forest patches in northern Chiloé (Echeverría et al., 2007; Jaña-Prado et al., 2006; Armesto et al., 2009), the finding of comparable densities of *D. gliroides* in OG and SL forest patches of the rural landscape constitutes an important result. If *D. gliroides* is negatively affected by habitat fragmentation, as shown for Andean forests in Argentina (Rodríguez-Cabal et al., 2008), and pastures constitute powerful barriers for movement between forest fragments (Rodríguez-Cabal et al., 2007; Fontúrbel et al., 2009), shrublands and logged forests could facilitate the movement of animals across the rural landscape. SL forests could become relevant by providing suitable habitat for *D. gliroides* in areas where land cover of OG forests has been greatly reduced. According to our results, shrublands that are contiguous to forest habitats could also mitigate restrictions on the movement of animals between forest fragments in the rural landscape. In this sense, recent studies in forest mammals communities, have shown a crucial role of the matrix in maintaining diversity in fragmented landscapes (Umetsu and Pardin, 2007). Consequently, assessing the capacity of remnant forest to support regional biodiversity and inclusion of matrix quality parameters in managing rural landscapes is of critical importance to enhance conservation, especially where agriculture frontiers are still expanding and limited land can be allocated to new reserves (Chan and Daily, 2008).

Populations phenology of *D. gliroides* varied between spring and summer each year, with a trend in both forested habitats toward higher densities in summer, associated to favorable weather. In spring 2008, we observed a drastic reduction in *D. gliroides* population (Fig. 2). We suggest that a severe drought of spring-summer 2007-2008 could explain this population pattern, but we did not have enough data to support this hypothesis.

Reproduction in marsupials is usually cued to environment temperature and resource abundance (Tyndale-Biscoe and Renfree, 1987; Quental et al., 2001). Accordingly, favorable conditions of food and climate during the austral spring-summer, result in synchronous reproductive patterns and maximum sex hormone levels during this period, associated with the development of secondary sexual traits (Muñoz-Pedreros et al., 2005). Based on the general climate of Chiloé Island (di Castri and Hajek, 1979), we suggest a slight delay in the female reproductive cycle in comparison to mainland populations (*e.g.* developmental morphology; see Muñoz-Pedreros et al., 2005), because mating, gestation and parturition in Chiloé Island generally took place from late October to early December. Higher and constant progesterone levels during gestation and lactation, in contrast to male testosterone, might be attributed to the luteal phase duration (Tyndale-Biscoe and Renfree, 1987). However, a second reproductive event or a delayed one could sustain higher hormones levels during summer periods, contrary to previous reports that *D. gliroides* breeds exclusively in spring (Mann, 1978; Muñoz-Pedreros et al., 2005). In males, melanistic scrotal coloration, such as found in males from Chiloé forest, has been described in species exposed to harsh environments, and thought to aid in regulating sperm temperature (Scott, 1979) and also suggested previously for *D. gliroides* (Grant and Temple-Smith, 1987).

In most marsupials, the culmination of lactation and weaning are critical periods for offspring survival (Lee and Cockburn, 1985), and in rural Chiloé forests, these two phases always occurred in summer, during the peak of arthropod and fruit abundance (Smith-Ramírez and Armesto, 1994; Díaz, 2008). Similar population densities in OGF and SLF in rural Chiloé Isalnd, suggest that modification of forest resources by limited logging of some tree species does not translate into demographical consequences (see chapter II).

Mann (1978) indicated that *D. gliroides* reached its sexual maturity during its second year. However, our data on sex hormone levels and secondary sexual traits (*e.g.* testicular size in males and pouch development and coloration in females), suggest that individuals born in mid-spring (November) could reach sexual maturity in the following breeding season (i.e., next November), if they have reached a minimum mass of 25 g and 27 g for males and females, respectively.

Adult males often have larger home ranges during mating periods (see Kraaijeveld et al., 2002; Belcher and Darrant, 2004). Considering that our home range estimates from captured animals in Chiloé forests were smaller than radio-tracking estimates (Fontúrbel et al., 2009; unpublished data), larger home ranges were documented in the spring, when mating occurs (Fontúrbel et al., 2009). Smaller home ranges based on mark-recapture methods, in comparison to radio-tracking estimates, can be equivalent to the core areas of radio-tracking estimates (Pavey et al., 2003). In this case, mean adult home range (0.2 ha) from Chiloé forests was similar to the core area defined in an Andean population (0.2 ha) (Fontúrbel et al., 2009).

Artificial nest boxes are widely used as ecological research tools and wildlife management resources, especially in field studies of arboreal marsupials (Lindenmayer et al., 2003). Although of controversial application in forest conservation (Lindenmayer et al., 1991; Gibbons and Lindenmayer, 2002), their use may reduce the negative effects of declines in nesting sites due to habitat loss (Lindenmayer et al., 1997; Cornelius 2007). In our case, nest boxes provided useful tools as a proxy to estimate animal abundances and to study nesting and reproductive behavior of a cryptic nocturnal and arboreal marsupial.

Mann (1978) reported solitary torpor of *D. gliroides*, and its physiological bases were analyzed by Bozinovic et al. (2004), who noted the first record of hibernation in a South American mammal. Nesting and torpor in tree hollows are common in Australasian marsupials, and appears to be a response to harsh environmental conditions to increase winter survival (Geiser 1994; Canals et al., 1997; Bozinovic et al., 2004). In marsupials, the number of animals nesting in the same place shows wide variation (Lee and Cockburn, 1985; Lindenmayer, 1997). We report the first record of communal nesting for a South American marsupial in the case of *Dromiciops* in Chiloé forests. Communal torpor or huddling is energetically advantageous for social thermoregulation (Lee and Cockburn, 1985; Bozinovic et al., 1988; Rhind, 2003). It is facilitated by reproductive cessation and low relatedness (Rhind, 2003; Ebensperger et al., 2004). Ontogeny and group size may influence the effectiveness of huddling, because early developmental stages and the number of animals reduce the surface/volume ratio, and hence individual metabolic rates (Canals et al., 1998). Accordingly, in rural populations of *D. gliroides*, communal nests are often composed of juveniles with lower body mass, as opposed to solitary nests, which are mostly occupied by adults. Communal nesting also reduces time needed for nest construction, so that one individual may use more than one nest within its home range (Lee and Cockburn, 1985). In this sense, in our studied area, we observed winter movement of torpid animals between nests; however, further analysis is needed to determine the relevance of communal nesting to sociality. Some authors suggest that the decline of large and old trees, as a result of logging may affect population abundance and winter survival of many arboreal mammals (Laurance, 1996; Lindenmayer et al., 1997) and forest specialist birds (Diaz et al., 2005; Cornelius, 2007), due to resource limitation (as is the case of the arboreal marsupial *Hemibeldeus lemuroids* in Australian tropical forest and *Aphrastura spinicauda*, a secondary-cavity forest bird in Chilean temperate rain forest; Laurance, 1996; Cornelius, 2007). Nevertheless, we found no evidence of a reduction in population abundance of *Dromiciops* residents in SL forests in Chiloé Island. This result suggests that the type of logging practiced in rural forest patches has not negatively affected nesting sites of *D. gliroides*; however, demographic consequences need to be assessed (see Chapter 2).

As other small marsupials, *D. gliroides* is omnivorous. Arthropods, particularly Coleoptera and Orthoptera, were common items in the diet, but fruits were also an important component of the animals' diet (Armesto et al., 1987; Amico et al., 2009). *D. gliroides* showed high consumption of a diversity of fleshy fruits, particularly in OG forest, some of which had no dispersal agent known (Table 5). Hence, *D. gliroides* may have a key role in forest patches, especially when dispersing seeds of epiphytes and vines (Rodríguez-Cabal et al., 2007; Amico et al., 2009). Among mammals, *D. gliroides* is responsible for the dispersal of up to 24% of all fleshy-fruited species in Chiloé Island forests (J. L. Celis, unpublished data). Considering that in temperate rainforests of Chiloé Island, about 70% of the woody flora bears fleshy fruits (Armesto and Rozzi, 1989), animal frugivores such as *D. gliroides* may facilitate the processes leading to seed dispersal and forest regeneration. The fruit-eating behavior of *D. gliroides* is exacerbated in summer, coinciding with the fruiting peak of many fleshy-fruited forest species (Smith-Ramírez and Armesto, 1994; Riveros and Smith-Ramírez, 1996). High arthropod abundance in Chiloé forests during spring-summer (Díaz, 2008) becomes particularly important for *D. gliroides* during the first months following hibernation, both in SL and OG forest patches. We propose that a higher proportion of arthropods in the diet of *D. gliroides* detected in SL forests can be related to greater bamboo cover in the understory of open canopy stands (Pérez et al., 2009). Bamboos sustain a great abundance of some arthropod groups (Reid et al., 2004). Alternatively, our data support the hypothesis of Amico et al. (2009), which suggests that the higher incidence of arthropods in *Dromiciops* diet in logged forests could be related to a reduction in the local abundances of epiphytes and vines after logging.

Although higher parasitism has been generally attributed to greater population stress and low habitat quality (Daszak et al., 1999), higher prevalence of ectoparasites in *D. gliroides* populations (55%) at SL forests compared to OG forests did not have noticeable effects on the animal's health condition, as there were no differences in body mass, recapture rate, or population density between habitats. This relict marsupial is the main host for *I. neuquenensis*, the only tick ever recorded on *D. gliroides* (Marín-Vial et al., 2007) and likely, the biological vector of a recently documented blood parasite of this species (Merino et al., 2009). Health consequences of these vector-parasite-host interactions in different forested habitats of northern Chiloé Island are still unknown.

In short, knowledge of the population density, behavior, and food habits of *D. gliroides* and other forest specialist species that use remnant forest patches in rural landscapes and lowland areas of the Chilean Lake District (Castellon and Sieving, 2006; Fontúrbel et al., 2009; Smith-Ramírez et al., ms), is urgently needed in the context of regional conservation of biodiversity outside protected areas, and also considering a rapidly changing rural scenario, due to unabated rates of forest cover loss, fragmentation and reduction of old-growth forest habitats, and climate change (Armesto et al., 1998; Echeverría et al., 2007; Jaña et al., 2007; Armesto et al., 2009). On the positive side, maintenance of *D. gliroides* populations in rural forest patches has been documented under current selective logging practices, which means that under regulated management of rural forest patches, conservation of forest dwelling species with strict habitat requirements is possible. Consequently, our report provides valuable guidance for *D. gliroides* conservation and for planning the sustainable management of rural areas.

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FIGURE LEGENDS

Figure 1. Satellite image (gray scale) of the rural landscape of northern Chiloé Island (Landsat 2001). Darker areas indicate wooded habitats, and lighter areas indicate shrublands and agricultural lands. Circles indicate studied sites where Old-growth forest (OGF), Selective-logged forest (SLD) and ESS are found.

Figure 2. Different habitats were *Dromiciops* was studied in a rural landscape of northern Chiloé. A and B = Old Growth forest, C and D = Selective Logged forest and D and E = shrubland.

Figure 3. Population density (based on mnka) of *Dromiciops gliroides* in the studied sites in northern Chiloe Island. (A) Densities in different sites SD = Senda Darwin OG forest; Caulín = Caulin OG forest; LL = Llanquihue SL forest; QU = Quilar SL forest; ESS1 = Senda Darwin shrubland 1; ESS2 = Senda Darwin shrubland 2, and (B) Densities in different habitat types. OFG = Old-growth forest; SLF = Selective logged forest and ESS= Shrublands.

Figure 4. Example of home range overlaps among two adult males (horizontal lines) and four adult females (vertical lines) at Senda Darwin OGF in Chiloé Island.

Figure 5. Characterization of the trophic niche of *Dromiciops gliroides* in forest habitats and during spring and summer, from the three-year of data in northern Chiloé Island.

Figure 6. Mean (± 1 SE) plasma testosterone and progesterone concentration (ng/ml) in blood samples from 44 males (black) and 19 females (white) of *D. gliroides* from pooled data for all rural forest sites in northern Chiloé Island.

Appendix 1. Monthly climatic records from Senda Darwin Biological Station for the studied periods (2005 – 2009).

Figure 1.

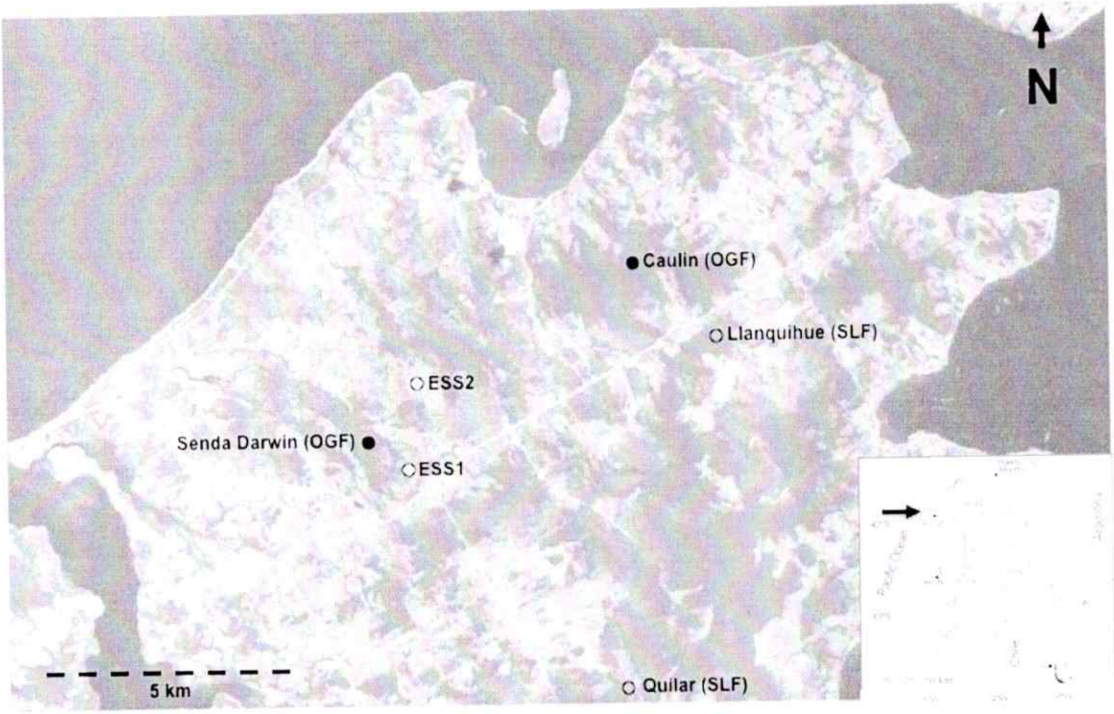

N
Caulin (OGF)
Llanquihue (SLF)
ESS2
Senda Darwin (OGF)
ESS1
5 km
Quilar (SLF)

Figure 2.

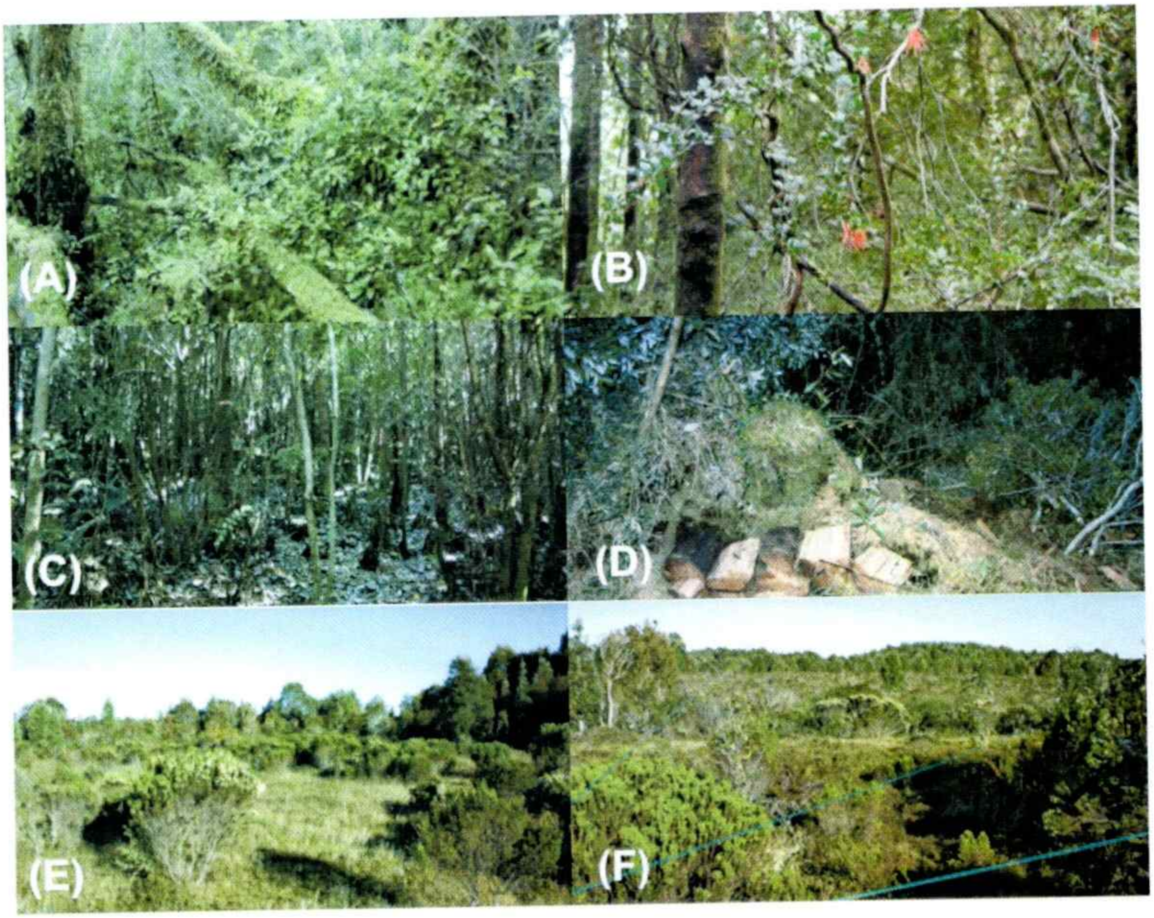


Figure 3.

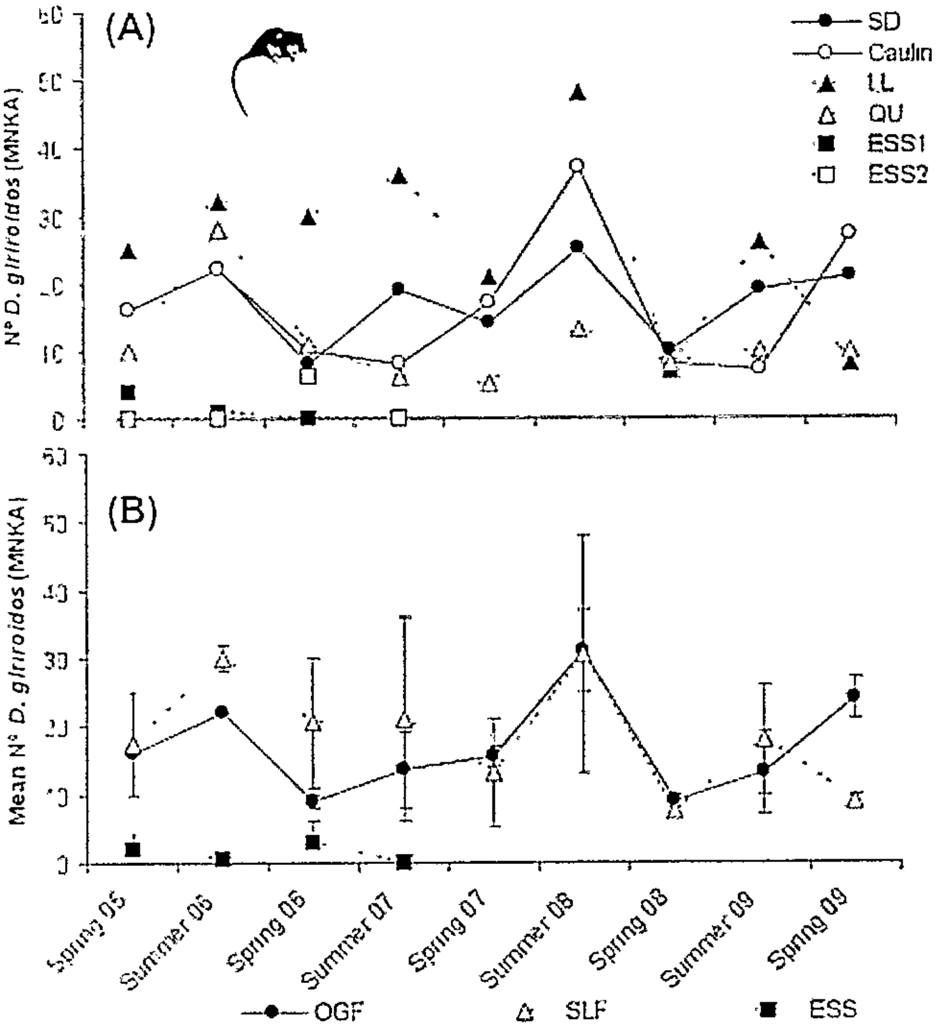

(A)
N° D. gliroides (MNKA)
0
10
20
30
40
50
60
SD
Caulin
LL
QU
ESS1
ESS2
(B)
Mean N° D. gliroides (MNKA)
0
10
20
30
40
50
60
Spring 05
Summer 06
Spring 06
Summer 07
Spring 07
Summer 08
Spring 08
Summer 09
Spring 09
OGF
SLF
ESS

Figure 4.

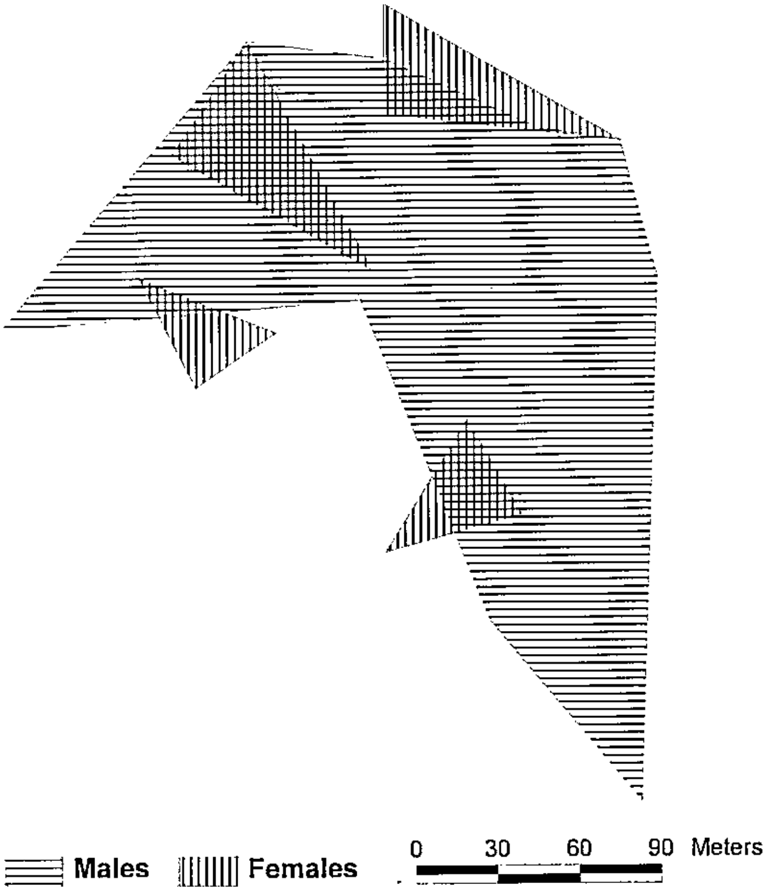


Figure 5.

