VARIACIÓN GEOGRÁFICA EN *Liolaemus pictus* (LIOLAEMINAE) DEL ARCHIPIÉLAGO DE CHILOÉ: EL ROL DE LAS GLACIACIONES Y LA INSULARIDAD EN LA DIVERGENCIA MOLECULAR, FISIOLÓGICA Y MORFOLÓGICA

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CONTENIDO

Capítulos

RESUMEN

Para reconstruir la historia biogeográfica de las especies es necesario considerar los factores que afectaron sus rangos de distribución y cómo estos han promovido o mantenido la diferenciación poblacional. Se han propuesto a escalas temporales recientes, entre otros, dos grandes promotores de divergencia: las glaciaciones pleistocénicas y la insularidad. El primero promueve la diferenciación por aislamiento, fragmentación de las poblaciones y desplazamientos a refugios glaciales alopátricos, mientras que el segundo origina divergencia por cambios bióticos y abióticos dados por el aislamiento.

El Archipiélago de Chiloé (entre 41° y 43°S) constituye un complejo de islas al Oeste de Chile continental que debe su conformación actual a cambios climáticos del Pleistoceno. Dado que el archipiélago ofrece la oportunidad de estudiar el efecto de las glaciaciones e insularidad conjuntamente, en este trabajo se examinó la contribución de ambos procesos sobre los patrones de variación molecular (*Citocromo b*), dietaria (composición), fisiológica (morfología digestiva, enriquecimiento isotópico, actividad digestiva) y morfológica (morfometría geométrica) en el complejo *Liolaemus pictus*.

La evidencia molecular sugiere que tanto la población continental como la Isla Gran de Chiloé presentan altos niveles de diferenciación genética debido a que no comparten haplotipos. Sin embargo, esta divergencia podría ser muy antigua lo cual reflejaría un aislamiento pasado y que no estaría relacionado con el Último Máximo Glacial. El "linaje continental" muestra cuatro haplogrupos generales lo cual sugiere que la fragmentación de *L. pictus* ocurrió en múltiples refugios. Por otra parte, la colonización de las islas pequeñas habría ocurrido desde "sitios fuente" cercanos.

La dieta de *L. pictus* es amplia en ítemes de presa, donde tanto poblaciones continentales como insulares, consumen frutos como un ítem relativamente común. A pesar que los individuos en las islas tienen un tracto digestivo más corto y muestran un mayor consumo de frutos que en el continente, la actividad enzimática es alta para trehalasa y N-aminopeptidasa lo cual sugiere una capacidad enzimática más eficiente para hidrolizar y quebrar tejidos de insectos. De la misma manera, los isótopos estables revelan que el

enriquecimiento de $\delta^{15}N$ de las islas es dos veces (dos niveles tróficos) más alto que en el continente lo cual indica una mayor incorporación de nutrientes provenientes de insectos.

Por otra parte, detectamos divergencia morfológica entre las localidades continentales y la Isla Grande de Chiloé. Los datos de la morfometría geométrica indican que la órbita de los ojos de los especímenes insulares es más extendida que en los continentales lo que probablemente se relaciona con a una capacidad diferencial del usar el sustrato. Además, la posición terminal de la boca es más posterior en los especímenes de la isla lo que sugeriría que la variación morfológica entre continente e isla podrían relacionarse a eventos históricos. A partir de los datos morfológicos, se sugiere que la evolución morfológica insular en esta especie es acelerada debido a la particularidad de ambientes ecológicos disponibles que favorecerían una evolución diferencial en un corto período de tiempo. Finalmente, debido a que la distribución geográfica de los haplotipos y fenotipos de las subespecies de *L. pictus* no son concordantes, se hace irrelevante el uso de esta nomenclatura.

ABSTRACT

To reconstruct the biogeographical history of species it is necessary to understand the factors that affect their distribution range and influence their population differentiation. Two main divergence agents can be identified: Pleistocene glaciations and insular effects. The first promotes differentiation through isolation, population fragmentation and displacement to allopatric glacial refuges, whereas the latter induces divergence through biotic and abiotic changes.

The chilotan archipelago (between 41° and 43°S) an island complex located west of continental Chile. Its present configuration is due to Pleistocene climatic changes. Since the archipelago offers the opportunity to study the effect of glaciations and insularity simultaneously, I examined the contribution of both processes on the patterns of molecular (*Cytochrome b*) variation, diet (composition), physiology (digestive morphology, isotopic enrichment, and digestive activity), and morphology (geometric morphometric) in the *Liolaemus pictus* complex.

The molecular evidence suggests that mainland and island population do not share haplotypes, which implies high levels of genetic differentiation. This divergence may reflect an ancient isolation which may procede the Last Glacial Maximum. The "mainland lineage" shows four general haplogroupes which suggests that fragmentation of *L. pictus* population occurred in multiple refugia. The colonization of the small islands would have occurred from near source.

The diet of *L. pictus* is broad in prey items. Fruit consumption in mainland and insular populations is a relatively common item. Although island individuals have a shorter digestive tract and show higher fruit consumption than mainland populations, the activity of trehalase and N-aminopeptidase is high, which suggests an efficient capacity to hydrolyze and breakdown insect products. Stable isotopes reveal that enrichment of ^{15}N on islands is twice (two trophic levels) higher than in the mainland.

In the morphological context, divergence exists only when mainland localities and Chiloé Island are considered. Geometric morphometric data indicate that the eye orbit of insular especimens is more extended than in mainland specimens, which is probably related to a different capacity to use substrate. In addition, the terminal position of the mouth is more posterior in island especimens, which would suggest that the morphology variation between mainland and island could be related to historical events. Finally, morphometric data suggests that insular morphological evolution has occurred at a higher rate, probably due to the peculiar ecological environments. Therefore, the geographic distribution of haplotypes and phenotypes for *L. pictus* populations are not compatible with the recognition of subspecies, suggesting that the usage of this nomenclature for *L. pictus* subspecies should be invalid

INTRODUCCION

Refugios glaciales vs. Recolonización post – glacial

Para reconstruir la historia biogeográfica de las especies, es importante considerar las barreras intrínsecas (Ehrlich 1961) y extrínsecas (Endler 1977) que pueden haber producido cambios en sus rangos distribucionales. Barreras intrínsecas son aquellas que modifican la distribución por rasgos ecológicos propios de las especies, tales como la interacción entre especies, vagilidad, disponibilidad de sitios de reproducción o disponibilidad de recursos (Schluter 2000, Crespi et al. 2003). Las barreras extrínsecas son aquellas que producen modificaciones distribucionales debido a cambios externos a macroescala, tales como la formación de ríos, las glaciaciones o las islas (Hewit 1996, Pounds & Jackson 1981, Anderson & Handley 2002). Dentro de las barreras extrínsecas, los ciclos glaciales del Pleistoceno (los últimos dos millones de años en la escala geológica del tiempo) son reconocidos como los eventos históricos más significativos que han ocurrido durante el último tiempo evolutivo de muchas especies (ver Hewitt 1996, 1999). Éstos han sido descritos como grandes expansiones de glaciares y ambientes asociados que, por efecto de los descensos generalizados de las temperaturas en un periodo prolongado de tiempo (Hewitt 1996, 1999), se extendieron desde altas latitudes y altitudes hacia sectores más ecuatoriales y de menor altitud, cuya extensión en esta última área fue cada vez más acotada (Denton et al. 1999). En este sentido, se ha descrito que el efecto de estos cambios sobre la biota fue promover la diferenciación en los patrones de variación geográfica de las especies (Hewitt 1996, 1999, Schmitt & Seitz 2001,

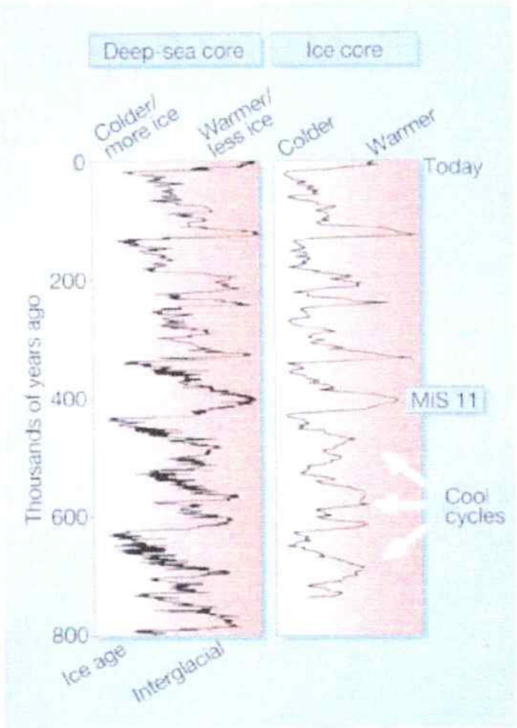

Figura 1. Variaciones en el volumen de hielo continental global y temperaturas atmosféricas durantes los últimos 800.000 años. Extraído de McManus (2004).

Janzen et al. 2002, Crespi et al.2003). Se ha estimado que al menos 6 glaciaciones (avance glacial, estabilización y retracción) ocurrieron durante los últimos 700.000 años (Martinson et al. 1987) y que cada una de ellas habría durado 100.000 años aproximadamente seguidos de posteriores periodos interglaciales con duración aproximada de 10.000 a 12.000 años (Dawson 1992, McManus 2004; Figura 1). Es objeto de debate el rol que tuvieron los ciclos glaciales en la promoción de divergencia en muchos grupos de animales. Por ejemplo, en *Melanoplus oregonensis* (Ortóptera) de Norteamérica se detectó que los patrones de variación genética poblacional se asocian a una divergencia histórica explicada por el aislamiento de sus poblaciones durante el último periodo glacial y que existiría una posterior diferenciación explicada por una colonización o expansión de su rango geográfico hacia las áreas que antes estuvieron cubiertas por la masa glacial (Knowles 2001) lo cual también ha sido demostrado en otras especies (Hewitt 1996, 1999, Schmitt & Seitz 2001, Janzen et al. 2002, Liebers & Helbig 2002), Sin embargo, la variación del rango geográfico de las especies no siempre puede se explicado por la ocurrencia de estos ciclos. En efecto, Crespi et al. (2003) demostraron que la diferenciación entre poblaciones de salamandras de Norteamérica muestran tiempos de divergencia pre-pleistocénicas y un caso similar se registra en las lagartijas del complejo *Liolaemus monticola* en Chile y *L. darwini* en Argentina (Torres-Pérez 2004, Morando et al. 2004).

Por otro lado, la expansión del rango geográfico de una especie durante el corto el periodo interglacial revela otros mecanismos asociados a la distribución actual de las especies. Así, el modo en que las áreas históricamente glaciadas son posteriormente colonizadas puede variar de acuerdo a características intrínsecas de las especies o a los cambios topográficos producidos durante el periodo glacial (Hewitt 1996). De esta manera, cuando la divergencia está asociada con los períodos glaciales donde las poblaciones se encuentran restringidas a areas geográficas alopátricas simples o múltiples, podría evidenciar la estructuración regional de la variación genética (Premoli et al. 2002). Contrariamente, los sucesivos eventos fundadores que ocurren durante la expansión del rango geográfico hacia áreas previamente glaciadas provocan que las poblaciones recientemente fundadas deriven de la población fuente y muestren reducidos niveles de diversidad (Hewitt 1999, Knowles 2001).

Teoría insular

Otra de las barreras extrínsecas a la dispersión lo constituye la insularidad (Irschick et al. 1997). La biogeografía de islas aborda los patrones de distribución de las especies en las islas, influenciado por procesos ecológicos y evolutivos como el aislamiento y el área (MacArthur & Wilson 1967, Lomolino 1986). A pesar de que esta teoría ha sido ampliamente discutida (Wu & Vankat 1995) y aplicada para entender los patrones de recambio insular derivados desde la fuente continental (p.e., ciclo del taxón, Schluter 2000), el efecto del aislamiento sobre la divergencia poblacional son actualmente debatidas (Anderson & Handley 2002, Renaud & Millien 2001). De esta manera, cuando las especies de vertebrados son comparadas con las especies equivalentes en el continente, se evidencian cambios en sus rasgos fisiológicos y morfológicos (Irschick et al. 1997) los que a su vez incluyen cambios en demografía, reproducción, conducta (Adler & Levins 1994) y pool genético (Hille et al. 2003). Un clásico ejemplo de esta divergencia es la evolución del tamaño corporal de vertebrados insulares, donde los taxa de gran tamaño evolucionan hacia tamaños pequeños en las islas y taxa pequeños hacia tamaños grandes (Lomolino 1985, Anderson & Handley 2002, Clegg & Owens 2002, Renaud & Michaux 2003).

Se han propuesto diferentes explicaciones para esta diferenciación, entre los que cuenta la presencia de pocos depredadores en islas, un aumento de la competencia intraespecífica, cambios en la ecología trófica y térmica (Clegg & Owens 2002), diferencias climáticas y el rol de los eventos de colonización sobre la estructura genética de las poblaciones insulares (Irschick et al. 1997, Calsbeek & Smith 2003). Dentro de estos factores, se ha sugerido que uno de los primeros agentes promotores de cambio morfológico en aves insulares es la divergencia trófica (Clegg & Owens 2002), debido a que los caracteres morfológicos y la dieta habitualmente están correlacionados (Ruber & Adams 2001). En reptiles, la divergencia insular en la ecología trófica ocurre porque las lagartijas de la isla alcanzan densidades muy altas (compensación de densidad), experimentan un menor riesgo de depredación que aquellos en el continente y por consiguiente, pueden extender su dieta para incluir néctar, polen y frutas (Olesen & Valido 2003).

Si bien estos cambios morfológicos sugieren la existencia de diferentes factores selectivos en las poblaciones insulares y continentales, la influencia de los modos de colonización juega un rol importante en la evolución al interior de las islas. Por ejemplo, en *Anolis sagrei* de Bahamas, la influencia de las corrientes marinas y la dirección del viento durante los huracanes, explicarían una correlación entre el flujo génico entre islas y los niveles de divergencia morfológica (longitud de las extremidades). A partir de estos resultados la migración de lagartijas podría corresponder a un modelo saltatorio o "stepping – stone" de una isla a otra por arrastre que requeriría una semana (rango de 2–15 días) para que ocurra la colonización (Calsbeek & Smith 2003).

El Archipiélago de Chiloé y su historia Pleistocénica

El Archipiélago de Chiloé (entre 41° y 43°S) constituye un complejo de islas al Oeste de Chile continental. Desde el punto de vista histórico y geológico, la zona ha sido diferencialmente afectada por los cambios climáticos del Pleistoceno, los cuales determinaron su conformación actual (Villagrán et al. 1986, Moreno & León 2003, Denton et al. 1999). De hecho, tanto la Isla Grande de Chiloé como las islas al E de ésta, muestran una cubierta de depósitos glaciales de la última glaciación (Denton et al. 1999). En la región, los glaciares cubrieron extensas zonas hoy cubiertas por bosques (Figura 2) en ambas vertientes andinas, parte de la depresión intermedia y los sectores NE y mitad sur de la Isla Grande de Chiloé (Mercer 1976, Heusser & Flint 1977). Durante el

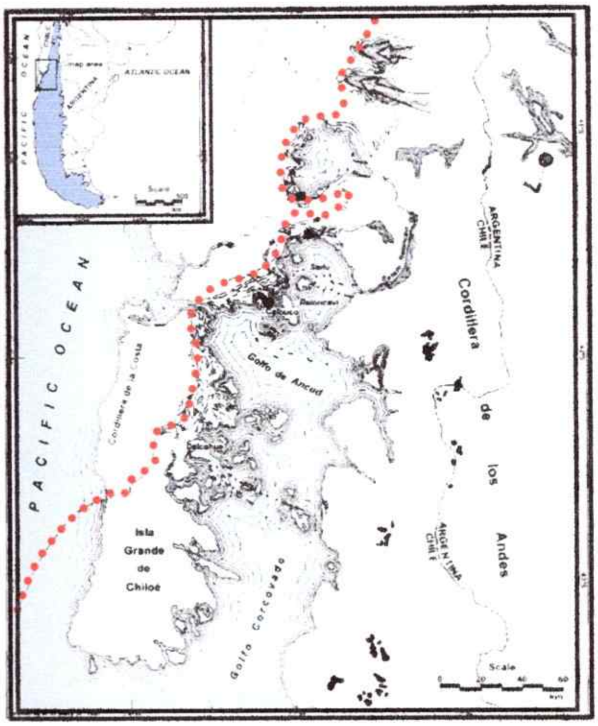

Figura 2. Mapa que indica los límites de la masa de hielo que cubrió la porción sur de la Región de Los Lagos durante el Último Máximo Glacial. El inserto muestra la extensión total en el cono sur de sudamérica. La línea roja punteada indica los límites en la zona del Archipiélago de Chiloé. Extraído y modificado de Denton et al. (1999).

Último Máximo Glacial, fechado entre 26.000 y 14.700 años ^{14}C AP las temperaturas medias de verano descendieron alrededor de 6-8°C (Heusser et al. 1999). Luego, durante la última terminación glacial (14.700 años ^{14}C AP, Denton et al. 1999), los glaciares se retiraron rápidamente de las zonas bajas, y en menos de 2.000 años, las temperaturas ascendieron hasta niveles cercanos a los actuales, comenzando la recolonización de la vegetación en las áreas perturbadas por el efecto glacial (Villagrán 1985, 1988, Moreno et al. 1999, Abarzúa et al. 2004). Debido a que sectores de la Isla Grande de Chiloé y del continente no estuvieron cubiertos por glaciares, se ha propuesto que éstas áreas pudieron –eventualmente- haber constituído refugios para la biota glacial (Heusser 1982, Villagrán et al. 1986, Formas 1989). Se plantea que la acción de los hielos hizo emigrar el bosque hacia el norte con un consecuente transporte de la fauna (Formas 1989, Villagrán et al. 1986), pero se desconoce cuál habría sido el modo de expansión del rango de distribución geográfico de los vertebrados luego del retroceso glacial al interior del continente, dentro de la Isla Grande y al resto de islas del archipiélago. Algunas especies de herpetozoos han sido registradas en este grupo de islas. En la Isla Grande de Chiloé es posible encontrar anfibios como *Eupsophus calcaratus*, *E. emiliopugini*, *Batrachyla leptopus* y *B. taeniata* (Formas 1989, Núñez et al. 1999, Formas & Brieva 2000, Brieva & Formas 2001) y reptiles como *Liolaemus cyanogaster*, *L. pictus* y *Pristidactylus torquatus* (Donoso-Barros 1966, Pincheira & Núñez, 2005) los cuales se encuentran también representados en Chile continental (Veloso & Navarro 1988). Una especie interesante que habita en el archipiélago es *Liolaemus pictus* (Duméril & Bibron 1837). Esta especie es una de las lagartijas chilenas con rango distribucional más austral; que se extiende desde Concepción hasta las islas del Archipiélago de Chiloé [37°S – 44°S] (Donoso-Barros 1966, 1970, Veloso & Navarro 1988) con una población aislada a los 34°S (Pincheira & Núñez, 2005) y se extiende su distribución a la Provincia de Río Negro en Argentina (Cei 1986) (Figura 3). Esta especie adquiere una especial relevancia dada la diversificación en términos de subespecies descritas en el archipiélago; de hecho, han sido descritas hasta la fecha seis subespecies, de las cuales tres son insulares: *L. p. chiloensis* en Isla Grande de Chiloé (Muller & Hellmich 1939), *L. p. codoceae* en las pequeñas islas al Este de Isla Grande y en Isla Guafo (Donoso–Barros 1966, Pincheira & Núñez, 2005) y *L. p. talcanensis* endémica de Isla Talcán (Urbina

& Zuñiga 1977); en tanto sus contrapartes continentales corresponden a *L. p. pictus*, *L. p. argentinus y L. p. septentrionales,* esta última corresponde a una población aislada al norte en la localidad de Altos de Vilches (Pincheira & Núñez, 2005).

El principal criterio usado para discriminar estas subespecies corresponde al tamaño corporal [*L. p. pictus* < *L. p. chiloensis*, (Donoso-Barros 1966)] y la coloración [*L. p. codoceae*, *L. p. argentinus* y *L. p. talcanensis* melánicas (Donoso-Barros 1970, Urbina & Zúñiga 1977)*; L. p. pictus* y *L. p. chiloensis* no melánicas (Donoso-Barros 1970)]. Debido a estas diferencias, Young (1998) en un análisis filogenético con isoenzimas, intentó determinar el estatus taxonómico de estas subespecies, pero la utilización de sólo algunas subespecies en sus análisis no le permitió definirlas con claridad. Lobo (2001) en un estudio morfológico, incorporó cuatro de las subespecies de *L. pictus* considerándolas especies efectivas (*L. pictus*, *L. argentinus*, *L. chiloensis* y *L. talcanensis)*, sin embargo, todas las subespecies conformaron un clado monofilético lo cual no justifica su elevación a estatus específico dado que la delimitación de especies requiere examinar también el polimorfismo y su base genética (Wiens 1999). Por otra parte, otro antecendente que reafirmó las diferencias subespecificas se relacionó a las diferencias de la dieta entre subespecies. De hecho, Ortiz (1974) describió los hábitos alimenticios de *L. p. pictus* provenientes del Parque Nacional Vicente Pérez Rosales en el continente, donde indica que la dieta está compuesta fundamentalmente de dípteros, homópteros, arácnidos, himenópteros y coleópteros. Sin embargo, Willson et al. (1995) y Willson et al. (1996) al analizar el contenido fecal de *L. p. chiloensis* provenientes del NE de Isla Grande de Chiloé

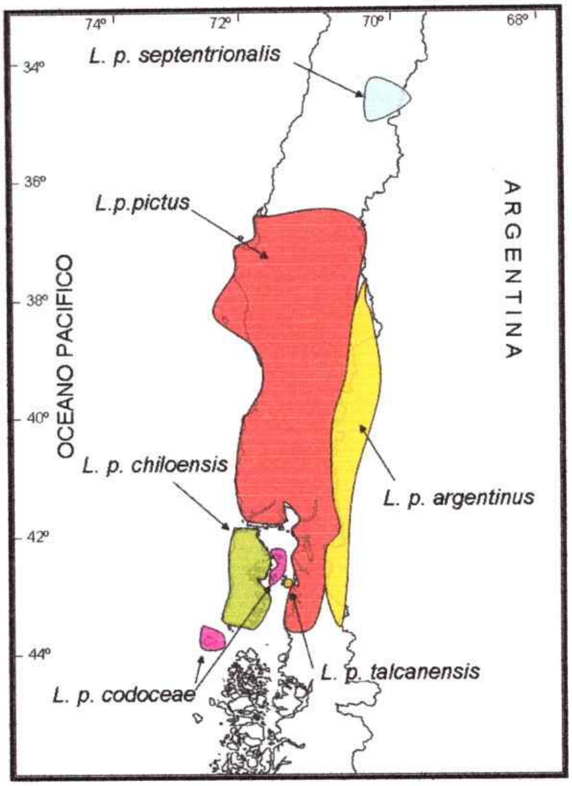

Figura 3. Mapa que muestra el rango de distribución geográfica aproximado de las subespecies de *Liolemus pictus* (ver Pincheira & Nuñez, 2005)

señalan que se alimenta de frutos de *Nertera granadensis* y *Relbunium hypocarpium* e indican que esta especie sería un agente dispersor de semillas.

