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Filogeografía, delimitación de especies y reconstrucción de relaciones filogenéticas del complejo *Liolaemus tenuis*

Tesis para optar al grado de Doctor en Sistemática y Biodiversidad

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RESUMEN

La identificación de linajes genealógicos utilizando ADN ha revelado mayores niveles de biodiversidad que los propuestos por la taxonomía clásica, tanto a nivel de especie como a nivel intraespecífico. Mediante el estudio de la distribución espacial de la diversidad genética, es posible comprender los procesos históricos que dieron origen a la diversidad biológica, delimitar unidades naturales y, de esta forma, diseñar estrategias eficientes para la conservación de la misma. En el sur de América del Sur, la disposición actual de los linajes puede explicarse mayormente por procesos orogénicos y climáticos pasados, como lo son el levantamiento de los Andes y las glaciaciones pleistocénicas, respectivamente. Actuando en conjunto, estos procesos han generado cambios en la conectividad del territorio y en los niveles de estabilidad de hábitat, afectando tanto la distribución y la demografía de las poblaciones, como la diversidad intraespecífica de las especies. De esta manera, las regiones históricamente estables son candidatas a albergar una mayor diversidad genética, mientras que las regiones históricamente inestables tienden a presentar signos de colonización reciente y por lo tanto un menor grado de diferenciación entre las poblaciones. Sin embargo, dentro de dichas áreas inestables, pudieron existir refugios (áreas estables) donde las especies pueden haberse diversificado producto de largos períodos de aislamiento, lo cual suele observarse en la forma de linajes de divergencias profundas. La filogeografía, mediante el estudio de la diversidad intraespecífica, ha logrado detectar linajes de divergencias profundas dentro de especies actualmente reconocidas, indicando que las mismas podrían contener múltiples especies morfológicamente crípticas. El desconocimiento de esta diversidad críptica puede llevar a conclusiones erróneas sobre, por ejemplo, tasas de diversificación, o sobre los procesos que están generando la biodiversidad observada. Por lo tanto, se vuelve necesario identificar estos linajes para inferir una filogenia robusta, que refleje la biodiversidad real y sirva por lo tanto como punto de partida de diversos estudios de índole biogeográfico y evolutivo. Los enfoques integrativos, particularmente aquellos que combinan evidencia molecular, ambiental y morfológica, han mostrado ser de utilidad para clarificar el status taxonómico de linajes que contienen altos niveles de diversidad críptica.

Chile es una región propicia a albergar altos niveles de biodiversidad sin documentar, debido a su peculiar situación geográfica (aislado al norte por el desierto de Atacama, al este por el Océano Pacífico y al oeste por la Cordillera de los Andes), compleja orografía e historia paleoclimática y geomorfológica. Durante los ciclos

pleistocénicos, esta región ha experimentado efectos diferenciales a lo largo de su geografía, siendo más afectada la zona centro-sur, desde los 33°S hasta aproximadamente los 54º S. Especies con una larga historia evolutiva, de vagilidad media a baja, con signos de variación fenotípica y que han estado sujetas a este tipo de escenarios, son candidatas a albergar altos grados de diversidad críptica, la cual en ciertas ocasiones amerita el status de especie. *Liolaemus tenuis* (Duméril & Bibron 1837) es una lagartija con una historia evolutiva de más de un millón y medio de años, y una amplia distribución que abarca varios biomas. A su vez, se la encuentra actualmente tanto en áreas que han sido históricamente cubiertas de hielo como en áreas históricamente estables. Lo anterior indica que *Liolaemus tenuis* probablemente contenga altos niveles de diversidad críptica, convirtiéndola en un buen modelo para estudios filogeograficos. Debido a la gran variación fenotípica que presenta, su historia taxonómica ha sido históricamente confusa; las dos subespecies descritas, *Liolaemus tenuis tenuis* (Duméril & Bibron 1837) y *Liolaemus tenuis punctatissimus* Müller & Hellmich 1933, no son reconocidas por todos los autores. *Liolaemus tenuis sensu lato* se encuentra clasificada dentro del subgénero *Liolaemus*, el cual se subdivide a su vez en dos secciones principales, sección "*chiliensis*" (en la cual se incluye a *L. tenuis*) y "*nigromaculatus*". Si bien la monofilia del género *Liolaemus* (ca. 250 especies) ha sido apoyada por datos tanto morfológicos como moleculares, estudios recientes han mostrado que las dos secciones descritas dentro del subgénero *Liolaemus* no son monofiléticas. A pesar de que varios trabajos han intentado abordar esta problemática, varios factores han dificultado la estimación de una filogenia robusta (e.g. la incongruencia entre filogenias inferidas a partir de datos morfológicos y genéticos, el uso de pocos marcadores y muestreos deficientes, entre otros). Lo anterior ha obstaculizado el desarrollo de trabajos biogeográficos, los cuales serían de gran utilidad para implementar estrategias de conservación adecuadas. Lo anterior cobra particular importancia en Chile, dado que alberga una alta riqueza de especies de *Liolaemus* (ca. 96 especies), de la cual gran parte son especies endémicas. Por lo tanto, se vuelve necesario inferir una filogenia robusta que permita clarificar la historia evolutiva de este grupo.