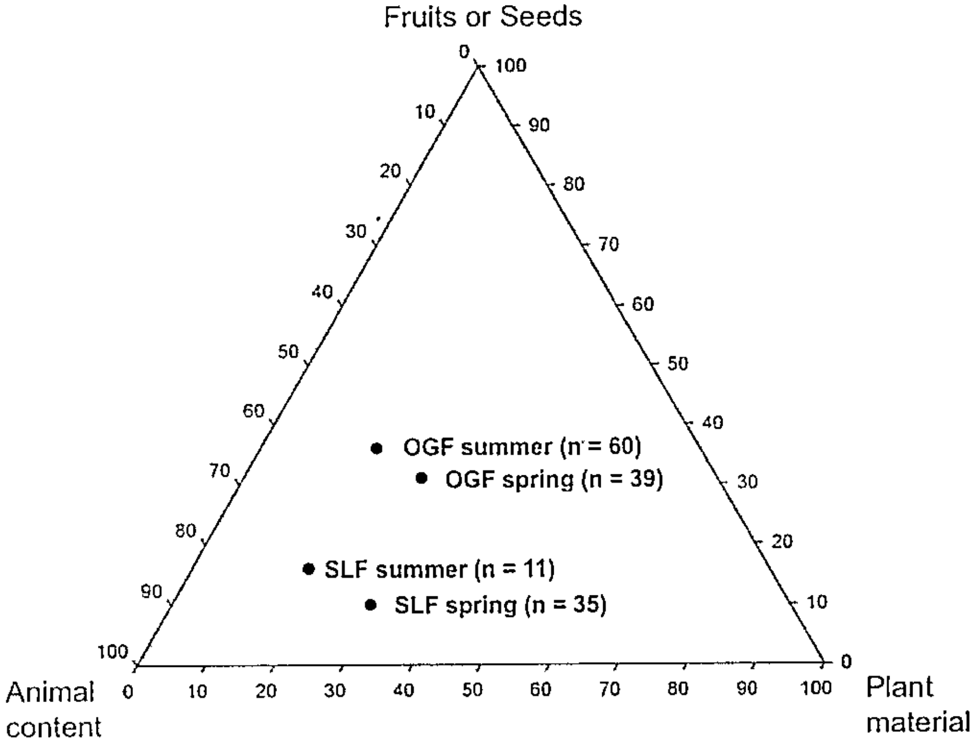

Fruits or Seeds
Animal content
Plant material
0
10
20
30
40
50
60
70
80
90
100
OGF summer (n = 60)
OGF spring (n = 39)
SLF summer (n = 11)
SLF spring (n = 35)

Figure 6.

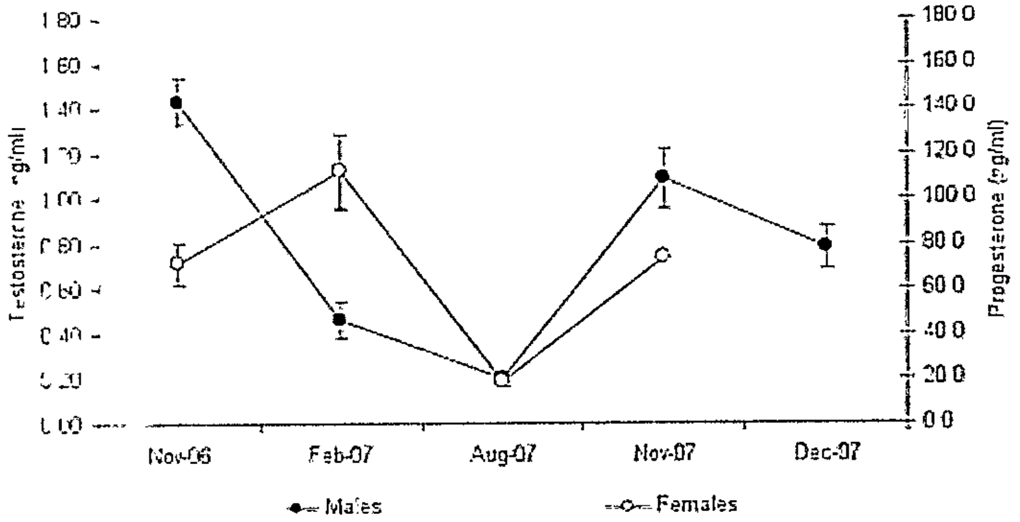

Testosterone (ng/ml)
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
Progesterone (ng/ml)
180.0
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Nov-06
Feb-07
Aug-07
Nov-07
Dec-07
Males
Females

Table 1. Number of captured (MNKA) and recaptured individuals of *D. gliroides* from spring 2005 to spring 2009 according to sex and age categories in all study sites (forests and shrublands) in the rural environment of northern Chiloé Island.

	OG forest													
	Senda Darwin							Caulin						
				Adult							Adullt			
Season	mnka	recaptures	dead	Males	Females	Pregnant females	Juveniles	mnka	recaptures	dead	Males	Females	Pregnant females	juveniles
Spring 05	16	12	1	12	5	4	0	16	2	0	12	3	1	1
Summer 06	22	27	1	8	3	0	12	22	7	0	6	6	0	10
Spring 06	8	2	0	5	3	0	0	10	7	2	6	2	0	4
Summer 07	19	19	1	7	2	0	11	8	2	0	2	4	0	2
Spring 07	14	13	1	11	3	2	0	17	12	0	12	5	3	0
Summer 08	25	33	1	7	4	0	15	37	41	2	11	10	0	17
Spring 08	10	5	0	9	1	0	0	8	0	0	5	3	0	0
Summer 09	19	6	0	5	2	2	12	7	1	0	1	1	0	5
Spring 09	21	27	0	15	7	0	0	27	13	0	20	6	0	1

Table 1. cont.

	SL forest													
	Llanquihue							Quilar						
				Adult							Adullt			
Season	mnka	recaptures	dead	Males	Females	Pregnant females	juveniles	mnka	recaptures	dead	Males	Females	Pregnant females	Juveniles
Spring 05	25	11	2	15	12	0	0	10	5	1	5	6	5	0
Summer 06	32	16	3	4	6	0	25	28	8	0	7	5	0	16
Spring 06	30	24	3	20	8	0	5	11	10	3	9	1	0	3
Summer 07	36	27	1	10	13	0	13	6	9	1	0	3	0	4
Spring 07	21	12	1	14	8	8	0	5	2	0	4	1	1	0
Summer 08	48	23	0	7	6	0	35	13	20	0	3	2	0	8
Spring 08	7	1	0	4	3	2	0	8	4	0	7	1	0	0
Summer 09	26	2	0	6	6	4	14	10	1	0	3	1	0	6
Spring 09	8	5	2	6	4	0	0	10	5	0	7	3	0	0

Table 1. cont.

	ESS													
	ESS1							ESS2						
				Adult							Adullt			
Season	mnka	recaptures	dead	Males	Females	Pregnant females	juveniles	mnka	recaptures	dead	Males	Females	Pregnant females	Juveniles
Spring 05	4	0	0	1	2	1	1	0	0	0	0	0	0	0
Summer 06	1	0	0	0	0	0	1	0	0	0	0	0	0	0
Spring 06	0	0	0	0	0	0	0	6	0	0	3	3	0	0
Summer 07	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2. Mean (± 1 SE) body mass (g) of *D. gliroides* during spring and summer, and differences between sexes and ages classes from 2005-2009 in different forest and shrubland habitats of northern Chiloe Island. nd = no data.

Habitat	Forest	Season	Sex											
			Male						Female					
			adult	SE	n	juvenile	SE	n	adult	SE	n	juvenile	SE	n
OGF	Senda Darwin	Spring	26.5	0.7	35	n/d	n/d	n/d	25.8	1.1	13	n/d	n/d	n/d
		Summer	29.1	1.2	12	18.0	0.7	34	31.1	1.4	9	18.9	1.0	16
	Caulin	Spring	26.3	0.6	46	17.5	4.1	1	24.9	1.2	11	20.3	2.4	3
		Summer	29.6	1.6	7	17.0	0.9	20	31.2	1.4	9	17.3	1.1	13
SLF	Llanquihue	Spring	27.0	0.6	42	27	4.1	1	28.4	0.9	19	20.3	2.1	4
		Summer	29.0	1.3	10	18.8	0.6	53	31.4	1.0	17	19.5	0.7	31
	Quilar	Spring	28.1	0.9	21	n/d	n/d	n/d	28.3	1.3	10	20.3	2.4	3
		Summer	28.1	1.5	8	19.9	0.8	27	37.4	1.6	7	18.4	1.7	6
ESS	ESS1	Spring	28.0	4.1	1	n/d	n/d	n/d	27.8	2.9	2	n/d	n/d	n/d
		Summer	n/d	n/d	n/d	22.5	4.1	1	n/d	n/d	n/d	n/d	n/d	n/d
	ESS2	Spring	28.8	2.4	3	n/d	n/d	n/d	36.3	2.4	3	n/d	n/d	n/d
		Summer	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d

Table 3. Mean home range areas (ha) for adult male and female *D. gliroides* in forested habitats of northern Chiloé Island. OGF = Old growth forest patches and SLF = Selective logged forest patches.

Habitat	Forest	Sex					
		Male	SE	n	Female	SE	N
OGF	Senda Darwin	0.35	0.05	15	0.13	0.06	9
	Caulin	0.12	0.06	11	0.15	0.06	9
	Mean	*0.26*	*0.04*	*26*	*0.14*	*0.05*	*18*
SLF	Quilar	0.18	0.07	8	0.23	0.09	5
	Llanquihue	0.27	0.06	12	0.12	0.06	11
	Mean	*0.23*	*0.05*	*20*	*0.15*	*0.05*	*16*
	Total mean	*0.26*	*0.03*	*46*	*0.14*	*0.04*	*80*

Table 4. Relative frequency of occurrence (FO%) of arthropods in feces of *Dromiciops gliroides* in rural forest habitats of northern Chiloé Island.

Arthropod order	FO % (n = 126)
Coleoptera	42.9
Orthoptera	24.6
Lepidoptera	11.9
Hymenoptera	2.4
Hemiptera	1.6
Aranea	0.8
Acari	1.6
Unidentified	14.3

Table 5. Relative frequency of occurrence (FO%), and relative abundance (%) of seeds over the total sample, and total number of seeds of 12 different fleshy-fruited forest species found in feces (n = 146) of *Dromiciops gliroides* in rural forest habitats of northern Chiloé Island. Number of fruits eaten (in parentheses) is estimated from number of seeds per fruit (Armesto et al., 1987) and personal observations*. na = not applicable. Known dispersal agent extracted from Armesto et al. (1987).

	Growth form	FO %	Abundance % (n = 359)	No. seeds (fruits)	Known dispersal agent
Tristerix corymbosus	Hemiparasite	43.08	25.07	90 (90)	*D. gliroides* – Birds
Gaultheria insane	Shrub	18.46	13.93	50 (na)	Unknown
Gaultheria mucronata.	Shrub	10.77	15.32	55 (6)	Birds
Luzuriaga polyphylla	Vine	7.69	4.46	16 (16)	Unknown
Sarmienta repens	Hollo-epiphyte	6.15	5.85	21 (na)	Unknown
Mitraria coccinea	Vine-Epiphyte	3.08	3.34	12 (1)	Unknown
Griselinia racemosa	Vine	3.08	5.29	19 (19)	Unknown
Asteranthera ovata	Vine-Epiphyte	1.54	2.23	8 (1)	Unknown
Raukaua laetervirens	Hemi-epiphyte	1.54	1.67	6 (2)	Unknown
Myrteola numularia	Shrub	1.54	22.28	80 (6)	Birds
Philesia magellanica	Shrub, vine	1.54	0.28	1 (1)	Birds
Amomyrtus luma	Tree	1.54	0.28	1 (1)	Birds

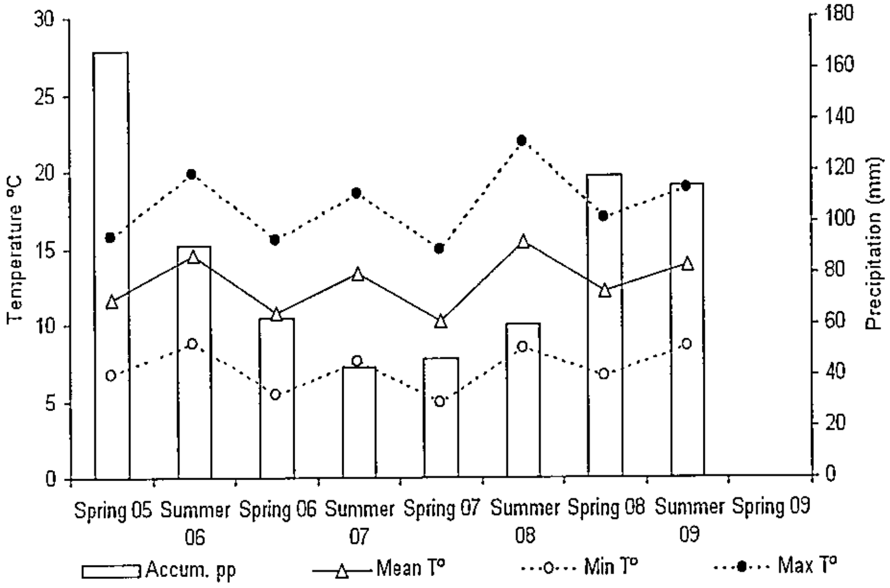
Table 6. Number of adult and juvenile individuals with and without ectoparasites in forest sites of rural environments in northern Chiloé Island.

Habitat	Forest	With		Without ectoparasites		Total
		Adults	Juveniles	Adults	Juveniles	
OGF	Senda Darwin	10	6	10	7	
	Caulin	10	4	15	5	
	Total OGF	*20*	*10*	*25*	*12*	*67*
SLF	Quilar	11	11	7	4	
	Llanquihue	27	20	21	9	
	Total SLF	*38*	*31*	*28*	*13*	*110*
ESS	ESS1	0	1	2	0	
	ESS2	2	0	3	0	
	Total ESS	*2*	*1*	*5*	*0*	*8*
	Total	*60*	*42*	*58*	*25*	

Table 7. Mean mass (± 1 SE) and number of individuals of *D. gliroides* found nesting in artificial nest boxes in rural forest patches of northern Chiloé Island.

Weight	Solitary				Communal			
	Males		Females		Males		Females	
	Adults	juveniles	adults	juveniles	adults	juveniles	adults	juveniles
Mean	34.67	28	32.2	27.67	32.44	26.88	34.56	26.98
n	12	1	5	3	51	16	24	27
SE	1.82	Na	1.55	0.44	0.85	0.75	1.04	0.54

Appendix 1. Monthly climatic records from Senda Darwin Biological Station for the studied periods (2005 – 2009).



CAPITULO 2

DEMOGRAPHY OF THE ARBOREAL MARSUPIAL *DROMICIOPS GLIROIDES* IN OLD GROWTH AND SELECTIVELY LOGGED FORESTS OF CHILOÉ ISLAND, CHILE

ABSTRACT

Demographic consequences of selective logging on the endemic arboreal marsupial Monito del monte (*Dromiciops gliroides*) were assessed in forest patches in a rural landscape of northeastern Chiloé Island (42 S), Chile. Two old growth (OG) and two selectively logged (SL) temperate rainforest fragments were studied as representative of remnant, forested areas, where populations of the relict and threatened *D. gliroides* occur. We expected that habitat changes due to logging could have negative effects on *D. gliroides* demography. Individuals were live-trapped for four consecutive years (2005–2009) during the mating (November) and non-mating (February) seasons. Differences in forest structure and food resources, such as arthropods and fruits, were measured between OG and SL forests. Demographic parameters were modeled using mark-recapture procedures. OG forests had a higher density of larger canopy trees and snags, and more fleshy-fruited epiphytes than SL forests. Cover of the understory native bamboo (*Chusquea* sp.) was higher in SL than in OG forests and higher arthropod abundances were associated with bamboo cover. Population sizes of *Dromiciops* varied seasonally but did not differ between the two forest habitats. Demographic parameters (e.g., monthly survival and recruitment) were not different between habitat types, but survival varied across years, and recruitment between seasons each year. Yearly population growth rate of *Dromiciops* was similar between logged and unlogged forests but showed inter-annual and forest fragment variation. In conclusion, small-scale, selective logging of remnant OG forest patches in northern Chiloé does not translate into demographical consequences for this arboreal marsupial, despite some changes in habitat structure. The

maintenance of understory bamboo cover is a key element for the persistence of the population, by providing nesting materials, protection from predators, and arthropod food resources. This result constitutes an important finding to support the conservation of this forest-dependent relict species in anthropogenic rural landscapes.

INTRODUCTION

Forestry practices such as logging can have profound implications for forest structure and wildlife habitat (Lindenmayer et al., 1990; Newton, 1994; Lindenmayer et al., 1997), depending on the degree of impact, along a gradient from clear-cutting to selective cutting of single trees (Emmingham et al., 1992; Laurance, 1996; Sullivan et al., 1999; Gagne et al., 1999). In anthropogenic landscapes, such as rural environments, forest-dependent species are especially susceptible to the often-invisible process of forest degradation, where forest area remains unchanged but there is a significant loss of forest structures and habitat quality due to firewood extraction, cattle grazing, local fires, and logging of remnant forest patches (Lambert et al., 2005; Sekercioglu et al., 2007). Depending on logging intensity, selective logging scenarios can substantially alter forest structure and resources for wildlife. However, most damage to vegetation is incidental to the forestry practices; thus the net effect is not "selective" removal of large canopy trees of a few timber species, but a broader impact across a nearly random cross-section of trees, understory, shrubs, epiphytes, vines (Johns 1988) as well as terrestrial and flying arthropods (Lambert et al., 2005). Arboreal mammal populations may be strongly influenced by changes in structure and composition of forests (Laurance, 1996; Wilson and Friend, 1999; Ecke et al., 2002; Simard and Fryxell, 2003; Lee, 2004; Renner et al., 2006; Isaac et al., 2008). For example, dead or over-mature trees which are characteristic of primary forest are a crucial source of den cavities for many arboreal vertebrates (Lindenmayer et al., 1997; Gibbs et al., 1999, Gibson and Lindenmayer, 2002). Alternatively, openings created within logged stands may provide new niches that could potentially increase biodiversity.

Habitat quality is often defined in relation to population density and resource availability. Moreover, population fluctuations are the result of demographic processes (Julliard et al., 1999) and the loss of habitat quality such as forest heterogeneity and resources due to logging could have long-term consequences, which are translated into demographic parameters rather than current population abundances (Mahan et al., 1996; Martin and Handasyde, 2006; Isaac et al., 2008).

In some cases, population density estimates are not adequate indicators of habitat quality (Van Horne, 1983; Pulliam, 1988; Brawn & Robinson, 1996). Consequently, habitat quality should be assessed directly by examining relationships between population survival or growth rate and variation in habitat characteristics (Loeb, 1999; Manning and Edge, 2004). However, few studies have investigated demographic responses to habitat degradation as a way of understanding the consequences of logging on forest-dwelling species.

Forest cover loss and fragmentation has been intense in South American temperate rain forests during the past 100 years (Willson and Armesto, 1996; Armesto et al., 1998; Jaña-Prado et al., 2006; Echeverria et al., 2007). Old-growth forests in lowland areas of Chiloé Island occupy today less than one third of its original distribution (Armesto et al., 2009) and consequently, Chile belongs to the group of countries with biodiversity hotspots that have increased overall deforestation rate from 1.02% during the 1980s to 1.76% during the 1990s (Jha and Bawa, 2006). Deforestation has continued to drive the loss of old-growth forest cover and greatly altered the landscape of this temperate forest region (Willson and Armesto, 1996; Echeverria et al., 2007; Armesto et al., 2009). It has been estimated that only 5% of logging of native forests is based on controlled silvicultural practices (Lara, 1996). In Chile, most forests have been clear cut or burned and replaced by exotic forestry plantations. However, in many rural areas remnant forests are usually subjected to selective logging, where individual trees of high timber value or high quality for firewood are removed, leaving large clearings in the canopy. Given the frequency of traditional selective logging by rural inhabitants and by small forestry operations, it is relevant to assess the impacts of such practices on habitat quality for forest dwelling species (Nystrand and Granstrom, 2000).

Dromiciops gliroides (Microbiotheria), a threatened arboreal marsupial (IUCN 2008) is endemic to South American temperate rainforests (Herskhovitz, 1999), and has been frequently cited as an inhabitant of extensive old-growth forest patches (see chapter 1; Mann, 1978; Kelt and Martinez, 1989; Rodriguez-Cabal et al., 2007, 2008; Fontúrbel and Jiménez, 2009). Recently, this arboreal species has been recorded with similar densities in secondary-growth forest as in old-growth forest patches (see chapter 1; Patterson et al., 1990; Fonturbel et al., 2009), and therefore it is interesting to know the consequences of habitat degradation due to logging on the long-term demography of *D. gliroides*. This should help assess the possible trends in *D. gliroides* populations of remnant forest patches in rural landscapes, as habitat quality may deteriorate over time leading to population declines. Here, we report a four-year study of *D. gliroides* populations in remnant forests of rural northern Chiloé Island, which differ in structure due to logging.

Our specific objectives were: (1) To characterize and compare the structure of old-growth (OG) and selectively logged (SL) forest stands, as habitats for *D. gliroides*, (2) to estimate *D. gliroides* population size in each habitat, and (3) to estimate the main demographic parameters (*i.e.* survival, recruitment and population growth rates) reflecting the status of *D. gliroides* populations in SL and unlogged forest patches in northern Chiloé. On the basis of previous reports, we expected to find similar densities in both forest types, but higher survival and recruitment rates in OG than in SL forest habitats, because the former should provide the best-quality habitat in terms of forest structure and availability of food resources for *D. gliroides*. Considering *D. gliroides* hibernation behavior, the greater abundance of hollowed trees (Diaz et al., 2005) and presumably the greater abundance of fleshy-fruited epiphytes and vines in OG forests, results in better habitat quality for *D. gliroides* than logged forests. Alternatively, SL forests may provide opportunities for higher survival of *D. gliroides* due to the expansion of native bamboo cover and associated arthropods that can serve as protective habitat and resources for *D. gliroides*.