Predicciones y objetivos

Si se considera la complejidad de la topografía y la historia geológica del Archipiélago de Chiloé, así como las variaciones poblacionales registradas en *L. pictus*, entonces cabe plantearse las siguientes preguntas:

- ¿las glaciaciones pleistocénicas (en este caso, el Último Máximo Glacial) promovieron divergencia morfológica y genética en *L. pictus*?,
- ¿exhibe menor variabilidad genética la población de lagartijas del propuesto refugio glacial (Isla Grande de Chiloé)?,
- ¿la expansión post – glacial de las restantes islas del archipiélago se realizó desde el continente o desde Isla Grande de Chiloé?,
- el aislamiento de algunas poblaciones en el archipiélago ¿promovió divergencia trófica?,
- ¿la divergencia trófica se asocia con cambios fisiológicos y/o morfológicos?,
- ¿se relaciona el cambio dietario, fisiológico y morfológico con el origen continente-isla de las poblaciones?

Análisis molecular

Para entender la variación geográfica de *L. pictus* se debe considerar que el eventual refugio glacial de la zona noroeste de Isla de Chiloé podría conformar una población con menor diversidad genética que las del continente. A su vez, las áreas que son posteriormente colonizadas mostrarían valores aún más bajos (Hewitt 1999, Knowles 2001). Por otro lado, si las subespecies de *L. pictus* descritas en las islas pequeñas del archipiélago han colonizado desde una población ancestral (continente o Isla Grande), el efecto fundador reduciría la variabilidad genética de la población producto de la deriva genética y el número de individuos colonizadores (Hartl & Clark 1997). Así, se esperaría encontrar una baja variabilidad genética y un cambio en la estructura poblacional de *L. pictus* en estas islas. Si la colonización ha ocurrido con un elevado número de fundadores, o en sucesivos eventos, el tamaño de la nueva población permitiría un menor efecto de procesos de cambio al azar (i.e. deriva genética), por lo que la fuerza de cambio será principalmente la selección natural (Futuyma 1998). Para explicar la historia de colonización y diferenciación en el archipiélago, el uso de ADN mitocondrial (mtDNA) como marcador, proporciona información acerca de la historia poblacional en un contexto evolutivo y biogeográfico (Avise 2000). Esta aproximación permitiría conocer la relación entre linajes y dar cuenta de la colonización en el archipiélago, así como detectar eventos demográficos del pasado y estimar los parámetros de cambio demográfico (Cook et al. 2001, Rosenberg & Nordborg 2002, Crespi et al. 2003).

En este trabajo se analiza la variación geográfica de *L. pictus* en el archipiélago de Chiloé. Se evalua el rol de las glaciaciones y el posterior efecto insular en la divergencia molecular, fisiológica y morfológica de esta especie. Desde el punto de vista genético poblacional (Capítulo 1) estudiado a través del uso de ADN mitocondrial los objetivos de este trabajo fueron: i) probar la hipótesis general de que el continente y la Isla Grande de Chiloé conformaron refugios glaciales durante el Ultimo Máximo Glacial, donde la disyunción entre ambos lugares mostrará diferencias predecibles en su estructura filogeográfica y ii) determinar cómo las pequeñas islas dentro del archipiélago fueron colonizadas desde las diferentes fuentes.

Análisis fisiológico

En el contexto insular, las posibles causas que explicarían una divergencia dietaria descrita entre poblaciones continentales e insulares de *L. pictus* corresponderían a selección natural que promueve fenotipos localmente adaptados y a la existencia de barreras físicas o biológicas al flujo génico. Esta diferenciación puede ser abordada por enfoques multidisciplinarios, desde la descripción detallada de la composición dietaria (Krebs 1999) hasta la determinación de los cambios fisiológicos y morfológicos asociados a la misma (Adams & Rohlf 2000, Herrel et al. 2004). Diversos estudios han utilizado el enriquecimiento de isótopos estables de ^{15}N para describir el flujo de energía dentro de una trama trófica (Cabana & Rasmussen 1994). La proporción de isótopos estables de nitrógeno ($\delta^{15}N = [^{15}N/^{14}N_{muestra}/^{15}N/^{14}N_{referencia} - 1] \times 1000$) muestra un enriquecimiento entre 3-4‰

entre el tejido de la presa y el depredador, lo que permite obtener una medida de la posición trófica del consumidor (Vander Zanden et al. 1999). De esta manera, los tejidos con alto contenido de Nitrógeno de *L. pictus* darían cuenta de un consumo mayor de insectos mientras que un bajo contenido de nitrógeno indicaría una dieta que incorpora menos insectos y más frutos.

Si bien la composición química de la dieta, la abundancia y disponibilidad de recursos afectan la habilidad del animal para adquirir, manipular e ingerir el alimento (Parker 1994), la eficiencia en hidrolizarlo y absorberlo está determinada por características morfológicas, fisiológicas y biomecánicas, así como por la plasticidad y restricciones del tracto digestivo (Bozinovic 1993). La capacidad de regular los mecanismos digestivos en vertebrados de acuerdo al tipo de dieta consumida ha sido analizada por su actividad enzimática (Hernández & Martínez del Río 1992, Sabat et al 1995, Sabat 2000). Dado que los insectos poseen altas concentraciones de lípidos y proteínas y bajas concentraciones de carbohidratos (Bell 1990 *fide* Sabat et al. 1993), en *L. pictus* una alta actividad de trehalasa que hidroliza trehalosa (azúcar de almacenamiento de insectos, Sabat et al. 1993) daría cuenta de un consumo importante de insectos en la dieta en poblaciones continentales, mientras que una alta actividad digestiva de maltasa y sacarasa, que hidrolizan maltosa y sacarosa, respectivamente (azúcar de los frutos), evidenciaría la utilización mayoritaria de frutos como recurso en las poblaciones insulares de esta especie (Sabat et al. 1995). Desde este punto de vista (Capítulo 2), en este trabajo se determina cómo la actual morfología digestiva y fisiología de esta especie puede ser modulada por sus hábitos dietarios en cinco poblaciones. Los objetivos principales fueron: i) analizar la dieta a través del análisis del contenido estomacal en diferentes poblaciones insulares y continentales; ii) determinar la posición relativa en la cadena trófica por medio de señales isotópicas de nitrógeno y carbono de hueso y músculo; y iii) determinar la morfología del tracto digestivo y algunas de las características asociadas a la digestión enzimática de carbohidratos (sacarasa, maltasa y trehalasa) y proteínas (N-aminopeptidasa). Se postula la existencia de variación geográfica significativa de la dieta entre localidades la cual puede ser tratada como una variable predictora de la expresión de rasgos digestivos.

Análisis morfológico

Aún cuando la determinación de parámetros fisiológicos permite comprender ajustes a un tipo de dieta, las diferencias morfológicas entregan información sobre el origen de la diversidad de fenotipos (Herrel et al. 2004). Desde el punto de vista del cambio morfológico asociado a la dieta, la morfometría geométrica permite analizar diferencias de la forma sin la influencia de variables de tamaño (Rohlf & Slice 1990; Rohlf et al. 1996, Bookstein et al. 1999) lo que permite obtener una visión geométrica de una estructura determinada (Rohlf & Slice 1990). A través de esta metodología se ha determinado el cambio de forma de estructuras craneales de acuerdo a un tipo particular de dieta. En dos especies de salamandras del género *Pletodon* de Norteamérica se detectó un desplazamiento de caracteres craneales cuando ambas se encontraban en simpatría producida por consumo de dietas de distinto tamaño (Adams & Rohlf 2000) y en el roedor *Apodemus* se plantea que el cambio de forma de las mandíbulas estaría determinado por su diferenciación genética poblacional y a un ajuste ambiental que produce cambios en los ítems de presa disponibles en cada localidad (Renaud & Michaux 2003). En este sentido, el origen putativo de las poblaciones de *L. pictus* en diferentes islas en el archipiélago (sean desde el continente o desde Isla Grande de Chiloé) permitiría determinar la existencia de adaptaciones locales de la morfología craneal hacia un tipo de dieta o la existencia de restricciones si estas provienen desde el continente. Por esta razón, el análisis conjunto de la variabilidad genética poblacional y los cambios fisiológicos y morfológicos asociados a la alimentación permitirán determinar el origen de esa variación en un contexto evolutivo.

De esta manera, en este estudio se evalúa la variación morfológica de la especie en diferentes localidades, continentales e insulares, por medio de morfometría tradicional de medidas lineales y morfometría geométrica. Es este punto, si se toma en cuenta que las variaciones dietarias entre el continente y Isla Grande de Chiloé han sido descritas previamente, se predice que la morfología de la cabeza podría variar de acuerdo a este patrón (Capítulo 3). Por otra parte, se pretende determinar si los cambios en la morfología (forma por análisis de morfometría geométrica) entre las diferentes poblaciones asignadas a las distintas subespecies de *L. pictus* del continente y las islas están correlacionados a la historia filogeográfica del archipiélago (Capítulo 4). Debido a que los procesos históricos y naturales influencian la diversidad genética y morfológica, se predice que las subespecies de las islas (*L. p. chiloensis, L. p. talcanensis* y *L. p. codoceae*) serán diferentes morfológicamente a la subespecie del continente (*L. p. pictus*) y que los linajes insulares están relaciondos a la población de origen.

Insular colonization of *Liolaemus pictus* (Squamata, Liolaeminae) in southern Chile

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Abstract

The area around the Chilotan Archipelago is relevant because mainland and low-elevation sites in the southeastern portion of Isla Grande de Chiloé were glaciated, and an important refuge for biota was formed in its northwestern portion. The relationships between the bioclimatic history of southern Chile and the *Liolaemus pictus* subspecies living in the mainland (*L. p. pictus*) and islands (*L. p. chiloensis, L. p. codoceae, and L. p. talcanensis*) are interesting because the influence of glaciations can be studied. In this study, we analize the phylogeographic structure (*Cytochrome b*) of *L. pictus* subspecies and specifically, we attempted to i) determine if *Liolaemus* populations survived in NW portion of Isla Grande de Chiloé and in the mainland during the last glaciation resulting in predictable differences in their phylogeographic structure; and ii) to determine the origin and colonization routes of small island population following deglaciation.

Our results indicate that mainland and Isla Grande de Chiloé populations do not share haplotypes and they are highly divergent haplogroups. This divergence might reflect an ancient isolation much older than the Last Glacial Maximum. Moreover, the existence of four divergent haplogroups among mainland *Liolaemus* populations, suggests the persistance of isolated populations during the last glaciation. Our results suggest that colonization of small islands occured from near source sites located both in the mainland and in Isla Grande de Chiloé after the Last Glacial Maximum.

Introduction

The archipelagean biota constitute useful models to study many evolutionary hypotheses including those pertaining to repeated cycles of colonization and extinction, adaptation and drift - induced population differentiation (Juste *et al.* 2001; Hille *et al.* 2003). Depending on the island group examined, different historical processes can be considered as the factors responsible for the observed patterns. In many archipelagos, tectonic and glacioeustatic variations have altered the surface areas and degrees of insularity (Cook *et al.* 2001; Hille *et al.* 2003; Parmakelis *et al.* 2003). In other cases, direct glacial influence has imposed additional variation (Cook *et al.* 2001; Fleming & Cook, 2002; Harlin-Cognato *et al.* 2006).

Liolaemus lizards are widely represented in South America (Donoso-Barros 1966; Frost & Etheridge 1989) with more than 160 living species in Argentina and Chile (Etheridge & Espinoza 2000; Schulte *et al.* 2000). Until now, most Chilean species of *Liolaemus* have been distinguished in terms of morphological variation, and the literature concerning genetic variation is relatively scarce (Vásquez 2001, Torres-Pérez *et al.* 2003, Vidal *et al.* 2004). *Liolaemus pictus* is the southernmost distributed lizard species in Chile (37°S-43°S) including mainland and island populations. Six subspecies have been described for this species on the basis of morphological traits and geographic distribution (Donoso-Barros 1966; 1970; Urbina & Zúñiga 1977, Pincheira & Nuñez 2005): *L. p. pictus* (Duméril & Bibron 1837) inhabiting the mainland from Concepción to Puerto Montt [37° - 41°S] (Donoso-Barros 1966; 1970; Veloso & Navarro 1988), *L. p. septentrionalis* Pincheira & Nuñez 2005 inhabiting Alto de Vilches (35°36'S - 71°03'W) (Pincheira & Nuñez 2005), *L. p. argentinus* Muller & Hellmich, 1939 found in the Río Negro Province of Argentina and adyacent areas in Chile (Donoso-Barros 1966; Cei 1986), *L. p. chiloeensis* Muller & Hellmich 1939 from Isla Grande de Chiloé (Muller & Hellmich 1939), *L. p. talcanensis* Urbina & Zúñiga 1977 in Isla Talcan (Urbina & Zúñiga 1977), and *L. p. codoceae* Boulanger 1885 from islands adyacent to Isla Grande de Chiloé and Isla Guafo (Donoso-Barros 1966; Pincheira & Nuñez 2005).

The Chilotan Archipelago (41° - 43°S), the area where subspecies occur, constitutes an island complex bounded by Isla Grande de Chiloé on the east and mainland areas located both to on the noth and west. This area has been affected by repeated glaciations during Plio-Pleistocene times, which determined its present biotic and geomorphological configuration (Villagrán *et al.* 1986; Denton *et al.* 1999; Moreno & León 2003). Piedmont glacier lobes covered the Andean slopes, vast areas of the intermediate depression the NE, and the southern half of Isla Grande de Chiloé during the Last Glacial Maximum (LGM)(from 26,000 to 14,700 ^{14}C yr BP) (Mercer 1976; Heusser & Flint 1977). Palynological studies suggest that mean summer temperatures descended up to 6-8ºC during the coldest intervals of the LGM (Heusser *et al.* 1999). Starting at 14,700 ^{14}C yr BP, (Denton *et al.* 1999) the glaciers retreated rapidly from the lowlands, and in less than 2,000 years the temperatures reached levels similar to the present climate (Heusser et al. 1999, Moreno et al. 1999).

Considering that sea level dropped ~120 meters below the present during the LGM, Isla Grande de Chiloé must have been connected with the mainland through a land bridge along the exposed continental shelf (Moreno et al. 1994). A possibility exists that ice-free areas distal to the piedmont glaciers may have served as refugia or corridors for the biota (Heusser 1982; Villagrán *et al.* 1986; Formas 1989; Formas & Brieva 2000), expanding towards the previously glaciated terrains following ice recession at 14,700 ^{14}C yr BP (Denton *et al.* 1999). The spatial and temporal patterns of expansion of vertebrates after the last glacial termination, however, remain largely unknown. A series of small islands located east of Isla Grande de Chiloé were formed as recessional moraine complexes sometime between 14,700 and 13,000 ^{14}C yr BP (Denton *et al.* 1999). Sea-level reconstructions indicate that a gradual rise commenced right after 14,700 ^{14}C yr BP, punctuated by two rapid pulses at ~13,000 and ~10,000 ^{14}C yrBP (Fairbanks 1989; Lambech & Chappell 2001; Sidall *et al.* 2003). Based on this evidence, it is thus possible that some of the small islands east of Isla Grande de Chiloé may have been connected to the mainland or Isla Grande de Chiloé through the exposed continental shelf during the time window between 14,700 to ~8,000 ^{14}C yr BP, depending on the battymetry of the interior sea.

At present, *L. p. pictus* (mainland) has been described as morphologically very different to *L. p. chiloensis* (Isla Grande de Chiloé) (Vidal *et al.* 2006). Therefore, the relationship between island and mainland, and their sudsequent separation, may be relevant to explain the morphological differences observed between these modern populations. Here we present a phylogeographic study using mtDNA data from mainland and island populations of *L. pictus*. We specifically attempt to test the hypothesis that populations from the mainland and Isla Grande de Chiloé were confined to eventual glacial refugia during the LGM. Disjunction between the two areas results in predictible differences in phylogeographic structure. In addition, we evaluated the mode to which the small islands of the archipelago have been colonized from different areas.

Materials and methods

Sampling and laboratory protocols

Tissue samples were obtained from 142 lizard tails from eight mainland and insular localities (Fig. 1; Table 1). Total genomic DNA was isolated from samples using a standard phenol chloroform extraction protocol. The partial Cytochrome b (*Cyt b,* 535 bp) gene was amplified and sequenced with two primers designed from tRNA-Glu and tRNA-Thr sequences of iguanid lizards *Iguana iguana* (AN: NC002793) and *Sceloporus occidentalis* (AN: NC005960) (LioGluFor: AAACCACCGTTGTTATTCAACTA and LioThrRev: CCATCTTTGGCTTACAAGACCAGAG). Five microliters of extraction product were electrophoresed on 1% agarose gel to estimate the quality and amount of genomic DNA, and sample dilutions were performed (100ng/µL) for polymerase chain reaction (PCR) amplification. The *Cyt b* gene region was amplified via PCR in 25µL of reaction volume containing 0.3µL Taq (Invitrogen), 2.5µL of PCR Buffer 10X, 1.6µL $MgCl_2$ 50mM, 2µL dNTPs 100mM, 0.5µL of each primer (10pmol), and 1µL of template DNA. The thermal cycling amplification conditions were as follows: initial denaturation at 94 °C for 5 min, followed by 35 cycles of strand denaturation at 94 °C for 30 s, annealing at 53.3 °C for 1.30 min, primer extension at 72 °C for 1.5 min, and a final 10 min extension at 72 °C. The size of the PCR products for a mitochondrial marker was checked by comparing with the help of 100bp DNA ladder (Invitrogen) in 2% agarose gel. Amplified DNA was purified with the QIAquick PCR Kit according to the supplier's protocol (Qiagen, USA). Finally, all samples were sequenced in the forward and reverse direction with an automated DNA-sequencer. Sequence alignments were accomplished using Proseq (Filatov 2002), and checked by eye. For phylogenetic reconstruction, partial sequences of the locality of Bariloche (*L. p. argentinus* [Accession number (AN): AY367791, and AY173795]) and outgroup (*L. tenuis* [AN AY851742], *L. chillanensis* [AN AY850622], and *L. villarricensis* [AN AY850629]) were obtained from GenBank.

Phylogeography of mainland and Isla Grande de Chiloé

For this analysis we focused on the phylogeographic patterns between the mainland and Isla Grande de Chiloé sites, excluding the islands that were formed after the LGM (Butachuques and Talcán islands). This approach will allow us to differentiate recent colonization events from older processes. We computed median-joining network using the program Network 4.1.0.0 (Bandelt *et al.* 1999) to decipher the relationships and geographical partitioning among haplotypes. To assess mitochondrial genetic diversity within *L. pictus*, a number of haplotypes (K), polymorphic sites (S), nucleotide diversity (H) and number of pairwise differences (Π) were calculated using the program DNAsp (Rozas et al., 2003). Tajima's D statistics (Tajima 1989) and Fu's Fs-test (Fu 1997) were calculated with the same program to test the selective neutrality. Significantly negative D-values can be interpreted as signatures of population expansion (Aris-Brosou & Excoffier 1996). Analysis of molecular variance (AMOVA, Excoffier *et al.* 1992) were performed using the SAMOVA program (Dupanloup *et al.* 2002) to study the proportion of total genetic variation attributable to different hierarchical levels based on the geographic distribution of haplotypes. Several groupings of populations were tested to maximize the among-group component of molecular variance, i.e. to determine the maximum degree of phylogeographical structure present in the data (Liebers & Helbig 2002). As a final assessment of population demographic histories, we performed mismatch analysis (with pairwise distances).

Colonization of the small islands

We computed median-joining network using the program Network 4.1.0.0 (Bandelt et al. 1999) to reveal the relationship between geographical partitioning of haplotypes, including the small island (Isla Butachauques and Isla Talcán). We constructed a tree based on phylogenetic methods. Maximum parsimony (MP) and Maximun Likelihood (ML) criteria was implemented using PAUP* 4.0b1 (Swofford 2002) in order to determine the phylogenetic position of haplotypes of the recent colonized island. For MP analysis all characters were equally weighted, and a search was performed with 1,000 replicates and maxtrees set to 5,000, followed by tree-bisection reconnection (TBR) branch swapping. For ML, the model of evolution TIM+I+G selected using ModelTest version 3.7 (Posada & Crandall 1998), was estimated using the Akaike Information Criterion (AIC) that employed a heuristic search with 10 replicates and TBR branch-swapping. Support for the resulting clades was estimated with nonparametric bootstrapping (Felsenstein 1985) using 1,000 pseudoreplicates. Bootstrap values > 90% are considered strong support for a clade (Hillis & Bull 1993). Partial sequences of *L. tenuis, L. chillanesis and L. villarricensis* were used as outgroups, and *L. p. septentrionalis* (from Alto de Vilches) and *L. p. argentinus* (from Bariloche) were included in this analysis within the ingroup.

Results

Phylogeography of mainland and Isla Grande de Chiloé

Sequences of 535pb of *Cytb* were obtained of 144 individuals from ten localities for *L. pictus*. Five main haplogroups within *L. pictus* were assorted by median-joining networks (Fig. 2). The first haplogroup included individuals from the mainland localities Antillanca and Las Cascadas. A second haplogroup included all specimens from Hornopirén, Chaitén and Bariloche (n = 2), and one haplotype from Los Muermos. A third haplogroup included Isla Grande de Chiloé (both the northern and southern populations). This haplogroup did not share haplotypes with the mainland. Haplotypes from Los Muermos are divided in two separate haplogroups.