Objetivos e hipótesis. Considerando entonces el escenario chileno y la historia evolutiva de *L. tenuis*, el presente trabajo tiene como objetivo estudiar la respuesta de esta especie frente a los cambios climáticos pasados, e indagar acerca de su diversidad intraespecífica, ya que resultados preliminares sugieren que *L. tenuis* podría ser en

realidad un complejo de especies compuesto de varios linajes crípticos. En este contexto, se busca evaluar la validez de las especies candidatas aquí propuestas mediante enfoques integrativos que se sirven de información genética, morfológica y ambiental. Adicionalmente, utilizando una submuestra de especies y un enfoque filogenómico, se infiere una filogenia robusta para el subgénero *Liolaemus*, y se pone a prueba la monofilia de las secciones "*chiliensis*" y "*nigromaculatus*" así como de los principales grupos de especies dentro de ambas secciones.

Se pondrán a prueba tres hipótesis en el marco de tres capítulos:

CAPÍTULO 1. Dado lo expuesto anteriormente, sería esperable que las poblaciones que hayan persistido en áreas históricamente estables (áreas libres de hielo o en refugios intraglaciales) muestren una mayor estructuración, signos de estabilidad demográfica, alta diversidad y singularidad haplotípica, en contraposición con áreas históricamente inestables (e.g. áreas que estaban anteriormente glaciadas) las cuales deberían presentar poblaciones menos estructuradas, signos de reducción poblacional y expansión reciente acompañados de una baja diversidad. Sería esperable también que quiebres filogenéticos entre clados de *L. tenuis* sean consistentes con barreras al flujo génico propuestas para otras especies de reptiles en la región del centro-sur de Chile.

HIPÓTESIS 1. Las oscilaciones climáticas del Pleistoceno afectaron la filogeografía de *Liolaemus tenuis*, con un mayor impacto en las poblaciones del rango sur de *Liolaemus tenuis*, que en las poblaciones del rango norte, las cuales a su vez estuvieron sujetas a más procesos vicariantes.

CAPÍTULO 2. Tal como se expuso anteriormente, *Liolaemus tenuis* ocurre en un área históricamente compleja debido a eventos orogénicos y fluctuaciones climáticas recurrentes. Como consecuencia, el aislamiento prolongado y repetitivo de poblaciones puede haber promovido la divergencia y especiación debido a la interrupción del flujo génico. En estos escenarios, y considerando la gran variación fenotípica que presenta la especie, en conjunto con altos niveles de diversidad genética y linajes de divergencias profundas, la ocurrencia de especies crípticas dentro del taxón nominal *Liolaemus tenuis* es muy probable.

El objetivo de este capítulo es evaluar, mediante un enfoque integrativo, si dichos linajes corresponden a especies válidas.

HIPÓTESIS 2: *Liolaemus tenuis* constituye un complejo de especies, formado por al menos tres linajes evolutivos independientes que ameritan estatus de especie por sí mismas.

CAPÍTULO 3. *Liolaemus* es uno de los géneros más diversos de lagartijas a nivel mundial, incluyendo más de 250 especies distribuidas en el sur de América del Sur. Si bien las especies que lo componen han servido como modelo para muchos estudios ecológicos y evolutivos, las mismas presentan una historia taxonómica compleja producto la gran variación fenotípica que presentan dentro y entre especies, sumado a las incongruencias entre datos genéticos y moleculares, muestreos deficientes, repartición incompleta de linajes, entre otros factores. Muestreos exhaustivos así como estudios moleculares que intenten resolver las relaciones entre estos taxa no han sido realizados.