MATERIAL AND METHODS

Study sites. — The study was conducted in four medium size (> 50 ha) forest fragments, representing two different levels of logging in northeastern Chiloé Island, Ancud County, Chiloe province (41° 50' S, 73° 36′ W; Fig. 1). The rural landscape mosaic has about 40% of forest fragments, including both old growth and secondary forest patches, embedded in an anthropogenic matrix of grazing pastures, agricultural fields, and dense humid shrublands (Willson and Armesto

1996; Aravena et al., 2002; Díaz et al., 2005). We sampled two selectively logged and two un-harvested forest patches, because of they represented two contrasting habitat for *D. gliroides*, due to their different vegetation structures. The patches selected were separated from each other by at least 5 km. Old-growth (OG) forest fragments were within Senda Darwin Biological Station and in Fundo Cisnes at Caulín (Fig.1), had no signs of human impact, and had large emergent trees in their canopies. Selectivelly-logged (SL) forests were at Llanquihue and El Quilar, where landowners had harvested valuable and relatively large timber trees with no defined silvicultural system (see Cornelius 2007). Thus, we had two replicates for logged and two for unlogged patches.

The two OG forest remnants were about 300 years old, based on tree-ring counts of dominant canopy trees, and had no visible signs of recent logging or fire (Aravena et al., 2002; Gutiérrez et al., 2004). These OG patches were characterized by a multi-layered canopy, with large emergent trees of *Nothofagus nitida* (Nothofagaceae), *Drimys winteri* (Winteraceae), *Podocarpus nubigena* and *Saxegothaea conspicua* (both Podocarpaceae), plus few individuals of *Eucryphia cordifolia* (Eucryphiaceae) and *Caldcluvia paniculata* (Cunoniaceae) in the lower canopy. Trees were often covered with vines and epiphytes such as ferns, mosses and bromeliads (Muñoz et al., 2003). Large amounts of dead biomass (snags and logs) were frequent within OG stands. The understory had a high density of tree saplings, few shrubs, and dense thickets of 2-3 m tall native bamboo (*Chusquea* spp.) within tree-fall gaps (Gutiérrez et al., 2004).

SL forest patches are frequent in the rural landscape, due to the widespread practice of small-scale harvesting, which generally results in an open canopy forest with remnant large *Nothofagus nitida*, which are left standing. The patches sampled had areas with a closed canopy of dense juvenile trees, 10–20 m tall, predominantly shade-intolerant *D. winteri*, and *Amomyrtus luma*. In SL forests, open areas with a dense cover of native bamboos, and a sparse presence of tree seedlings and saplings due to shading, were frequent (Aravena et al., 2002). Both SL patches endured limited logging at the end of summer during the study period (Quilar in 2007 and Llanquihue in 2008); nevertheless, the two sites showed no evidence of cattle impact or fire.

Habitat structure and food resources. —At each forest fragment thirty 10-m radius circular plots were established. Each plot was assigned randomly to a trapping station within each 100 m radial line (see below). We divided habitat variables into three groups: habitat structures, vines, and resources. At each plot we counted or measured the following: (a) The number of live trees, snags and fallen logs with a DBH (diameter at breast height) >30 cm as a proxy for availability of potential hollowed-trees or potential nest sites for secondary-cavity nesters (Newton, 1994; Escobar et al., 2002; Cornelius 2007). This arbitrary cut-off diameter was defined considering the smallest diameter of holes used by *D. gliroides* to built nests (unpublished data), and was consistent with dimensions of nest-boxes used by *D. gliroides*. (b) Within a 2-m radius circle at the center of each vegetation plot, we measured native bamboo cover (*Chusquea* spp.), canopy, and non-bamboo understory cover (i.e., shrubs and sapling). These different covers were estimated visually as the percentage of ground covered by vegetation above 10 m (canopy) and between 0,5 and 3 m (understory), assigning each point to one of five cover classes: 0%, 1–25%, 26–50%, 51–75% and 75-100%. (c) As plant resource variables we measured: presence of fleshy-fruited vines and epiphytes consumed and probably dispersed by *D. gliroides* (i.e., *Tristerix corymbosus*, *Luzuriaga poliphylla, Mitraria coccinea, Asteranthera ovata*, *Sarmienta repens*, *Gaultheria insana;* see chapter 1; Amico et al., 2009). (d) Abundance of arthropods (>5 mm long), was estimated as the number trapped by with 2 m height interception traps, in one half of the circular plot at each forest fragment. Variables (a) and (b) are descriptors of habitat structures while (c) and (d) are rough estimators of food resources used by this arboreal marsupial. Differences between logged and OG forests in habitat and resource variables were analyzed using a permutational multivariate analysis of variance through PERMANOVA software (version 1.6 2005; Anderson, 2005). Data were previously transformed using log(x+1) for habitat variables and presence/absence distance matrix for occurrence of fleshy–fruited epiphytes (Anderson, 2005).

Animal trapping. — *Dromiciops gliroides* at each forest patch were studied from spring 2005 to spring 2009 (four consecutive years). To estimate demographic parameters, trapping was conducted over 10 consecutive nights in two seasons each year: (a) in mid-spring (November-December), after animals became active following winter torpor and before the onset of the breeding season, and (b) late austral summer (February-March), after the reproductive season ended. Trapping webs were established in both OG and SL forests, consisting of twelve radial lines of 100 m long. Each trapping line had 12 stations; the first four were at 5 m intervals from the center (5, 10, 15, and 20 m), and the following eight were at 10 m intervals (30, 40, 50, 60, 70, 80, 90, and 100 m). With this web design, 144 traps were set in each forest patch covering 3.46 ha (Parmenter et al., 2003). We used Tomahawk-like traps (baited with banana) fixed on tree branches between a minimum height of 1 m and maximum of 3 m. Traps were checked daily between 06:00 and 08:00 hours. For all captured animals we recorded sex, age, breeding status (based on scrotum size, pouch morphology and mammary gland development), and body mass, using a Pesola ® (Baar, Switzerland) spring balance, 0.1 g precision. In late summer, animal weight from adult individuals was compared between sexes and both forest patches, because before entering in winter torpor, weight can be a good proxy of habitat quality through health status. To avoid pseudo replication we considered body mass for only new unmarked adult individuals. Statistical analyses were performed using two-way ANOVA in Statistica software (version 6.0, 2001, Statsoft Inc.).

Model selection and demographic parameters. — Population parameters were estimated for OG and SL forests by capture-mark-recapture (CMR) procedures (Lebreton et al., 1992). Our data consisted of captured-recaptured history from nine primary occasions. We combined closed population models to estimate population size within each trapping session (10 days per trapping period as secondary sessions), with open population models to estimate survival between primary sessions (Quental et al., 2001). Time between primary sessions was three months in summer and nine months in winter. Survival estimation was standardized to one month following Yoccoz et al. (1998). Population abundance was estimated by software DENSITY (version 4.1, 2007; Efford et al., 2004), using a Jackknife estimator that does not assume homogeneity of capture probabilities among individuals (Burnham and Overton, 1979). This method was adopted because, including heterogeneity in model selection we obtain a better adjustment (Δ AIC > 2) because many individuals were caught just once (n = 260 from a total of 494) and few were caught 5 times or more (n = 43). Relative population densities were estimated as the ratio of population abundance to the effective area estimated as the trapping web area plus a buffer area of a width equal to half of the largest recapture distance (i.e., MDA; Otis et al., 1978; Parmenter et al., 2003: Fontúrbel et al., 2009). Because we expected differences in population densities between seasons (see chapter 1), we performed a two-way ANOVA in Statistica software (version 6.0, 2001, Statsoft Inc.), to test differences in population densities between habitat types and seasons.

A Goodness of fit test for the general model was applied with U-Care software (version 2.02; Choquet et al., 2005), searching for transients, as an estimate of migration, and trap shy-happy individuals.

For all demographic parameters, we constructed a model set to test the hypothesis of difference among forest patch sites (i.e., Senda Darwin, Caulin, Llanquihue, Quilar), between OG and SL forests, between seasons (summer vs. spring), and among years (from 2005 to 2009). Capture probabilities were also tested for spatial and temporal variation. Survival (φ) and recruitment rates (1-γ) were analyzed by CMR statistical modeling based on Akaike's information criterion (AIC; Lebreton et al., 1992) using M-SURGE software (version 1.8.5, 2008; Choquet et al., 2004).

We selected models with the lowest AIC (Δ AIC > 2); otherwise models were considered statistically identical (Burnham and Anderson, 1998), in which case we selected the model with the smallest number of parameters, assuming a parsimonious criterion (Crespin et al., 2006).

Recruitment rate was estimated with the method developed by Pradel (1996), "reading" the capture histories backwards (reverse-time capture-recapture methods). This makes it possible to estimate the probability that an individual caught at time t_{+1} was present in the population at time *t*. These probabilities are called seniority probabilities (γ; Pradel, 1996) and represent a fraction of the population. Seniority probabilities can be used to estimate other related demographic parameters, such as population growth rate (λ) (Lima et al., 2001).

Reproductive rate was estimated from adult females as a fraction from reproductively active individuals (pregnant or lactating) at both habitat types.

Finally, population growth rate (Lambda: λ) was estimated using Pradel's estimator through MARK software (version 5.1; White and Burnham, 1999). For this analysis we considered only one primary capture session per year in mid-spring (i.e., in November).

RESULTS

Habitat structures. — OG and SL forests differed markedly in: (a) number of large tree, snags, canopy cover, woody understory cover, and bamboo cover (ANOVA $F_{1,\,119}$ = 54,44, $P < 0.001$), (b) food resources such as the presence of fleshy-fruited vines and epiphytes (ANOVA $F_{1,\,119}$ = 14,99, $P < 0.001$), and (c) arthropods per unit of understory vegetation (ANOVA $F_{1, 59}$ = 4,22, P = 0.015). The number of large trees and snags was greater in OG than in SL forests (Table 1). Understory cover of shrubs and tree saplings was also higher in OG than in SL forests, while bamboo cover was larger in the more open SL than in OG forest patches (Table 1). Forest canopy cover also differed between forests, being denser in SL than in OG patches (Table 1).

The frequency and species composition of fleshy-fruited epiphytes and vines also differed between logged and unharvested forests. More fleshy-fruited vines were found in OG forests. Finally, we recorded higher abundances of arthropods at SL forests (Table 1). Arthropod abundances were positively and significantly correlated with volume of understory bamboo in both logged and unlogged stands (Spearman rank correlation R = 0.20, n = 60, P = 0.025).

Abundance and population parameters of Dromiciops gliroides.— From spring 2005 to summer 2009, we recorded a total of 1054 captures, representing 494 different individuals in OG and SL forests combined. A total of 119 animals were trapped at Senda Darwin, 111 at Caulín (both being OG forests), while 181 were trapped at Llanquihue and 83 at Quilar (SL forests). On average, each animal was captured 2.13 times. In mid-late summer, there was no significant difference in adult body mass between habitat types (ANOVA $F_{1, 75}$ = 0.16, P = 0.69), but we did detect marginal differences between both sexes (ANOVA $F_{1, 75}$ = 3.81, P = 0.054).

Overall, mean population densities were not statistically different between OG and SL forests (i.e., OGF mean = 6.18 ± 1 SE = 1.26, n = 9; SLF mean = 8.8 ±

1 SE = 1.26, n = 9; GLM W = 3.15, P = 0.07; Fig. 2), however, we observed a slight trend towards higher values in SL forests. Population density varied seasonally in all habitats (Fig. 2). In both forested habitats (i.e., OGF and SLF), we observed higher *Dromiciops* densities in the austral summer (February), which corresponded to the juvenile recruitment period, than in the previous spring (November), when mating and breeding took place (summer mean = 9.73 ± SE = 2.09, n = 8; SLF mean = 5.3 ± SE = 1.33; GLM W = 10.6, P = 0.01; Fig. 2).

The highest and lowest *Dromiciops* densities were observed at SL forests with a maximum of 20.9 individuals/ha in Quilar (e.g., summer of 2006) and a minimum of 1.5 individuals/ha in the same forest patch (e.g., summer and spring of 2007; Fig. 2). In spring 2008, we recorded a drastic reduction in population in all forest patches.

Model selection and demographic parameter estimation. — Based on the AIC criterion, neither logging nor season had significant effects on survival rates of *Dromiciops* populations (Δ AICc < 2; Table 2). However, we did detect an inter-annual variation in survival (Table 2). With the exception of 2007, we observed a trend towards higher survival rates during summer than in winter, yielding no overall difference between seasons. We detect a small proportion of transient individuals in Senda Darwin OG forest. Testing model selection assumptions, no effect on survival was detected for the general model. Capture probabilities were significantly different between each studied sessions, but neither selective logging nor season had significant effects (Table 2).

As expected, recruitment rates (1- γ) showed strong seasonal variability with higher values in summer (monthly recruitment = 0.33) than in spring (monthly recruitment = 0.02; Table 3). Neither timber harvesting nor year had significant effects on recruitment (Table 3). Reproductive rate, as the proportion of reproductive females, was not different between both habitat types (0.17 in OG vs. 0.22 in SL forests; $\chi 2 = 0.4$, df = 1, P = 0.53).

The estimation of annual population growth rate (λ) ranged from 0.8 to 1.11 in a given forest fragment with a mean of 1.15 and 0.97 at OG and SL forest, respectively. Annual population growth rate did not differ between OGF and SLF (Table 3). However it did differ between each forest stand with the lowest value at Llanquihue SL forest (Table 3). Overall annual population growth rates were 1.11 ± 0.09, 1.19 ± 0.09, 1.10 ± 0.12 and 0.83 ± 0.06 for Senda Darwin, Caulin, Quilar and Llanquihue, respectively (Table 3), indicating slow population growth rate during the four years of study.

DISCUSSION

Most studies on habitat loss have focused on landscape-level processes, such as patch size, the spatial configuration of patches, and dispersal patterns of organisms, paying less attention to habitat degradation within fragments (Harrison and Bruna, 1999, Jaña-Prado et al., 2006).

Selective logging usually modifies food-resource availability, and shape life history traits of forest-dwelling species (see chapter 1; Wheatley et al., 2002; Isaac 2005; Herbers & Klenner 2007) through changes in vegetation structure of remnant forest patches (Pérez et al., 2009). This traditional logging practice by landowners typically target large trees of selected timber species (Aravena et al., 2002; Lambert et al., 2005; Diaz et al., 2005; Pérez et al., 2009), thus reducing the abundance of tree holes, snags and understory trees. As a consequence of the opening of the canopy, SL forests present higher cover of the understory bamboo, *Chusquea* sp. (Pérez et al., 2009), especially under canopy openings created within logged stands. These canopy gaps provide new opportunities for bird species that can take advantage of the protection of bamboo cover, such as tapaculos (Reid et al., 2004) and the Des Murs' Wiretail (Díaz et al., 2006). In this regard, higher arthropods abundances documented for SL forests, associated with denser understory bamboo cover or woody debris (Niemelä et al., 1993; Reid et al., 2004), translate into increases in the frequency of arthropods in the diet of *D. gliroides* at the expense of fleshy fruits, in comparison with individual from OG forest (see chapter 1). Increased arthropod abundance represents an important food resource for Chilean marsupials because of their higher energy content (Lima et al., 2001) (see chapter 1; Meserve et al. 1988), as well as for specialized understory birds (Reid et al., 2004; Diaz et al., 2005; Díaz 2008). In addition bamboo cover also provides effective protection against natural predators for both birds and mammals (Manning and Edge, 2004: Reid et al., 2004).

This dietary shift at SL forest could have consequences for seed dispersal by *D. gliroides* of many vines and epiphytes, with potential cascading effects throughout the rest of the plant community (see chapter 1; Armesto et al., 1987; Rodriguez-Cabal et al., 2007; Salinas, 2008; Amico et al., 2009). In summary, our results, documented that the consumption of fleshy fruits vs. arthropods (see chapter 1), was related to the relative abundances of these food resources in each forest type, SL vs. OG.

Considering that *D. gliroides* like other arboreal mammals, depends on natural cavities in large canopy trees for shelter, hibernation and reproduction, and that selective logging reduces the availability of cavities in the stand (Lindenmayer et al., 1997; Cornelius, 2007), we expected a reduction in winter survival rate that could translate into overall population survival, as has been documented for many other arboreal mammal species (Lindenmayer et al., 1997; Pilastro et al., 2003), and for the forest bird *Aphrastura spinicauda* in the same study area (Cornelius, 2007). However, despite a reduction in the density of old trees in SL forests compared to OG forests, winter survival probabilities remained the same in both types of forest over four years. One possible explanation is that the denser bamboo cover developed after tree harvesting in the understory of SL stands may compensate for the lack of tree holes. It is known that bamboo foliage and culms are frequently used by *D. gliroides* to construct woven spherical nests, which protect the animals against unfavorable weather conditions (Mann, 1978, Patterson et al., 1990). In this regard, more than 80% of nests found in artificial nest boxes (see chapter 1) had bamboo foliage as construction material. Moreover, although hibernation is energetically challenging, we did not found differences in survival between winter and summer seasons (Schaub & Vaterlaus-Schlegel, 2001; Pilastro et al., 2003).

Dromiciops gliroides populations have often been thought to depend greatly on the amount of land covered by OG forest patches along its geographical distribution (see chapter 1, Mann, 1978; Kelt and Martínez, 1989; Hershkowitz, 1999; Rodriguez-Cabal et al., 2008). Most published evidence indicates that arboreal mammals, and especially nocturnal marsupials in Australia, are highly susceptible to logging damage (McNab, 1978; Johns, 1985, 1986; Laurance, 1990; Lindenmayer et al., 1997) or logging intensity (Herbers & Klenner 2007) due to reductions in population density and/or survival. Nevertheless, our results show that OG and SL forests have similar population densities, and there is even a trend toward higher densities at SL stands considering all sampling dates. Other works have also shown no differences in *Dromiciops* densities between forest patches with different vegetation structures such as OG and secondary forests or tree-fall gaps (see chapter 1; Patterson et al., 1989; Fontúrbel et al., 2009; Smith-Ramirez et al., ms). Variable densities in SL forests could be attributable to differences in logging intensity (Fig. 2). For example, Laurance (1996) recorded higher abundance of green ringtail possums in logged forests, while others arboreal marsupials did not differ significantly between logged and unlogged forests. Moreover, harvesting intensity was the more important determinant of squirrel density (Herbers & Klenner 2007).

Dromiciops densities in logged and unlogged forest patches in the rural landscape of Chiloé were considerable lower (overall mean density = 7.3 individual/ha) than mean densities from Andean populations in OG forests of mainland Chile (ca. 19 individual/ha) (Fontúrbel et al., 2009). However, it is important to note serious time-restrictions (one season or one year of sampling) and methodological shortcomings (see Parmenter et al., 2003; small sample size and less powerful trap spatial arrangements) of previous studies (see Rodriguez-Cabal et al., 2008; Fontúrbel et al., 2009).

Habitat selection models predict that higher quality habitats will be preferred over those of poor quality (Fretwell and Lucas, 1970). We initially hypothesized that OG forests had higher habitat quality than logged forests. However, our results show that *D. gliroides* densities did not differ between logged and unlogged stands, which did not translate into differences in demographic parameters and population growth rate. We propose that bamboo cover and arthropod abundances are key structural elements and food resources respectively for the maintenance of viable *D. gliroides* populations and for some understory-specialist birds (Willson and Armesto, 2003), making SL forests relatively high quality habitats. Telleria et al. (1991), Sullivan and Sullivan (2001) and Ecke et al. (2002) have also documented higher abundance of small mammals in remnant forests in anthropogenic landscapes. Moreover, in some temperate rainforests of South America, rodent species can be more abundant in forest remnants compared large protected areas due to increased availability of food resources and reduced predator abundance (Saavedra and Simonetti, 2005). Coincidentally, we also found that the abundance of *Irenomys tarsalis*, a nocturnal arboreal rodent, was higher in SL forest (JL Celis-Diez, personal observation). Therefore, forests subjected to limited logging may also serve as relatively high quality habitats for some small mammal species, in contrast to large vertebrate predators (Sergio et al., 2005).

Because top predators are particularly sensitive to habitat disruption (Sergio et al., 2005), we expect that they exert stronger negative pressure on prey survival in OG stands. The main predators of *D. gliroides* in temperate rainforests are Rufous-legged owls (*Strix rufipes*) and Kodkod cats (*Leopardus guigna*). Both species are especially vulnerable to habitat degradation and are less commonly found in open SL forests, small forest fragments and exotic pine plantations (Martinez and Jaksic, 1996; Sanderson et al., 2002: Acosta-Jamett and Smonetti 2004). Lower predator densities in SL forests should result in higher survival of *Dromiciops* in these stands, compared to OG stands, where predators remain present, as a possible explanation of higher population densities at least in one SL forest patches. However, a predator release hypothesis (Ferguson and Fox, 1984) to explain the higher survival of *Dromiciops* in SL stands has to be rejected, because we did not observe differences in survival rates of between OG and SL forests.

This work is the longest demographic study of *D. gliroides* in South America (four years; see Rodriguez-Cabal et al., 2008 for a previous record), but it is not yet sufficient to provide reliable documentation of population dynamic and to predict the fate of *D. gliroides* in remnant forests in anthropogenic landscapes. Annual variation in survival has been documented to depend on food resources (Cezilly et al., 1996; Lahti et al., 1998; Gauthier et al., 2001) and in temperate rain forets of Chiloé, arthopods abundances vary according to season and years (Díaz 2008).

Dromiciops populations both in logged and unlogged forests did not seem to be declining, and because of the limited time series, we could not ascertain whether their population dynamics is regulated by denso-dependent or denso-independent factors (Lima et al., 2001). Indeed, we suggest that the drastic population drop recorded in all forest patches in the spring 2008 was possibly a consequence of the severe drought of the summer 2007-2008. This highlights the need to expand long-term demographic studies of this and other forest species (Carmona et al., ms)

With our results, we can be certain that populations of the threatened arboreal marsupial are not so rare as previously thought (Kelt, 2000), and that the species can be found in both logged and unlogged forest remnants in the rural landscape, despite the severe loss of forest cover in the recent decades (Echeverria et al. 2007). Further, the impact of non-industrial logging, when limited to the removal of few harvestable large trees from the forest, and the ensuing proliferation of bamboo, does not harm the populations of this arboreal marsupial, and enhances the potential for the conservation of biodiversity in rural areas under productive management of remnant forests and OG forest patches.

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of Chile regarding trapping and manipulation of live animals. This is a contribution to the Research Program of Senda Darwin Biological Station and LTSER, Ancud, Chiloé, Chile.

FIGURE LEGENDS

Figure 1. Satellite image (gray scale) of the rural landscape of northern Chiloé Island (Landsat 2001). Darker areas indicate wooded habitats, and lighter areas indicate shrublands and agricultural land use. Circles indicate studied sites where OGF and SLD are found.

Figure 2. Population density of the arboreal *Dromiciops gliroides* in the studied area in northern Chiloe Island. (A) Mean densities (± 1 SE) of animals in both habitat types. Circles OGF = Old-growth forest and triangles SLF = Selective logged forest, and (B) Mean densities (± 1 SE) of animals in all studied sites, SD = Senda Darwin OG forest; Caulín = Caulin OG forest; LL = Llanquihue SL forest; QU = Quilar SL forest.

Appendix 2. Number of live captures (line, not including recaptures) and proportion of adult males, females and juveniles (bars) in forest fragments in a rural landscape of northern Chiloé Island. OGF sampled at Senda Darwin (a) and Caulín; (b) SLF sampled at Quilar (c) and Llanquihue (d).

Figure 1.