The number of haplotypes fluctuated between four (Hornopirén and Chaitén) and nineteen (Isla Grande de Chiloé). Higher haplotypic diversity was in Antillanca, Los Muermos and Isla Grande de Chiloé than in the other localities. Eighty-nine nucleotid sites (18.3%) were found to be polymorphic, with Antillanca, Las Cascadas, and Los Muermos in mainland, and Isla Grande de Chiloé showing the highest values. The mean number of pairwise sequence differences ranged from 0.7 to 13.2 (Table 2). Tajima's test showed negative and non-significant ($p < 0.05$) values in all localities, except in Los Muermos where D>0. Fu's test also showed negative values, with statistically significant values in Antillanca and Isla Grande de Chiloé (Table 2). A nested analysis of variance found several models that maximize the variation among groups. One model of five groups applied to the L. pictus localities accounted for 73% of the molecular variance among groups and 19.5% of variance within groups (Table 3). Figure 3 shows the mismatch distribution for each group.

Colonization of the small islands

When the small islands were included, the same five main haplogroups within *L. pictus* were assorted by median-joining networks (Fig. 4). Haplotypes from Isla Talcán were assorted in two haplogroups: two haplotypes associated to Antillanca + Las Cascadas clade, and two haplotypes associated to the Chaitén clade. The Isla Butachauques samples form a haplogroup with samples from Los Muermos, while one haplotype was assorted to Isla Grande de Chiloé.

The MP analyses recovered 541 equally parsimonious trees (L = 361, CI = 0.66, RI = 0.86), and a strict consensus tree was generated (Fig. 5). The ML analyses recovered one tree (ln L = -1759.61617) and, because MP and ML analyses produced very similar results, the single tree is presented here (Fig. 5).

A *L. pictus* haplotype tree rooted with the sequences of *L. tenuis*, *L. villarricensis,* and *L. chillanensis,* revealed two principal clades supported by a 100% bootstrap: a clade (corresponding to *L. p. septentrionalis* subspecies) with individuals of the northernmost distributional range of the species, and another clade, with the ingroup (localities around and within the chilotan archipelago). In this last group, five clades were strongly supported by bootstrap values >90%. A first clade included haplotypes from Antillanca and Las Cascadas, both described as *L. p. pictus* (Donoso-Barros 1966), and from Isla Talcán previously assigned to *L. p. talcanensis* by Urbina & Zuñiga (1977) and Pincheira & Nuñez (2005). A second clade included all localities from Isla Grande de Chiloé assigned to *L. p. chiloensis*, two haplotypes from Isla Guafo, and one haplotype from Isla Butachauques [illegible] to *L. p. codoceae*. A third clade includes haplotypes from Los Muermos, one [illegible] adas (*L. p. pictus*), and haplotypes from Isla Butachauques (*L. p.* [illegible] e). A fourth clade includes mainland haplotypes attributed to *L. p. pictus* [illegible] pirén, Chaitén, and one haplotype from Los Muermos), and haplotypes attributed as [illegible] *rgentinus* (Bariloche), as well as haplotypes from Isla Talcán (*L. p. talcanensis*). [illegible] ly, a fifth clade includes only haplotypes from Los Muermos (assigned to *L. p. pictus*) (Fig. 5).

Discussion

The origin of differentiation (e.g. morphology, or genetic diversity) is often associated with Late Pleistocene glacial cycles (Hewitt 1996; 1999) involving (i) fragmentation of a widespread ancestral species into refugia during periods of glacial advance, and (ii) subsequent genetic divergence among small isolated populations, followed by (iii) range expansion during interglacials. Typically, one [beginning ca. 125,000 years ago] or two (ca. 250,000 years ago) of such cycles are invoked (Klicka & Zink 1997). Our results indicate that populations from the mainland and Isla Grande de Chiloé exhibit high levels of genetic divergence. These results suggest that the divergence time could predate the last glaciation, reflecting an ancient lineage split that could have migrated into the region during the waxing and waning of Pleistocene glaciations.

In several cases, the role of glacial cycles has been demostrated to promote or maintain divergence (Hewitt 1996; 1999; Schmitt & Seitz 2001; Janzen *et al.* 2002; Liebers & Helbig 2002). For example, the patterns of population genetic variation found in *Melanoplus oregonensis* (Orthoptera) are associated to a historical divergence explained by displacements of multiple glacial refugia, and a later differentiation explained by a colonization of individuals from these sites (Knowles 2001). Nevertheless, glacial cycles do not always allow us to explain divergent processes. In fact, Crespi *et al.* (2003) demonstrated that the differentiation between populations of salamanders of North America shows pre-Pleistocene times of divergence, and a similar case seemed to ocurr in *Liolaemus monticola* complex in Chile, and *L. darwini* in Argentina (Torres-Perez *et al.* 2007; Morando *et al.* 2004).

Our results indicate that the "mainland lineages" identified by mitochondrial sequences are found in four general haplogroups (e.g. Antillanca-Las Cascadas, Hornopirén-Chaitén-Bariloche, and two haplogroups in Los Muermos), suggesting that fragmentation of *L. pictus* species occurred in multiple loci. Within species, the independent lineages were apparently originated or maintained in separate ice-free regions during glaciations (Cook et al. 2001). In fact, the high levels of intraspecific differentiation shown by several lineages (e.g., Los Muermos, Las Cascadas) suggest the possibility that their origin predates the Pleistocene (Klicka & Zink 1997; Cook *et al.* 2001).

The results from Isla Butachauques, Isla Guafo and Isla Talcán suggest that they were colonized both from mainland sources and from Isla Grande de Chiloé. The population from Isla Butachauques is related to the mainland by individuals collected from Los Muermos. The limited relationship with the populations from Isla Grande de Chiloé (one haplotype) suggests that waif dispersals may have reached Isla Butachauques via rafting from the mainland. Meanwhile, the Isla Guafo population shared haplotypes only with Isla Grande de Chiloé. The Isla Talcán case is particularly interesting. Our results suggest intermingling of two "mainland lineages": the Antillanca-Las Cascadas in the north and the Hornopirén-Chaitén in the south. In this case, both mainland lineages do not have haplotypes in common, but they were assorted in the island. We suggest that Isla Talcán was colonized by individuals that had haplotypes from a northern source (Antillanca), and these haplotypes were preserved in the island which may have acted as a postglacial refuge. A second wave of inmigrants might have reached Isla Talcán with haplotypes characteristic from Hornopirén and Chaitén, and populations from the other side of the Andean range (*L. p. argentinus* subspecies). The absence of haplotypes from Isla Talcán in the Chaitén populations suggests local extinction. Intensive disturbance by fire and volcanic activity on the Andean foothills (Moreno 2000, 2004) during the early Holocene, coupled with profound landscape and vegetation changes (Moreno 1997), might have decimated lizard populations in the Chaitén area. Isla Talcán might have escaped the effects of disturbance owing to its isolation from the mainland, along with its larger distance to the active volcanic centers.

Taxonomic implications

The subspecies concept was originally conceived as a population genetically distinct, geographically separate populations belonging to the same species and therefore interbreeding freely at the zones of contact (Mayr 1942; 1969). Mayr also extended the racial category to include closely related but geographically isolated populations; particularly those inhabiting archipelagoes (see Wilson & Brown 1953). In the *L. pictus* case, although the morphological differences are evident among the subspecies (Donoso-Barros 1966, Urbina & Zúñiga 1977, Pincheira & Núñez 2005), the molecular inference has many taxonomic implications. The mainland subspecies involving this area (*L. p. pictus* and *L. p. argentinus*) could correspond to a single taxonomic entity nominated as *L. p. pictus* because haplotypes are shared (see haplogroups related Antillanca-Las Cascadas and Hornopiren-Chaiten-Bariloche). Similarly, individuals from Isla Grande de Chiloé (*L. p. chiloensis*) shared haplotypes with two individuals from Isla Butachauques and Isla Guafo (*L. p. codoceae*) these corresponding to *L. p. chiloensis*. A major part of individuals from Isla Butachuques are related to Los Muermos in the mainland, therefore, this corresponds to *L. p. pictus*. Finally, Isla Talcán (*L. p. talcanensis*) shares haplotypes with two mainland lineages (Antillanca and Hornopirén-Chaitén) that correspond to *L. p. pictus*. Therefore, the geographic distribution of haplotypes and phenotypes for *L. pictus* populations are not compatible with the recognition of subspecies, suggesting that the usage of this nomenclature for *L. pictus* subspecies should be invalid. Disparities between mtDNA haplotypes and subspecific designations could be due in part to selection on morphology; consequently, a critical comparison of both approaches is required to determine whether distinct haplogroups separately denote evolving morphological lineages (Thorpe & Malhotra, 1996; Janzen et al., 2002; Parmakelis et al. 2003). Finally, this study is only based on a single mitochondrial marker but still clearly highlights the need for future work on chilotan species, as they may reveal additional patterns of population dynamics and climate-induced genetic changes.

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Table 1 Number of individuals of *Liolaemus pictus* sequenced in this study from each locality. Locality number (in parentheses) match those in Fig. 1.

Places	Subspecies	Locality	n	coordenates
Mainland	*L. p. pictus*	Antillanca	25	40°46’S; 72°16’W
		Las Cascadas	5	40°58’S; 72°20’W
		Los Muermos	12	41°20’S; 73°25’W
		Hornopiren	18	41°56’S; 72°23’W
		Chaiten	7	42°57’S; 72°38’W
	L. p. septentrionalis	Altos de Vilches	4	35°36’S; 71°03’W
Isla Grande de Chiloé	*L. p. chiloensis*	North		
		Route to Abtao	22	42°23’S; 73°51’W
		Quemchi	9	42°52’S; 73°48’W
		South		
		Yerbaloza	5	42°08’S; 73°23’W
		Quellon	3	43°03’S; 73°35’W
Small island	*L. p. codoceae*	Isla Butachauques	14	42°18’S; 73°08’W
		Isla Guafo	5	43°35’S; 74°41’W
	L. p. talcanensis	Isla Talcan	17	42°44’S; 72°57’W

Table 2 Diversity parameters for nine *Liolaemus pictus* localities estimated from mtDNA Cytb sequences. Number of individuals (n); number of haplotypes (K), haplotypic diversity (H), number of polymorphic sites (S), mean number of pairwise sequences differences (Π). * $P < 0.05$.

Locality	n	K	H	S	Π	D	Fu
Antillanca	25	12	0.843	14	2.3	-1.28	-5.40*
Las Cascadas	5	5	1.000	28	11.6	-1.02	-0.15
Los Muermos	12	11	0.985	36	13.2	0.50	-2.05
Hornopirén	18	4	0.575	3	0.7	-0.72	-1.09
Chaitén	7	4	0.714	4	1.1	-1.42	-1.22
I. G. Chiloé	39	19	0.906	31	3.7	-1.73	-8.48*
I. Guafo	5	2	0.400	5	2.0	-1.12	2.64
I. Butachauques	14	6	0.813	23	6.0	-0.73	2.09
I. Talcán	17	5	0.757	35	13.4	1.06	9.04*
Total	142	67	0.975	98	16.6	-0.34	-18.42*

Table 3 Analysis of molecular variance for seven *Liolaemus pictus* populations for six different models or agroupment. Chiloé Island population was divided in north (N) and south (S) populations.

							% Variance	
Model	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Inter	Intra
2	Antillanca Las Cascadas Los Muermos Hornopirén Chaitén	I. Chiloé N I. Chiloé S I. Guafo					34.10	18.24
3	Antillanca Las Cascadas	Hornopirén Chaitén	I. Chiloé N I. ChiloéS Los Muermos I. Guafo				57.80	18.17
4	Antillanca Las Cascadas	Hornopirén Chaitén	I. Chiloé N I. Chiloé S I. Guafo	Los Muermos			70.47	19.00
5	Antillanca Las Cascadas	I. Chiloé N I. Chiloé S	Hornopirén Chaitén	Los Muermos I. Guafo			72.66	19.51
6	Hornopirén Chaitén	I. Chiloé N I. Chiloé S	Antillanca	Las Cascadas	Los Muermos	I. Guafo	71.90	19.90

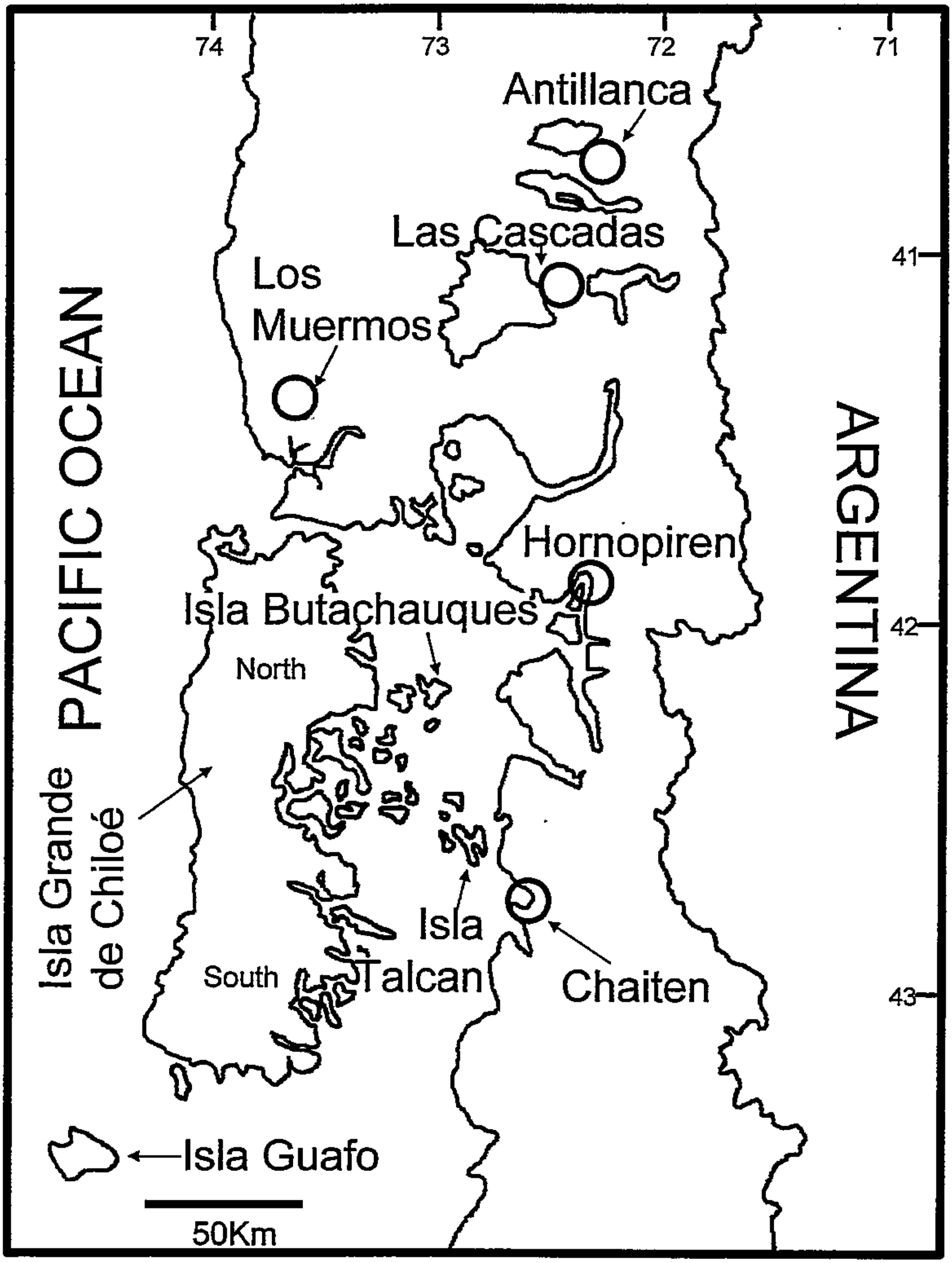

Fig. 1 Geographic distribution of *Liolaemus pictus* included in this study within archipelago area. Locality numbers and letters correspond to those in Table 1.

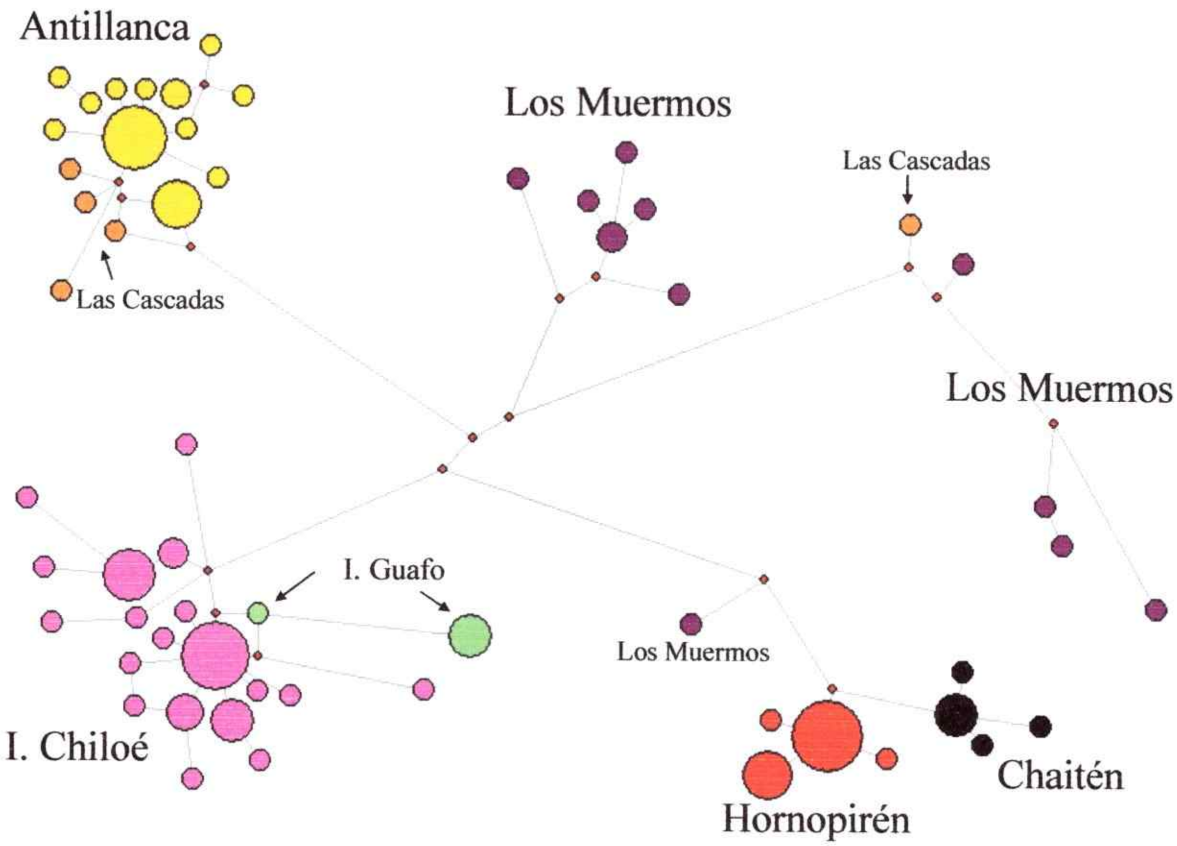

Fig. 2 Median-joining networks of mitochondrial Cytochrome b (*Cytb*) haplotypes of seven *Liolaemus pictus* populations. Size of circles is proportional to frequency.

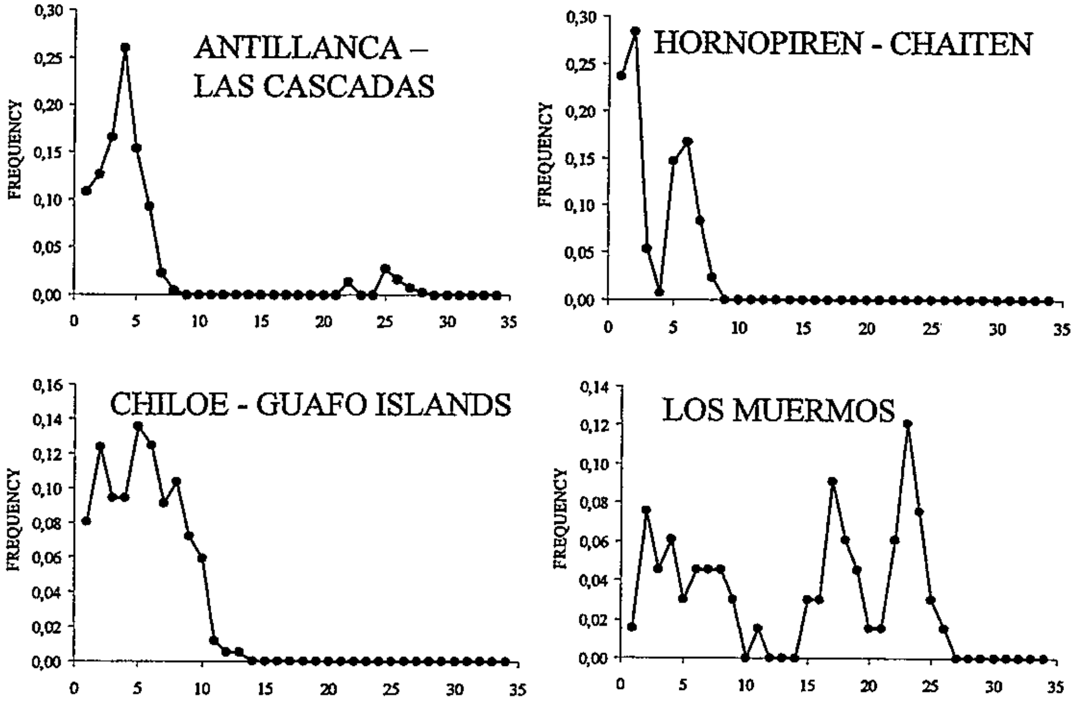

Fig. 3 Mismatch distribution of pairwise differences between sequences of L*iolaemus pictus* from different groups (obtained by AMOVA).