El objetivo de este capítulo es inferir una filogenia robusta, utilizando una submuestra de lagartijas representando el subgénero *Liolaemus*, y poner a prueba la monofilia de las secciones *chililensis* y *nigromaculatus* así como de algunos de los principales grupos de especies descritos.

HIPÓTESIS 3: Las secciones *nigromaculatus* y *chiliensis* dentro de *Liolaemus sensu stricto* no son monofiléticas.

INTRODUCCIÓN GENERAL

La heterogeneidad espacial y las fluctuaciones climáticas son consideradas como uno de los principales promotores de diversificación, tanto a nivel intraespecífico como supra-específico (Levy et al. 2012; Brown et al. 2014; Wang & Yan 2014). Por el contrario, la homogeneidad en el paisaje es asociada a una mayor conectividad entre las poblaciones, y por lo tanto, a una menor diferenciación genética entre ellas (Chan & Zamudio 2009). Ha sido sugerido que el levantamiento de los Andes, en conjunción con las glaciaciones pleistocénicas, han sido clave en la generación de la biota Neotropical actual (Rull 2011). Adicionalmente, otros factores (tanto bióticos como abióticos) que afectan el nivel de flujo génico entre poblaciones, como la baja vagilidad de algunas especies y barreras vicariantes entre poblaciones (cadenas montañosas, presencia de ríos) propician la especiación alopátrida, que pueden evidenciarse mediante la presencia de linajes estructurados de divergencias profundas dentro de las especies (Rodriguez et al. 2015). Con el fin de mejorar el entendimiento tanto de los patrones globales de diversidad como de los procesos que la generan, es necesario, en una primera etapa, cuantificar la diversidad real, y en una segunda etapa, inferir filogenias robustas ya que las mismas servirán como punto de partida de estudios de sistemática, biogeografía, ecología y conservación.

Patrones y procesos determinantes de la biodiversidad en el sur de América del Sur. América del Sur presenta diversas regiones biogeográficas y climas contrastantes (e.g. Morrone 2006), y alberga la mayor cantidad de biodiversidad del planeta (Myers et al. 2000). Sin embargo, los estudios enfocados en evaluar procesos de diversificación en América del Sur se encuentra muy subrepresentados en comparación a aquellos realizados en el hemisferio norte, los cuales constituyen más del 77% (Beheregaray 2008; Turchetto-Zolet et al. 2012). En el sur de América del Sur, la diversificación de las especies así como la distribución de la diversidad genética han sido afectadas principalmente por eventos que ocurrieron entre el Terciario y el Cuaternario, asociándose con la acción conjunta de las oscilaciones climáticas del Pleistoceno (~2.6 millones de años - 14,000 años) y eventos orogénicos del Mioceno-Pleistoceno (Rull 2011; Turchetto-Zolet et al. 2012). El levantamiento de los Andes, el cual comenzó hace unos 23 millones de años y continúa hasta el presente (Ramos 1989), ha influenciado en gran magnitud la organización de la biodiversidad mediante la generación de relieves complejos, sobre todo al oeste de