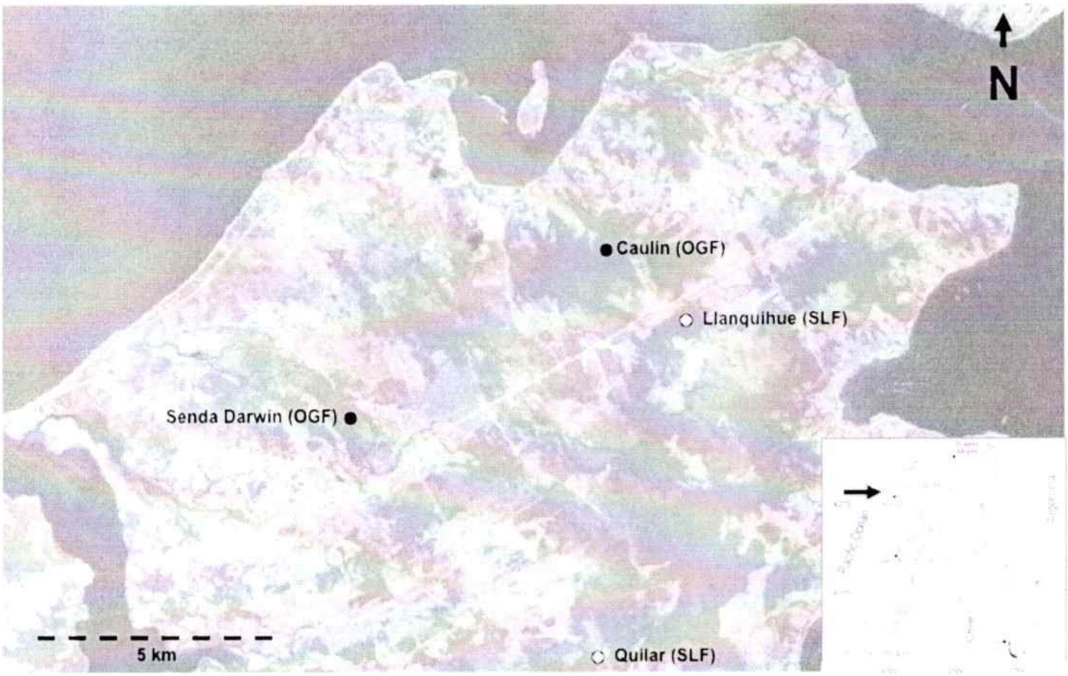
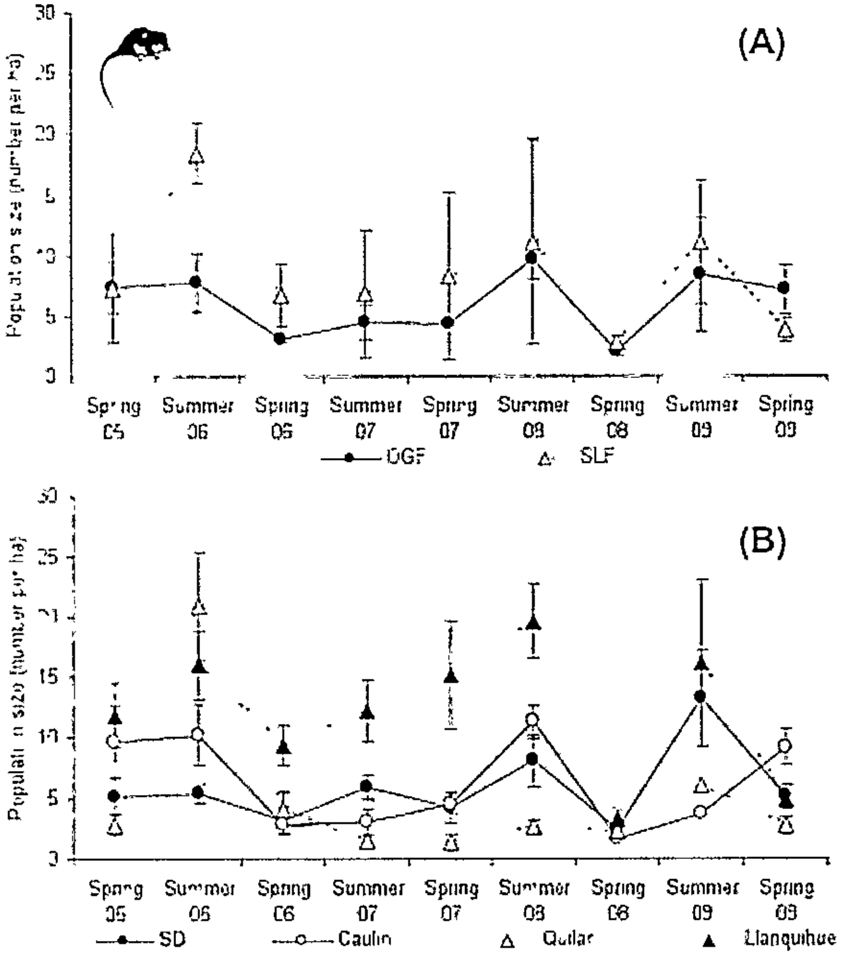
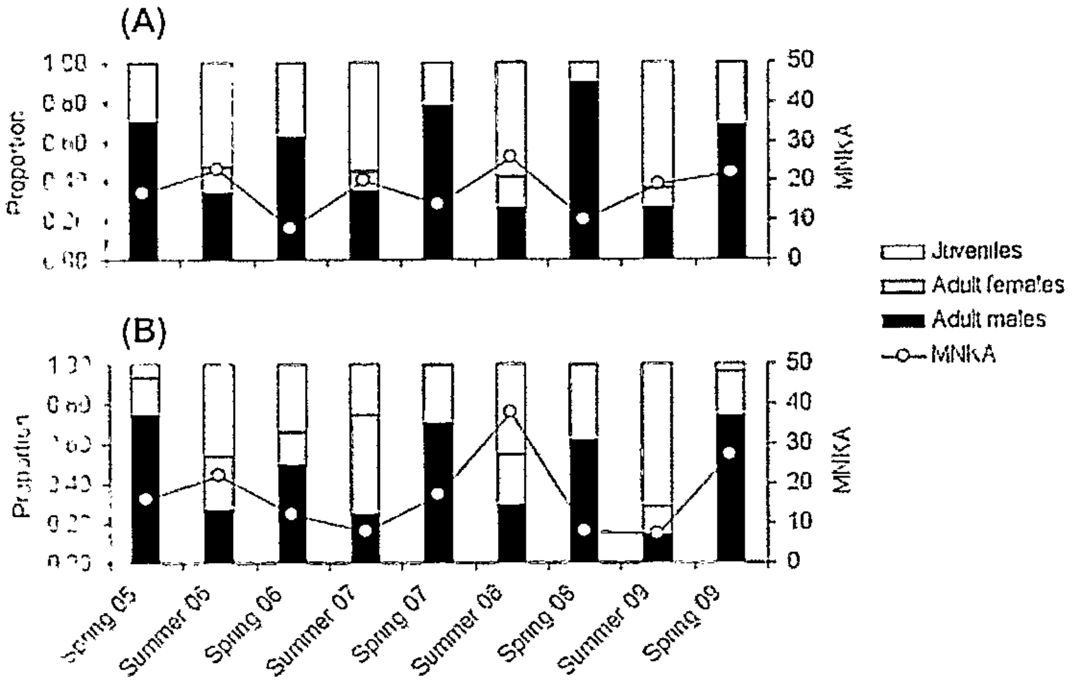


Figure 2.


(A)
Population size (number per ha)
0
5
10
15
20
25
30
Spring 05
Summer 06
Spring 06
Summer 07
Spring 07
Summer 08
Spring 08
Summer 09
Spring 09
OGF
SLF
(B)
Population size (number per ha)
0
5
10
15
20
25
30
Spring 05
Summer 06
Spring 06
Summer 07
Spring 07
Summer 08
Spring 08
Summer 09
Spring 09
SD
Caulin
Quilar
Llanquihue

Appendix 2a.


(A)
(B)
Proportion
MNKA
1.00
0.80
0.60
0.40
0.20
0.00
50
40
30
20
10
0
Juveniles
Adult females
Adult males
MNKA
Spring 05
Summer 05
Spring 06
Summer 07
Spring 07
Summer 08
Spring 08
Summer 09
Spring 09

520 **Appendix 2b.**

521

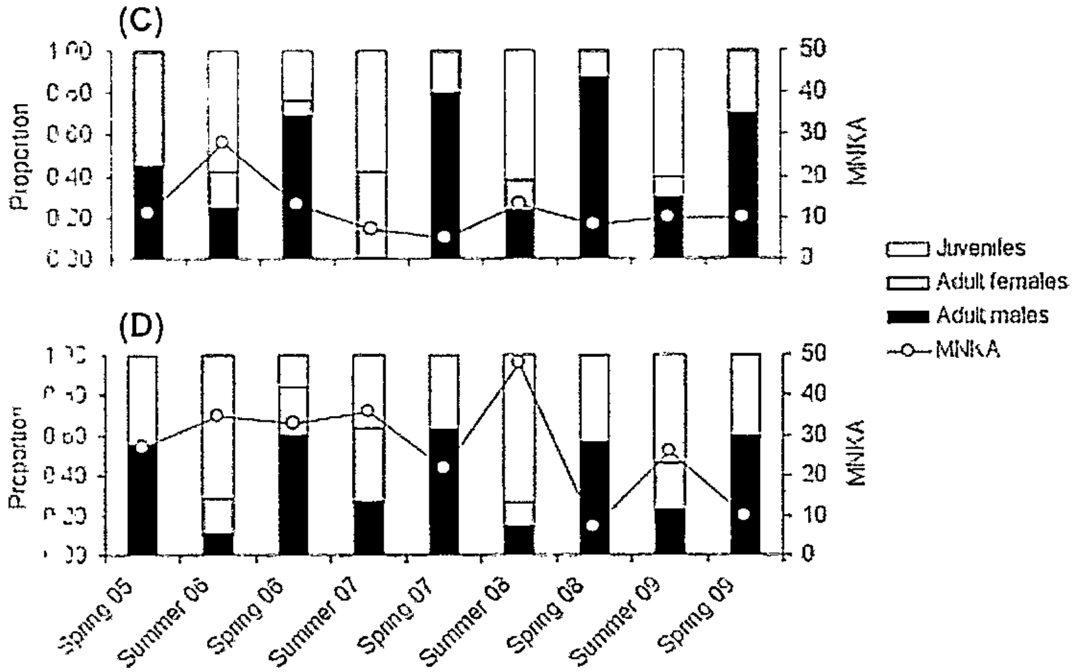


Table 1. Vegetation structure and food resources characteristics for both studied habitat types. OGF: Old growth forest; SLF: Selective logged forest; dbh: diameter at the breast height; Tc: *Tristerix corymbosus*; Lp: *Luzuriaga poliphylla*; Gi: *Gaultheria insana*; Mc: *Mitraria coccinea*; Sr: *Sarmienta repens*; Ao: *Astheranthera ovata*; (*) P < 0.05.

Habitat	**Mean number (per plot)**				**Frequency of occurrence (n = 60)**						**Median category**		
	No. trees (dbh > 50 cm (*)	No. snags (*)	No. logs	Mean number arthropods/trap (*)	Tc (*)	Lp	Gi (*)	Mc (*)	Sr (*)	Ao (*)	% Canopy cover	% Understory cover (*)	% bamboo cover (*)
OGF	3.80	0.50	2.08	2.43	0.42	1	0.32	0.13	0.27	0.47	25	75	50
SLF	1.45	0.25	1.70	5.40	0.07	0.98	0.07	0.45	0.07	0.25	50	25	75

Table 2. The statistical model tested denoted according to each model-specific variation in survival (Φ) and capture probability (*p*). i denotes constant parameter; time denotes fulltime specific variation; forest denote habitat type (e.g., OGF vs. SLF); site denotes all studied forest sites (e.g., Senda Darwin, Caulin, Llanquihue and Quilar); season denotes seasonal time-specific variation (e.g., summer vs. winter) and year denotes between year variation (e.g., 2005-2009). AICc is the estimated Akaike's information criterion: models with lower values and marked with asterisk indicate more parsimonious models. *ne* indicate not estimated parameter.

Model	**AICc**	**# Parameters**	**Deviance**	**Monthly parameter values** (for the significant model)
Capture (*p*)				
Φ i p time	884.89 (*)	9	866.63	p1 = 0.32; p2 = 0.60; p3 = 0.41; p4 = 0.82; p5 = 0.65; p6 = 0.37; p7 = 0.38; p8 = 0.64
Φ i p season	893.63	3	887.59	
Φ i p i	896.68	2	892.66	
Φ i p year	901.03	5	890.94	
Survival (Φ)				
Φ year p time	879.17 (*)	12	854.70	*Φ* 2005- 2006 = 0.85 *Φ* 2006- 2007 = 0.89 *Φ* 2007- 2008 = 0.85 *Φ* 2008- 2009 = *ne*
Φ time p time	881.05	15	850.33	
Φ forest+year p time	881.24	13	854.70	
Φ season p time	882.93	10	862.61	
Φ forest.year p time	883.49	16	850.68	
Φ forest+season p time	883.89	11	861.50	
Φ i p time	884.89	9	866.63	
Φ forest p time	885.76	10	865.44	
Φ site p time	887.78	12	863.31	

Table 3. The statistical model tested denoted according to each model-specific variation in seniority (γ) (recruitment rate = 1-γ) and population growth rate (Lambda = λ). i denotes constant parameter; time denotes fulltime specific variation; forest denote habitat type (e.g., OGF vs. SLF); site denotes all studied forest sites (e.g., Senda Darwin, Caulin, Llanquihue and Quilar); season denotes seasonal time-specific variation (e.g., summer vs. winter) and year denotes between year variation (e.g., 2005-2009). AICc is the estimated Akaike's information criterion: models with lower values and marked with asterisk indicate more parsimonious models. *ne* indicate not estimated parameter.

Model	**AICc**	**# Parameters**	**Deviance**	**Monthly parameter values** (for the significant model)
Seniority (γ) (recruitment rate = 1-γ)				
γ season p time	*889.4*	*10*	*869.05*	γ spring = 0.98 γ summer = 0.67
γ fores+season p time	*891.4*	*11*	*869.02*	
γ time p time	*896.2*	*15*	*865.5*	
γ year p time	*943.4*	*12*	*918.9*	
γ i p time	*944.9*	*9*	*926.60*	
γ forest p time	*946.9*	*10*	*926.52*	
Growth rate (λ)				
λ site	989.59	9	58.6	λ SD = 1.11 ± 0.09 λ Caulin = 1.19 ± 0.09 λ Quilar = 1.10 ± 0.12 λ Llanquihue = 0.83 ± 0.06
λ forest	992.68	8	63.8	
λ forest+time	995.9	13	56.3	
λ constant	998.7	7	71.9	
λ time	1002.3	9	71.3	

CONCLUSION

Los resultados de esta tesis aportan al conocimiento de la mastozoología de los bosques templados de nuestro país, en particular respecto de una especie de marsupial endémica de los bosque templados de Sudamérica, cuya historia natural, biología, estado de conservación y dinámica poblacional en paisajes antropogénicos habían sido escasamente estudiados. La especie en muchos catálogos nacionales e internacionales se considera aún inadecuadamente conocida (Redford & Eisenberg 1992; Glade 1993; Muñoz-Pedreros & Palma 2000; www.redlist.org). La mayor parte de los datos poblacionales sobre pequeños mamíferos de los bosques templados del sur de Chile, incluido *Dromiciops gliroides*, ha sido generada prácticamente en tres localidades, La Picada (Puerto Montt), Las Cascadas (Osorno), y Fundo San Martín (Valdivia) (véase revisión en Kelt, 2006), dos de ellas andinas y la última costera, por lo que dado los actuales cambios en el uso del suelo, se hace urgente conocer el estado de las poblaciones en zonas antroponegizadas.

Datos de censos poblacionales de cuatro años (2005-2009), mediante métodos de captura-recaptura, del marsupial arbóreo *Dromiciops gliroides* en parches de bosque antiguos, expuestos a tala selectiva y en matorrales, que conforman los principales tipos de parches del paisaje rural del norte de Chiloé (Echeverria et al., 2007), registran la presencia de individuos en todos los hábitat, a pesar de las grandes diferencias en la estructura de la vegetación y en los tipos y abundancia de recursos tróficos. El principal resultado de este trabajo es que, en comparación con otros micromamíferos del bosque templado, *Dromiciops gliroides* fue más común en el norte de Chiloé de lo que había sido previamente descrito en otras localidades del sur de Chile (Kelt & Martínez, 1989; Saavedra & Simonetti, 2005; Kelt, 2006). La densidad poblacional fue considerablemente mayor en hábitats de bosque antiguo y sujeto a tala selectiva que en matorrales secundarios; sin embargo no se observaron diferencias en densidad poblacional entre los dos tipos de bosques comparados en este trabajo.

En ambos bosques, independientemente del impacto e la tala, la densidad poblacional mostró una fuerte variación estacional con mayores densidades en verano que en primavera. También hubo variación entre los sitios de bosque sujetos a tala selectiva, atribuible a diferencias en intensidad de tala, lo que es muy difícil de controlar en experimentos naturales. Sin embargo, no se observaron tendencias de declinación del tamaño poblacional en ninguno de los bosques en el período de cuatro años de monitoreo (2005 a 2009). Se podría inferir que en el período de estudio las poblaciones han permanecido relativamente estables con variaciones atribuibles a la fenología de la población (Cezilly et al., 1996).

Por otra parte, a pesar de nuestras predicciones originales, la sobrevivencia y reclutamiento poblacional no difirieron entre las poblaciones de bosques antiguos y expuestos a tala selectiva, observándose también una fuerte variación interanual en la sobrevivencia, sumada a una variación estacional en el reclutamiento. En consecuencia, al combinar estos parámetros demográficos no se registraron diferencias en la tasa anual de crecimiento poblacional de *Dromiciops* en ninguno de los bosques comparados. Dada las actuales tasas de perdida y degradación del hábitat de bosques remanentes en la zona rural de Chiloé (Echeverria et al., 2007), estos resultados permiten sugerir que la conservación de parches de bosques remanentes en paisajes rurales pueden sostener poblaciones viables de *D. gliroides* por períodos largos de tiempo, asegurando su viabilidad futura y complementando el esfuerzo de conservación en áreas protegidas.

Considerando que frecuentemente existen diferencias metodológicas asociadas con las estimaciones de densidad poblacional en mamíferos pequeños (Van Horne, 1983; Efford, 1992), el uso de modelos demográficos, más que las estimaciones de densidad per se, es una importante herramienta para evaluar el estado de conservación y tendencias de largo plazo de las poblaciones de especies amenazadas. Debido a que esta metodología demanda censos sistemáticos durante varios años de estudio sucesivos, es necesario implementarlos como parte de un programa de estudios de largo plazo. Mediante este tipo de información es posible entender las consecuencias de las perturbaciones antrópicas del paisaje, incluyendo cambios de cobertura y degradación de los parches, sobre la calidad de hábitats para los taxa de interés.

La mantención de áreas con bosque antiguo, asegura la conservación de esta especie arbórea de marsupial en el paisaje rural, pero la tala selectiva, como explotación local de madera de los bosques, asociada a la remoción selectiva de árboles de mayor tamaño, al promover la presencia de quila, mantiene una adecuada cobertura vegetal en el sotobosque que favorece algunas especies restringidas a fragmentos de bosque tales como *D. gliroides* junto a otras aves del sotobosque (Reid et al., 2004; Castellon and Sieving, 2006a y b)

Nuestros datos demuestran que *Dromiciops gliroides*, al igual que algunas especies de aves del bosque, es un importante vector de semillas de árboles y epífitas de los bosques templados (Armesto et al., 1987; Amico & Aizen, 2000; Rodriguez-Cabal et al., 2007; Amico et al., 2009)., de las cuales no existía registro previo de sus dispersantes bióticos (Salinas, 2008). Estos datos amplían el espectro de frutos y semillas consumidos por este marsupial. En el contexto de la trama de interacciones planta-frugívoro en bosques templados del sur de Chile, la disminución o aislamiento de las poblaciones de *Dromiciops gliroides* de los parches de bosque remanentes en la zona rural podría afectar negativamente la regeneración y sobrevivencia de una alta diversidad de especies de bosque (Rodriguez-Cabal et al., 2007).

Considerando el ámbito de hogar de *D. gliroiodes* se puede inferir los tamaños de parches mínimos o las dimensiones de corredores biológicos que permiten mantener poblaciones viables de esta especie en el ámbito rural. El registro en este estudio, de la presencia de *Dromiciops gliroides* en matorrales secundarios del norte de Chiloé, constituye un importante hallazgo, debido a que este tipo de matorrales no sería una barrera infranqueable al movimiento de individuos de *D. gliroides* entre fragmentos de bosque, favoreciendo la conectividad, a escala de paisaje, entre parches de bosques. También registramos la presencia en las fecas de este marsupial de semillas de *Myrteola numularia,* una especie frecuente en bordes y matorrales, lo que podría indicar que estos matorrales pueden proveer recursos para esta especie durante sus desplazamientos o incluso proponer dinámicas fuente-sumideros entre parches de bosques y matorrales. Al respecto, se ha documentado depredación de nidos de *Aphrastura spinicauda* en matorrales por *D. gliroides* (S. Ippi comunicación personal y datos no publicados). Sin embargo, faltan estudios más detallados sobre el valor de la matriz como lugar de tránsito entre parches de bosque, para modelar la distribución de especies en distintos escenarios de paisajes rurales o dinámicas metapoblacionales (Umetsu & Pardini, 2007; Umetsu et al., 2008).

En base a la información ecológica generada en esta tesis, y en conjunto con recientes trabajos (Fontúrbel et al. 2009; Smith-Ramírez et al. ms), se propone revisar la clasificación actual de *Dromiciops gliroides*, incluido en la categoría "insuficientemente conocida" propuesta para los vertebrados de Chile (D.S. N° 151, 2007) a "vulnerable", a nivel nacional, de manera de concordar con la clasificación internacional de "Casi Amenazada" (Díaz & Teta 2008) debido al actual conocimiento de sus densidades poblacionales en Chiloé y en el continente, y la rápida tasa de deforestación y degradación del bosque nativo (Echeverria et al. 2008), que constituye el principal hábitat de esta especie.

Finalmente, en base a los datos de esta tesis, se sugiere considerar las siguientes medidas en el manejo forestal para asegurar la viabilidad de las poblaciones de *D. gliroides* en zonas rurales: (a) mantener, cuando sea posible, los troncos muertos en pie o árboles grandes y viejos sin valor económico en los rodales, (b) evitar el ingreso de ganado al bosque, manteniendo el sotobosque denso y la cobertura de quila en los claros (*Chusquea* spp.), (c) favorecer la presencia de corredores de vegetación nativa arbórea como cercos vivos a través de las praderas, y cobertura arbustiva (matorrales) favoreciendo la conectividad entre fragmentos de bosque o con áreas protegidas (Willson, 2004; Díaz et al., 2005), (d) proteger y monitorear la presencia de

D. gliroides en las áreas con bosques primarios remanentes en ambientes rurales. Con las primeras tres medidas, incorporadas en los requisitos para la obtención de planes de manejo en la nueva legislación forestal (CONAF, 2008) o de producción y protección agrícola (SAG, 1998), se proveería de un paisaje más favorable para la sobrevivencia de especies como *Dromiciops gliroides* y otras restringidas a los fragmentos de bosque, que pueden ser indicadores del impacto del manejo sobre la calidad de los hábitats (Willson & Armesto, 2003; Díaz et al., 2005; Cornelius, 2007).