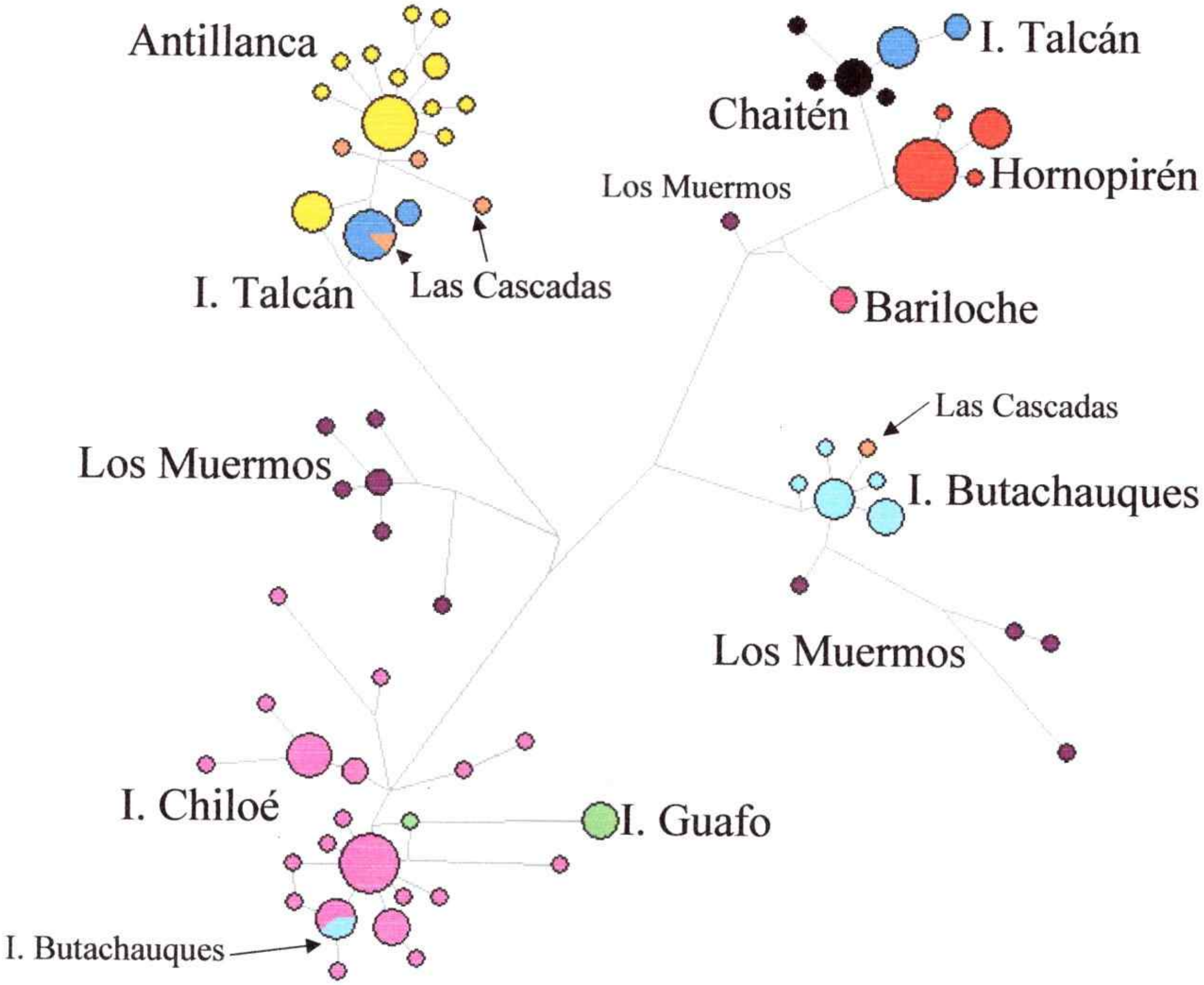

Fig. 4 Median-joining networks of mitochondrial Cytochrome b (*Cytb*) haplotypes of ten *Liolaemus pictus* populations. Size of circles is proportional to frequency.

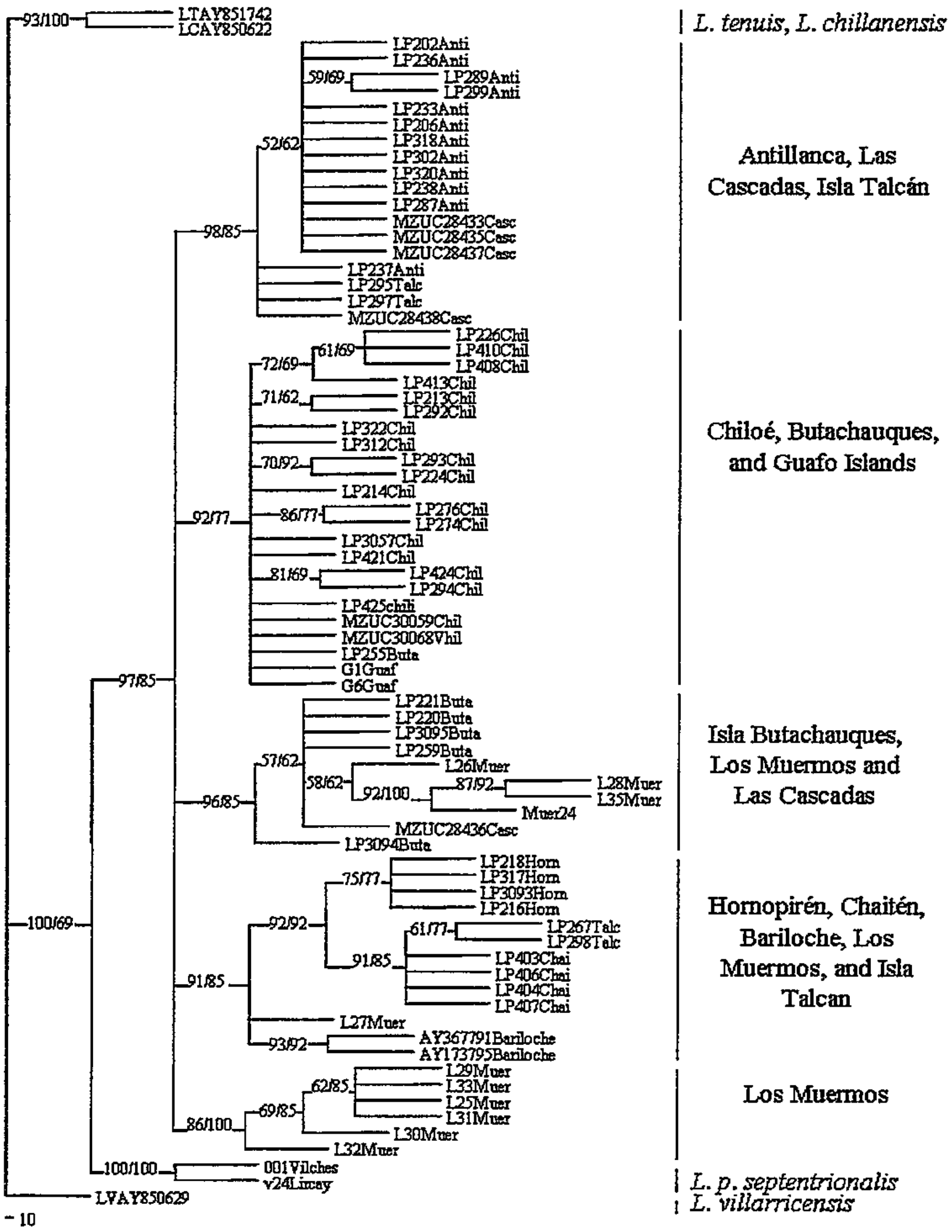

Fig. 5 Single ML tree (lnL = -1759.61617) for nonredundant *Cytochrome b* haplotypes of the *Liolaemus pictus* populations. Numbers in the branches represent bootstrap for MP and ML.

Trophic adaptability of island and mainland populations of *Liolaemus pictus* from Southern Chile

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Running title: Trophic analysis in *Liolaemus pictus*

CON FORMATO PARA REVISTA "Comparative Biochemistry and Physiology, Part A"

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Abstract

The foraging of the ecology behaviour of reptiles is characterized by adaptive flexibility and trophic adaptability which is often associated with species at low or intermediate trophic levels. On the insular context, the dietary divergence is the main promoter of the island – mainland change (e.g. insular lizards have a tendency towards a more herbivorous diet than lizards on mainland) *Liolaemus pictus* has different subspecies on island and mainland. These different types of habitats on which this species inhabits, suggests that dietary variation (diet, intestinal activity, intestinal morphology) may be influenced by local ecological factors. In this study, we examine the stomach content and also a proxy variable that indicates the relative position of this species in a food web (^{15}N). We also determined the morphology of the digestive tract and some biochemical features associated to the enzymatic digestion of carbohydrates and proteins. We specifically postulate that geographic dietary variation differs among populations and that this variation can be traced through a continuous variable which in turn may predict the expression of digestive features.

Our results indicate that the diet of *L. pictus* was characterized by the consumption of a relatively wide spectrum of food items and that the diet of the mainland and all island populations contain fruit as an item relatively common, although vegetal diets have been described as of low importance. For mainland localities, invertebrate orders and seeds consumed by lizards were different to those found on island localities. Although insular individuals on island have a shorter intestinal tract and show a higher fruit consumption than in mainland specimens, the enzyme activity of trehalase and N-aminopeptidase was high, suggesting that the enzymatic ability was more efficient to hydrolyze and breakdown insect products. These results are consistent with stable isotopes that revealed enrichment of $\delta^{15}N$ which was two-fold higher (two trophic leveles) on island than mainland populations. Finally, the difference among populations of *L. pictus* could be interpreted as the outcome of demands imposed by different diets on the morphology and function of the feeding system. Physiological specialization resulting in an improved ability to deal with invertebrate and fruit matter as a food resource suggests that functional trade-offs have not constrained the evolution of omnivory in *Liolaemus* lizards.

1. Introduction

The foraging behavior and the feeding ecology of reptiles are generally characterized by adaptive flexibility and trophic adaptability (Pough et al., 1998; Rocha et al., 2004). More than for many other groups of animals, the trophic ecology of many lizard species allows them to feed on a variety of prey and shift among prey types as the relative food availability and/or nutrient requirements change (Hurtubia, 1973; Ortiz and Riveros, 1976; Hirai, 2002). This high degree of feeding plasticity is often associated with species at low or intermediate trophic levels (Melville and Connolly, 2003; Spence and Rosenhein, 2005), whose preys are often smaller and less mobile than those of omnivorous lizard species (Rocha et al., 2004). On the insular context, it has been suggested that the dietary divergence is the main promoter of the island – mainland change (Clegg and Owens, 2002, Rocha et al., 2004). Due to the changes in density compensation, diet expansion and low predation levels (Olesen and Valido 2003), lizards inhabiting island habitats have a tendency towards a more herbivorous diet than lizards on mainland (Van Damme, 1999; Cooper and Vitt, 2002, Valido and Nogales, 2003);

Liolaemus lizards are widely distributed in South America (Donoso-Barros, 1966; Frost and Etheridge, 1989) and more than 160 living species have been described (Etheridge and Espinoza, 2000; Schulte et al., 2000). Given the variety of habitats throughout Chile (di Castri, 1968), it is possible that different selective forces have promoted diet and morphological variation among *Liolaemus* populations (Vidal et al., 2004; for *Liolaemus tenuis*). *Liolaemus pictus* is one of the southerly most distributed lizard species in Chile (37°S-43°S). Five subspecies have been described according to morphological traits: *Liolaemus pictus pictus* inhabiting the mainland from Concepción to Puerto Montt [37°-41°S] (Donoso-Barros, 1966, 1970; Veloso and Navarro, 1988), *L. p. argentinus* from Río Negro Province in Argentina (Donoso-Barros, 1966; Cei, 1986), *L. p. chiloensis* from Isla Grande de Chiloé (Müller and Hellmich, 1939), *L. p. talcanensis* in Isla Talcan (Urbina and Zúñiga, 1977); *L. p. codoceae* from islands in the vicinity of Isla Grande de Chiloé (Donoso-Barros, 1966, Pincheira and Nuñez, 2005) and an isolated population (*L. p. septentrionalis*) from Altos de Vilches (Pincheira and Nuñez, 2005).

Because subspecies of *L. pictus* inhabit different types of habitats (island and mainland), it is likely that dietary variation (diet, intestinal activity, intestinal morphology) is influenced by local ecological factors. While Ortiz (1974) indicated that *L. p. pictus* from the mainland fed on dipterans, homopterans, arachnids, hymenopterans and coleopterans,Willson et al. (1995) and Willson et al. (1996) indicated that *L. p. chiloensis* from Isla Grande de Chiloé is frugivorous, eating *Nertera granadensis* and *Relbunium hypocarpium*. Those authors suggested that this subspecies acted as a seed disperser agent. A recent study on *L. pictus* (Vidal et al., 2006) suggests that the morphometric differences among mainland and Isla Grande de Chiloé may support the hypothesis of insular divergence associated with dietary changes (Ortiz, 1974; Willson et al., 1996). Information on the feeding ecology in *L. pictus* indicates a wide geographic variation of the diet (Ortiz, 1974; Willson et al., 1996) and that morphological differentiation between mainland and island populations do exist (Vidal et al., 2006). The main objective of this study is to determine the extent to which morphology and physiology could be modulated by dietary habits in five populations of *Liolaemus pictus*. We performed a traditional dietary analysis through the examination of stomach contents in different island and mainland populations and also estimated the relative position in a food web based on a proxy variable through the isotopic signature of nitrogens, in bone collagen and leg muscle. We also determined the morphology of the digestive tract and some biochemical features associated to the enzymatic digestion of carbohydrates (sucrase, maltase and trehalase) and proteins (aminopeptidase-N). We specifically postulate that dietary differs among populations and that this variation can be traced through a continuous variable which in turn may predict the expression of digestive features.

2. Material and methods

Animals were collected in five localities (see Fig. 1) from Chilotan Archipelago: *L. p. pictus* from Antillanca (40°46'S, 72°15'W), and Hornopirén (41°56'S; 72°23'W); *L. p. chiloensis* from Isla Grande de Chiloé (42°23'S; 73°51'W); *L. p. talcanensis* from Isla Talcan (42°44'S; 72°57'W), and *L. p. codoceae* from Isla Butachauques (42°18'S; 73°08'W). Animals were sacrified and stored in liquid nitrogen for subsequent morphological analysis. Samples were transported to the Laboratory of Ecophysiology, Universidad de Chile in Santiago where all analyses were performed.

2.1. Intestinal morphology

Preserved specimens were thawed enough to allow us to perform ventral dissection. The entire digestive tract was removed from the specimen. The length of the digestive tract and its subdivisions was determined for every lizard. The length of the entire digestive tract was measured including the stomach and the large intestine. The length of the stomach, the small intestine and the large intestine were measured separately. Additionally, we measured the snout-vent length of all specimens. All measurements were taken using a digital caliper (±0.01 mm). After morphological measurements, the stomach content was removed for diet analysis and the small intestine was used on enzyme analysis.

2.2. Diet composition

Stomach contents were voided in a Petri capsule, sorted taxonomically and counted. For each sample, the frequency of occurrence and the percentage composition of prey taxa by number (percent number) were calculated. The relative importance index, RI (George and Hadley 1979), is the mean of the two diet measures for each prey taxon and reduces the potential bias that may occur if measures are used separately (Wallace, 1981). For a given population the RI of prey taxon *i* is calculated as: $RI_i = 100\ AI_i / \sum AI_i$ where AI_i (absolute importance of prey taxon *i*) = frequency of occurrence + percent number; n is the number of different prey types; frequency of occurrence is the percentage of all non-empty stomachs containing food in which prey taxon *i* was found, percent number is the percentage that prey taxon *i* contributes to the total number of food items in all stomachs (Bowen, 1996). For lizards, the frequency of occurrence and percent number were determinated and used in RI calculations.

2.3. Enzyme activity measurements

Small intestine sections were thawed and homogenized (30 s in an Ultra Turrax T25 homogenizer at maximum setting) in 20 vol of 0.9% NaCl solution. Disaccharidase activity was determined according to the method described by Martínez del Río et al. (1995). We measured enzyme activity in a whole-tissue homogenate to avoid the underestimation of activity. Hence, the activities of all enzymes are presented as standardized hydrolytic activity (UI total, where UI=μmol hydrolyzed/min). Briefly, tissue homogenates 100μL of 56 mmol L-1 sugar solutions (maltose, sucrose and trehalose) were added in 0.1 M maleate/NaOH buffer, pH 6.5. After 10 min incubation, reactions were stopped by adding 3 mL of a stop/develop glucose-Trinder (one bottle of Glucose Trinder 500 reagent (Sigma, St Louis, MO, USA) in 250 mL 0.1 mol L^{-1} TRIS/HCL, pH 7 plus 250 mL of 0.5 NaH_2PO_4, pH 7). Absorbance was measured to 505 nm with a spectrophotometer after 18 min at 20°C. Aminopeptidase-N assays were done with L-alanine-*p*-nitroanilide as a substrate. Briefly, 100 μL of homogenate diluted with 0.9% NaCl solution was mixed with 1 mL of assay mix (2.04 mmol L-1 L-alanine-*p*-nitroanilide on 0.2 mol L-1 NaH_2PO_4/Na_2HPO_4, pH 7). The reaction was incubated at 25°C and arrested after 10 min with 3 mL ice-cold acetic acid 2 N, and absorbance was measured at 384 nm. On the basis of absorbance, standardized intestinal enzymatic activities were calculated. The selected pHs for measuring the activities were the optimum for each enzyme, which were determined previously by measuring enzyme activities in a range of pH from 4.0 to 9.0.

2.4. Isotopic composition

Muscle and bone tissues were extracted for analysis of isotopes. Muscle dried up during 3 days at 70 °C and bone was cleaned to remove visible tar. Both muscle and bone were ground to a fine powder. Samples of powder were placed in heat-sealed Ankom filter bags and then refluxed with petroleum ether in a soxhlet extractor to remove hydrocarbons and lipids. Bones were demineralized in 1.0N HCl at air temperature. Collagen extracts were rinsed with distilled water and dried under vaccuum. Samples were analyzed for stable carbon and nitrogen ratios with a continuous flow isotope ratio mass spectrometer. Stable isotope data were presented as the relative difference between ratios of the sample and standards. Stable isotope ratios were expressed using standard delta notation (δ, Beaudoin et al., 1999; Vizzini and Mazzola, 2003) in parts per thounsand (‰) as:

$$\delta X = (R_{sample}/R_{standard} - 1) X 1000$$

where R_{sample} and $R_{standard}$ are the molar ratios of the heavy/light isotope of the sample and the reference, respectively. Samples were referenced against the international standard, the Vienna Pee Dee Belemnite (VPDB) for $\delta^{13}C$ and atmospheric nitrogen (AIR) for $\delta^{15}N$.

2.5. Statistical analysis

All data were examined for assumptions of normality and homogeneity of variance, using the Kolmogorov–Smirnov and Levene tests, respectively, prior statistical analyses. Differences on diet composition among localities and mainland-island were tested by one-way ANOVA, followed by Tukey test (Sokal and Rohlf, 1995). Differences of intestinal morphology among populations were evaluated by MANCOVA, using body mass as covariate, followed by Tukey's test. The relationship between body mass and intestinal measurement are evaluated from the slopes of the linear regressions, and were tested by one-way ANOVA. For enzyme activity, a correlation matrix was performed to examine a potential relationship among disaccharidases and N-aminopeptidase. Changes in enzyme activity were evaluated in MANCOVA, using body mass as covariate and tested by one-way ANOVA. The differences in isotopic composition between bone and muscle and among localities were evaluated by 10,000 random permutations. Finally, the correlational evidence was evaluated by a correlation matrix among the more significant variables (Sokal and Rohlf, 1995). All statistical analyses were conducted using SYSTAT software (SPSS, 2001).

3. Results

3.1. Intestinal morphology

A relationship between body mass and these intestinal measurements (Fig. 2) were found for stomach length ($R^2 = 0.11$; $P < 0.002$ [intercept different from zero $P < 0.0001$]), small intestine ($R^2 = 0.22$; $P < 0.001$ [intercept different from zero $P < 0.0001$]), large intestine ($R^2 = 0.34$; $P < 0.001$ [intercept different from zero $P < 0.0001$]) and total length ($R^2 = 0.43$; $P < 0.001$ [intercept different from zero $P < 0.0001$]). A MANCOVA analysis for morphometric measurements of digestive tract, which included locality (mainland – island) as factor and body mass as covariate indicated significant differences between localities

(Wilks`Lambda = 0.624; $P < 0.001$; Table 2). When all localities are considered, significant differences were found (Wilks`Lambda = 0.498; $P < 0.001$) where the differences were found for small intestine length between Hornopirén and all island localities (HSD-Tukey, $P < 0.001$) and for large intestine length were Antillanca-I. Chiloé ($P < 0.001$),Antillanca-I. Talcán ($P < 0.01$), Hornopiren-I. Chiloé ($P < 0.01$), I. Chiloe-I. Butachauques ($P < 0.001$) and I. Talcan-I. Butachauques ($P < 0.001$) (See Table 2).

The ratio between the small and large intestine was higher in mainland (1.49±0.09) than in island (1.39±0.09) individuals (ANOVA, $F_{1,83} = 23.51$, $P = 0.001$). This ratio was also different among localities (ANOVA, $F_{4,80} = 12.95$; $P = 0.0001$). The *a posteriori* test indicated the following significant differences: Antillanca and Hornopiren – Butachauques, Talcan Islands, Butachauques – Chiloé islands, and Chiloé – Talcan islands ($P < 0.001$). The ratio Log small intestine / Log stomach considering mainland and island groups indicated differences between them (ANOVA, $F_{1,83} = 25.54$, $P = 0.00001$) with a higher ratio on mainland (1.52±0.08) than island lizards (1.43±0.06). The proportion also showed significant differences among localities (ANOVA, $F_{4,80} = 7.01$; $P = 0.0001$) and a *posteriori* test indicated differences among Antillanca and Hornopiren with all islands ($P < 0.001$) but no significant differences were found among islands ($P > 0.1$).

3.2. Diet composition

The diet composition of *L. pictus* included 19 taxonomic groups (Table 1). The 88.7% corresponds to invertebrate prey and the remainder corresponds to plant material (plant remains, fruits, and seeds). Of these, mainlandlizards consume 89.1% of invertebrate and 10.9% of vegetal material, and island lizards consume 88.5% of invertebrate and 11,5% of vegetal material. No differences were found between mainland and island specimens for invertebrate (ANOVA, $F_{1,95} = 1.27$; $P >0.1$) and fruits (ANOVA, $F_{1,95} = 0.07$; $P >0.1$). However, significant differences were found when all localities are analyzed (ANOVA, $F_{4,92} = 4.24$; $P < 0.001$) where the differences were found between Antillanca- Hornopiren (HSD-Tukey, $P < 0.01$) and Antillanca-I. Talcan ($P < 0.01$). For each locality, the RI varied in the taxonomic item prey consumed. For mainland localities, Dermaptera, Diptera, Thysanoptera, and Coleoptera were the more represented items, whereas seeds of *Nertera* *granadensis* were of low importance. For island localities, Odonata, Diptera, Coleoptera, Hymenoptera, Quilopoda, Crustacea, seeds of *Galium hypocarpium*, *Gaultheria* sp and plant remains were the more consumed prey (Table 1). Although lizards consumed a higher number of invertebrates than fruit, lizards from Isla Talcan showed a higher RI of fruit contents than other localities (Table 1).