la cordillera (Hoorn et al. 1995, 2010; Ruzzante et al. 2006). Adicionalmente, los recurrentes ciclos glaciales e interglaciales habrían alterado tanto el paisaje como sus niveles de conectividad (debido al avance de los hielos, aumento del caudal de los ríos durante las desglaciaciones y efectos periglaciales). De esta manera, algunas poblaciones habrían sido restringidas a refugios (zonas de estabilidad histórica), a partir de los cuales las mismas probablemente expandieron sus rangos durante los interglaciares. El escenario anterior se habría dado de forma cíclica, en especial durante el último millón de años, y esto podría haber promovido procesos de diversificación (Diniz-Filho et al. 2008). Si bien el rol de los refugios en la diversificación de la biota tropical ha sido bastante explorado (e.g. Haffer 2008; Carnaval et al. 2009; Fouquet et al. 2012), no ha recibido la misma atención en el resto de los biomas (Beheregaray 2008; Sérsic et al. 2011). Entender cómo las especies han respondido a eventos climáticos pasados y dónde persistieron durante climas adversos, es de gran importancia para generar modelos que permitan predecir cómo las especies responderán a cambios climáticos futuros (e.g. Duckett & Stowe 2013). La vertiente occidental de la cordillera de los Andes se encuentra aislada al norte por el desierto de Atacama, al oeste por el Océano Pacífico y al este por los Andes, exhibiendo como consecuencia un alto endemismo de especies en múltiples grupos (Smith-Ramirez 2004). Su gran extensión latitudinal ofrece un gradiente climático acompañado de un gradiente de conectividad del territorio, que aumenta de norte a sur debido a que la Cordillera de los Andes exhibe cumbres más altas y una mayor fragmentación hacia el norte. Durante el Último Máximo Glacial (UMG) la extensión de los hielos cubrió gran parte del sur de Chile, desde los 36º hasta los 56º como límite norte (aunque el casquete de hielo en los Andes se extendió hasta los 28º) pero dejando parte de la costa libre de hielo, entre los 36º y los 43º grados aproximadamente (Figura 2) (Hulton et al. 2002; Heusser et al. 2003). De esta forma, la biota se vio excluida de gran parte del territorio, o bien fue restringida a pequeños refugios intraglaciares o a regiones costeras sin hielo (Xu et al. 2009; Sérsic et al. 2011; Vera-Escalona et al. 2012). Si bien las respuestas a las glaciaciones difieren ampliamente entre grupos (e.g. entre plantas y roedores, Palma et al. 2002; Muellner et al. 2005), en general se ha destacado un vínculo entre ambientes históricamente estables con alta diversidad intraespecífica (Victoriano et al. 2008; Carnaval et al. 2009; Valdez & D'Elia 2013; Gehara et al. 2013). Los paradigmas filogeográficos postulan que existe una asociación positiva entre grado de fragmentación de hábitat (e.g. relieve, presencia de ríos) y el grado de estructuración poblacional, y que los niveles de estabilidad

histórica del hábitat se asocian con altos niveles de variabilidad genética y con poblaciones en equilibrio demográfico.

En conclusión, debido a características topográficas de Chile y a los efectos diferenciales del avance de los hielos a lo largo del territorio, existiría i) hacia el rango norte, un gradiente caracterizado por hábitat más estable, con mayor cantidad de procesos vicariantes debido a fragmentación del hábitat, y por lo tanto poblaciones locales más variables y más estructuradas respecto a las demás poblaciones; ii) hacia el rango sur, por el contrario, se presentaría un hábitat históricamente más inestable con más procesos de dispersión acompañados de poblaciones menos estructuradas. Estos patrones cíclicos de fragmentación, expansión y contracción de las poblaciones posiblemente hayan facilitado procesos de diversificación, lo que haría de ésta región un área propicia a albergar altos niveles de diversidad sin documentar. Estos atributos hacen de Chile un escenario ideal para estudios filogeográficos y microevolutivos, ya que permiten evaluar el efecto conjunto de los cambios topográficos y las fluctuaciones climáticas en la evolución de las poblaciones en un gradiente latitudinal.

La filogeografía como enfoque integrativo. Históricamente, la filogeografía ha basado sus inferencias en árboles de genes e indicadores demográficos estimados a partir de ADN mitocondrial. Debido a la potencial discordancia entre árboles de genes y árboles de especies (e.g. a raíz de la repartición incompleta de linajes, Funk & Omland 2003), se hace necesario utilizar múltiples loci independientes para realizar inferencias filogenéticas (Godinho et al. 2008). Aproximaciones tales como modelajes de distribución de las especies (SDM) han sido muy útiles para clarificar distribuciones actuales de especies (e.g. Panzera et al. 2017), identificar hotspots de diversidad (e.g. García 2006), así como comprender los procesos ecológicos, geológicos y climáticos que dieron lugar a los patrones de biodiversidad observados (e.g. Graham et al. 2004; Rissler & Apodaca 2007). Por lo tanto, la integración de este tipo de metodologías con datos genéticos podría ayudar a identificar, por ejemplo, posibles barreras vicariantes (e.g. áreas inestables, donde cambios climáticos hubieran vuelto a esta área inhabitable durante un período particular) o distribuciones pasadas (e.g. durante las glaciaciones) que podrían explicar quiebres genéticos actuales (Beatty & Provan 2010; Lawson 2010). Los SDM, al constituir evidencia independiente, permiten corroborar hipótesis filogeográficas sobre los procesos que generan los patrones de