REFERENCIAS

ACOSTA-JAMETT, G . & J.A. SIMONETTI .2004. habitat use by *Oncifelis guigna* and *Pseudalopex culpaeus* in a fragmented forest landscape in central Chile. Biodiversity and Conservation 13: 1135–1151,

AGÜERO, T. & SIMONETTI, J.A. 1988. Home range assessment: a comparison of five methods. Revista Chilena de Historia Natural 61: 223–229.

ALARCÓN, M.E. 2003. Sifonapterofauna de tres especies de roedores de Concepción, VIII región-Chile. Gayana 67: 16-24.

ALEIXO A. 1999. Effects of selective logging on a bird community in the Brazilian Atlantic forest. The Condor 101: 537–548

AMICO, G., & AIZEN, M. 2000. Mistletoe seed dispersal by a marsupial. Nature 408: 929-930.

AMICO, G.C., RODRIGUEZ-CABAL, M.A. & AIZEN, M.A. 2009. The potential key seed-dispersing role of the arboreal marsupial Dromiciops gliroides. Acta Oecologica 35: 8-13.

ANDERSON, M.J. 2005. PERMANOVA. Permutational multivariate analysis of variance. A computer program.

ANDREN, H. 1994. Effects of habitat fragmentation on birds and mammals in landscapes with different proportions of suitable habitat: a review. Oikos 71: 355-366.

ANDREWARTHA, H.G., & BIRCH, L.C. 1954. The Distribution and Abundance of Animals. The University of Chicago Press, Chicago, Illinois.

ARAVENA, J.C., CARMONA, M.R., PÉREZ, C.A. & ARMESTO, J.J. 2002. Changes in tree species richness, stand structure and soil properties in a succesional chronosequence in northern Chiloé Island, Chile. Revista Chilena de Historia Natural 75: 339-360.

ARMESTO, J.J., MANUSCHEVICH, D., MORA, A., SMITH-RAMIREZ, C., ROZZI, R., ABARZÚA, A.M & MARQUET, P.A. 2009b. From the Holocene to the Anthropocene: A historical framework for land cover change in southwestern South America in the past 15,000 years. Land Use Policy ion press

ARMESTO JJ & R ROZZI (1989) Seed dispersal syndromes in the rain forest of Chiloé: evidence for the importance of biotic dispersal in a temperate rain forest. Journal of Biogeography 16: 219-226.

ARMESTO, J.J., ROZZI, R. & CASPERSEN, J. 2001. Temperate forest of North and South America. Pp. 223-250 in Global biodiversity in a changing environment. Springer-Verlag, NewYork

ARMESTO, J.J., ROZZI, R., MIRANDA, P. & SABAG, C. 1987. Plant/frugivore interactions in South American temperate forests. Revista Chilena de Historia Natural 60: 321-336.

ARMESTO, J.J., ROZZI, R., SMITH-RAMIREZ, C. & ARROYO, M.T.K. 1998. Conservation targets in South American temperate forests. Science 282: 1271–1272.

ARMESTO, J.J., SMITH-RAMÍREZ, C., CARMONA, M., CELIS-DIEZ, J.L., DÍAZ, I., GAXIOLA, A., GUTIÉRREZ, A.C., NÚÑEZ-AVILA, M., PÉREZ, C. & ROZZI, R.

2009a. Old-growth temperate rain forests of South America: Conservation, plant-animal interactions, and baseline biogeochemical processes In Wirth, C, M, Heimann and G, Gleixner (Eds) Ecosystem functioning of old-growth forest. Springer Verlag, NY, en prensa

BELCHER, C.A. & DARRANT, J.P. 2004. Home range and spatial organization of the marsupial carnivore, *Dasyurus maculatus maculatus* (Marsupialia : Dasyuridae) in south-eastern Australia. Journal of Zoology 262: 271-280.

BOWMAN, J. C., D. SLEEP, G. J. FORBES, AND M. EDWARDS. 2000. The ssociation of small mammals with coarse woody debris at log and stand scales. Forest Ecology and Management 129:119–124.

BOZINOVIC, F., RUIZ, G & ROSENMANN, M. 2004. Energetics and torpor of a South American living fossil, the microbiotheriid Dromiciops gliroides. Journal of Comparative Physiology B 174: 293-297.

BRAGAGNOLO, C. NOGUEIRA, A.A. PINTO-DA-ROCHA, R. & PARDINI R. 2007. Harvestmen in an Atlantic forest fragmented landscape: Evaluating assemblage response to habitat quality and quantity. Biological Conservation XX: 389-400

BRAWN J., ROBINSON S. (1996) Source-sink population dynamics may complicate the interpretation of long-term census data. Ecology 77: 3-12.

BURNHAM, K.P. & ANDERSON, D.R. 1998. Model selection and inference. A practical information theoretic approach. Springer, Verlag, New York, USA. 353 pp.

BURNHAM, K.P. & OVERTON, W.S. 1979. Robust estimation of population size when capture probabilities vary among animals. Ecology 60: 927-936.

BUSH, A.O., LAFFERTY, K.D., LOTZ, J.M. & SHOSTAK, A.W. 1997. Parasitology meets ecology on its own terms: Margolis et al., Revisited. Journal of Parasitology 83: 575-583.

CANALS, M., ROSENMANN, M. & BOZINOVIC, F. 1997. Geometrical aspects of the energetic effectiveness of huddling in small mammals. Acta Theriologica 42: 321-328.

CANALS, M., ROSENMANN, M., NOVOA, F.F. & BOZINOVIC, F. 1998. Modulating factors of the energetic effectiveness of huddling in small mammals. Acta Theriologica 43: 337-348.

CASTELLÓN, T.D. & SIEVING, K.E. 2006a. An experimental test of matrix permeability and corridor use by an endemic understory bird. Conservation Biology 20: 135-145.

CASTELLÓN, T.D. & SIEVING, K.E. 2006b. Landscape history, fragmentation, and patch occupancy: models for a forest bird with limited dispersal. Ecological Applications 16: 2233-2234.

CARMONA, M.R., J.C. ARAVENA, M.A. BUSTAMANTE-SANCHEZ, J.L. CELIS-DIEZ, A. CHARRIER, I.A. DÍAZ, J. DÍAZ-FORESTIER, M.F. DÍAZ, A. GAXIOLA, A.G. GUTIÉRREZ, C. HERNANDEZ-PELLICER, L. ROJAS, S. IPPI, R. JAÑA-PRADO, P. JARA-ARANCIO, J. JIMENEZ, D. MANUSCHEVICH, M. NUÑEZ-AVILA, C. PAPIC, C. PÉREZ, F. PÉREZ, S. REID, B. SALGADO, C. SMITH-RAMÍREZ, A. TRONCOSO, R.A. VÁSQUEZ, M.F. WILLSON, R. ROZZI & J.J. ARMESTO. 2010. Estación Biológica Senda Darwin: investigación ecológica de largo plazo en la interfase ciencia-sociedad. Revista chilena de Historia Natural in press.

CEZILLY, F., A. VIALLEFONT, V. BOY & A. R. JOHNSON. 1996. Annual Variation in Survival and Breeding Probability in Greater Flamingos. Ecology: Vol. 77: 1143-1150.

CHAN, K.M.A. & DAILY, G.C. 2008. The payoff of conservation investments in tropical countryside. PNAS 105: 19342–19347.

CHOQUET, R, REBOULET, A.M., LEBRETON, J.D., GIMÉNEZ, O. & PRADEL, R. 2005. U-CARE 2.2 User's manual. CEFE/CNRS, Montpellier.

CHOQUET, R., REBOULET, A.M., PRADEL, R., GIMENEZ, O. & LEBRETON, J.D. 2004. M–SURGE: new software specifically designed for multistate capture–recapture models. Animal Biodiversity and Conservation 27: 207-215.

CONAF-CONAMA-BIRF (1999) Catastro y evaluación de recursos vegetacionales nativos de Chile. Informe Nacional con Variables Ambientales. Corporación Nacional Forestal (Chile).

CORNELIUS, C. 2007. Genetic and demographic consequences of human-driven landscape changes on bird populations: the case of Aphrastura spinicauda (Furnariidae) in the temperate rainforest of South America. Tésis de Doctorado, University of Missouri, St. Louis, USA.

CORNELIUS, C. 2008. Spatial variation in nest-site selection by a secondary cavity-nesting bird in a human-altered landscape. The Condor 110:615–626.

CORNELIUS, C., COCKLE, K., POLITI, N., BERKUNSKY, I., SANDOVAL, L., OJEDA, V., RIVERA, L., HUNTER JR., M. & MARTIN, K. 2008. Cavity-nesting birds in Neotropical forests: cavities as a potentially limiting resource. Ornitología Neotropical 19:253–268.

CRESPIN, L. & LIMA, M. 2006. Supervivencia adulta y dinámica poblacional del lanchón orejudo (Phyllotis darwini) en Chile Central. Revista Chilena de Historia Natural 79: 295-308.

DASZAK, P. & CUNNINGHAM, A.A. 1999. Extinction by infection. Trends in Ecology and Evolution 14: 279.

DAILY, G.C, CEBALLOS G., PACHECO, J., SUZAN G., SANCHEZ-AZOFEIFA, A. 2003. Countryside biogeography of Neotropical mammals: conservation opportunities in agricultural landscapes of Costa Rica. Conservation Biology 17:1814–1826.

DÍAZ, I. 2008. Epiphyte, bird interactions in Chilean rainforest. PhD thesis, University University of Florida, Gainesville.

DÍAZ, I.A., J.J. ARMESTO & M.F. WILLSON. 2006. Mating success of the endemic des Murs' Wiretail (Sylviorthorhynchus desmursii, Furnariidae) in fragmented Chilean rainforests. Austral Ecology 31: 13–21.

DÍAZ, I., J.J ARMESTO, S. REID, K.E. SIEVING, & M.F. WILLSON. 2005. Linking forest structure and composition: avian diversity in successional forests of Chiloé Island, Chile. Biological Conservation 123: 91–101.

DÍAZ, I., K.E. SIEVING, M.E. PEÑA-FOXON, J. LARRAÍN & J.J. ARMESTO. 2010. Epiphyte diversity and biomass loads of canopy emergent trees in Chilean temperate rain forests: A neglected functional component. Forest Ecology and Management in press.

DÍAZ, M. & TETA, P. 2008. *Dromiciops gliroides.* In: IUCN 2009. IUCN Red List of Threatened Species. Version 2009.2. <www.iucnredlist.org>. Downloaded on 03 March 2010.

DI CASTRI, F. & HAJEK, E.R. 1976. Bioclimatología de Chile. Editorial de la Universidad Católica de Chile, Santiago.

EBENSPERGER, L.A., HURTADO, M.J., SOTO-GAMBOA, M., LACEY E.A. & CHANG, A.T. 2004. Communal nesting and kinship in degus (Octodon degus). Naturwissenschaften 91: 391-395.

ECHEVERRÍA, C., COOMES, D., NEWTON, A., SALAS, J., REY, J.M. & LARA, A. 2006. Rapid fragmentation and deforestation of Chilean Temperate Forests. Biological Conservation 130: 481-494.

ECHEVERRÍA, C., COOMES, D., NEWTON, A., REY-BENAYAS, J.M. & LARA, A. 2007. Impacts of forest fragmentation on species composition and forest structure in the temperate landscape in southern Chile. Global Ecology and Biogeography 16: 426-439.

ECHEVERRÍA, C., COOMES, D., HALL, M., NEWTON, A. 2008. Spatially explicit models to analyze forest loss and fragmentation between 1976 and 2020 in southern Chile. Ecological Modelling 212: 439-449.

EFFORD, M. 1992. Comment-Revised estimates of the bias in "minimum number alive" estimator. Canadian Journal of Zoology 70: 628-631.

EFFORD, M.G., DAWSON, D.K. & ROBBINS, C.S. 2004. DENSITY: software for analyzing capture-recapture data from passive detector arrays. Animal Biodiversity and Conservation 27: 217-228.

ECKE, F., LÖFGREN, O. & SÖRLIN, D. 2002. Population dynamics of small mammals in relation to forest age and structural habitat factors in northern Sweden Journal of Applied Ecology 39: 781–792.

FAO. 2009. State of world's forest. Rome.

FERGUSON, G.W. & S.F. FOX. 1984. Annual Variation of Survival Advantage of Large Juvenile Side-Blotched Lizards, *Uta stansburiana*: Its Causes and Evolutionary Significance. Evolution 38: 342-349.

FONTÚRBEL, F.E., et al. 2009. Spatial ecology of monito del monte (Dromiciops gliroides) in a fragmented landscape of southern Chile. Mammalian Biology in press.

FONTUÚRBEL, F.E. & J.E. JIMÉNEZ. 2009. Underestimation of abundances of the monito del monte (Dromiciops gliroides) due to asampling artifact. Journal of Mammalogy in press.

FRETWELL S. & LUCAS H.J. 1970. On territorial behavior and other factors influencinghabitat distribution in birds. Acta Biotheoretica 19: 16-36

GANNON, W.L. & SIKES, R.S. 2007. The animal care and use committee of the American Society of Mammalogists. 2007. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy 88: 809–823.

GAUTHIER, G., R. PRADEL, S. MENU & J-D. LEBRETON. 2001. Seasonal survival of greater snow geese and effect of hunting under dependence in sighting probability. Ecology 82: 3105-3119

GEISER, F. 1994. Hibernation and daily torpor in marsupials: a review. Australian Journal of Zoology 42: 1–16.

GENTILE, R., D'ANDREA, P. S., CERQUEIRA, R. & MAROJA, L.S. 2000. Population dynamics and reproduction of marsupials and rodents in a Brazilian rural area: a five-year study. Studies on Neotropical Fauna and Environmment 35: 1–9.

GIBBONS, P. & LINDENMAYER, D.B. 2002. Tree hollows and wildlife conservation in Australia. CSIRO Publishing, Melbourne.

GLADE, A. 1993. Libro Rojo de los Vertebrados Terrestres de Chile. 2ª ed. Corporación Nacional Forestal. Santiago, 68 pp.

GUGLIELMONE, A.A., VENZAL, J.M., AMICO, G., MANGOLD, A.J. & KEIRANS, J.E. 2004. Description of the nymph and larva and redescriptions of the female of Ixodes neuquenensis Ringuelet, 1947 (Acari: Ixodidae), a parasite of the endangered Neotropical marsupial Dromiciops gliroides Thomas (Microbiotheria: Microbiotheriidae). Syst Parasitol 57: 211-219.

GIBBS, J.P., M.L. HUNTER & S. M. MELVIN. 1993. Snag availability and communities of cavity nesting birds in tropical versus temperate forests. Biotropica 25: 236-241.

GRANT T.R. & TEMPLE-SMITH, P.D. 1987. Observations on torpor in the small marsupial Dromiciops australis (Marsupialia: Microbiotheriidae) from southern Chile. In: Archer M (ed) Possums and opossums: studies in evolution. Surrey Beatty and Royal Zoological Society of New South Wales, Sydney, pp 273–277

GUTIERREZ, A.G., ARMESTO, J.J. & ARAVENA, J.C. 2004. Disturbance and regeneration dynamics of an old-growth North Patagonian rain forest in Chiloé Island, Chile. Journal of Ecology 92: 598-608.

GUTIERREZ, A.G., ARMESTO, J.J., ARAVENA, J.C., CARMONA, M., CARRASCO, N.V., CHRISTIE, D.A., PEÑA, M.P., PEREZ, C. & HUTH, A. 2009. Structural and environmental characterization of old-growth temperate rainforests of northern Chiloe´ Island, Chile: Regional and global relevance. Forest Ecology and Management in press.

HARRISON, S. & BRUNA, E. 1999. Habitat fragmentation and large-scale conservation: what do we know for sure? Ecography 22: 225-232.

HEIDEMAN, P.D., BIERL, C.K. & GALVEZ, M.E. 2000. Inhibition of reproductive maturation and somatic growth of fisher 344 rats by photoperiods shorter than L14:D10 and by gradually decreasing photoperiod. Biology of Reproduction 63: 1525-1530.

HERSKHOVITZ, P. 1999. Dromiciops gliroides Thomas 1894, last of the Microbiotheria (Marsupialia) with a review of the family Microbiotheriidae. Fieldiana Zoology 93: 1-60.

HINOJOSA, L.F. & C. VILLAGRÁN. 1997. Historia de los bosques del sur de Sudamérica, I: antecedentes paleobotánicos, geológicos y climáticos del Terciario del cono sur de América. Revista Chilena de Historia Natural 70: 225-239.

ISAAC, J.L. 2005. Life history and demographics of an island possum. Australian Journal of Zoology 53: 195–203.

ISAAC, J.L., VALENTINE, L.E. & GOODMAN, B.A. 2008. Demographic responses of an arboreal marsupial, the common brushtail possum (Trichosurus vulpecula), to a prescribed fire. Population Ecology 50: 101-109.

JAÑA-PRADO, R.C., CELIS-DIEZ, J.L., GUTIÉRREZ, A.G., CORNELIUS, C. & ARMESTO, J.J. 2006. Diversidad en bosques fragmentados de Chiloé ¿son todos los fragmentos iguales? Pp. 59-189 en Biodiversidad en ambientes fragmentados: patrones y procesos a diferentes escalas (Grez, A.A., Bustamante, R. & Simonetti, J.A., eds.). Editorial Universitaria, Santiago.

JHA, S. & BAWA, K.S., 2006. Population growth, human development, and deforestation in biodiversity hotspots. Conservation Biology 20: 906–912.

JIMÉNEZ, J. & RAGEOT, R. 1979. Notas sobre la biología del "Monito del Monte", Dromiciops australis Philippi 1893. Anales del Museo de Historia Natural de Valparaíso Chile 12: 83-88.

JIMÉNEZ, J. 2007. Ecology of a coastal population of the critically endangered Darwin's fox, Pseudalopex fulvipes on Chiloé Island, southern Chile. Journal of Zoology 271: 63–77.

JULLIARD, R., LEIRS, H., STENSETH, N.C., YOCCOZ, N.G., PRÉVÔT-JULLIARD, A.C., VERHAGEN, R. & VERHEYEN, W. 1999. Survival-variation within and between functional categories of the African multimammate rat. Journal of Animal Ecology 68: 550-561.

KELT, D.A. 2000. Small mammal communities in rainforest fragments in Central Southern Chile. Biological Conservation 92: 345-358.

KELT, D.A. 2006. Efectos de la fragmentación del bosque lluvioso templado sobre los pequeños mamíferos. Pp. 115- 142 en Biodiversidad en ambientes fragmentados: patrones y procesos a diferentes escalas (Grez, A.A., Bustamante, R. & Simonetti, J.A., eds.). Editorial Universitaria, Santiago.

KELT, D.A. & MARTINEZ, D. 1989. Notes on the distribution and ecology of two marsupials endemic to the Valdivian forests of southern South America. Journal of Mammalogy 70: 220-224.

LAHTI, K., M. ORELL, S. RYTKÖNEN & K. KOIVULA. 1998. Time and food dependence in willow tit winter survival. Ecology 79: 2904-2916

KOZAKIEWICZ, M. 1995. Resource tracking in space and time. Pp. 136–147 in Mosaic landscapes and ecological processes (L. Hansson, L. Fahrig, and G. Merriam, eds.). Chapman and Hall, London, United Kingdom.

KRAAIJEVELD-SMIT, F. J. L., S. J. WARD, AND P. D. TEMPLE-SMITH. 2002. Multiple paternity in a field population of a small carnivorous marsupial, the agile antechinus, Antechinus agilis. Behavioral Ecology and Sociobiology 52: 84-91.

THOMAS, D., J.R. MALCOLM, B.L. ZIMMERMAN. 2005. Effects of mahogany (Swietenia macrophylla) logging on small mammal communities, habitat structure, and seed predation in the southeastern Amazon Basin. Forest Ecology and Management 206: 381–398

LARA, A. & VEBLEN, T. 1993. Forest plantations in Chile: a successful model? In: Mather, A. (ed.) Afforestation. Policies, Planning and Progress. Belhaven Press, London, UK, pp. 118–139

LAURANCE, W.F. 1990. Comparative response of five arboreal marsupials to tropical forest fragmentation. Journal of Mammalogy 71: 641 – 653

LAURANCE, W.F. & LAURANCE, S.G. 1996. Responses of Five Arboreal Marsupials to Recent Selective Logging in Tropical Australia. Biotropica 28: 310-322

LAURANCE, W.F., NASCIMENTO, E.M.H., LAURANCE, S.G., ANDRADE, A., EWERS, R.M., HARMS, K.E., LUIZAO, R.C.C. & RIBEIRO, J.E. 2007. Habitat Fragmentation, Variable Edge Effects, and the Landscape-Divergence Hypothesis Plos One 10: e1017

LEBRETON, J.D., BURNHAM, K.P., CLOBERT, J. & ANDERSON, D.R. 1992. Modeling survival and testing biological hypotheses using marked animals: a unified approach with case studies. Ecological Monographs 62: 67-118.

LEE, A.K., & COCKBURN, A. 1985. Evolutionary Ecology of Marsupials. Cambridge University Press, London.

LIMA, M. & JAKSIC, F.M. 1998. Population variability among three small mammal species in the semiarid Neotropics: The role of density-dependent and density-independent factors. Ecography 21: 175-180.

LIMA, M., STENSETH, N.C., YOCCOZ, N.G. & JAKSIC, F.M. 2001. Demography and population dynamics of the mouse opossum (Thylamys elegans) in semi-arid Chile: seasonality, feedback structure and climate. Proceedings of the Royal Society of London series B 268: 2053–2064.

LINDENMAYER, D.B. 1997. Differences in the biology and ecology of arboreal marsupials in forests of Southeastern Australia. Journal of Mammalogy 78: 1117-1127.

LINDENMAYER, D.B., TANTON, M.T. & CUNNINGHAM, R.B. 1991. A critique of the use of nest boxes required for the conservation of Leadbeater's Possum, Gymnobelideus leadbeateri. Wildlife Research 18: 619-624.

LINDENMAYER, D., CUNNINGHAM, R., DONNELLY, C., TRIGGS, B. & BELVEDERE, M. 1994. The conservation of arboreal marsupials in the montane ash forest of the central highlands of Victoria, south-eastern Australia. V Patterns of use and the microhabitat requirements of the mountain brush tail possum Trichosurus caninus ogilby in retained linear habitat (wild life corridors). Biological Conservation 68: 43 – 51

LINDENMAYER, D.B., CUNNINGHAM, R.B. & DONNELLY, C.F. 1997. Decay and collapse of trees with hollows in eastern Australian forest: impacts on arboreal marsupials. Ecological Applications 7: 625–641.

LINDENMAYER, D. B., CUNNINGHAM, R.B, TANTON, M.T., SMITH, A.P. & NIX, H.A. 1990. The conservation of arboreal marsupials in the montane ash forests of the central highlands of Victoria, south-east Australia: III. The habitat requirements of lead-beater's possum Gymnobelideus leadbeateri and models of the diversity and abundance of arboreal marsupials. Biol. Conserv 56:295–315.