3.3. Enzyme activity

A correlation matrix between the enzyme activity of digestive tract indicates that all dissacharidases are positively correlated (Sucrase – Maltase: $r = 0.58$; $P = 0.0001$; Sucrase – Trehalase: $r = 0.38$, $P = 0.0002$; Maltase – Trehalase: $r = 0.49$, $P = 0.0001$). Aminopeptidase-N activity did not correlate with disaccharidase activity (with Sucrase: $r = 0.10$, $P > 0.05$; Maltase: $r = 0.16$, $P > 0.05$, and Trehalase: $r = 0.2$, $P > 0.05$). When data are pooled as mainland and island groups, significant differences are found (MANCOVA, Wilks`Lambda = 0.770; $P < 0.0004$) where the overall mean activity was higher on the island for Sucrase, Trehalase and N-aminopeptidase. Besides, mainland and island individuals showed different levels of association among disaccharidases (Table 3). A MANCOVA analysis for enzyme activity, that included locality as factor and body mass as covariate indicated significant differences between localities (UI total: Wilks`Lambda = 0.630, $P < 0.003$) where Antillanca (with highest values) differed from Isla Grande de Chiloé and Isla Butachauques for Maltase (HSD-Tukey, $P<0.001$).

Considering only the ratio between the total maltase activity and the total Aminopeptidase - N activity, the ANCOVA revealed significant differences among localities ($F_{4,78} = 3.865$; $P = 0.006$). The a *posteriori* test revealed differences between Antillanca and all islands ($P < 0.01$). Besides, significant higher ratios were found in mainland (198.4) than in island individuals (115.4) (ANCOVA, $F_{1,81} = 10.87$, $P = 0.001$).

3.4. Isotopic composition

When comparing isotopic composition of ^{15}N and ^{13}C between muscle and bone (Table 3), we did find significant differences (10,000 random permutations, $P = 0.413$). In both, mainland and island locality, $\delta^{15}N$ signature was 4.83±2.1‰ (mean ± SE) that ranged from

2.04 to 9.27‰, indicating considerable individual variation that spanned approximately two trophic levels. $\delta^{15}N$ values were lower in the mainland (2.75 ± 0.4‰) than the island (6.46 ± 1.3‰) (Table 3, 10,000 random permutations, $P = 0.0001$). Values of $\delta^{13}C$ in all localities were -25.15±0.66‰ (range: -24.20 to -26.14‰). This signature was similar between mainland (-25.08±0.51) and island (-25.19±0.75) (10,000 random permutations, $P = 0.0001$).

3.5. Correlational evidence

$\delta^{15}N$ was negatively correlated with the ratios: Maltase/Aminopeptidase-N, small intestine length/large intestine and small intestine/ stomach. The ratio Maltase/ Aminopeptidase-N was positively correlated with the following ratios: Small intestine/ large intestine and Small intestine/ stomach. Also the small intestine/ large intestine ratio was correlated with Small intestine/ stomach ratio (Table 4).

4. Discussion

The diet of *L. pictus* was characterized by the consumption of a relatively broad spectrum of food items (19 prey types) which characterizes the diet of an opportunistic predator (Rocha et al., 2004). Ortiz (1974) indicated that *L. p. pictus* from the mainland feed on dipters, homopters, arachnids, hymenopters and coleopters, yet Willson et al. (1995) and Willson et al. (1996) indicated that *L. p. chiloensis* from Chiloé Island is frugivorous, eating *Nertera granadensis* and *Relbunium hypocarpium*. In this context, our results indicate that the diet of the mainland and all island populations contain fruit as a relatively common item, although plant diets have been described as of low importance in the lizards of the genus *Liolaemus* (Fuentes and di Castri, 1975). On the other hand, *Liolaemus* lizards, in general, are specialized predators of arthropods (e.g., *L. fuscus, L. lemniscatus, L. tenuis*; Hurtubia, 1973). The consumption of a wide array of prey types, including both relatively sedentary (e.g., larvae) and highly mobile preys (e.g., spiders, orthopterans), has also been found for some *Liolaemus* species from Chile (see Fuentes, 1981 and references therein). The variation in the consumption of invertebrate and fruit by *L. pictus* was remarkable. In fact, for mainland localities, invertebrate orders and seeds consumed were different to the island localities. Although lizards consumed higher numbers of invertebrates than fruit, Isla Talcan individuals consumed more fruit than any of the remainder populations. Urbina and Zuñiga (1977) indicated that this insular population (assigned to *L. p. talcanensis* subspecies), also consumes grasses, which suggests that the trophic spectrum of *L. pictus* could be even wider if other localities are included in the analysis. Since we do not have data on prey availability at each locality, we do not know if this differential consumption of invertebrates and fruit reflects selectivity of this prey type in the mainland and island populations.

Several studies have described the intestinal morphology and diet in a wide range of lizard species (Greene, 1982; Schwenk, 2000; Herrel et al. 2004). Those studies have suggested that the length of the small intestine tends to be negatively associated with the amount of animal prey on the diet. However, we did not detect clear relationship between intestinal length and diet across the mainland and island populations of *L. pictus*. Mainland populations, which consume a low amount of *Nertera granadensis* fruits, have relatively longer intestines than the island populations that consume more fruits and plants (e.g., *Galium hypocarpium*, *Gaultheria sp.*). Although the size of an individual`s intestine may change in response to diet in vertebrates (Sabat and Bozinovic 2000; Elliott and Bellwood, 2003; Castañeda et al., 2006), an alternative hypothesis is the lizards analyzed do not need special anatomical traits like true herbivorous lizards (Iverson, 1982; Cooper and Vitt, 2002), because the main parts in the diet are fleshy fruit which have a relatively high digestibility (Valido and Nogales, 2003).

The levels of many digestive enzymes in vertebrates appear to be modulated reversibly according to the substrate levels (Stevens and Hume, 1995). Also, the overall ability of a lizard to extract energy from its prey items depends on kinetic behavior of digestive enzymes (Andrews and Asato 1977; Beaupre et al., 1993). In the *L. pictus* case, the shift in digestive physiology suggests that in insular populations there is an increase in the activity of enzyme that hydrolyze carbohydrates (e.g., trehalase, which hydrolyzes the sugar trehalose present in the insect, and sucrase, which hydrolyzes sucrose, a disaccharide present mainly in fruits and other terrestrial plant tissues), and an increase in enzyme activity associated with the breakdown of proteins (i.e., aminopeptidase-N activity). In this sense, although island individuals have a shorter intestinal tract and show a higher consumption of fruits than mainland individuals, their enzyme activity is higher for trehalase and N-aminopeptidase, suggesting that enzymatic ability is more efficient to hydrolyze and breakdown insect products. Consistently with this, the proportion maltase / N-aminopeptidase was higher in mainland than island, indicating high content of maltase and low N-aminopeptidase in mainland, contrary to that, low content of maltase and high content of N-aminopeptidase in island. Our results suggest that mainland and island *L. pictus* populations show phenotypic flexibility to the enzyme expression in the digestive tract (Starck, 1999).

$\delta^{15}N$ measurements revealed enrichment in island populations (two trophic levels) relative to two-fold mainland population. This result can be interpreted as island lizards that tend to be more "insectivorous" and in mainland to be "herbivorous". This type of intraspecific variation in enrichment values has been found in seasonal changes of diet (Darimont and Reimchen, 2002; Vizzini and Mazzola, 2003) or changes in assemblage of item prey of diet (Beaudoin et al. 1999). In this sense, we hypothesize that the differences observed in the isotopic composition between localities reflect differences in resource use (i.e. relative importance of some taxonomy item preys). These changes in lizard diets could reflect the high flexibility in each site along the distribution range. These results are consistent with those of digestive enzymes in which the island populations show high enzyme activity for trehalase and N-aminopeptidase, suggesting more efficient ability to hydrolyze and breakdown insect products.

Furthermore, island populations of *L. pictus* show similar features related to feeding ecology (Fig. 3). This suggests that environmental conditions may be more similar between them than between island and mainland localities. It has been suggested that the presence of few predators and competitors on islands, climatic differences, and the role of colonization events of island populations may often explain the observed divergence in morphology, physiology, diet and genetic variability between island and mainland populations (Irschick et al., 1997; Clegg and Owens, 2002; Calsbeek and Smith, 2003). In this vein, it has been suggested that the dietary divergence is the main promoter of the island - mainland change (Clegg and Owens, 2002). Concerning lizard populations, Olesen and Valido (2003) suggested that island lizards tend to eat fruits. In conclusion, the differences among *L. pictus* populations could be interpreted as the result of demands imposed by different diets on the morphology and function of the feeding system; physiological specializations resulting in an improved ability to deal with invertebrate and fruit matter as a food resource would suggest that functional trade-offs have not constrained the evolution of omnivory en *Liolaemus* lizards.

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Table 1

Frequency of occurrence (%FO), percentage by number (%Number) and Index of relative importance (RI) of prey taxa in the diets of *Liolaemus pictus* from island and mainland localities. Isla Grande de Chiloé (CHI, n = 30); Isla Talcan (TAL, n = 14); Isla Butachauques (BUT, n = 17), Antillanca (ANT, n = 22) and Hornopiren (HOR, n = 14). Samples sizes (n) refer to the total number of lizard stomachs examined.

	%FO					%NUMBER					RI				
Prey item	CHI	TAL	BUT	ANT	HOR	CHI	TAL	BUT	ANT	HOR	CHI	TAL	BUT	ANT	HOR
Homoptera	23.3	21.4	23.5	22.7	14.3	38.2	7.3	10.9	40.0	3.6	7.4	6.8	4.9	5.7	4.5
Dermaptera	0	0	0	4.5	0	0	0	0	100	0	0	0	0	9.6	0
Lepidoptera	10.0	0	0	18.2	0	37.5	0	0	62.5	0	5.7	0	0	7.4	0
Odonata	3.3	0	11.8	0	0	33.3	0	66.7	0	0	4.4	0	11.3	0	0
Ortoptera	10.0	7.1	0	31.8	7.1	23.8	4.8	0	66.7	4.8	4.1	2.8	0	9.0	3.0
Diptera	36.6	35.7	58.8	54.5	78.6	27.6	5.7	21.8	31.0	13.8	7.7	9.8	11.6	7.8	23.3
Thysanoptera	0	0	0	9.1	0	0	0	0	100	0	0	0	0	10.0	0
Hemiptera	26.7	7.1	29.4	31.8	14.3	26.4	3.8	32.1	34.0	3.8	6.4	2.6	8.8	6.0	4.6
Coleoptera	83.3	85.7	94.1	86.4	78.6	34.8	12.9	18.3	25.6	8.4	14.2	23.3	16.2	10.3	21.9
Hymenoptera	53.3	0	0	40.9	7.1	31.6	0	0	49,1	19.3	10.2	0	0	8.2	6.7
Aracnidae	16.7	7.1	17.6	4.5	7.1	44.4	5.6	33.3	11.1	5.6	7.4	3.0	7.3	1.4	3.2
Quilopoda	3.3	0	0	0	0	100	0	0	0	0	12.5	0	0	0	0
Gastropoda	0	0	5.9	13.6	0	0	0	16.6	83.3	0	0	0	3.2	8.9	0

Insecta larvae	0	14.3	17.6	18.2	14.3	0	25.0	25.0	33.3	16.6	0	9.3	6.1	4.7	7.8
Crustacea	0	0	23.5	0	14.3	0	0	80	0	20	0	0	14.9	0	8.6
Galium hypocarpium	0	50.0	11.8	4.5	7.1	0	42.9	22.4	12.2	22.4	0	21.9	4.9	1.5	7.4
Nertera granadensis	10.0	7.1	5.9	18.2	21.4	20	2.9	25.7	37.1	14.3	3.6	2.4	4.5	5.1	9.0
Gaultheria sp	3.3	0	0	0	0	100	0	0	0	0	12.5	0	0	0	0
Vegetal rest	6.6	71	11.8	9.1	0	25	6.3	31.3	37.5	0	3.8	18.2	6.2	4.3	0
Total Invertebrates	100	100	100	100	100	31.5	8.8	18.8	31.4	9,6	80.1	57.5	84.3	89.1	83.6
Total Fruit	13.3	57.1	17.6	18.2	28.6	12.5	25	22.7	21.6	18.2	19.9	42.5	15.7	10.0	16.4

Table 2

Morphometic measurements of the digestive tract of *Liolaemus pictus* from mainland and island populations. n: sample size.

			Length (mm)			
Locality	n	Body mass (g)	Total	Stomach	Small intestine	Large intestine
Antillanca	21	5.29±1.91	115.77±16.38	18.16±2.47	79.19±12.85	18.70±3.76
Hornopirén	17	6.78±2.01	123.27±18.41	18.13±2.75	84.50±15.55	20.34±3.61
I. Chiloé	19	5.60±1.78	109.17±15.79	18.97±3.01	71.89±11.69	18.67±3.86
I. Butachauques	17	7.55±4.41	114.90±22.39	19.91±3.39	70.71±15.81	24.28±6.20
I. Talcan	16	6.60±3.18	111.26±24.57	19.80±5.47	68.26±17.23	22.76±4.87

Table 3

Relationship between intestinal Sucrase, Maltase and Trehalase activity in *Liolaemus pictus* from mainland and island localities. r: Spearman correlation coefficient ** indicate $p < 0.01$.

	Mainland		Island	
	r	Intercept	r	Intercept
Sucrase - Maltase	0.73**	0.085	0.53**	0.322
Sucrase – Trehalase	0.15	1.47**	0.41**	0.913
Maltase - Trehalase	0.37	7.14**	0.53**	3.66**

Table 4

Mean (±SE) of $\delta^{15}N$ and $\delta^{13}C$ signature in mainland and island localities of *Liolaemus pictus*.

		$\delta^{15}N$		$\delta^{13}C$	
Locality	n	Bone	Muscle	Bone	Muscle
Antillanca	13	3.36±0.44	2.81±0.47	-24.57±0.66	-25.09±0.56
Hornopirén	5	3.37±0.00	2.59±0.05	-24.74±0.24	-25.05±0.05
I. Chiloé	13	6.90±0.86	6.31±0.63	-24.60±0.60	-25.05±0.86
I. Butachauques	5	9.09±2.11	7.89±1.84	-24.42±0.49	-25.19±0.38
I. Talcán	5	6.24±0.08	5.31±0.86	-24.94±0.33	-25.83±0.45

Table 5

Correlation matrix of six feeding variables of *Liolaemus pictus*. * indicate $P < 0.05$.

	Invertebrate	Fruit	Maltase/ Aminopeptidase-N	Small intestine/ large intestine	Small intestine/ Stomach
$\delta^{15}N$	0.31	0.01	-0.53*	-0.50*	-0.58*
invertebrate	-	-0.33	0.03	0.30	0.04
Fruit		-	-0.29	-0.38	-0.44
Maltase/ Aminopeptidase-N			-	0.51*	0.61*
Small intestine/ large intestine				-	0.54*

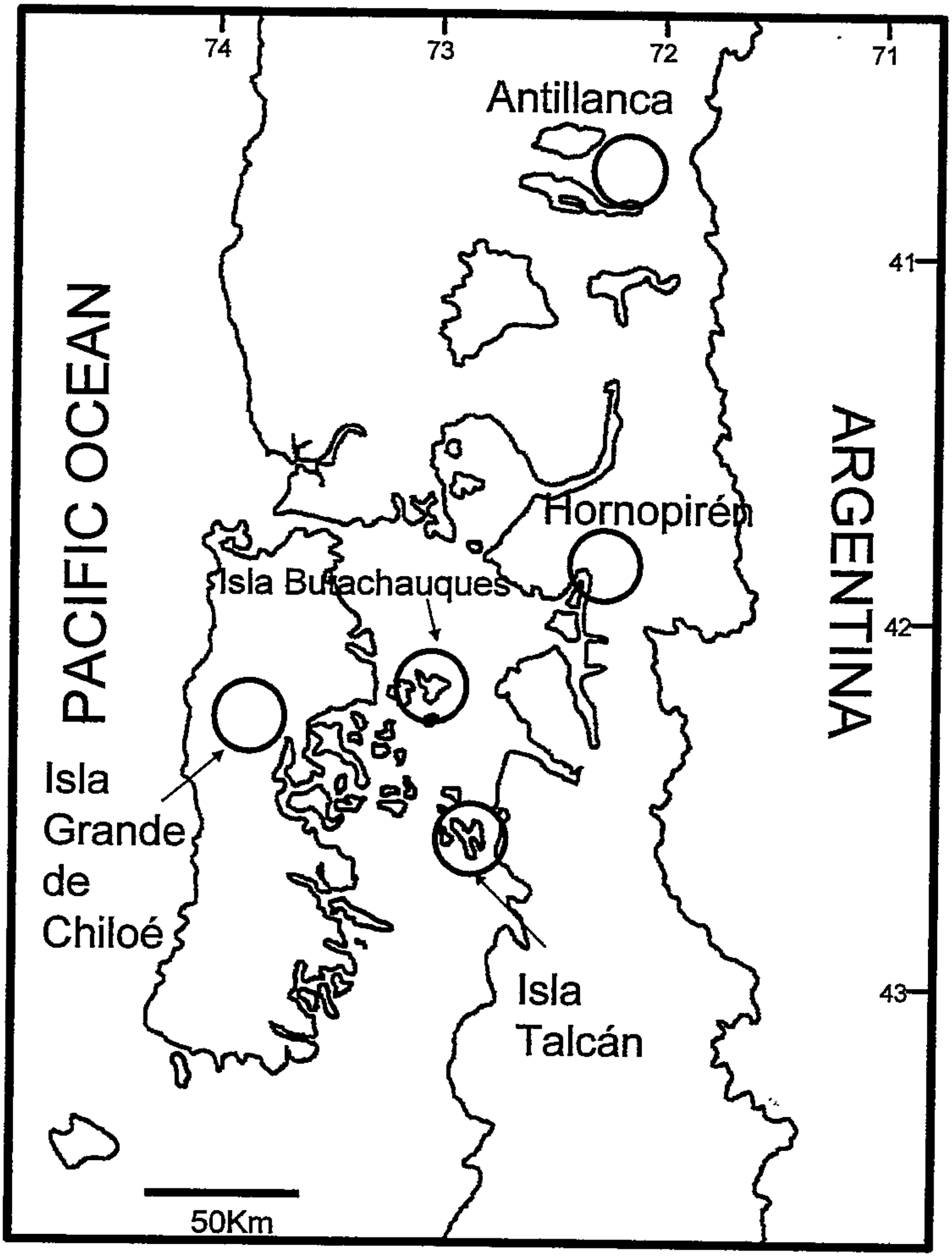

Fig. 1. Geographic distribution of *Liolaemus pictus* included in this study within archipelago area.

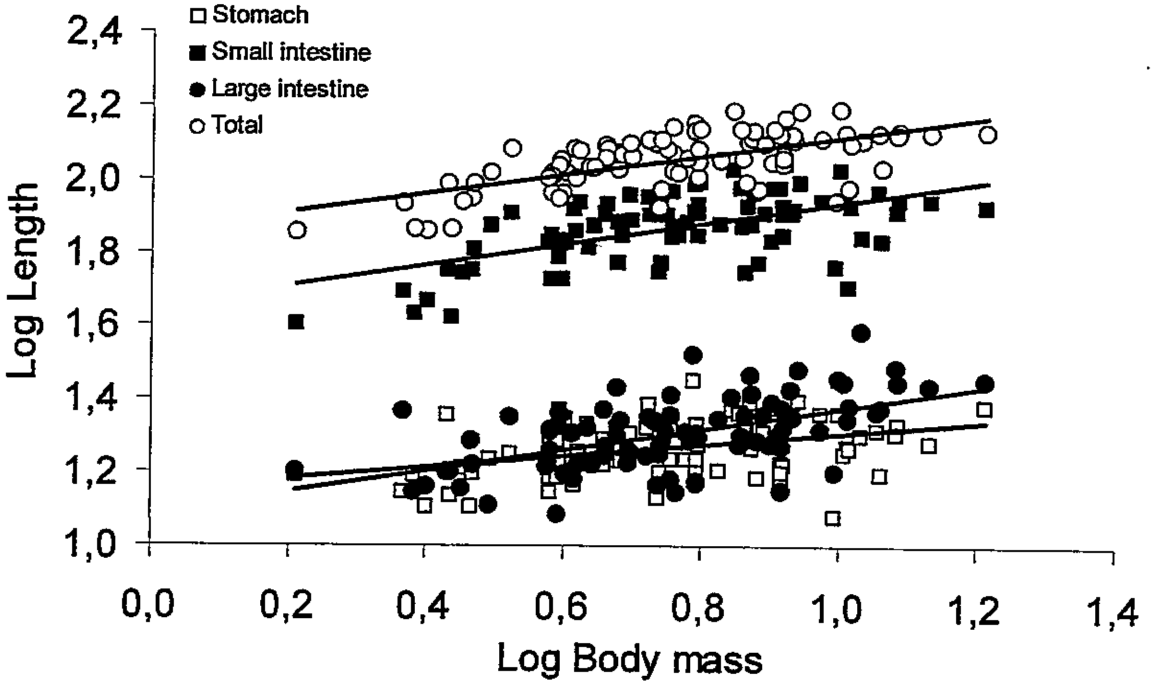

Fig. 2. Relationship between Log body mass (g) and Log stomach, small and large intestine and total length of digestive tract of *Liolaemus pictus*.

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Insular morphological divergence in the lizard *Liolaemus pictus* (Liolaemidae)

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Abstract. *Liolaemus pictus* is a widely distributed arboreal lizard species in southern Chile. Within this species, mainland and island subspecies have been described mainly based on external morphological characters, however, their diagnostic characters have not been accurately demarcated. We used both traditional and geometric morphometrics to study the morphological divergence in *L. pictus* subspecies. Since geographic isolation can be related to environmental variation (e.g., variation in abundance and richness of insects or fruits), we predict that the *L. pictus* morphology could vary in head shape between mainland and island localities.

Our results show morphological divergence in all variables analyzed among the island and mainland localities. The traditional morphometric approach reveals differences between populations from the mainland and island localities, where the mainland populations show a larger head size. Geometric morphometric data indicate that the eye orbits of the island specimens are more extended than in the mainland specimens in the dorsal view; this is probably related to a different capacity of substrate used. Also, in the lateral view, the eye orbits are extended and the head shape is less dorso-ventrally compressed in the island locality than in mainland populations and the terminal mouth position is more posterior in island specimens. We suggest that the morphological variation between mainland and island localities could be related to the fact that these populations were isolated during the last maximum glacial period in southern Chile, a period in which geomorphologic and bioclimatic features of this area were modified forming the present archipelago.