diversidad genética, cómo por ejemplo, la concordancia de áreas con alta y baja diversidad genética (inferidas a partir de indicadores demográficos) con áreas estables e inestables respectivamente (inferidas a partir de SDM). En este caso, se estaría indicando que las diferencias en diversidad genética a lo largo del rango de distribución de la especie estarían reflejando diferencias en cuanto a la persistencia de las poblaciones durante períodos glaciares (e.g. Buckley et al. 2009). Trabajos que han incorporado este tipo de aproximaciones han destacado la importancia de procesos de vicarianza y divergencia genética mediada por barreras ambientales (Hewitt 2004) (y en algunos casos, la acción sinérgica de barreras como ríos y montañas; Smissen et al. 2013; Victoriano et al. en preparación), y la fuerte relación entre áreas que históricamente albergaron refugios con regiones de alta diversidad y haplotipos exclusivos (Araújo et al. 2008; Carnaval et al. 2009).

Estimación de la biodiversidad real: diversidad críptica. La zona mediterránea en general, y la de Chile (centro-sur) en particular, presenta altos niveles de endemismos y diversidad críptica debido a que se encuentra ubicada entre dos regiones climáticas contrastantes (la templada y la desértica), a su alto grado de heterogeneidad del territorio comparado con otros biomas, a la marcada estacionalidad que resulta en una variabilidad estacional de las comunidades bióticas, y al impacto de las glaciaciones pleistocénicas, que tuvo como consecuencia que muchos componentes de la biota utilizaran la región mediterránea como área refugial (Dallman 1998; Hewitt 2004; Koniak & Noy-Meir 2009). La filogeografía ha mostrado una alta prevalencia de diversidad críptica (Beheregaray & Caccone 2007; Bock et al. 2014; Fennessy et al. 2016), lo cual ha cambiado antiguos paradigmas, tanto sistemáticos como de los patrones y procesos relativos a la distribución geográfica y generación de la biodiversidad (Brown et al. 2007; Elmer et al. 2007), sugiriendo que muchos grupos quedarían por ser explorados (Bickford et al. 2007; Oliver et al. 2009; Trontelj & Fišer 2009).

La práctica de delimitar especies no se ha desarrollado sin dificultades, y el debate respecto a que metodologías producen resultados más precisos continúa hasta el día de hoy (Marshall et al. 2006; Fujita & Leaché 2011; Flot 2015; Rannala 2015). Cuando la diversidad morfológica no es acompañada de diversidad genética y viceversa (radiación adaptativa y especies crípticas, respectivamente) los límites entre las especies no pueden ser resueltos mediante enfoques tradicionales (Funk et al. 2012; Wagner et al. 2012; Blair et al. 2013). En el caso de las