LINDENMAYER, D.B., MACGREGOR, C.I., CUNNINGHAM, R.B., INCOLL, R.D., CRANE, M., MICHAEL, D.R., RAWLINS, D. & TRIBOLET, C.R. 2003. On the use of nest boxes by arboreal marsupials in the forests of the Central Highlands of Victoria. Wildlife Research 30: 259-264.

LOBOS, G., CHARRIER, A., CARRASCO, G. & PALMA, R.E. 2005. Presence of Dromiciops gliroides (Microbiotheria: Microbiotheriidae) in the deciduous forests of central Chile. Mammalian Biology 70: 376-380.

LOEB, S. C. 1999. Responses of small mammals to coarse woody debris in a southeastern pine forest. Journal of Mammalogy 80: 460–471.

LOMOLINO, M.V. & PERAULT, D.R. 2000. Assembly and disassembly of mammals communities in a fragmented temperate rain forest ecosystem. Ecology

LOMOLINO, M.V. & PERAULT, D.R. 2000. Island biogeography and landscape ecology of mammals inhabiting fragmented, temperate rain forests Global Ecology and Biogeography 10: 113-132 Published: MAR 2001

MACARTHUR, R.H. & MACARTHUR, J.W. 1961. On bird species diversity. Ecology, 42: 594–598.

MANNING, J.A. & EDGE, W.D. 2004. Small mammal survival and downed wood at multiple scales in managed forests. Journal of Mammalogy, 85: 87–96

MAHAN, C.G. & YAHNER, R.H. 1996. Effects of forest fragmentation of burrow-site selection of the Eastern chipmunk (Tamias striatus). American Midland Naturalist 136: 352-357

MANN, G. 1955. Monito del Monte, Dromiciops australis. Investigaciones Zoologicas Chilenas 2: 159-166

MANN, G. 1978. Los pequeños mamíferos de Chile. Gayana Zoología 40: 1–342.

MARÍN-VIAL, P., GONZÁLEZ-ACUÑA, D., CELIS-DIEZ, J.L., CATTAN, P.E. & GUGLIELMONE, A.A. 2007. Presence of Ixodes neuquenensis Ringuelet, 1947 (Acari: Ixodidae) on the endangered Neotropical marsupial Monito del monte (Dromiciops gliroides Thomas, 1894, Microbiotheria: Microbiotheriidae) at Chiloé Island, Chile. European Journal of Wildlife Research 53: 73-75.

MARTIN J.K & HANDASYDE, K.A. 2006. Comparison of bobuck (Trichosurus cunninghami) demography in two habitat types in the Strathbogie Ranges, Australia. Journal of Zoology 271: 375–385.

MARTÍNEZ DR, JAKSIC FM. 1996. Habitat, relative abundance, and diet of rufous-legged owls (Strix rufipes King) in temperate forest remnants of southern Chile. Ecoscience 3: 259-263.

MERINO, S., R.A. VÁSQUEZ, J. MARTÍNEZ, J.L. CELIS-DIEZ, L. GUTIÉRREZ, S. IPPI, I. SÁNCHEZ-MONSALVEZ, J. MARTÍNEZ DE LA PUENTE. 2009. Molecular characterization of an ancient Hepatozoon species parasitizing the 'living fossil' marsupial "Monito del Monte" Dromiciops gliroides from Chile. Biological Journal of the Linnean Socienty, 99: 568 – 576

MESERVE, P., LANG, B.K. & PATTERSON, B.D. 1988. Trophic relationship of small mammals in a Chilean temperate rain forest. Journal of Mammalogy 69: 721-730.

MORENO, J., MERINO, S., VÁSQUEZ, R.A. & ARMESTO, J.J. 2005. Breeding Biology of the Thorn-tailed Rayadito (Furnariidae) in south-temperate rainforests of Chile. The Condor, 107: 69-77.

MORRIS, D. W. 1996. Coexistence of specialist and generalist rodents via habitat selection. Ecology 77: 2352–2364.

MUÑOZ, A., CHACON, P., PÉREZ, F., BARNERT, E.S. & ARMESTO, J.J. 2003. Diversity and host tree preferences of vascular epiphytes and vines in a temperate rainforest in southern Chile. Australian Journal of Botany 51: 381-391.

MUÑOZ-PEDREROS, A., LANG, B.K., BRETOS, M. & MESERVE, P.L. 2005. Reproduction and development of Dromiciops gliroides (Marsupialia: Microbiotheriidae) in temperate rainforests of southern Chile. Gayana Zoología 69: 225-233.

MUÑOZ-PEDREROS, A. & PALMA, R.E. 2000. Marsupiales. Pp 43-51 in Mamíferos de Chile (Muñoz-Pedreros, A., and J. Yañez, eds.). Ediciones CEA, Valdivia, Chile.

MURÚA, R.E. 1996. Comunidades de mamíferos del bosque templado de Chile. Pp 113-133 in Ecología de los bosques nativos de Chile (Armesto, J.J., C.Villagrán, C. & Arroyo, M.T.K.eds.). Editorial Universitaria, Santiago,

NIEMELÄ, J., LANGOR, D., AND SPENCE, J.R. 1993. Effects of clear-cut harvesting on boreal ground-beetle assemblages (Coleoptera: Carabidae) in western Canada. Conservation Biology. 7: 551–561.

NEWTON, I. 1994. The role of nest sites in limiting the numbers of hole nesting birds: a review. Biological Conservation 70: 265–276

NOUR, N., MATTHYSEN, E. & DHONDT, A.A. 1997. Effects of habitat fragmentation on foraging behaviour of tits and related species: does niche space vary in relation to size and degree of isolation of forest fragments?. Ecography 20: 281-286

OTIS, D.L., BURNHAM, K.P., WHITE, G.C., ANDERSON, D.R., 1978. Statistical inference from capture data on closed animal populations. Wildlife Monographs 62, 1–135.

PALMA, R.E. & SPOTORNO, A.E. 1999. Molecular systematics between marsupials based on the rRNA 12S mitochondrial gene: the phylogeny of Didelphimorphia and of the living fossil microbiotheriid Dromiciops gliroides Thomas. Molecular Phylogenetics and Evolution 13: 525-535.

PARDINI, R. 2004. Effects of forest fragmentation on small mammals in an Atlantic Forest landscape. Biodiversity and Conservation 13: 2567–2586

PARMENTER, R.R., YATES, T.R., R.ANDERSON, D., BURNHAM, K.P., DUNNUM, J.L., FRANKLIN, A.B., FRIGGENS, M.T., LUBOW, B.C., MILLER, M., OLSON, G.D., PARMENTER, C., POLLARD, A.J., REXSTAD, E., SHENK, T.M., STANLEY, T.R. & WHITE, G.C. 2003. Small-mammal density estimation: a field comparison of grid-based vs. web-based density estimators. Ecological Monographs 73: 1–26.

PATTERSON, B.D., MESERVE, P.L. & LANG, B.K. 1989. Distribution and abundance of small mammals along an elevational transect in temperate rainforests of Chile. Journal of Mammalogy 70: 67-78.

PATTERSON, B.D., MESERVE. P.L. & LANG, B.K. 1990. Quantitative habitat associations of small mammals along an elevational transect in temperate rainforests of Chile. Journal of Mammalogy 71:620-633

PAVEY, C.R., GOODSHIP, N. & GEISER, F. 2003. Home range and spatial organization of rock-dwelling carnivorous marsupial, Pseudantechinus macdonnellensis. Wildlife Research 30:135-142.

PEREZ, C.A., M.R. CARMONA, J.M. FARIÑA, J.J. ARMESTO. 2009. Selective logging of lowland evergreen rainforests in Chiloe´ Island, Chile: Effects of changing tree species composition on soil nitrogen transformations. Forest Ecology and Management 258: 1660–1668

PHILLIPS, M.J., MCLENACHAN, P.A., DOWN, C., GIBB, G.C. & PENNY, D. 2006. Combined mitochondrial and nuclear DNA sequences resolve the interrelations of the major Australasian marsupial radiations. Systematic Biology 55: 122–137.

PILASTRO, A, TAVECCHIA, G. & MARIN, G. 2003. Long living and reproduction skipping in the fat dormouse. Ecology, 84: 1784–1792.

PINHEIRO, P.S., CARVALHO, F.M.V., FERNANDEZ, F.A.S. & NESSIMIAN, J.L. 2002. Diet of the marsupial Micoureus demerarae in small fragments of Atlantic Forest in southeastern Brazil. Studies on Neotropical Fauna and Environment 37: 213–18.

PRADEL, R. 1996. Utilization of Capture-mark-recapture for the study of recruitment and population growth rate. Biometrics 52: 703-709.

QUENTAL, T.B., FERNANDEZ, F.A.S, DÍAS, A.T.C. & ROCHA, F.S. 2001. Population dynamics of the marsupial Micoureus demerarae in small fragments of Atlantic coastal forest in Brazil. Journal of Tropical Ecology 17: 339–352.

RANGANATHANA,J., RANJIT DANIELS, R.J., SUBASH CHANDRANC, M.D., EHRLICH, P.R. & DAILY G.C. 2008. Sustaining biodiversity in ancient tropical countryside. PNAS 105: 17852–17854.

RAPHAEL, M.G. & WHITE, M. 1984. Use of snags by cavity-nesting birds in the Sierra Nevada. Wildlife Monographs 86: 1-66.

REDFORD, K.H. & EISENBERG, J.F. 1992. Mammals of the Neotropics: the Southern Cone: Chile, Argentina, Uruguay, Paraguay, Vol. 2, University of Chicago Press, Chicago.

REID, S., DÍAZ, I.A., ARMESTO, J.J. & WILLSON, M.F. 2004 Importance of native bamboo for understory birds in Chilean temperate forests. Auk 121: 515-525.

REIG O. A. 1955. Noticia preliminar sobre la presencia de Microbiotherinos vivientes en la fauna Sudamericana. Investlgaciones Zoologicas Chilienas 2:121-130.

RENDIGS, A., RADESPIEL, U., WROGEMANN, D. & ZIMMERMANN, E. 2003. Relations between microhabitat structure and distribution of Mouse lemurs (Microcebus spp.) in northwestern Madagascar. International Journal of Primatology 24: 47-64.

RHIND, S.G. 2003. Communal nesting in the usually solitary marsupial, Phascogale tapoatafa. Journal of Zoology 261: 345-351.

RIBBLE, D.O., WURTZ, A.E., MCCONNELL, E.K., BUEGGE, J.J. & WELCH, K.C.Jr. 2002. A comparison of home ranges of two species Peromyscus using trapping and radiotelemetry data. Journal of Mammalogy 83: 260-266.

RIVERO, M. 1991. Aspectos sobre la biología reproductive en dos comunidades del sur de Chile, 40 _S. Tesis de Doctorado, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.

RODRÍGUEZ-CABAL, M. A., AIZEN, M. A. & NOVARO, A.J. 2007. Habitat fragmentation disrupts a plant-disperser mutualism in the temperate forest of South America. Biological Conservation 139: 195-202.

RODRÍGUEZ-CABAL, M.A., AMICO, G.C., NOVARO, A.J. & AIZEN, M.A. 2008. Population characteristics of Dromiciops gliroides (Philippi, 1893), an endemic marsupial of the temperate forest of Patagonia. Mammalian Biology 73: 74-76.

ROSENZWEIG M. 1991. Habitat selection and population interactions: the search for mechanisms. American Naturalist 137: S5-S28

SAAVEDRA, B & SIMONETTI, J.A. 2005. Small mammals of Maulino forest remnants, a vanishing ecosystem of south-central Chile. Mammalia 69: 337-348.

SALINAS, F. 2008. Diferenciación de nichos ecológicos de tres especies de gesneriáceas epífitas del bosque templado del sur de Chile. Tésis de Doctorado, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.

SALA, E.O., CHAPIN III, F.S., ARMESTO, J.J., BERLOW, E., BLOOMFIELD, J., DIRZO, R., HUBER-SANWALD, E., HUENNEKE, L.F., JACKSON, R.B., KINZIG, A., LEEMANS, R., LODGE, D.M., MOONEY, H.A., OESTERHELD, M., LEROY POFF, N., SYKES, M.T., WALKER, B.H., WALTER, M., & WALL, D.H. 2000. Global biodiversity scenarios for the year 2100. Science 287: 1770-1774.

SANDERSON, J., SUNQUIST, M.E. & IRIARTE, A. 2002. Natural history and landscape-use of guignas (Oncifelis guigna) on Isla Grande de Chiloé, Chile. Journal of Mammalogy 83: 608-613.

SAUNDERS, D.A., HOBBS, R.J. & MARGULES, C.R. 1991. Biological consequences of ecosystem fragmentation: a review. Conservation Biology 5: 61-67.

SCHOENER, T.W. 1974. Resource partitioning in ecological communities. Science 185:27-39.

SCOTT, J.L. 1979. Non pigmented tunica vaginalis testis in the opossum. Ohio Journal of Science 79: 79-80.

SEKERCIOGLU, C.H., LOARIE, S.R., OVIEDO BRENES, F., EHRLICH, P.R. & DAILY, G.C. 2007. Persistence of Forest Birds in the Costa Rican Agricultural Countryside Conservation Biology Volume 21: 482–494

SERGIO, F, I. NEWTON & L. MARCHESI. 2005. Top predators and biodiversity. Nature 436:192

SCHAUB, M. & C. VATERLAUS-SCHLEGEL. 2001. Annual and seasonal variation of survival rates in the garden dormouse (*Eliomys quercinus*). Journal of Zoology 255: 89-96.

SIEVING, K.E., WILLSON, M.F. & DE SANTO, T.L. 1996. Habitat barrier movement of understory birds in fragmented south-temperate rainforest. Auk 113: 944-949.

SIMONETTI, J.A. & J.E. MELLA. 1997. Park size and the conservation of Chilean mammals. Revista Chilena de Historia Natural 70: 213-220.

SMITH-RAMÍREZ, C. & ARMESTO, J.J. 1994. Flowering and fruiting patterns in the temperate rain forest of Chiloé: ecologies and climatic constraints. Journal of Ecology 82: 353-365.

SPRINGER, M.S., WESTERMAN, M., KAVANAGH, J.R., BURK, A., WOODBURNE, M.O., KAO, D.J. & KRAJEWSKI, C. 1998. The origin of the Australasian marsupial fauna and the phylogenetic affinities of the enigmatic Monito del Monte and marsupial mole. Proceedings of the Royal Society of London series B265: 2381-2386.

STATSOFT, INC. 2001. STATISTICA (data analysis software system), version 6. www.statsoft.com.

STENSETH N. C. 1980 – Spatial heterogeneity and population stability: some evolutionary consequences – Oikos, 35: 165–184.

SULLIVAN, T.P. & SULLIVAN, D.S. 2001. Influence of variable retention harvests on forest ecosystems. II. Diversity and population dynamics of small mammals. Journal of Applied Ecology 38: 1234–1252.

TELLERIA, J.L., SANTOS, T.M. & ALCANTARA, M. 1991. Abundance of food searching intensity of wood mice Apodemus sylvaticaa in fragmented forest. Journal of Mammalogy 72: 183-187

TEWS, J., BROSE, U., GRIMM, V., TIELBÖRGER, K., WICHMANN, M.C., SCHWAGER, M. & JELTSCH, F. 2004. Animal species diversity driven by habitat heterogeneity/diversity: the importance of keystone structures. Journal of Biogeography 31: 79-92.

TILMAN, D., MAY, R.M., LEHMAN, C.L. & NOWAK, M.A. 1994. Habitat destruction and extinction debt. Nature 371: 65-66.

TOGNELLI, M.F., RAMIREZ DE ARELLANO, P.I. & MARQUET, P.A. 2008. How well do the existing and proposed reserve networks represent vertebrate species in Chile? Diversity and Distributions 14: 148-158.

TURCHIN P. 1999. Population regulation: a synthetic review. Oikos 84: 153-159

TYNDALE-BISCOE, C.H. & RENFREE, M.B. 1987. The female urogenital tract and oogenesis. Pp 172-257 en Reproductive Physiology of Marsupials (Tyndale-Biscoe, C.H., eds.). University Press, Cambridge, London.

UMETSU, F & PARDINI, R. 2007. Small mammals in a mosaic of forest remnants and anthropogenic habitats—evaluating matrix quality in an Atlantic forest landscape Landscape Ecology 22: 517–530.

UMETSU, F., METZGER, J.P. & PARDINI, R. 2008. Importance of estimating matrix quality for modeling species distribution in complex tropical landscapes: a test with Atlantic forest small mammals. Ecography 31: 359-370

VAN HERCK, H., BAUMANS, V., BRANDT, C.J.W., BOERE, H.A.G., HESP, A.P.M., LITH, H.A., SCHURINK, M. & BEYNEN, A.C. 2001. Blood sampling from the retro-orbital plexus, the saphenus vein and the tail vein in rats: comparative effects on selected behavioural and blood variables. Laboratory Animals 35: 131-139.

VAN HORNE, B. 1983. Density as a misleading indicator of habitat quality. Journal of Wildlife Management 47: 893-901

VIVEIROS DE CASTRO, E.B. & FERNÁNDEZ, F.A.S. 2004. Determinants of differential extinction vulnerabilities of small mammals in Atlantic forest fragments in Brazil. Biological Conservation 119: 73-80.

WATSON, J.E.M., WHITTAKER, R.J. & DAWSON, T.P. 2004. Habitat structure and proximity to forest edge affect the abundance and distribution of forest-dependent birds in tropical coastal forests of southeastern Madagascar. Biological Conservation 120: 311-327

WELLS, K., KALKO, E.K.V., LAKIM, M.B., & PFEIFFER, M. 2007. Effects of rain forest logging on species richness and assemblage composition of small mammals in Southeast Asia. Journal of Biogeography 34: 1087–1099.

WILLSON, M.F. 2004. Loss of habitat connectivity hinders pair formation and juvenile dispersal of Chucao tapaculos in Chilean rainforest. The Condor 106: 166-171.

WILSON, B.A. & FRIEND, G.R. 1999. Responses of Australian mammals to disturbance factors: a review. Australian Mammalogy 21: 87–105

WILLSON, M.F. & ARMESTO, J.J. 1996. The natural history of Chiloé: on Darwin's trail. Revista Chilena de Historia Natural 69: 149-161.

WILLSON, M.F. & ARMESTO, J.J 2003. Efectos de la fragmentación de bosques para las aves de los bosques australes chilenos. Ambiente & Desarrollo 19: 54-59.

WILLSON, M.F. & ARMESTO, J.J. 2006. Is natural history really dead? Toward the rebirth of natural history. Revista Chilena de Historia Natural 79: 279-283.

WILLSON, M.F., DE SANTO, T.L., SABAG, C. & ARMESTO, J.J. 1994. Avian communities in fragmented south-temperate rainforest in Chile. Conservation Biology 8: 508–520.

WILLSON, M.F., MORRISON, J.L., SIEVING, K.E., DE SANTO, J.L., SANTISTEBAN, L. & DÍAZ, I. 2001. Predation risk and survival of bird nests: patterns in a Chilean agricultural landscape. Conservation Biology 15:447-456.

WILLSON, M.F. & PENDLETON, G. 2008. Survival of Chucaos, Scelorchilus rubecula, in rainforest fragments on Isla Chiloé, Chile. Boletin Chileno de Ornitologia 14: 2–7.

WHITE, G.C., BURNHAM, K.P. 1999. Program MARK: Survival estimation from populations of marked animals. Bird Study 46, 120–138.

YOKOZ, N., NAKATA, K., STENSETH, N.C. & SAITOH, T. 1998. The demography of Clethrionomys rufocanus: form mathematical statistical models to further fiel studies. Res. Pop. Ecol. 40: 107-121.

ZAR, J.H. 1999. Biostatistical Analysis. Prentice Hall, New Jersey.

ANEXO I. Artículo publicado en European Journal of Wildlife Research (2007)

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SHORT COMMUNICATION

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Presence of *Ixodes neuquenensis* Ringuelet, 1947 (Acari: Ixodidae) on the endangered Neotropical marsupial Monito del monte (*Dromiciops gliroides* Thomas, 1894, Microbiotheria: Microbiotheriidae) at Chiloé Island, Chile

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Abstract The presence of *Ixodes neuquenensis* (Ringuelet, *Notas Mus la Plata* 12:207–216, 1947) (Acari: Ixodidae) parasitizing populations of *Dromiciops gliroides* Thomas, 1894 (Microbiotheria: Microbiotheriidae) at Chiloé Island confirms that this tick species is established in Chile. No preference of the ticks for sex or age of the host was observed.

Keywords Ticks · Marsupials · Ectoparasites · Chile

Introduction

The "Monito del monte", *Dromiciops gliroides* (Microbiotheria: Microbiotheriidae), is the only living species of the Order Microbiotheria and considered a fossil of the South American fauna (Hershkowitz 1999). This small and rare mouse opossum is an endemic marsupial of the temperate rainforests of South America which extends along the Pacific rim in Chile and adjacent parts of Argentina and in western Neuquén and southwestern Río Negro provinces. These rainforests hold endemic and endangered flora and fauna (Armesto et al. 1998). In addition, this marsupial has an enigmatic origin, being closely related to Australian marsupials rather than the highly diversified American ones (Kirsh et al. 1991). *D. gliroides* is confined to southern South America, from Talca province (region VII, ca. 35°30′) to Chiloé Island (region X, ca. 44°00′) in Chile and east to Argentina near Lago Nahuel Huapi (Hershkowitz 1999; Saavedra and Simonetti 2001; Lobos et al. 2005). *D. gliroides* is a tree climber species with nocturnal habits, associated to southern genus *Nothofagus* (Fagaceae) and *Chusquea* bamboo (Jiménez and Rageot 1979). According to IUCN, this species is threatened due to habitat loss, and its population has declined over the previous years (Nowak 1999). Its parasites are also consequently at risk of extinction (Guglielmone et al. 2004).

In continental Chile, *D. gliroides* was found infested with the fleas *Chiliopsylla allophyla* Rothschild 1908 and *Plocopsylla diana* Beaucournu et al. 1986 (Beaucournu and Gallardo 1991, 1992). In Argentina, Ringuelet (1947) presents a description of the holotype female of *Ixodes neuquenensis* on *D. gliroides*, and Guglielmone et al. (2004) recently described the nymph and larvae and redescribed the female of *I. neuquenensis*, collected from *D. gliroides* in Llao-Llao Reserve (Río Negro, Argentina). González-Acuña and Guglielmone (2005) previously concluded that, if *I. neuquenensis* was endemic to *D. gliroides*, this parasite–host interaction could also be found in Chile. In this paper, we present information that supports such inference together with additional information on the *D. gliroides*–*I. neuquenensis* relationship at Chiloé Island.

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Materials and methods

The study area is located northeast of Chiloé Island, Ancud city (41°50′ S, 73°36′ W). This area is characterized by a wet temperate weather with a strong ocean influence. The annual mean temperature is 12°C, with monthly maximum and minimum means of 16°C (January) and 5°C (July), respectively (Aravena et al. 2002). The rainfall amount is 2,000–2,500 mm yearly, with a dry period during January–February (Di Castri and Hajek 1976). The present-day rural landscape is characterized by a mosaic of remnant forest fragments, woodlands, and grazing pastures in rolling hills with altitudes ranging from 50 to 100 m (Aravena et al. 2002).