Introduction

The size of organisms has long been recognized to display important biogeographical variations between and within populations (Malho-tra and Thorpe, 1991; Renaud and Michaux, 2003). Island vertebrate species, when compared to equivalent mainland species, are often characterized by peculiar morphological traits, a classical example being the evolution of body size toward gigantism or dwarfism (Renaud and Millien, 2001; Anderson and Handley, 2002; Clegg and Owens, 2002). According to the literature, the presence of few predators and competitors on islands, climatic differences and the role of colonization events of island populations have been invoked to explain divergence in morphology, diet and genetic variability between island and mainland populations (Irschick et al., 1997; Clegg and Owens, 2002; Calsbeek and Smith, 2003). In this context, it has been suggested that the dietary divergence is the main promoter of the island – mainland morphological change (Clegg and Owens, 2002). Concerning lizard populations, Olesen and Valido (2003) suggested, based on density compensation, diet expansion and low predation levels, that island lizards tend to eat fruits and that these dietary changes could produce divergence given that morphological characters and diet are correlated (Ruber and Adams, 2001). For example, morphological changes have been described in two sympatric salamander species due to biomechanical differences in jaw closure associated with the differences in prey consumption (Adams and Rohlf, 2000).

Liolaemus lizards are widely distributed in South America (Donoso-Barros, 1966; Frost and Etheridge, 1989) and more than 160 living species have been described (Etheridge and Espinoza, 2000; Schulte et al., 2000). Given the

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variety of habitats throughout Chile (di Castri, 1968), it is possible that different selective forces can promote morphological variation among populations (Vidal et al., 2004; for *Liolaemus tenuis*). Although some Chilean species of *Liolaemus* have been characterized in terms of their population variation (Lamborot, 1993, 1998; Torres-Perez et al., 2003), the literature concerning morphological variation is relatively scarce (Labra, 1997; Schulte et al., 2004).

Liolaemus pictus is one of most southerly distributed lizard species in Chile (37°S-43°S). Five subspecies have been described for this species according to morphological traits: *Liolaemus pictus pictus* (Duméril and Bibron, 1837) inhabiting the mainland from Concepción to Puerto Montt [37·-41·S] (Donoso-Barros, 1966, 1970; Veloso and Navarro, 1988), *L. p. argentinus* Müller and Hellmich, 1939 from Río Negro Province in Argentina (Donoso-Barros, 1966; Cei, 1986), *L. p. chiloensis* Müller and Hellmich, 1939 from Chiloé Island (Müller and Hellmich, 1939), *L. p. talcanensis* Urbina and Zúñiga, 1977 in Talcan Island (Urbina and Zúñiga, 1977) and *L. p. major* Boulanger, 1885 from islands in the vicinity of Chiloé Island (Donoso-Barros, 1966).

Because subspecies of *L. pictus* live in different types of habitats, it is possible that morphological variation observed could be influenced by local ecological factors. While Ortiz (1974) indicated that *L. p. pictus* from the mainland feed on dipters, homopters, arachnids, hymenopters and coleopters,Willson et al. (1995) and Willson et al. (1996) indicated that *L. p. chiloensis* from Chiloé Island is frugivorous, eating *Nertera granadensis* and *Relbunium hypocarpium*, suggesting that this subspecies is a seed disperser agent. If these ecological factors are present both in mainland and island populations of *L. pictus*, differences between the two regions may lead to morphological divergence, particularly in head shape when dietary differences are involved.

Traditional morphometric analyses are frequently used to test the hypotheses related to morphological polymorphism in a microevolutionary context (Endler, 1977; Scolaro and Cei, 1987; Quatrini et al., 2001). However, due to scale problems with linear measurements, when used in morphometric analyses they do not take into account the morphological complexity of biological structures. (Humphries et al., 1981; Rohlf and Bookstein, 1987; Mousseau, 1991; Warheit, 1992). On the other hand, the geometric morphometric approach is a tool which allows the analysis of differences in shape without the influence of size variables (Rohlf and Slice, 1990; Rohlf et al., 1996; Bookstein et al., 1999; Rohlf, 1999; Rohlf and Corti, 2000). This second approach offers a geometric vision of a given structure (Rohlf and Slice, 1990; Adams and Rohlf, 2000), registering information regarding shape that allows a more complete biological interpretation of morphological variation (Rohlf and Marcus, 1993).

The aim of this study is to evaluate the morphological variation of *L. pictus* in different localities, comparing mainland and island populations applying both traditional and geometric methods. Given that dietary variation for *L. pictus* between mainland and island populations has been described, we predicted that head morphology could show variation according to this pattern.

Materials and Methods

All materials used in this study belong to the collection of the Museum of Zoology of the Universidad de Concepción (MZUC). Traditional morphometric analyses were performed on 88 adult *Liolaemus pictus* specimens from four localities (fig. 1): *L. p. pictus* from Antillanca [40°46_S, 72°12_W (8 males, 11 females)], Nahuelbuta [37°20_S, 73°30_W (10 males, 14 females)], Puesco [39°25_S, 71°-40_W (10 males, 11 females)] and *L. p. chiloensis* from Ancud, Chiloé Island [41°56°S, 73°53°W (11 males, 13 females)].

Twelve morphological characters were studied: (SVL) snout-vent length, (ISD) interparietal scale-snout distance, (IS) interparietal scale size, (MHW) maximum head width, (ID) interocular distance, (HL) head length measured from the tympanic cavity to the snout, (ML) mouth length, (EL) eye length, (END) anterior eye-nose distance, (NSD) nose-snout distance, (ESD) anterior eye-snout distance, and (PERD) post-eye-rostral distance. All measurements were

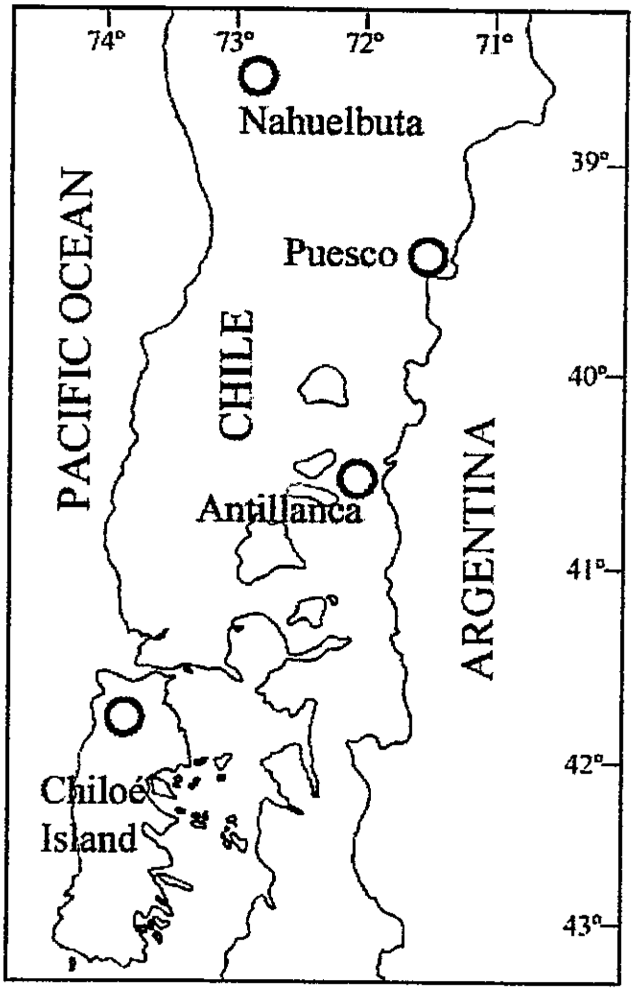

Figure 1. Geographic locations of the populations studied.

taken with a digital micrometer Mitutoyo (±0.01 mm). These morphological variables were analyzed with a oneway multivariate covariance analysis (MANCOVA); geographic locations were included as a factor and SVL as a covariate. An *a posteriori* Tukey test (Sokal and Rohlf, 1995) was used to determine differences between pairs of localities. A cluster analysis using the unweighted pair group average method (UPGMA) was performed on the Euclidean distances in order to show hierarchically the morphometric differences among populations. Samples had a normal distribution for all analyses performed. The SYSTAT 10.0. software for Windows (SPSS, 2000) was used in all analysis.

A geometric morphometric analysis (Bookstein, 1991) was used to assess the variation attributed exclusively to shape. Dorsal and lateral views of heads of 88 adult specimens were registered with a Sony-Mavica digital camera. Ten homologous landmarks, coincident with the intersections of the cranial scales and soft tissues were digitalized in the dorsal view and ten homologous landmarks in the lateral view (fig. 2) were analyzed following procedures of Rohlf and Slice (1990) and Rohlf et al. (1996). The *x*, *y* coordinates of biologically homologous landmarks were aligned and superimposed using the least squares method based on the generalized procrustes analysis (GPA), allowing for the removal of variations not due to shape (e.g. rotation, movement). Digitalization was performed with Tpsdig version 1.22 and further superimposition was performed with TpsSuper version 1.06 (Rohlf, 2003a, 2003b).

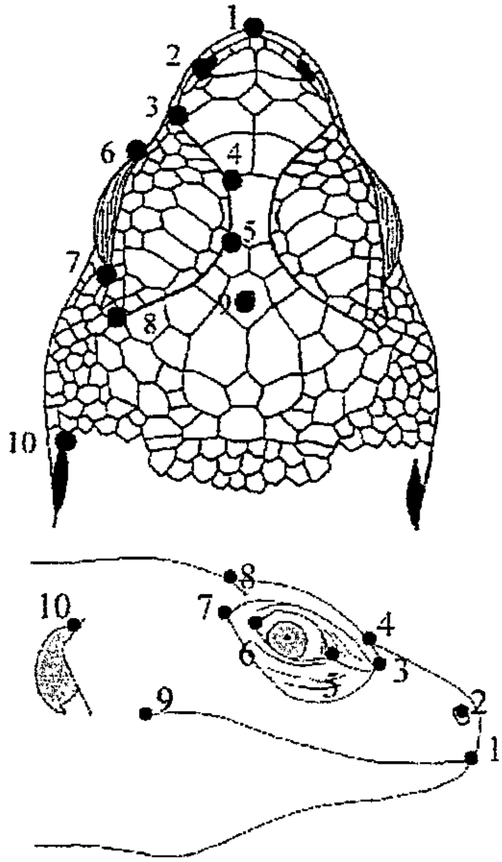

Figure 2. Location of landmarks used in this study in the dorsal and lateral views of the head.

To obtain shape variables, optimally aligned individuals were compared using TpsRelw version 1.21 (Rohlf, 2003c), which performed a thin-plate spline interpolation function to project the data onto a Euclidean plane. Thus, shape variables with both uniform and non-uniform components (total of $2+2k-4$ variables, where k is the number of landmarks used) were obtained. These variables were then used in multivariate statistical analyses (Rohlf et al., 1996; Adams and Rohlf, 2000). TpsRelw version 1.21 (Rohlf, 2003c) was used to perform a principal component analysis (Relative warp analysis). A one-way multivariate analysis of variance (MANOVA) (comparing by locality) and a cluster analysis was performed using Euclidean distances in order to hierarchically depict the shape differences among populations.

In order to determine if the shape incorporated an allometric effect, regression analysis was performed between the first axis of the principle components analysis (shape variables) and the centroid size (CS), which is defined as the sum of squared Euclidean distances from each landmark to the centroid of the shape (Bookstein, 1991). The centroid size (Log [CS]) of all specimens was compared using oneway analysis of variance (ANOVA), with locality as a factor.

Results

The morphometric measurements by locality are show in table 1. Because not sexual dimorphism were found (Wilks' Lambda = 0.884; $P > 0.1$), male and female data were pooled. MANOVA analysis, which included geographic location as a factor for all the analyzed variables, indicates significant differences among

Table 1. Morphometric variables of *Liolaemus pictus* that showed significant differences among localities by traditional morphometric method. Data are shown as mean and standard error in parenthesis. See Materials and Methods for meaning of the variables.

Locality	SVL	ISD	IS	MHW	ID	HL	ML	EL	END	NSD	ESD	PERD
Antillanca	60.57	10.58	1.61	11.15	7.85	13.81	9.01	3.26	2.60	1.97	4.67	9.66
	(5.13)	(0.62)	(0.20)	(0.84)	(0.40)	(1.04)	(0.67)	(0.20)	(0.32)	(0.25)	(0.54)	(0.69)
Chiloé	56.37	9.67	1.56	10.14	7.19	12.24	8.44	2.96	2.38	1.79	4.24	8.64
	(5.85)	(0.85)	(0.23)	(0.83)	(0.56)	(1.07)	(0.66)	(0.45)	(0.28)	(0.26)	(0.45)	(0.64)
Nahuelbuta	57.34	10.36	1.88	10.97	7.84	12.88	8.95	3.77	2.89	1.82	4.59	9.14
	(3.66)	(0.60)	(0.24)	(0.72)	(0.53)	(0.78)	(0.70)	(0.36)	(0.30)	(0.19)	(0.42)	(0.52)
Puesco	61.20	10.99	1.53	11.90	8.58	14.14	9.41	3.45	3.11	1.95	5.05	10.15
	(4.46)	(0.52)	(0.23)	(0.76)	(0.51)	(0.62)	(0.74)	(0.31)	(0.45)	(0.21)	(0.36)	(0.66)

Table 2. One-way analysis of variance for variables that showed significant differences using locality as a factor and SVL as a covariate for *Liolaemus pictus.* (IS) interparietal scale size, (MHW) maximum head width, (ID) interocular distance, (HL) head length measured from the tympanic cavity to the snout, (ML) mouth length, (EL) eye length, (END) anterior eye-nose distance, (ESD) anterior eye-snout distance, and (PERD) post-eye-rostral distance.

Source variation	Degrees of freedom	Variable	*F*-value	*P*-value
Locality	3, 79	IS	12.47	0.001
		MHW	8.75	0.001
		ID	24.32	0.001
		HL	5.83	0.001
		ML	3.54	0.018
		EL	27.8	0.001
		END	14.93	0.001
		ESD	4.50	0.005
		PERD	6.81	0.001

Table 3. Variables of traditional morphometric analysis performed in *Liolaemus pictus* showing significant differences ($P < 0.01$) in the *post-hoc* test (HSD-tukey). See Materials and Methods for meaning of the variables.

Localities	Antillanca	Chiloé	Nahuelbuta	Puesco
Antillanca	---	MHW. ID. HL. PERD	IS. ID. EL. END	MHW. ID. HL. END. ESD. PERD
Chiloé		---	IS. MHW. ID. HL. ML. EL. END. PERD	MHW. ID. HL. ML. EL. END. ESD. PERD
Nahuelbuta			---	IS. ID. HL. EL. ESD. PERD
Puesco				---

the geographic locations (Wilks' Lambda = 0.135; $P < 0.0001$). Differences were found among localities for nine variables – IS, MHW, ID, HL, ML, EL, END, ESD, and PERD (table 2) – and lower average values were registered for Chiloé Island (table 1). Differences were found between mainland and island populations (Wilks' Lambda = 0.631;$P < 0.0003$); Chiloé Island showed great morphological differentiation when compared with Nahuelbuta and Puesco (table 3). Cluster analysis showed two groups: Antillanca, Nahuelbuta and Puesco forming one, and the Chiloé Islands forming another (fig. 3A).

Multivariate analysis of shape variables showed differences among localities when dorsal and lateral head shapes were compared (Wilks Lambda = 7.03; $P < 0.001$, Wilks, Lambda = 5.92;$P < 0.001$, respectively). The dorsal view landmarks 3, 4, 5, 6, 7 and 8, and

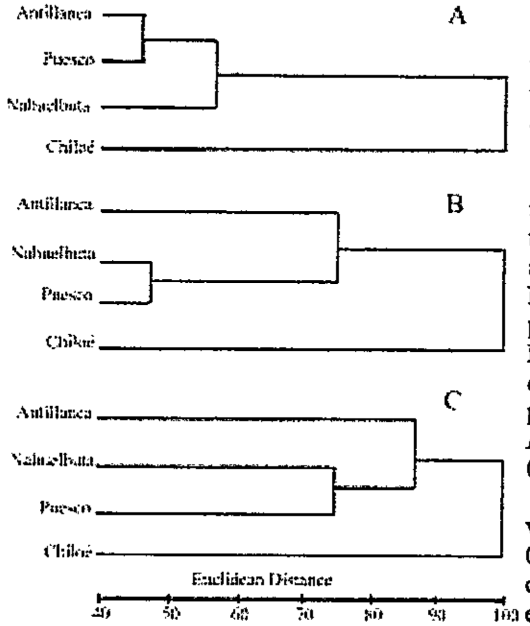

Figure 3. UPGMA phenogram constructed from Euclidean Distances computed on (A) traditional morphometric variables and geometric morphometric variables in (B) dorsal view and (C) lateral view.

in the lateral view landmarks 3, 5, 6, and 7, showed a higher contribution in differentiating localities. All these landmarks were related to eye shape, and in the dorsal view eye orbits were more extended in lizards from Antillanca and Chiloé than in those from Nahuelbuta and Puesco, the most northern localities. These differences were also observed in comparisons between lizards from Antillanca – Chiloé and Puesco – Nahuelbuta, respectively (Tukey Test-HSD, $P < 0.001$). However, the cluster analysis showed that studied populations were assigned to different groups according to their respective origin. Localities within the mainland (Antillanca, Nahuelbuta and Puesco) were grouped together in the phenogram, while Chiloé Island was allocated in a separate cluster (fig. 3B).

In the lateral view, the head shape is less dorso-ventrally compressed in populations from Chiloé than in those from the mainland. Although the relative contribution of landmark 9 was low, in populations from Chiloé island its position is more posterior than in those from other localities. Also, cluster analysis showed two very distinct clusters according to their respective area. Localities within the mainland (Antillanca, Nahuelbuta and Puesco) were grouped together in the phenogram, while Chiloé Island was separated (fig. 3C). Furthermore, when data were pooled, we found differences in head shape between mainland and island populations in the lateral view (Wilks' Lambda = 0.472; $P < 0.001$), but not in the dorsal view (Wilks' Lambda = 0.848; $P = 0.358$).

An allometric effect was not found for the shape variables since no significant relationship ($r = 0.002$; $P = 0.98$) was found between centroid size and the first component in the principal components analysis (31% explained variance) in the dorsal view. Single classification ANOVA, however, indicates a significant difference in centroid size ($F3,87 = 12.42$, $P = 0.0001$) among the localities; a *post-hoc* test showed that specimens from Puesco differed from those from the other localities (Tukey Test- HSD, $P < 0.001$). In the lateral view there was no allometric effect ($r = 0.17$; $P = 0.10$) between centroid size and the first component of the principal components analysis (44% explained variance). ANOVA suggested a significant difference in centroid size ($F_{3,87} = 10.14$, $P = 0.0001$) among the localities and a *posthoc* test showed that Puesco is different from Chiloé Island and Nahuelbuta. This test also showed that population from Antillanca was different from this in Chiloé Island (Tukey Test- HSD, $P < 0.001$). When data were pooled in mainland and island populations, differences in the centroid size were found in both dorsal (ANOVA, $F_{1,86} = 13.51$; $P = 0.0004$) and lateral views (ANOVA, $F_{1,86} = 6.12$; $P = 0.015$). Island populations showed lower centroid size in both views (centroid size in dorsal view: island = 255.6 ± 5.6, mainland = 277.7 ± 2.89; centroid size in lateral view: island = 279.4 ± 9.9, mainland = 299.9 ± 4.6).

Discussion

In this study, we examined variation in both head measurements and head shape in *L. pictus* from mainland and island populations. Although there are also some differences in head measurements and shape among specimens from the mainland localities, however, major trends in morphological variation pointed out by traditional and geometric morphometric analysis show that *L. pictus* specimens from Chiloé Island are divergent from mainland localities (Antillanca, Nahuelbuta and Puesco). Populations from the mainland show a continuous distribution range, but the connection between island and mainland was interrupted with the last glacial maximum (18,000 Bp; Abarzúa et al., 2004). Pleistocene events, such as glaciations, could determine different island and mainland refuges that would explain the present distribution of many species (Moreno et al., 1994; Formas and Brieva, 2000). The connection between island and mainland, and their posterior separation, may be relevant to explain present morphological differences between disjunct Chiloé island and mainland populations.

Other species inhabiting this region also show changes in morphology but that seem to be more conservative than in *L. pictus*. Anuran amphibians, including *Eupsophus calcaratus*, *E. emiliopugini*, *Batrachyla leptopus* and *B. taeniata* show moderate population differentiation between both areas of insular divergence (Formas, 1989; Núñez et al., 1999; Formas and Brieva, 2000; Brieva and Formas, 2001). Populations on Chiloé Island could afford different ecological pressures than those from mainland concerning intraspecific competence, food availability and absence of predator (Clegg and Owens, 2002; Olesen and Valido, 2003), and could, therefore evolve towards a distinct morphological differentiation.

In the morphological context, various patterns of size variation have been recognized such as gigantism of insular mammal and bird populations (Lomolino, 1985; Clegg and Owens, 2002). Our results are unexpected because all variables from traditional morphometrics and centroid size (geometric morphometric) for island *L. pictus* show lower values. Donoso- Barros (1966) indicated that island *L. pictus* has a larger body size than other mainland populations, however, this may be due to sampling effects. An alternative explanation for our results could be the existence of a different pattern for island vertebrate evolution, as was pointed out by studies on island birds where it was shown that large birds evolve toward a smaller size while small birds evolve toward a larger size (Roth, 2001; Clegg and Owen, 2002).

Head size is related to the size of prey consumed (Schöener, 1967; Schöener et al., 1982). Morphometric results derived from *L. pictus* may support the hypothesis of insular divergence associated with dietary changes because individuals from mainland populations feed on insects while island population are frugivorous (Ortiz, 1974; Willson et al., 1996). However, since *L. pictus* is a semi-arboreal lizard (Donoso-Barros, 1966), the ocular extension in the island population could also be related to a better capacity to view food while perching. In fact, several studies indicate an association between morphological characteristics and type of habitat (Jaksic and Nuñez, 1979; Jaksic et al., 1980; Losos and Irschick, 1996). According to Schulte et al. (2004), a significant relationship was found between perch variables and escape in *Liolaemus* species, suggesting that this behaviour may also have a possible role in phenotypic evolution (Huey et al., 2003).