especies crípticas, las primeras aproximaciones se han basado en la reconstrucción de árboles de genes para la detección de clados de divergencias profundas, y posteriormente en el análisis del "barcode gap" de secuencias de ADN mitocondrial (e.g. Hebert et al. 2004). Estos últimos métodos necesitan establecer un porcentaje de distancia genética para distinguir diversidad inter- de intraespecífica, pero dado que este umbral varía incluso entre especies muy emparentadas, puede resultar en inferencias erróneas. Si bien métodos más recientes (e.g. GMYC, Pons et al. 2006; PTP, Zhang et al. 2013) no requieren establecer un valor a priori, la probable incongruencia entre el árbol de genes y el árbol de especies (debido a que estas aproximaciones utilizan un solo locus) sigue siendo problemático para la correcta determinación del número real de especies (Pamilo & Nei 1988; Nichols 2001; Edwards et al. 2005; Bensch et al. 2006; Edwards & Bensch 2009). Como consecuencia del aumento en la capacidad de generar secuencias, se comenzaron a utilizar varios genes de forma concatenada, aproximación que tampoco consideraba la incongruencia entre árboles de genes y árboles de especies (Funk & Omland 2003; Kubatko & Degnan 2007; Edwards et al. 2016). Métodos más recientes basados en la teoría del coalescente (e.g., Fujita et al. 2012) son más adecuados para estimar filogenias robustas así como límites entre especies, ya que evitan el uso de umbrales arbitrarios de distancia genética y el requerimiento de monofilias recíprocas. Dichos métodos consideran tanto la estocasticidad coalescente como la repartición incompleta de linajes (Rannala &Yang 2003) para calcular la probabilidad asociada a los distintos modelos de delimitación de especies, teniendo en cuenta a su vez la incerteza en la filogenia (e.g. BPP, Yang & Rannala 2010). Sin embargo, sobre todo en casos complejos (i.e. radiaciones adaptativas, especies crípticas), se ha recomendado adoptar enfoques que integren información genética con evidencia independiente, de naturaleza morfológica y ecológica (Wiens & Servedio 2000; Wiens 2007; Barley et al. 2013; Edwards & Knowles 2014; Sukumaran & Knowles 2017). Lo anterior haría más robustas las inferencias a la vez que facilitaría la descripción formal de las especies (e.g. Aguilar et al. 2013; Glaw et al. 2013). En este sentido, la morfometría geométrica ha mostrado ser capaz de distinguir especies en grupos morfológicamente conflictivos (e.g. triatominos, Dujardin et al. 2009), con lo cual su aplicación en taxa crípticos podría aportar poder resolutivo en conjunción con datos genéticos. La diferencia fundamental con la morfología clásica es que la morfometría geométrica elimina todo lo que no sea informativo acerca de la forma (Mutanen & Pretorius 2007), y es capaz de detectar diferencias fenotípicas que escapan a la morfología clásica,

logrando un gran poder resolutivo a nivel de especie y subespecie (e.g. Villegas et al. 2002; Nolte & Sheets 2005). Por lo tanto, el uso conjunto de marcadores moleculares con caracteres morfológicos constituye una excelente aproximación para identificar linajes evolutivos, ayudando además a discernir los procesos adaptativos que generan la variación morfológica.

Reconstrucción de las relaciones evolutivas. La filogenia constituye una parte integral y esencial para comprender la historia del paisaje, estudiar las causas de los patrones actuales de riqueza de especies, así como modelar la evolución de caracteres como tamaño corporal, modo de reproducción o de alimentación (Schulte et al. 2000; Espinoza et al. 2004; Pincheira-Donoso et al. 2007). Como se ha destacado anteriormente, en los últimos años se evidenciado que la robustez de las inferencias filogenéticas aumenta al incrementar el número de loci utilizados (e.g. Felsenstein 2006; Camargo et al. 2012) debido a que los árboles de genes no suelen coincidir con los árboles de especies (Pamilo & Nei 1988; Avise 1989; Maddison 1997; Nichols 2001; Gadagkar et al. 2005). Sin embargo, el costo y esfuerzo en amplificar y secuenciar marcadores mediante métodos tradicionales (amplificación por PCR y secuenciación Sanger) son elevados ya que solo puede amplificarse y secuenciarse un locus a la vez. Nuevos procedimientos aplicados a la reconstrucción de relaciones evolutivas (McCormack et al. 2013) han logrado grandes avances en cuanto al tiempo de secuenciación, permitiendo de esta forma caracterizar genomas enteros (e.g. *Anolis*, Alföldi et al. 2011). El secuenciamiento masivo (MPS) o Next-Generation Sequencing (NGS) mediante el secuenciamiento en paralelo de varias reacciones ha permitido identificar miles de marcadores esparcidos en el genoma, conocidos como elementos ultra-conservados (UCEs) (Bejerano et al. 2004; Faircloth et al. 2012). Estas regiones, si bien son altamente conservadas, se encuentran flanqueadas por regiones de alta variabilidad, lo cual las hace idóneas para estudios evolutivos a diferentes niveles (Faircloth et al. 2012; Smith et al. 2014). Los UCEs han ayudado a responder preguntas históricamente controversiales, como la posición filogenética de las tortugas respecto a archosaurios y lepidosaurios (Crawford et al. 2012), las relaciones entre las especies que comprenden Neoaves (McCormack et al. 2013) y el patrón de divergencia de los teleosteos (Faircloth et al. 2013), entre otros (e.g. Gilbert et al. 2015; Blaimer et al. 2016; Bryson et al. 2016; Streicher & Wiens 2016). Asimismo, ya se ha destacado su superioridad por sobre métodos multi-locus para la estimación de

filogenias, tanto a nivel de divergencias antiguas como someras (Blaimer et al. 2015). Por lo tanto, esta metodología tiene gran potencial para resolver las relaciones filogenéticas en grupos históricamente controversiales. Además, al tener poder resolutivo a nivel tanto poblacional como multiespecífico, es idóneo para resolver la posición filogenética de especies cercanamente emparentadas como lo son por ejemplo aquellas que forman complejos de especies.