The physiognomy of these temperate rainforests is characterized by a multi-layer canopy, with large emergent trees such as *Nothofagus nitida* (Nothofagaceae), *Eucryphia cordifolia* (Eucryphiaceae), *Saxegothaea conspicua* (Podocarpaceae), and *Aextoxicon punctatum* (Aextoxicaceae) and large amounts of dead logs biomass and stump covered with ephyphites and vines. The understory is quite abundant, composed of trees, shrubs, and bamboo (Aravena et al. 2002).

Captures of *D. gliroides* were carried out during February to March 2005 at two old-growth forest fragments located in the northeastern area of the island. One of the sites was located inside the private protected area of the "Fundación Senda Darwin" (41° 52′ S, 73° 40′ W) and the second one was in Fundo "Los Cisnes" located in Bahía Caulín (41° 50′ S, 73° 36′ W). To capture *D. gliroides* individuals, Tomahawk-like traps (baited with banana) were used as *D. gliroides* are not prone to be caught with Sherman-like traps (Kelt and Martínez 1989). The traps were set up on tree branches at a minimum height of 1 m and disposed in 10×10 grid, with ten lines of 100 m each and 10 m distance between traps. The trapping period was performed in two occasions in both study sites for 15 and 10 nights, respectively (trapping effort=1,500 and 1,000 traps/night, respectively). Each trap was checked daily between 06:00 and 08:00 A.M. To avoid re-sampling, the animals captured were tagged with a 2-mm-long aluminum tag on the right ear, identified according to sex (i.e., female or male) and age (i.e., juvenile or adult), weighed, and finally tick specimens collected. After 10 min of handling, the animals were released at the same capture site.

The site of attachment on the body of the animal was recorded for each tick. All specimens were immediately preserved in 70% alcohol. The description and redescriptions of *I. neuquenensis* by Guglielmone et al. (2004) were used to confirm the specific status of the ticks.

Exact Fisher's test was applied to test differences in tick parasitism on *D. gliroides* in relation to sex and age. Tick abundance according to sex and age was also evaluated with a non-parametric ANOVA for ranking data (Sokal and Rohlf 1995).

Results and discussion

Twenty seven (40.3%) from a total of 67 captured *D. gliroides* were infested with *I. neuquenensis* (Table 1). *I. neuquenensis* is the only tick ever reported from *D. gliroides*. A total of 56 larvae, 10 nymphs, and 3 female ticks were collected; 87% of them were observed attached to the ears or nearby. These results confirm the hypothesis proposed by González-Acuña and Guglielmone (2005); *I. neuquenenis* is a tick species present in Chile.

No significant differences were found in tick prevalence according to the sex or age of the hosts (Fisher $P=0.66$). The abundance of *I. neuquenensis* in relation to sex (males vs females $F_{1,63}=0.85$, $P=0.39$) and age (juveniles vs adults $F_{1,63}=0.57$, $P=0.45$) were also non-significant, as well as the interaction between the two mentioned variables ($F_{1,63}=44.25$, $P=0.70$).

These results show that *I. neuquenensis* is quite abundant at the study site but independent of the age and sex of its hosts in contrast to observations in Argentina where ticks were only observed on male hosts (Guglielmone et al. 2004). The male of *I. neuquenensis* remains still unknown. This probably means that this tick species is nidicolous with a non-parasitic male which mates female(s) of the host. An alternative explanation could be that this tick species is parthenogenetic. Further sampling of *D. gliroides*' nests may provide data about the existence of *I. neuquenensis* males. Larvae, nymphs, and female ticks were found at the same time of the year, indicating that this tick needs at least 3 years to complete its life cycle.

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Table 1 Prevalence of infestation of 67 *D. gliroides* by the tick *I. neuquenensis*

Sex	Age								
	Juvenile			Adult			Total		
	Total	Positive	%	Total	Positive	%	Total	Positive	%
Female	17	5	29.4	10	4	40	27	9	33.3
Male	24	10	41.6	16	8	50	40	18	45.0
Total	41	15	36.6	26	12	46.2	67	27	40.3

References

Aravena JC, Carmona MR, Pérez CA, Armesto JJ (2002) Changes in tree species richness stand structure and soil properties in a succesional chronosequence in northern Chiloé Island, Chile. Rev Chil Hist Nat 75:339 360

Armesto JJ, Rozzi R, Smith-Ramírez C, Arrollo MTK (1998) Conservation targets in South American temperate forest. Science 282:1271 1272

Beaucournu JC, Gallardo MH (1991) Catalogue provisoire des puces du Chili (Insecta; Siphonaptera) (1ére partie). Bull Soc Fr Parasitol 9:237 270

Beaucournu JC, Gallardo MH (1992) Catalogue provisoire des puces du Chili (Insecta; Siphonaptera) (2éme partie). Bull Soc Fr Parasitol 10:93 130

Beaucournu JC, Gallardo MH, Launay H (1986) Puces (Siphonaptera) nouvelles ou peu connues du Chili: description de *Plocopsylla diana* n. sp. (Stephanocircidae). Ann Parasitol Hum Comp 61:359 366

Di Castri F, Hajek ER (1976) Bioclimatología de Chile. Editorial de la Universidad Católica de Chile, Santiago

González-Acuña DA, Guglielmone AA (2005) Ticks (Acari: Ixodidae: Argasidae, Ixodidae) of Chile. Exp Appl Acarol 35:147 163

Guglielmone AA, Venzal JM, Amico G, Mangold AJ, Keirans JE (2004) Description of the nymph and larva and redescriptions of the female of *Ixodes neuquenensis* Ringuelet, 1947 (Acari: Ixodidae), a parasite of the endangered Neotropical marsupial *Dromiciops gliroides* Thomas (Microbiotheria: Microbiotheriidae). Syst Parasitol 57:211 219

Hershkowitz P (1999) *Dromiciops gliroides* Thomas, 1894. Last of the Microbiotheria (Marsupialia), with a review of the Family Microbiotheriidae. Fieldiana Zool 93:1 60

Jiménez J, Rageot R (1979) Notas sobre la biología del "Monito del Monte" (*Dromiciops australis* Philippi 1893). An Mus de Hist Nat Valparaiso (Chile) 12:83 88

Kelt DA, Martínez D (1989) Notes on the distribution and ecology of two marsupials endemic to the Valdivian forests of southern South America. J Mammal 70:220 224

Kirsh JAW, Dickerman AW, Reig OA, Springer MS (1991) DNA hybridization evidence for the Australasia affinity of the American marsupial *Dromiciops gliroides*. Proc Natl Acad Sci USA 88:10465 10469

Lobos G, Charrier A, Carrasco G, Palma RE (2005) Presence of *Dromiciops gliroides* (Microbiotheria: Microbiotheriidae) in the deciduous forests of central Chile: range expansion and comments on its conservation status. Mamm Biol 70:376 380

Nowak RM (1999) Walkers mammals of the world, 6th edn. The John Hopkins University Press, Baltimore

Ringuelet R (1947) La supuesta presencia de *Ixodes brunneus* Koch en la Argentina y descripción de una nueva garrapata *Ixodes neuquenensis* nov. sp. Notas Mus la Plata 12:207 216

Rothschild NC (1908) A new flea from Chili. Rev Chil Hist Nat 13:104 106

Saavedra B, Simonetti JA (2001) New records of *Dromiciops gliroides* (Microbiotheria: Microbiotheriidae) and *Geoxus valdivianus* (Rodentia: Muridae) in central Chile: their implications for biogeography and conservation. Mammalia 65:96 100

Sokal RR, Rohlf FJ (1995) Biometry, 3rd edn (editorial). Freeman, San Francisco, USA, p 896

ANEXO 2. Artículo publicado en Biological Journal of the Linnean Society (2009)

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Molecular characterization of an ancient *Hepatozoon* species parasitizing the 'living fossil' marsupial 'Monito del Monte' *Dromiciops gliroides* from Chile

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The Microbiotheriid *Dromiciops gliroides*, also known as 'Monito del Monte', is considered to be a threatened species and the only living representative of this group of South American marsupials. During the last few years, several blood samples from specimens of 'Monito del Monte' captured at Chiloé island in Chile have been investigated for blood parasites. Inspection of blood smears detected a *Hepatozoon* species infecting red blood cells. The sequences of DNA fragments corresponding to small subunit ribosomal RNA gene revealed two parasitic lineages belonging to *Hepatozoon* genus. These parasite lineages showed a basal position with respect to *Hepatozoon* species infecting rodents, reptiles, and amphibians but are phylogenetically distinct from *Hepatozoon* species infecting the order Carnivora. In addition, the *Hepatozoon* lineages infecting *D. gliroides* are also different from those infecting other micro-mammals living in sympatry, as well as from some that have been described to infect an Australian species of bandicoot. The potential vector of this parasite appears to be the host-specific tick *Ixodes neuquenensis* because the sequencing of a long amplicon determined the presence of one of the two lineages found in the marsupial. © 2009 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2009, **98**, 568–576.

ADDITIONAL KEYWORDS: hemoparasites – host-parasite coevolution – phylogeny – rodents – South America – tick.

INTRODUCTION

Among the American marsupials, there exists a species, the so-called 'Monito del Monte', *Dromiciops gliroides* Thomas, 1894, which is the last living representative of the Order Microbiotheria (Redford & Eisenberg, 1992; Herskhovitz, 1999). This small mammal is a tree climber species with nocturnal activity, which lives in southern forest of *Nothofagus* (Fagaceae) and *Chusquea* Bamboo in southern Chile and Argentina (Herskhovitz, 1999; Lobos *et al.*, 2005). The oldest fossil record from microbiotherids comes from the Early Paleocene of Bolivia (Gayet, Marshall & Sempere, 1991), and phylogenetic analyses of marsupials situated *Dromiciops* as being more closely related to Australidelphian than to Ameridelphians marsupials (Palma & Spotorno, 1999). It is clear that 'Monito del Monte' is a 'living fossil', being the last representative of a point of union between Australian and American marsupials.

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The protozoan blood parasites under the genera *Hepatozoon* are known to infect a variety of vertebrates, from anurans to mammals (Smith, 1996). These parasites are found in peripheral blood, where they infect white blood cells, except in marsupials and some rodents, where red blood cell infections are common (Smith, 1996). These parasites are transmitted to vertebrate hosts by ingestion of infected haematophagous ectoparasites, where the parasite is developed in the haemocoel. The variety of ectoparasites acting as definitive hosts of *Hepatozoon* parasites is also very diverse, varying from mosquitoes to mites, bugs, flies, lice, ticks, and fleas (Smith, 1996).

In the present study, we characterized, at the molecular and morphological level, *Hepatozoon* infections from a wild population of 'Monito del Monte' in Southern Chile. We also explored the phylogenetic position of these parasite lineages with respect to other previously described *Hepatozoon* species. In addition, we looked for the presence of these lineages infecting other micro-mammals cohabiting in the same area. Finally, we searched for potential parasite vectors using molecular tools.

MATERIAL AND METHODS

The study was conducted at Senda Darwin Biological Station and surrounding forest patches in northern Chiloé Island, Southern Chile. *Dromiciops gliroides* individuals were captured inside bird nest-boxes or by using Tomahawk-live traps (baited with banana), and blood samples were collected in August 2005 (for details of the study area, see Moreno *et al.*, 2005). Each animal was individually marked with numbered or coloured ear-tags to avoid resampling. Approximately 50 μL of blood were obtained with a heparinized hematocrit capillary tube from the tail vein or the infraorbital sinus (van Herck *et al.*, 2000). One part of the blood was immediately smeared on a slide, air dried, and later fixed with 96 ° ethanol and stained with Giemsa (1 : 10, v/v) for 40 min. Blood smears were scanned in search of blood parasites in accordance with previously described methods (Merino, Potti & Fargallo, 1997). The rest of the blood was transferred with the aid of a capillary to a sterilized tube containing a buffer composed of 100 mM Tris (pH 8.0), 100 mM ethylenediaminetetraacetic acid (EDTA), 2% sodium dodecyl sulphate (SDS) (Jarvi, Schultz & Atkinson, 2002) to preserve blood samples for DNA analyses, or were stored on FTA classic cards (Whatman International Ltd.; Gutiérrez-Corchero *et al.*, 2002). To identify potential vectors of the *Hepatozoon* infections, animals were also captured three times a year in 2007 and 2008 (i.e. August, November, and February) and examined in search of ectoparasites. Ticks were the most common ectoparasite detected and collected (see Results). Ticks and two unidentified fleas were maintained alive for 24 h to allow the blood digestion and then conserved in 70% ethanol. Subsequently, ectoparasites were lyophilized to facilitate transport to the laboratory in Spain and DNA extraction. In addition, during the summer of 2007, in the same study area, we also captured and blood sampled the following rodents; two *Oligoryzomys longicaudatus* Bennett, 1832, 11 *Abrotrix (Chroeomys) olivaceus* Thomas, 1916, and four *Abrotrix sanborni* Thomas, 1916, to check whether the *Hepatozoon* species detected in *D. gliroides* were also infecting other micro-mammals in the area of study. Only blood samples for DNA analyses were obtained from these animals and were analysed as described below.

Parasite morphometric measurements were made with the aid of image analyser software (Scion Image) from pictures of parasites taken under a ×100 oil immersion objective using an Olympus BX41 optic microscope. The length and width of the parasite and parasite nucleus were measured for each parasite (Table 1). Infected and uninfected erythrocytes were also measured.

DNA ANALYSIS

Genomic DNA from *D. gliroides* samples conserved in lysis buffer was obtained using the UltraClean DNA BloodSpin kit (MO BIO Laboratories, Inc.). On the other hand, genomic DNA present in FTA cards was extracted to a soluble solution before polymerase chain reaction (PCR) using the following protocol: cored samples were transferred to collection vials

Table 1. Measurements of parasites and erythrocytes found in 35 *Dromiciops gliroides*

	Parasite	Parasite nucleus	Infected erythrocyte	Uninfected erythrocyte (ø)
Length (μm)	9.39 (0.64)	3.42 (0.43)	11.33 (2.07)	5.86 (0.50)
Width (μm)	2.59 (0.34)	1.95 (0.47)	4.57 (1.13)	–
Sample size	23	23	21	69

The diameter of uninfected erythrocytes is also presented. Standard deviations are shown in parenthesis.

Table 2. List of primers used in the present study

Primers	Sequences (5′- to 3′)
NBA1b	GTT GAT CCT GCC AGT AGT
NBA2	GCC TGC TGC CTT CCT TA
HEP1	CGC GCA AAT TAC CCA ATT
HEP4	TAA GGT GCT GAA GGA GTC GTT TAT
HPF1	CTA TGC CGA CTA GAG ATT G
HPF2	GAC TTC TCC TTC GTC TAA G
NBA1	GGT TGA TCC TGC CAG TAG T

with 250 μL of SET buffer (0.15 M NaCl, 0.05 M Tris, 0.001 M EDTA; pH 8) at 4 °C for 6 h. Subsequently, SDS 20% (7 μL) and proteinase K (50 μg) were added to the vials and incubated at 55 °C overnight. After incubation, ammonium acetate 4 M (250 μL) was added to the vials at room temperature for 30 min. Subsequently, vials were centrifuged at 13 000 **g** for 10 min. After removing the pellet, DNA was precipitated with ethanol and resuspended in sterile water.

Amplification of 18S rDNA gene (1769 bp) was accomplished by PCR using the primers NBA1b/HPF2 (Table 2). PCR reactions consisted of 25-μL reaction volumes containing 20 ng of template DNA, 50 mM KCl, 10 mM Tris-HCl, 1.5 $MgCl_2$, 0.2 mM of each dNTP, 1 μM of each primer, and 1.25 U of AmpliTaq Gold (Applied Biosystems). The reactions were cycled at the following parameters using a thermal cycler (MasterCycler Personal, Eppendorf): 94 °C for 10 min (polymerase activation), 40 cycles at 95 °C for 40 s, 54 °C for 1 min, 72 °C for 2 min, and a final extension at 72 °C for 10 min. The amplicons obtained after PCR assays were recovered from agarose gels and subjected to direct sequencing using the primers NBA1b, NBA2, HEP1, HEP4, HPF1, and HPF2 (Table 2). The sequencing was carry out using an ABI 3130 (Applied Biosystems) automated sequencer. DNA sequences were aligned and edited using CLUSTALW (Thompson, Higgins & Gibson, 1994) and BIOEDIT software (Hall, 1999), respectively. The phylogenetic analyses were performed using three different methods of inference after removing all columns containing gaps or missing data. The Neighbour-joining method (maximum composite likelihood model), the maximum likelihood method (GTR model; the gamma distribution parameter, the proportion of invariable sites, and the transition/transversion ratio were estimated) and Bayesian estimation (Markov chain Monte Carlo algorithm) were carried out using MEGA, version 4.0 (Tamura *et al.*, 2007), PHYML, version 3.0 (Dereeper *et al.*, 2008) and MrBayes (Ronquist & Huelsenbeck, 2003), respectively. Tree consistency was estimated by bootstrap analysis with 100 replications. All *Hepatozoon* sequences in GenBank with a similar size to those lineages found in *D. gliroides* were included in the phylogenies (Table 3). Although with some limitations, small subunit rDNA (SSU) is generally considered to be a good marker for addressing relationships of apicomplexan species and their history (Morrison & Ellis, 1997; Zhu, Keithly & Philippe, 2000; Šlapeta *et al.*, 2003). In addition, only SSU rDNA sequences are available for sufficient *Hepatozoon* species to make evolutionary inferences of parasites within this genus.

To detect the presence of the parasite in potential ectoparasite vectors, we followed the method of Schall & Smith (2006). This method aims to avoid the amplification of DNA from parasites digested with the blood meal. This allows the discrimination of whether a particular species acts as vector of a particular parasite because only parasites that survive to digestion are transmitted. Arthropods were maintained alive for 24 h, thus allowing for the blood meal to be digested, including the parasite DNA. Thus, it would be very difficult to amplify a long DNA fragment from the parasite, except if the arthropod is the vector. In this case, the parasites would be located in the haemocele, which is a safe place from digestive endonucleases.

The ticks and two fleas achieved from 'Monito del Monte' were maintained alive for 24 h before storage in 70% ethanol. DNA from lyophilized ectoparasites was extracted using the method previously described for FTA cards. Amplification of 18S rDNA gene (1096 bp) was accomplished by PCR using the primers NBA1/HEP4 (Table 2). PCR reactions were carried out using the conditions described above.

STATISTICAL ANALYSIS

To identify potential differences in the prevalence of infection by *Hepatozoon* between individuals of different sexes and ages, we conducted statistical analyses using Yates corrected chi-square tests (STATISTICA, version 6.0; StatSoft, Inc.).

RESULTS

Overall, we captured 77 *D. gliroides*, comprising 44 males (39 adults and four juveniles; one male was not aged), 29 females (11 adults and 18 juveniles) and four individuals not sexed and aged. Excluding the last four individuals, blood smears were obtained from all animals. Also, with the exception of two juvenile females, blood for DNA analyses was obtained for a total of 75 individuals.

Thirty-five out of 73 (47.9%) blood smears from different individuals were found infected by a *Hepa-*

Table 3. Protozoan species used for phylogenetic analyses indicating their hosts, Genbank accession numbers, location, and reference when available

Parasite	Host	Genbank accession number	Location	Reference
Hepatozoon catesbianae	*Rana catesbiana*	AF 130361	USA	Carreno, Martin & Barta (1999)
Hepatozoon boigae	*Boiga fusca*	AF 297085	Australia	Unpublished
Hepatozoon sp.	*Isoodon obesulus*	EF152218 to EF152230	Australia	Wicks *et al.* (2006)
Hepatozoon sp.	*Abrotrix olivaceus*	FJ719815, FJ719817, FJ719818	Chile	Present study
Hepatozoon sp.	*Abrotrix sanborni*	FJ719816 FJ719819	Chile	Present study
Hepatozoon sp.	*Bandicota indica*	AB181504	Thailand	Unpublished
Hepatozoon sp.	*Clethrionomys glareolus*	AY 600625, AY 600626	Spain	Criado-Fornelio *et al.* (2006)
Hepatozoon felis	*Felis catus*	AY 620232, AY 628681	Spain	Criado-Fornelio *et al.* (2006)
Hepatozoon americanum curupira	*Dusycion thous*	AY 461377	Brazil	Criado-Fornelio *et al.* (2006)
Hepatozoon canis	*Canis familiaris*	AY 461378	Spain	Criado-Fornelio *et al.* (2006)
Hepatozoon canis	*Vulpes vulpes*	AY731062, AY 150067	Spain	Criado-Fornelio *et al.* (2006)
Hepatozoon canis curupira	*Dusycion thous*	AY 461375	Brazil	Criado-Fornelio *et al.* (2006)
Hepatozoon canis curupira	*Pseudalopex gymnocercus*	AY471615, AY 461376	Brazil	Criado-Fornelio *et al.* (2006)
Hepatozoon sp.	*Dromiciops gliroides*	FJ719813, FJ719814	Chile	Present study
Adelina bambarooniae	*Dermolepida albohirtum*	AF494059	Australia	Unpublished

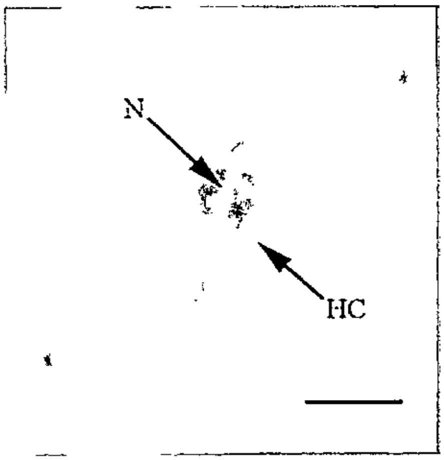

Figure 1. Photomicrograph of a blood smear from the 'Monito del Monte' (*Dromiciops gliroides*) showing an erythrocyte infected with *Hepatozoon*. N, parasite nucleus; HC, host cell. Scale bar = 5 μm.

tozoon species. Parasites infect red blood cells and show the typical banana shape of these parasite species (Fig. 1). Positive slides are deposited at the collection of Museo Nacional de Ciencias Naturales, Madrid, Spain (accession numbers: MNCN 35.02/33, MNCN 35.02/34, and MNCN 35.02/35 blood smears from *D. gliroides*). Molecular methods increased the prevalence of infection detected up to 86.7% (i.e. 65 out of 75 individuals infected).

There were no significant differences between sexes in the proportion of infected individuals estimated either by molecular ($\chi^2 = 1.52$, $P = 0.22$) or microscopic methods ($\chi^2 = 0.08$, $P = 0.78$). Significant differences between age classes were only found for data based on blood smears ($\chi^2 = 14.20$, $P < 0.001$), indicating that more adults than juveniles were uninfected. The fact that this difference was not significant for molecular data on infection ($\chi^2 = 0.01$, $P = 0.96$) indicates that the intensity of infection is higher in juveniles because many infections were not detected in adults by microscopic as compared to molecular methods. Differences in the prevalence of infection between sexes by age classes were not significant (data not shown, $P > 0.05$).