Both traditional and geometric morphometric approaches reveal a divergence pattern in *L. pictus*. Unlike traditional morphometrics, the second approach gives information regarding shape and allows for a more complete biological interpretation (Rohlf and Marcus, 1993). However, the use of both traditional and geometric methods allows for a better exploration of morphological variations (Rohlf and Marcus, 1993), in this case, changes in head size (traditional approach) and head shape (geometric approach). The existence of *L. pictus* populations on other small islands from the Chiloé Archipelago offers the prospect to explore the processes of insular divergence in a wider scope.

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Morphological evolution of *Liolaemus pictus* within the Chiloé Archipelago in Southern Chile

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Abstract

When island vertebrate species are compared to equivalent mainland species, they are often characterized by peculiar morphological traits, a classical example being the evolution of body size towards gigantism or dwarfism. Various selective forces are thought to explain the size evolution in island as reduced predation risk and interspecific competition, and resource limitation.

Liolaemus pictus is one of the southernmost - distributed lizard species in Chile and six subspecies have been described according to morphological traits in mainland and island populations. In this paper, we examined whether changes in morphology of different subspecies of *L. pictus* from islands and mainland are congruent with their phylogeographic history in the archipelago. Our results support the hypothesis that morphological evolution of *L. pictus* can be attributed to different selective pressures in island and mainland populations but is not related to their phylogeographic structure following the last glaciation.

Keywords: Geometric morphometrics, mtDNA phylogeography, Last Glacial Maximum, Liolaemidae.

Introduction

Mitochondrial DNA sequences have been used intensively for phylogeographic studies, in which the distribution of haplotype clades across the range of a species or species complex is used to infer the history of that distribution (Beheregaray et al., 2003; Godoy et al. 2004; Vidya et al., 2005). In some cases, a strong geographical pattern in mtDNA haplotype distribution may mask the fact that the populations involved as part lineage (e.g. Palumbi & Baker, 1994; Thorpe et al., 1996) and a combination of molecular data, such as mtDNA sequences (which minimally confounded by selection), and morphology can provide evidence for the driving forces of the morphological and genetic diversity of populations (Thorpe & Malhotra, 1996; Puorto et al. 2001; Parmakelis et al. 2003). Consequently, a critical comparison of both approaches is required to determine whether distinct haplogroups separately denote evolving morphological lineages (Thorpe & Malhotra, 1996; Janzen et al., 2002; Parmakelis et al. 2003). In another context, island biota provides useful models to study many evolutionary hypotheses including those pertaining to repeated cycles of colonization and extinction, adaptation, and drift-induced population differentiation (see Lomolino, 1985). In this sense, the size of organisms has long been recognized to show important biogeographical variation between and within populations (Malhotra & Thorpe, 1991; Renaud & Michaux, 2003). Island vertebrate species, are often characterized by peculiar morphological traits, a classical example being the evolution of body size towards gigantism or dwarfism when compared to equivalent mainland species (Foster, 1964; Mayr, 1967; Renaud & Millien, 2001; Anderson & Handley, 2002).

Liolaemus pictus is one of the southernmost lizard species in Chile (37°S-43°S). Six subspecies have been described according to body size and body pigmentation (Donoso-Barros 1966, 1970, Urbina & Zúñiga 1977). *L. p. pictus* inhabiting the mainland from Concepción to Puerto Montt [37° - 41°S] (Donoso-Barros, 1966, 1970; Veloso & Navarro, 1988), *L. p. septentrionalis* inhabiting the Alto de Vilches (35°36'S - 71°03'W) (Pincheira & Nuñez, 2005), *L. p. argentinus* from Río Negro Province in Argentina (Donoso-Barros, 1966; Cei, 1986), *L. p. chiloeensis* from Isla Grande de Chiloé (Muller & Hellmich, 1939), *L. p. talcanensis* in Isla Talcan (Urbina & Zúñiga, 1977) and *L. p. codoceae* found in islands close of Isla Grande de Chiloé and Isla Guafo (Donoso-Barros, 1966, Pincheira &

Nuñez, 2005). The last three subspecies are distributed within the Chilotan Archipelago which is an island complex located between 41°S and 43°S in southern Chile. The Chilotan Archipelago (41° - 43°S) constitutes an island complex bounded by Isla Grande de Chiloé on the east and the mainland on the noth and west to mainland Chile. This area has been affected by repeated glaciations during the Plio-Pleistocene times, which determined its present biotic and geomorphological configuration (Villagrán *et al.* 1986; Denton *et al.* 1999; Moreno & León 2003).

During the last glacial period, Isla Grande de Chiloé was connected with the mainland through a land bridge along the exposed continental ice-free areas distal to the piedmont glaciers may have served as refuges or corridors for the biota (Heusser 1982; Villagrán *et al.* 1986; Formas 1989; Formas & Brieva 2000), expanding towards the previously glaciated terrains following ice recession at 14,700 ^{14}C yrBP (Denton *et al.* 1999). The spatial and temporal patterns of expansion of vertebrates after the last glacial termination, however, remain largely unknown. In the present, *L. p. pictus* (mainland) has been morphologically described very different to *L. p. chiloensis* (Chiloé Island) (Vidal *et al.*, 2006). Therefore, the relationship between island and mainland, and their posterior separation, may be relevant to explain present morphological differences between both populations. On the other hand, a series of small islands located east of Isla Grande de Chiloé were formed as recessional moraine complexes sometime between 14,700 and 13,000 ^{14}C yrBP (Denton *et al.* 1999). A gradual rise in sea level commenced after 14,700 ^{14}C yrBP (Fairbanks 1989; Lambech & Chappell 2001; Sidall *et al.* 2003). Based on this evidence, it is thus possible that some of the small island east of Isla Grande de Chiloé may have been connected to the mainland or Isla Grande de Chiloé it self during the time window between 14,700 to ~8,000 ^{14}C yrBP, depending on the battymetry of the interior sea. A question arises which is that the colonization of these recent islands by *L. pictus* population from some areas (mainland or Chiloé Island) and the subsequent isolation has promoted in a so short time a morphological differentiation (*L. p. talcanensis* and *L. p. codoceae*).

In this study, we evaluate whether changes in morphology (shape by geometric morphometric analysis) of different subspecies of *L. pictus* follows the phylogeographic history within the archipelago. Because the historical and natural processes influence genetic and morphological diversity, we predict that the island subspecies (*L. p. chiloensis, L. p. talcanensis, and L. p. codoceae*) are different morphologically than the relative subspecies from the mainland (*L. p. pictus*) and the insular lineages (Isla Butachauques and Isla Talcan) are related to population origin (mainland and/or Isla Grande de Chiloé).

Materials and methods

Sampling and laboratory protocols

Specimens were collected from five localities (see Fig. 1) in the Chilotan Archipelago and areas around them: *L. p. pictus* from Antillanca (40°46'S, 72°15'W), and Hornopirén (41°56'S; 72°23'W); *L. p. chiloensis* from Isla Grande de Chiloé (42°23'S; 73°51'W); *L. p. talcanensis* from Isla Talcan (42°44'S; 72°57'W), and *L. p. codoceae* from Isla Butachauques (42°18'S; 73°08'W). Tissue samples were obtained from 96 lizard tail for genetic analysis. Total genomic DNA was isolated from samples using a standard phenol chloroform extraction protocol. The partial Cytochrome b (*Cyt b,* 930 bp) gene was amplified and sequenced for each mainland and insular specimens. Partial sequences of the individuals from of Alto de Vilches (*L. p. septentrionalis*) were used as outgroup. The partial gene of lizard specimens were amplified and sequenced with two primers designed from tRNA-Glu and tRNA-Thr sequences of iguanids lizards *Iguana iguana* (AN: NC002793) and *Sceloporus occidentalis* (AN: NC005960) (LioGluFor: AAACCACCGTTGTTATTCAACTA and LioThrRev: CCATCTTTGGCTTACAAGACCAGAG). Five microliters of extraction product were electrophoresed on 1% agarose gel to estimate the quality and amount of genomic DNA, and sample dilutions were performed (100ng/µL) to polymerase chain reaction (PCR) amplification. The *Cyt b* gene region was amplified via PCR in 25µL of reaction volume containing 0.3µL Taq (Invitrogen), 2.5µL of PCR Buffer 10X, 1.6µL $MgCl_2$ 50mM, 2µL dNTPs 100mM, 0.5µL of each primer (10pmol), and 1µL of template DNA. The thermalcycling amplification conditions were as follows: initial denaturation at 94 °C for 5min, followed by 35 cycles of strand denaturation at 94 °C for 30 s, annealing at 53,3 °C for 1.30 min, primer extension at 72 °C for 1.5min, and a final 10min elongation at 72 °C.

The size of the PCR products for mitochondrial marker was checked by comparing with a 100bp DNA ladder (Invitrogen) in 2% agarose gel. Amplified DNA was purified with the QIAquick PCR Kit according to the supplier's protocol (Qiagen, USA). Finally, all samples were sequenced in the forward and reverse direction with an automated DNA-sequencer. Sequence alignments were accomplished using Proseq (Filatov, 2002), and were checked by eye.

Phylogeographic analysis

The complete set of *Cyt b* sequences (930bp) was used for analyses. In order to reveal relationships and geographical partitioning among haplotypes, median-joining networks were computed using the program Network 4.1.0.0 (Bandelt *et al.*, 1999). To assess mitochondrial genetic diversity within *L. pictus*, the number of haplotypes (K), polymorphic sites (S), nucleotide diversity (H) and pairwise differences (∏) were calculated using the program DNAsp (Rozas et al., 2003). Tajima's D statistics (Tajima, 1989) and Fu's Fs-test (Fu, 1997) were calculated in the same program to test for selective neutrality. Furthermore, significant negative D-values can be interpreted as signatures of population expansion (Aris-Brosou & Excoffier, 1996). Analyses of molecular variance (AMOVA, Excoffier *et al.*, 1992) were performed using SAMOVA program (Dupanloup *et al.*, 2002) to study the proportion of total genetic variation attributable to different hierarchical levels based on the geographic distribution of haplotypes. Several grouping of populations (including and excluding Butachauques, and Talcan Islands because these islands have been recently colonized) were tested to maximize the among-group component of molecular variance, i.e. to determine the maximum degree of phylogeographical structure present in the data (Liebers & Helbig, 2002). As a final assessment of population demographic histories, we performed mismatch analyses (with pairwise distances).

On the other hand, to determine the phylogenetic position of each population, only nonredundant haplotypes were used for the tree - based phylogenetic method. Maximum parsimony (MP) criteria were implemented using PAUP* 4.0b1 (Swofford, 2002). For MP analyses, all characters were equally weighed, and one search was performed with 1,000 replicates and maxtrees set to 5,000, followed by a tree-bisection reconnection (TBR) branch swapping. Support for the clades was estimated with nonparametric bootstrapping (Felsenstein, 1985) using 1,000 pseudoreplicates. Bootstrap values > 50% were considered strong support for a clade (Hillis & Bull, 1993).

Geometric morphometric analysis

A geometric morphometric analysis (Bookstein, 1991) was used to assess the variation attributed exclusively to shape. Lateral views of head of 139 adult specimens were registered with an Olympus digital camera. Ten homologous landmarks, coincident with the intersections of the cranial scales and soft tissues were digitized from a lateral perpective (Fig. 2), and analyzed with the procedures of Rohlf & Slice (1990) and Rohlf *et al.* (1996). Digitalization was performed with TpsDig version 1.22 (Rohlf, 2003a). The x and y coordinates of landmarks were aligned and superimposed using the least squares method based on the generalized procrustes analysis (GPA), allowing for the removal of variations not attributable to shape (e.g. rotation, traslation). Superimposition was performed with TpsSuper version 1.06 (Rohlf, 2003b).

Optimally aligned individuals were compared using TpsRelw version 1.21 (Rohlf, 2003c), which performed a thin-plate spline interpolation function to project the data onto an Euclidean plane. Thus, shape variables with both uniform and non-uniform components (total of $2+2k-4$ variables, where k is the number of landmarks used) were obtained. These variables were then used in multivariate statistical analyses (Rohlf *et al.*, 1996; Adams & Rohlf, 2000). A one-way multivariate analysis of variance (MANOVA) (comparing by locality) was used to determine the differences. To determine an allometric effect in shape, regression analysis was performed between the first axis of the principle components analysis (shape variables) and the centroid size (CS), which is defined as the sum of squared Euclidean distances from each landmark to the centroid of the shape (Bookstein, 1991). The centroid size (Log [CS]) of all specimens was compared using one-way analysis of variance (ANOVA), with locality as a factor.

Phylogenetic Effect

A test for serial independence was used to assess for the phylogenetic independence of the geometric configuration of head shape in *L. pictus*. The program PI (Phylogenetic Independence, v2.0; Abouheif, 1999) was used to conduct the test for serial independence. To test the hypothesis of phylogenetic independence, the topology and associated distribution of shape was randomized 1,000 times and the T statistic was calculated for each randomized topology. The observed T statistic was compared to the randomized distribution to calculate its level of significance (Millien, 2006).

Results

Phylogeographic analysis

A 930pb sequence of *Cytb* was determinated for 96 individuals representing five localities for *L. pictus*. Four main haplogroups within *L. pictus* were assorted by median-joining networks. A first haplogroup included Antillanca from mainland and two haplotypes from Isla Talcan. A second haplogroup included Hornopiren from mainland, and another two haplotypes from Isla Talcan. A third haplogroup included all the localities from Isla Grande de Chiloe. This haplogroup did not share haplotypes with the mainland. Finally, a fourth haplogroup included haplotypes from Isla Butachauques. One haplotype from this island was shared with isla Grande de Chiloé (Fig. 3)

The number of haplotypes fluctuated between four (Isla Talcan) and eighteen (Isla Grande de Chiloé). The higher haplotypic diversity value corresponde to Antillanca, Isla Butachauques, and Isla Grande de Chiloé. One hundred thirty one sites (14.1%) were found to be polymorphic.The mean number of pairwise sequence differences ranged from 2.7 to 20.1 (Table 1). Tajima's test showed negative and non significant values ($p > 0.05$) in all localities, except Isla Talcán. Fu' test also showed negative values except in Isla Talcán, but was statistically significant in Isla Grande de Chiloé and Isla Talcán (Table 1). A nested analysis of variance found two models that maximized the variation among *L. pictus* localities. One model (including small islands) by two groups indicates that 54% of the molecular variance among localities, only 23.2% was accounted for by differences within localities. Another model (excluding small islands) by two groups indicates 79.2% of variance among groups and 13.9% of variance within groups. The mismatch distribution for each group is shown in Figure 4.

The MP searches recovered 351 equally parsimonious trees (L = 319, CI = 0.71, RI = 0.85), and a strict consensus tree was generated (Fig. 5). A *L. pictus* tree of 58 non-redundant haplotypes rooted with the sequences of *L. p. septentrionalis* revealed two principal clades. A clade (corresponding to *L. p. septentrionalis* subspecies) with individuals of the northernmost distribution of the species, and other clade, with the ingroup (localities around and within the archipelago). Within the main clade there are recoverings with strong support (Fig. 5). A first clade included haplotypes from Antillanca assigned to *L. p. pictus* (Donoso-Barros, 1966), and Isla Talcán previously assigned to *L. p. talcanensis* by Urbina & Zuñiga (1977) and Pincheira & Nuñez (2005). A second clade, which includes mainland haplotypes from Hornopirén, also includes Isla Talcán haplotypes. A third clade includes haplotypes from Isla Butachauques assignated to *L. p. codoceae*. Finally, a fourth clade, which includes several localities from Isla Grande de Chiloé assigned to *L. p. chiloensis*, and also two haplotypes from lizards from Isla Butachauques corresponding to *L. p. codoceae* (Fig. 5).

Geometric morphometric analysis

Multivariate analysis of shape variables showed differences between localities when the lateral head shapes were compared (Wilk's Lambda = 0.679; $P < 0.001$). The landmarks 2, 3, 5, and 9, showed a higher contribution in differentiating localities that contributed with the 17%, 16%, 24%, and 12% of variance, respectively. All these landmarks were related to frontal head shape, with thin rostral shape in insular populations (Fig. 3, 5). No significant differences were found between Antillanca and Hornopirén in the mainland (Tukey Test-HSD, $P = 0.069$). However, all islands differ from mainland population (P < 0.005). The three insular localities were similar among them (Isla Grande de Chiloé – Isla Butachauques: P = 0.360; Isla Grande de Chiloé – Isla Talcán: P = 0.053; Isla Butachauques – Isla Talcán: P = 0.145).

We did not detect an allometric effect in shape variables between centroid size and the first component in the principal component analysis ($r = 0.044$; $P = 0.98$; 32,4% explained variance). Single classification ANOVA, however, revealed a significant difference in centroid size among localities ($F_{4,134} = 12.07$, $P = 0.0001$). Specimens from

Antillanca, Hornopirén and Isla Grande de Chiloé differed from those of the other localities (Tukey Test- HSD, $P < 0.001$). When data were pooled in mainland and island populations, differences in centroid size were found (ANOVA, $F_{1,137} = 4.55$; $P = 0.034$) with island populations showing a higher centroid size than in mainland populations (centroid size (mean ± standard error): island = 840.11 ± 28.61, mainland = 761.38 ± 23.38).

Phylogenetic Effect

Because the data encompasses the mainland (Antillanca and Hornopirén) and Chiloé island lineages that corresponds to putative glacial refuges, the phylogenetic effect was assessed in the distribution of geometric morphometric data on the phylogenetic relationship among localities. According to this method, there was no phylogenetic autocorrelation in the dataset ($T = 7.3$, $p = 0.652$, Fig. 5). Thus, this suggests that morphological variation in *L. pictus* is not phylogenetically conserved among lineages and that small island populations (e.g. Butachauques and Talcán) have been evolved independently within the islands after the last glaciation.

Discussion

We employed two complementary (morphologic and genetic) approach to examine the morphological evolution among populations of *L. pictus* is parallel to the phylogeographic history of the group. The origin of the species differentiation is often associated with Late Pleistocene glacial cycles (Hewitt, 1996; 1999) involving (i) fragmentation of a widespread ancestral species into refugia during periods of glacial advance, and (ii) subsequent genetic divergence among small isolated populations, followed by (iii) range expansion during interglacials. Typically, one [beginning ca. 125,000 years ago] or two (ca. 250,000 years ago) of such cycles are invoked (Klicka & Zink 1997). Our results suggest that the mainland and Chiloé Island conformed glacial refugia during the Last Glacial Maximum, and consequently, the disjunction between places resulted in predictable differences in the phylogeographic structure of *L. pictus*. Our results indicate that populations from the mainland and Isla Grande de Chiloé exhibit high levels of genetic divergence and these are congruent with the morphological differenciation (*L. p. pictus* and *L. p. chiloensis*) by a geometric morphometric approach.

Our results indicate that the "mainland lineages" identified by mitochondrial sequences are found in two general haplogroups (e.g. Antillanca and Hornopirén), suggesting that fragmentation of *L. pictus* species occurred in multiple refugia. Contrarily to that, morphological analysis suggest that both populations are similar, which probably indicate that selective pressures can be promoting the same head morphology. In the case of the small islands, our results suggest that they were colonized both from mainland sources and from Isla Grande de Chiloé. For example, the population from Isla Butachauques is related to the mainland by Los Muermos (41°20'S; 73°25'W; Vidal, Moreno & Poulin, unpubl. data). The limited relationship with the populations from Isla Grande de Chiloé (one haplotype) suggests that waif dispersal may have reached Isla Butachauques via rafting from the mainland (Calsbeek & Smith, 2003). However, there is no morphological relationship between them. In fact, Isla Butachauques individuals have a significant higher centroid size than the mainland (Antillanca and Hornopirén) individuals. For the other case, the Isla Talcan case is particularly interesting. Our results suggest intermingling of two "mainland lineages": the Antillanca in the north and the Hornopirén-Chaitén in the south. In this case, both mainland lineages do not have haplotypes in common, but they were assorted in the island. We suggest that Isla Talcán was colonized by individuals that had haplotypes from a northern source (Antillanca) where part of these haplotypes were left as a refuge in the island. Although this island was influenced by these volcanic events and forest fire, the population probably did not disappear. A second wave of inmigrants might have reached Isla Talcan with haplotypes characteristics from the Hornopirén and Chaitén, and populations from the other side of the Andes range (*L. p. argentinus* subspecies). The absence of haplotypes from Isla Talcan in the Chaiten populations suggests local extinction. Intensive disturbance by fire and volcanic activity on the Andean foothills (Moreno 200, 2004) during the early Holocene, coupled with associated vegetation changes (Moreno 1997), might have decimated lizard populations in the Chaiten area. Isla Talcan might have escaped the effects of disturbance owing to its isolation from the mainland, along with its larger distance to the active volcanic centers. Interestly, the individuals examined from Isla

Talcán show significant morphological differences related to mainland individuals although their mainland origin, suggests morphological divergence after the colonization from mainland.

Considering the phylogeographic history of *L. pictus*, one can ask whether there is a historical effect that promotes morphological divergence among island and mainland populations following isolation. In these senses, island populations (Isla Grande de Chiloé, Isla Butachauques, and Isla Talcán) of *L. pictus* show similar features related to feeding ecology (Vidal & Sabat, unpubl data). This suggests that environmental conditions may be more similar among them than between island and mainland localities. The major trend in morphological variation pointed out that geometric morphometric analysis of *L. pictus* from island populations diverge from mainland localities (Antillanca, and Hornopirén). In this context, various patterns of size variation have been recognized such as gigantism of insular mammal and bird populations (Lomolino, 1985; Clegg & Owens, 2002). Our results showed low values of centroid size for mainland compared to island population. However, this is not consistent with results obtained by Donoso- Barros (1966) that indicated larger body size in mainland populations of *L. pictus* and Vidal *et al* (2006) that indicated larger centroid size in mainland for lateral view. An alternative explanation for our results could be the existence of a different pattern for island vertebrate evolution, as suggested by studies on island birds where large birds evolve toward a small size and small birds evolve toward a large size (Roth, 2001; Clegg & Owen, 2002). In fact, the lack of phylogenetic autocorrelation among morphological and genetic data (Millien, 2006) detected in this study, suggests that independent of lineage or refugia origin, the specimens from island differ morphologically than mainland population.

Vidal et al. (2006) suggests that the head morphometric results may support the hypothesis of insular divergence associated with dietary changes because individuals from mainland populations feed on insects while island population are frugivorous (see Ortiz, 1974; Willson *et al.*, 1995; Willson *et al.* 1996). However, the diet of the mainland and all island populations in this study contain fruit as an item relatively common and similar in relative abundance, although the variation in other physiological features for these localities show the same variation exhibited for geometric morphometric data. Thus, the present data support the hypothesis that insular morphological evolution vary in *L. pictus*. Commonly, it has been proposed that founder events govern evolution on island (Millien, 2006), and our results suggest that the peculiar ecological environment on island (e.g., few predators and competitors, climatic differences, colonization events; Irschick *et al.*, 1997; Clegg & Owens, 2002; Calsbeek & Smith, 2003) favors faster evolution in a short time (Millien, 2006).

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Table 1. Diversity parameter for nine *Liolaemus pictus* localities estimated from mtDNA *Cytochrome b* sequences. Number of individuals (n); number of haplotypes (K), haplotypic diversity (H), number of polymorphic sites (S), mean number of pairwise sequences differences (∏). * P < 0.05.

Locality	n	K	H	S	∏	D	Fu
Antillanca	26	14	0.868	25	3.75	-1.65	-4.84
Hornopirén	18	8	0.843	16	2.68	-1.61	-1.49
I. Chiloé	22	18	0.983	34	6.26	-1.27	-8.84*
I. Butachauques	14	12	0.978	53	12.92	-0.98	-1.82
I. Talcán	16	4	0.725	39	20.06	2.96*	14.35*
Total	96	56	0.977	131	30.17	0.38	-5.50*

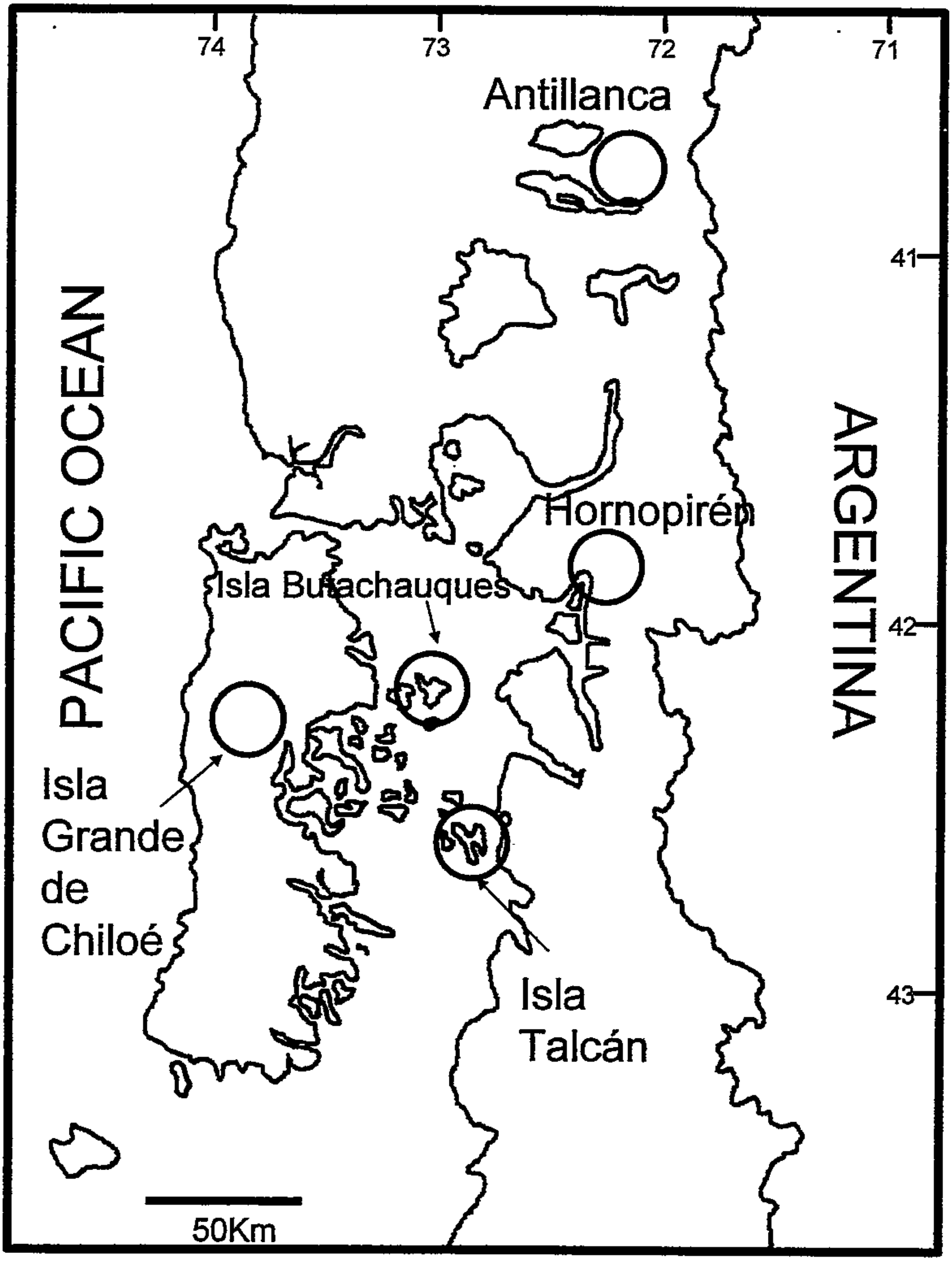

Fig. 1. Geographic distribution of *Liolaemus pictus* included in this study within archipelago area.

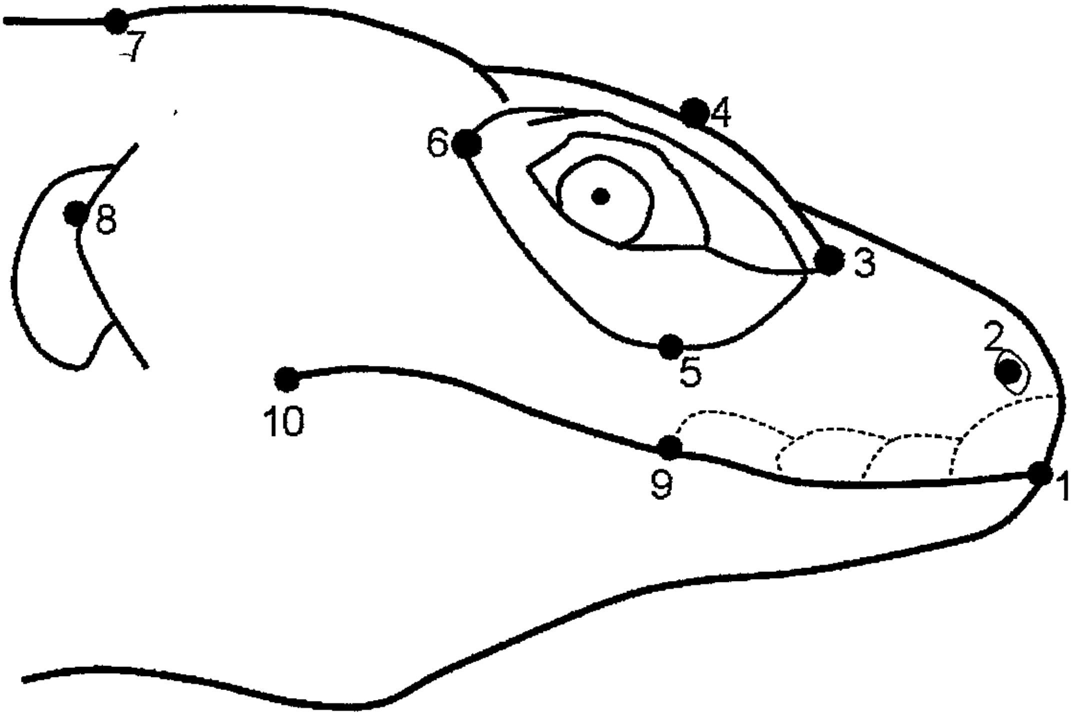

Fig. 2. Location of landmarks used in this study in the lateral view of the head.

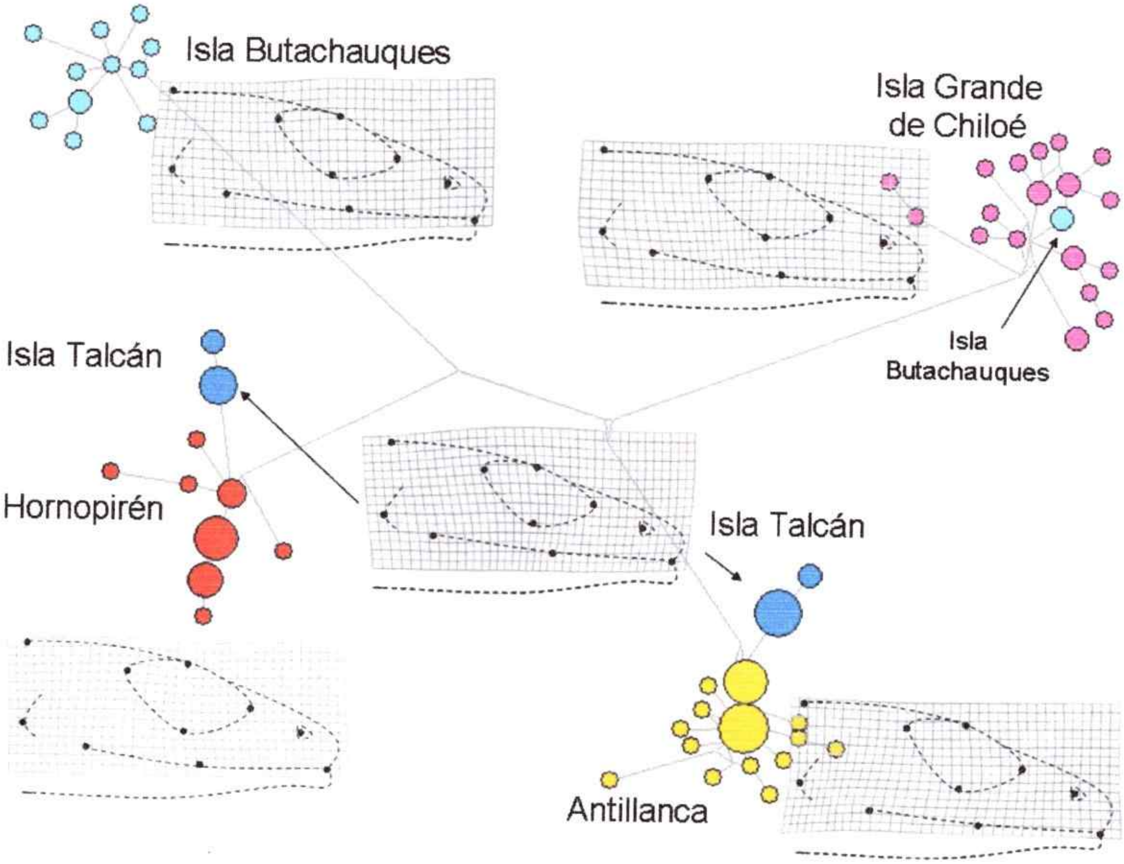

Fig. 3. Median-joining networks of mitochondrial Cytochrome b (*Cytb*) haplotypes of five *Liolaemus pictus* populations. Size of circles is proportional to frequency. Deformation grids (2X) indicate mean head shapes of each locality.

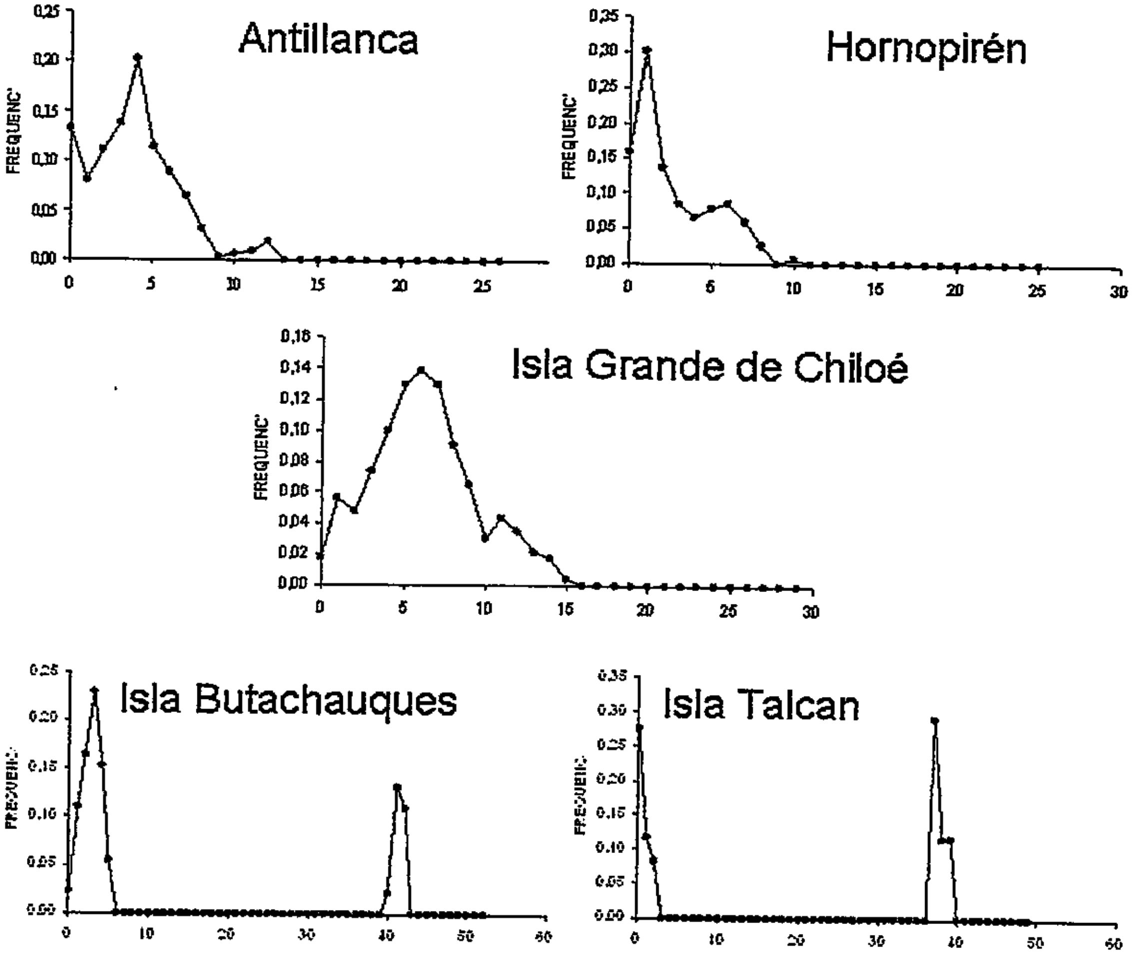

Fig. 4. Mismatch distribution of pairwise differences between sequences of *Liolaemus pictus* from different groups (obtained by AMOVA).

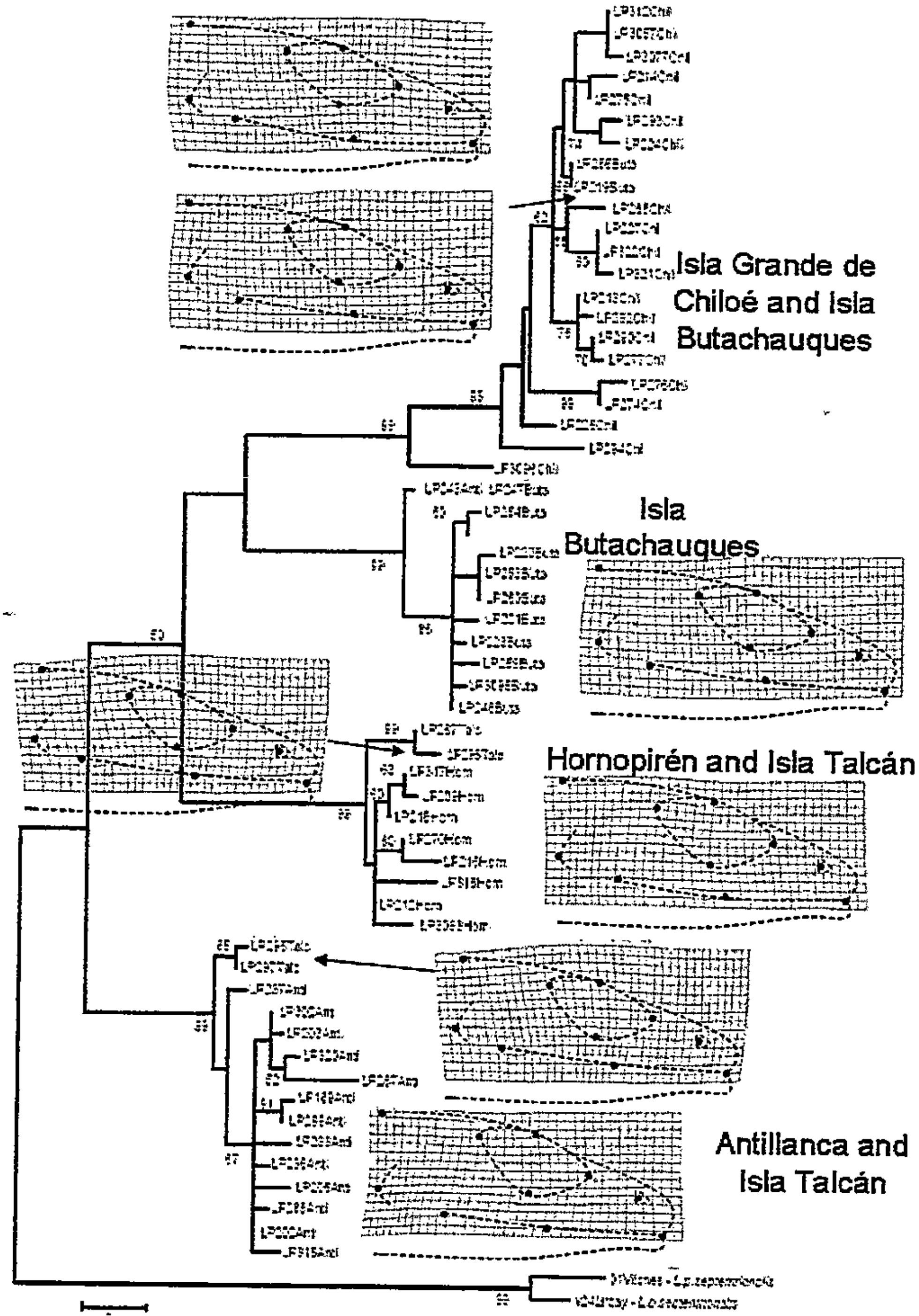

Fig. 5. Parsimony tree (strict consensus) for nonredundant *cytochrome b* haplotypes of the *Liolaemus pictus* populations. Numbers in the branches represent bootstrap values. Deformation grids indicate general head shapes of each locality.

Conclusiones

Desde el punto de vista molecular, los resultados obtenidos en este trabajo sugieren que el continente e Isla Grande de Chiloé muestran altos niveles de diversidad genética debido a que éstos no muestran haplotipos en común. En este sentido, el tiempo de divergencia para las poblaciones de esta especie podría ser más antiguo y no se relacionaría con el Último Máximo Glacial. Los "linajes continentales" muestran cuatro haplogrupos generales, lo cual sugiere que la fragmentacion ocurrió en multiples regiones. Por otra parte, los resultados sugieren que la colonización de las islas pequeñas al interior del archipiélago habría ocurrido desde "sitios fuente". Toda esta estructuración poblacional mostrada por *L. pictus* son consistentes con los cambios producidos durante el Pleistoceno y por los cambios más recientes ocurridos durante el actual interglcial.

Por otra parte, a pesar de las diferencias morfológicas evidenciables entre las subespecies de *L. pictus*, la inferencia molecular tiene implicancias sobre el estatus taxonómico de éstas: las subespecies continentales del área analizada (*L. p. pictus* and *L. p. argentinus*) podrían corresponder a una simple entidad taxonómica nominada como *L. p. pictus* debido a una mezcla de haplotipos. De la misma manera, los individuos de la Isla Grande de Chiloé (*L. p. chiloensis*) comparten haplotipos con individuos de las islas Butachauques y Guafo (*L. p. codoceae*) por lo que corresponderían a *L. p. chiloensis*. Sin embargo, la mayor parte de los individuos provenientes de la Isla Butachauques están relacionados a la localidad de Los Muermos en el continente, por lo que éstos corresponderían a *L. p. pictus*. Por último, los individuos de Isla Talcán (*L. p. talcanensis*) comparten haplotipos con dos linajes continentales (Antillanca y Hornopirén-Chaitén) lo que permite concluir que corresponde también a *L. p. pictus*.

Desde el punto de vista fisiológico, las poblaciones insulares mostraron similares caracteristicas en su ecología trófica lo cual sugiere que las condiciones ambientales (bióticas y abióticas) podrían ser más similares entre las islas que entre continente e islas. En este sentido, las diferencias encontradas entre las poblaciones de *L. pictus* podrían ser interpretadas como el resultado de demandas impuestas por diferentes dietas sobre la morfología (e.g., tracto digestivo) y función (e.g., actividad de enzimas digestivas, incorporación de nutrientes) del sistema de alimentación. Todas estas especializaciones serían el resultado de una habilidad con que los recursos (invertebrados y frutos) pueden ser obtenidos, lo cual sugiere que existe un compromiso funcional más que una restricción producto de la evolución de la omnivoría en *Liolaemus*.

Desde el punto de vista morfológico, existen diferencias entre los especímenes provenientes de las islas respecto de las provenientes del continente (e.g., bajos valores de tamaño del centroide para las localidades contientales). A pesar de que en términos generales, la dieta del continente y de todas las islas incluidas en el análisis es similar, las variaciones morfológicas (incluso las fisiológicas) muestran claramente esta tendencia. Sin embargo, una conclusión interesante de este trabajo sugiere que independientemente de linaje al que pertenezca cada población, los especímenes de las islas muestran tasas de evolución morfológica diferentes a las del continente. Esto último se sustenta en el hecho de que la colonización de éstas ha ocurrido muy recientemente (<10.000 años) y muestran morfologías claramente divergentes.

Finalmente, es posible concluir que las poblaciones insulares de *L. pictus* se han diferenciado fisiológica y morfológicamente de las poblaciones continentales, a pesar de que la evidencia genética muestra relaciones haplotípicas diferentes. De hecho, estas poblaciones se han aislado recientemente, lo cual sugiere que esta diferenciación ha ocurrido rápidamente como el resultado de procesos adaptativos convergentes (e.g. similares rasgos morfológicos y fisiológicos en los individuos insulares). En este sentido, futuros estudios podrían generar nueva evidencia que permitan determinar como estos ajustes en los rasgos fenotípicos de *L. pictus* se relacionan a cambios ambientales.

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