The tick *Ixodes neuquenensis* Ringuelet 1847 was collected from *D. gliroides* (two adults and 15 nymphs in 2007 and 13 adults and 12 nymphs in 2008). Long sequences from the *Hepatozoon* lineages infecting 'Monito del Monte' were recovered from three adult *I. neuquenensis* (one from 2007 and two from 2008). We

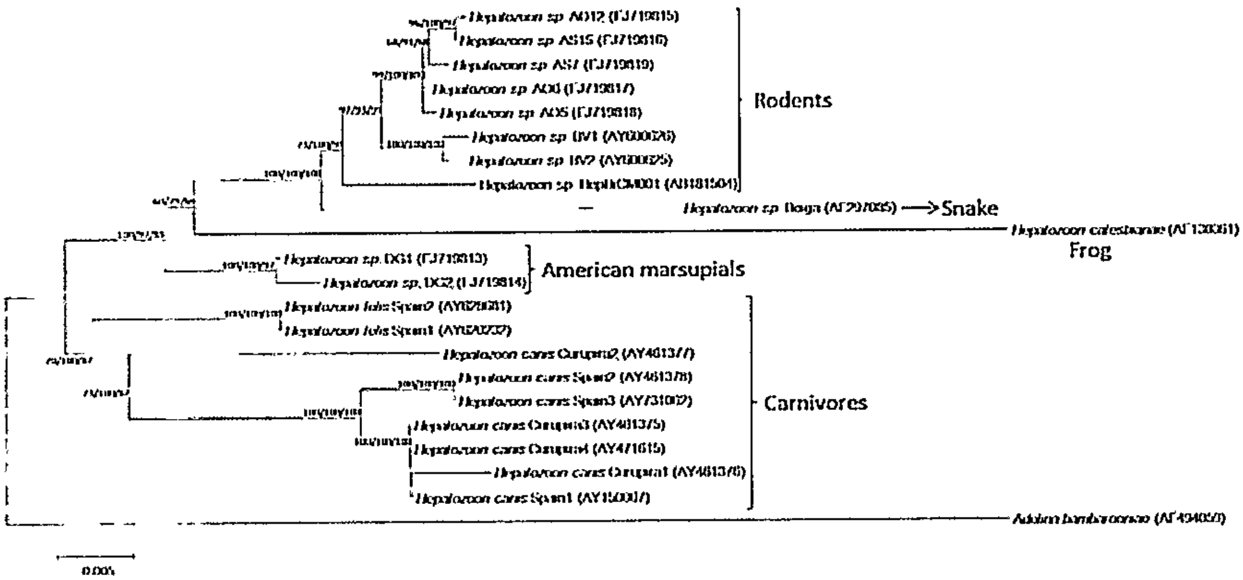

Figure 2. Phylogenetic tree obtained with the MEGA, version 4 software, using the Neighbour-joining method. Bootstrap values are shown at the corresponding nodes. When three values appear, they correspond to maximum composite likelihood, Bayesian, and maximum likelihood methods, respectively. Figures in parentheses after the species name indicate the GenBank accession number of the isolate. The hosts where *Hepatozoon* species were recovered are indicated. Samples from 'Monito del Monte' comprise those from American marsupials. This analysis is based on 1627 bp.

were unable to recover long segments of *Hepatozoon* from the two unidentified fleas.

Finally, we detected *Hepatozoon* infections in two of the three rodent species co-existing in the same area with 'Monito del Monte'. We found six *Abrotrix olivaceus* and two *A. sanborni* infected by five different *Hepatozoon* lineages. Three of them were present in *A. olivaceus* and two in *A. sanborni*. None of them were found infecting the 'Monito del Monte' (see sequences with accession numbers FJ719815 to FJ719819 in Fig. 2; Table 3).

PHYLOGENIES

The tree including the short sequences of *Hepatozoon* species from the Australian bandicoot are based on 743 bp after removing columns containing gaps or missing data. This tree is used to determine the phylogenetic relationships between *Hepatozoon* lineages isolated from 'Monito del Monte' and those from the bandicoot (Fig. 3), and we infer phylogenetic relationships among parasites infecting different host taxa from the tree based on 1627 bp (see below; Fig. 2) although conclusions did not vary considerably by using either of them except for bootstrap support. The *Hepatozoon* lineages found in both marsupial species are not closely related between them and the lineages found in Australian marsupials, American marsupials/anurans, rodents/reptiles, and carnivore species form clearly separated groups (Fig. 3). To infer a sound phylogenetic relationship, we consider the more robust tree based on 1627 bp after removing columns containing gaps or missing data (Fig. 2). The topology of the tree indicates the grouping of the lineages in two clades supported with a high bootstrap value. One of them is formed by all *Hepatozoon* lineages found in carnivore species and the other by the lineages found in rodents, amphibians, reptiles, and the American marsupial. In this case, the lineages found in marsupials have a basal position in the clade.

DISCUSSION

The *Hepatozoon* species infecting 'Monito del Monte' appears to be clearly separated from lineages recovered from the Australian bandicoot. This may be interpreted as indicating that different *Hepatozoon* lineages developed independently in marsupials in both continents because the lineages recovered from *D. gliroides* appear to evolve from a common ancestor, previous to the separation of *Hepatozoon* species infecting rodents and *Isoodon obesulus*, although this tree topology lacks strong support (Fig. 3). Indeed, it has been recently suggested that parasite–host coevolution implying Apicomplexa follows the definitive hosts, namely those where sexual reproduction appear (Šlapeta *et al.*, 2003). Based on molecular and paleontological data, Palma & Spotorno (1999) pro-

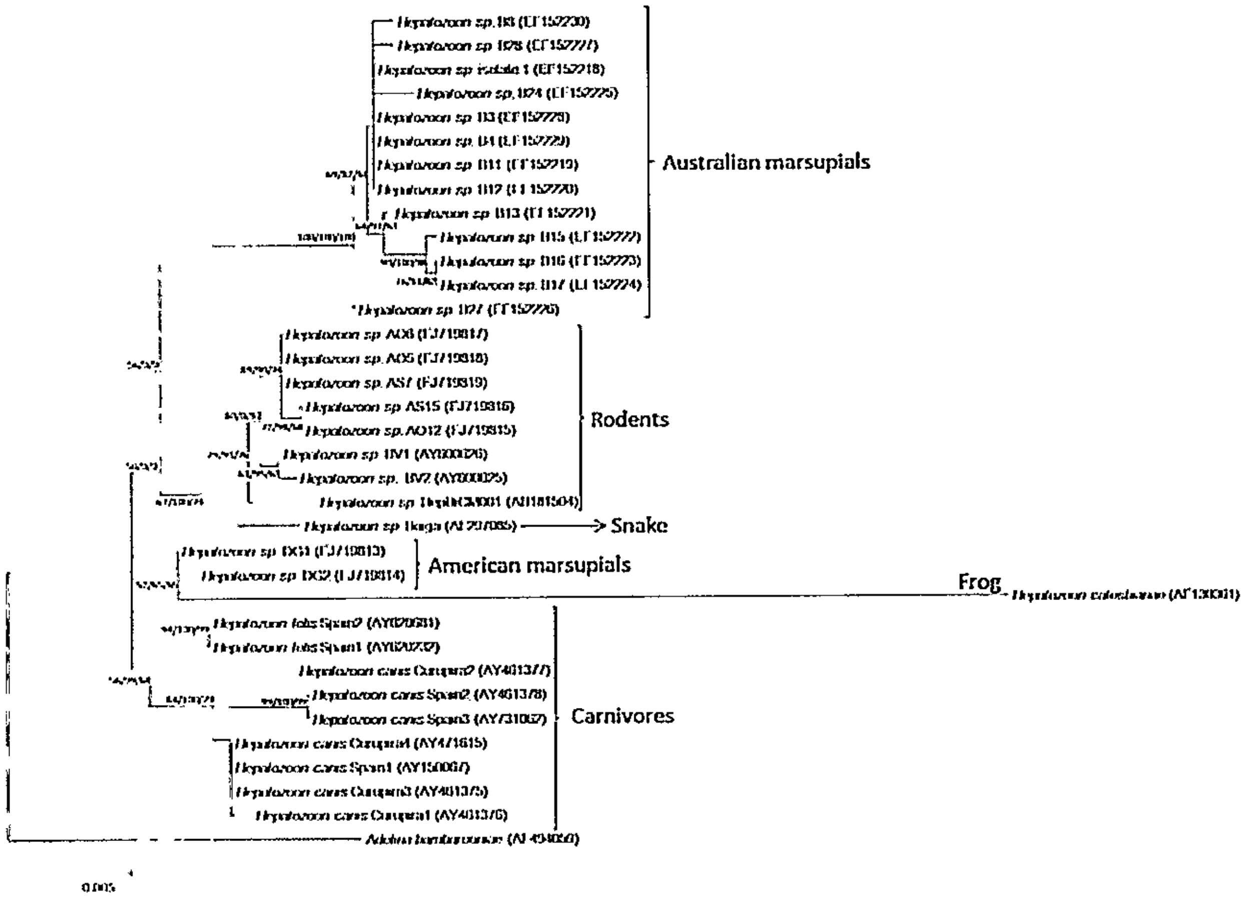

Figure 3. Phylogenetic tree obtained with the MEGA, version 4 software, using the Neighbour-joining method. Bootstrap values are shown at the corresponding nodes. When three values appear, they correspond to maximum composite likelihood, Bayesian, and maximum likelihood methods, respectively. Figures in parentheses after the species name indicate the GenBank Accession number of the isolate. The hosts where *Hepatozoon* species were recovered are indicated. Samples from 'Monito del Monte' are those from American marsupials. Samples from Australian marsupials are reported by Wicks *et al.* (2006). This analysis is based on 743 bp.

posed that Microbiotherids evolve in South America and later migrate to Australia across Antarctica prior to the separation of continents. If *Hepatozoon* species evolve with their definitive hosts and if *Hepatozoon* in *D. gliroides* separated from a common ancestor of other *Hepatozoon* species infecting Australian marsupials (Fig. 3), we can propose that the relationships *Hepatozoon–D. gliroides* is the result of a long coevolving relationship between host and parasite. That is, the relationship may have existed for a long time subsequent to the separation of continents. The complete sequence of 18S rDNA of *Hepatozoon* lineages from Australian marsupials may help to solve the position of the common ancestor of these parasites.

The possibility that *Hepatozoon* species infecting American marsupials were the ancestor to species infecting other mammals except carnivores obtains some reinforcement from the tree based on a longer fragment (1627 bp) of the small subunit ribosomal RNA gene, because the *Hepatozoon* lineages infecting *D. gliroides* appear in a basal position with respect to species infecting anurans, ophidians, and rodents, including lineages isolated from sympatric rodents. The *Hepatozoon* species parasitizing carnivores appear to be clearly separated in another clade (Fig. 2). Thus, the lineages isolated from *D. gliroides* appear to be host-specific and to be an ancient species of *Hepatozoon* with respect to those infecting rodents and lower vertebrates. The basal position of *Hepatozoon* in 'Monito del Monte' with respect to those in anurans and ophidians may indicate that these lower vertebrates were infected secondarily from mammals. *Hepatozoon* species present in rodents in the area also form a robust clade; thus, lineages of this parasite in these mammals also appear to be endemic to the area.

The basal position of *Hepatozoon* infecting 'Monito del Monte' prompted us to consider that the relationship between the marsupial and the parasite should also be mediated by an ancient and host-specific ectoparasite. In this respect, we recovered long segments of *Hepatozoon* DNA corresponding to the species infecting *D. gliroides* from three ticks using the method described above (Schall & Smith, 2006). Therefore, *I. neuquenensis* appears to be a potential vector of the *Hepatozoon* species infecting 'Monito del Monte'. This is interesting because *I. neuquenensis* is considered to be a host-specific ectoparasite of this vertebrate (Guglielmone *et al.*, 2004; Guglielmone & Nava, 2005; Marín-Vial *et al.*, 2007). However, this tick did not appear as a very ancient species compared to tick species from other vertebrates (Guglielmone *et al.*, 2006), although these data are only based on phylogenetic analyses of sequences of 460 bp. This may imply that the *Hepatozoon* parasite has adapted to *I. neuquenensis* later than to *D. gliroides*, and that either we failed to sample the original vector or it has now disappeared. The possibility that *I. neuquenensis* may have had a broad specificity and only recently invaded *D. gliroides* is not supported by the lack of reports of this tick species infecting other hosts (Guglielmone & Nava, 2005). It is possible that other vectors of the parasite exist because the prevalence of these *Hepatozoon* lineages in *I. neuquenensis* is very low compared to the prevalence in the vertebrate host. However, this may be related to host susceptibility and not necessarily to a relationship between high prevalences of infection in vertebrate hosts and a high abundance of vectors (Sol, Jovani & Torres, 2000).

Several species of *Hepatozoon* have been described infecting marsupials both in Australia and America, and all of them infect erythrocytes. A common species infecting American marsupials and described as *Hepatozoon didelphydis* was recently found to be a Sarcocystidae (Merino *et al.*, 2008), and a similar case has been recently reported for a marsupial species in Australia (Zhu *et al.*, 2009). Criado-Fornelio *et al.* (2006) failed to find *Hepatozoon* infections in 15 opossums (*Didelphis albiventris*) from Brazil either by molecular methods or microscopy, in contrast to the 25% prevalence detected by microscopy in the same species from French Guyana (de Thoisy *et al.*, 2000). *Hepatozoon* infections have also been reported infecting other neotropical marsupials, including *Didelphys marsupialis*, *Philander opossum*, and *Metachirus nudicaudatus* (d'Utra e Silva & Arantes, 1916; Regendanz & Kikuth, 1928; Garnham & Lewis, 1958; Deane & Deane, 1961; Ayala *et al.*, 1973). However, these reports are only based in microscopy and some of them are attributed to *H. didelphydis*, and may correspond to infections by an erythrocytic sarcocystidae (Merino *et al.*, 2008).

The high prevalence of these parasites in 'Monito del Monte' implies that it is a common parasite in the area of study. The infection is more difficult to detect in blood smears from adult individuals, thus indicating that juveniles suffer from more intense infections. This implies that juveniles are more susceptible to the parasite and adults probably control the infection better. This pattern agrees with an increase in immune responses as animals age, although, in most cases, the disease is not completely cleared, as demonstrated by the molecular data. Alternatively, highly-infected young individuals could die as a result of infection or associated causes and only individuals that are able to control infection reach adulthood. Information on the effects of infection on *D. gliroides* and on variation in the prevalence between populations, as well as on other potential vectors, will help to reveal the real impact of these parasites on this threatened 'living fossil' species of marsupial because *Hepatozoon* infections have been shown to be pathogenic with respect to their hosts (Ewing & Panciera, 2003).

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REFERENCES

Ayala SC, D'Alessandro A, Mackenzie R, Angel D. 1973. Hemoparasite infections in 830 wild animals from the eastern Llanos of Colombia. *Journal of Parasitolgy* **59:** 52–59.

Carreno RA, Martin DS, Barta JR. 1999. *Cryptosporidium* is more closely related to the gregarines than to coccidia as shown by phylogenetic analysis of apicomplexan parasites inferred using small-subunit ribosomal RNA gene sequences. *Parasitology Research* **85:** 899–904.

Criado-Fornelio A, Ruas JL, Casado N, Farias NAR, Soares MP, Müller G, Brum JGW, Berne MEA, Buling-Saraña A, Barba-Carretero JC. 2006. New molecular data on mammalian *Hepatozoon* species (Apicomplexa: Adeleorina) from Brazil and Spain. *Journal of Parasitology* **92:** 93–99.

Deane LM, Deane MP. 1961. Sobre dois hemocitozoários encontrados em mamíferos silvestres da Regiaõ Amazónica. *Revista do Instituto de Medicina Tropical de São Paulo* **3:** 107–110.

Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, Dufayard J-F, Guindon S, Lefort V, Lescot M, Claverie J-M, Gascuel O. 2008. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research* **36:** web Server issue W465–W469.

Ewing SA, Panciera RJ. 2003. American canine hepatozoonosis. *Clinical Microbiology Reviews* **16:** 688–697.

Garnham PCC, Lewis DJ. 1958. Demonstration. Some parasites from British Honduras. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **52:** 295–296.

Gayet M, Marshall LG, Sempere T. 1991. The Mesozoic and Paleocene vertebrates of Bolivia and their stratigraphic context: a review. *Revista Técnica Yacimientos Petrolíferos Fiscales Bolivianos Santa Cruz, Bolivia* **12:** 393–433.

Guglielmone AA, Nava S. 2005. Las garrapatas de la familia Argasidae y de los géneros *Dermacentor, Haemaphysalis, Ixodes* y *Rhipicephalus* (Ixodidae) de la Argentina: Distribución y hospedadores. *Revista de Investigaciones Agropecuarias* **34:** 123–141.

Guglielmone AA, Venzal JM, Amico G, Mangold AJ, Keirans JE. 2004. Description of the larva and redescription of the nymph and female of *Ixodes neuquenensis* Ringuelet, 1847 (Acari: Ixodidae), a parasite of the endangered species *Dromiciops gliroides* Thomas, 1847 (Microbiotheria: Microbiotheriidae). *Systematic Parasitology* **57:** 211–219.

Guglielmone AA, Venzal JM, González-Acuña D, Nava S, Hinojosa A, Mangold AJ. 2006. The phylogenetic position of *Ixodes stilesi* Neumann, 1911 (Acari: Ixodidae): morphological and preliminary molecular evidences from 16S rDNA sequences. *Systematic Parasitology* **65:** 1–11.

Gutiérrez-Corchero F, Arruga MV, Sanz L, García C, Hernández MA, Campos F. 2002. Using FTA cards to store avian blood samples for genetic studies. Their application in sex determination. *Molecular Ecology Notes* **2:** 75–77.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41:** 95–98.

van Herck H, Baumans V, Boere HAG, Hesp APM, van Lith HA, Beynen AC. 2000. Orbital sinus blood sampling in rats: effects upon selected behavioural variables. *Laboratory Animals* **34:** 10–19.

Herskhovitz P. 1999. *Dromiciops gliroides* Thomas 1894, last of the Microbiotheria (Marsupialia) with a review of the family Microbiotheriidae. *Fieldiana Zoology* **93:** 1–60.

Jarvi SI, Schultz JJ, Atkinson CT. 2002. PCR diagnostics underestimate the prevalence of avian malaria (*Plasmodium relictum*) in experimentally-infected passerines. *Journal of Parasitology* **88:** 153–158.

Lobos G, Charrier A, Carrasco G, Palma RE. 2005. Presence of *Dromiciops gliroides* (Microbiotheria: Microbiotheriidae) in the deciduous forests of central Chile. *Mammalian Biology* **70:** 376–380.

Marín-Vial P, González-Acuña D, Celis-Diez JL, Cattan PE, Guglielmone AA. 2007. Presence of *Ixodes neuquenensis* Ringuelet, 1947 (Acari: Ixodidae) on the endangered Neotropical marsupial Monito del monte (*Dromiciops gliroides* Thomas, 1894, Microbiotheria: Microbiotheriidae) at Chiloé Island, Chile. *European Journal of Wildlife Research* **53:** 73–75.

Merino S, Potti J, Fargallo JA. 1997. Blood parasites of some passerine birds from central Spain. *Journal of Wildlife Diseases* **33:** 638–641.

Merino S, Vásquez RA, Martínez J, Celis-Diez JL, Martínez-de la Puente J, Marín-Vial P, Sánchez-Monsalvez I, Peirce MA. 2008. A Sarcocystid misidentified as *Hepatozoon didelphydis*: molecular data from a parasitic infection in the blood of the southern mouse opossum (*Thylamis elegans*) from Chile. *Journal of Eukaryotic Microbiology* **55:** 536–540.

Moreno J, Merino S, Vásquez RA, Armesto JJ. 2005. Breeding biology of the thorn-tailed rayadito (Furnariidae) in south-temperate rainforests of Chile. *Condor* **107:** 69–77.

Morrison DA, Ellis JT. 1997. Effects of nucleotide sequence alignment on phylogeny estimation: a case study of 18S rDNAs of Apicomplexa. *Molecular Biology and Evolution* **14:** 428–441.

Palma RE, Spotorno AE. 1999. Molecular systematics of marsupials based on the rRNA 12S mitochondrial gene: the phylogeny of Didelphimorphia and of the living fossil microbiotheriid *Dromiciops gliroides* Thomas. *Molecular Phylogenetics and Evolution* **13:** 525–535.

Redford KH, Eisenberg JF. 1992. *Mammals of the neotropics: the southern cone: Chile, Argentina, Uruguay, Paraguay*, Vol. 2. Chicago, IL: University of Chicago Press.

Regendanz R, Kikuth W. 1928. Sur les Hémogrégarines de 'gamba', (*Haemogregarina didelphydis*), de la 'quica', (*Haemogregarina metachiri* n. sp.) et sur *l'Haemogregarina ratti. Comptes Rendus des Séances de la Société de Biologie et de Ses Filiales* **98:** 565–567.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19:** 1572–1574.

Schall JJ, Smith TC. 2006. Detection of a malaria parasite (*Plasmodium mexicanum*) in ectoparasites (mites and ticks), and possible significance for transmission. *Journal of Parasitology* **92:** 413–415.

Šlapeta JR, Modrý D, Votýpka J, Jirků M, Lukeš J, Koudela B. 2003. Evolutionary relationships among cyst-forming coccidian *Sarcocystis* spp. (Alveolata: Apicomplexa: Coccidea) in endemic African tree vipers and perspective for evolution of heteroxenous life cycle. *Molecular Phylogenetics and Evolution* **27:** 464–475.

Smith TG. 1996. The genus *Hepatozoon* (Apicomplexa: Adeleina). *Journal of Parasitology* **82:** 565–585.

Sol D, Jovani R, Torres J. 2000. Geographical variation in blood parasites in feral pigeons: the role of vectors. *Ecography* **23:** 307–314.

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software, Version 4.0. *Molecular Biology and Evolution* **24:** 1596–1599.

de Thoisy B, Michel J-C, Vogel I, Vie J-C. 2000. A survey of hemoparasite infections in free-ranging mammals and reptiles in French Guiana. *Journal of Parasitology* **86:** 1035–1040.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22:** 4673–4680.

d'Utra e Silva O, Arantes JB. 1916. Sobre uma hemogregarina da gambá. *Hemogregarina didelphydis* n. sp. *Memorias do Instituto Oswaldo Cruz* **8:** 61–64.

Wicks RM, Spencer PB, Moolhuijzen P, Clark P. 2006. Morphological and molecular characteristics of a species of *Hepatozoon* Miller, 1908 (Apicomplexa: Adeleorina) from the blood of *Isoodon obesulus* (Marsupialia: Peramelidae) in Western Australia. *Systematic Parasitology* **65:** 19–25.

Zhu BY, Hartigan A, Reppas G, Higgins DP, Canfield PJ, Šlapeta J. 2009. Looks can deceive: molecular identity of an intraerythrocytic apicomplexan parasite in Australian gliders. *Veterinary Parasitology* **159:** 105–111.

Zhu G, Keithly JS, Philippe H. 2000. What is the phylogenetic position of *Cryptosporidium*? *International Journal of Systematic and Evolutionary Microbiology* **50:** 1673–1681.