El modelo de estudio. Los reptiles escamados (lagartijas, serpientes y anfisbenas) constituyen una de las mayores radiaciones de vertebrados, incluyendo más de 10051 especies (Uetz & Hošek 2016). Las lagartijas en particular, son utilizadas como organismos modelo en diversas áreas de estudio como evolución, etología, fisiología, filogeografía y especiación (Huey & Bennet 1987; Camargo et al. 2010). Dentro de los taxa más estudiados en este aspecto en América del Sur se encuentran las lagartijas del género *Liolaemus* Wiegmann 1834, que con más de 250 especies descritas (Uetz & Hošek 2016) conforma uno de los géneros más ricos de reptiles (Pincheira-Donoso et al. 2013). Su origen se ha datado en unos 18-22 millones de años (Schulte et al. 2000; Fontanella et al. 2012). Las especies de este género presentan una amplia distribución, desde los 14º -53º S y altitudinalmente desde el nivel del mar hasta los 4800 metros de altura. Ocurren en una gran variedad de hábitats, desde el árido desierto de Atacama, en matorrales mediterráneos, bosques templados de *Notophagus* y estepas (Cei 1986, 1993; Donoso-Barros 1966). *Liolaemus* fue dividido en dos subgéneros (Laurent 1983, 1985) correspondientes a un clado "chileno" (*Liolaemus*, especies en su mayoría del oeste de la Cordillera de los Andes) y uno "argentino" (*Eulaemus*, especies al este de la Cordillera de los Andes), clasificación apoyada tanto por caracteres morfológicos cómo moleculares (e.g. Schulte et al. 2000; Abdala 2007). En Chile, *Liolaemus* está representado por aproximadamente 96 especies (Ruiz de Gamboa 2016), las cuales exhiben una gran diversidad ecológica, morfológica y de atributos de historia de vida (e.g. especies ovíparas y vivíparas, "*sit and wait*" y forrajeadoras activas). Históricamente han habido grandes controversias respecto a su taxonomía, con varias especies descritas por año pero también sinonimizadas; se estima que la riqueza real del género podría alcanzar las 320 especies (Morando et al. 2004). Las hipótesis sobre las relaciones evolutivas en *Liolaemus* han cambiado mucho en los últimos años debido mayormente a discordancias entre filogenias estimadas a partir de datos moleculares (Schulte

et al. 2000; Vidal & Labra 2008) y morfológicos (Lobo 2001, 2005). En Chile, los trabajos enfocados en *Liolaemus* son principalmente filogeográficos (Victoriano et al. 2008; Torres-Pérez et al. 2009; Vera-Escalona et al. 2012; Cianferoni et al. 2013), no habiendo estudios recientes que intenten resolver las relaciones dentro del subgénero *Liolaemus* como si los hay para el subgénero *Eulaemus* en Argentina (e.g. Camargo et al. 2012; Avila et al. 2013). Disponer de una filogenia robusta permitiría, entre otras cosas, re-evaluar hipótesis sobre el origen de *Liolaemus sensu lato*, ya que existe evidencia que apoya tanto un origen andino-patagónico como un origen andino-peruano (Cei 1979; Diaz-Gómez & Lobo 2006).

El *hotspot* chileno (Myers et al. 2000) se extiende desde la costa del Pacífico hasta los Andes, entre los 25º y los 47º de latitud sur, y alberga una gran cantidad de especies de *Liolaemus*, la mayoría de ellas endémicas (Vidal & Labra 2008). Cómo se expuso anteriormente, ésta región es geográfica y temporalmente compleja debido a eventos orogénicos como el levantamiento de la cordillera de los Andes y a las variaciones climáticas causantes del avance y retroceso de los hielos en el último millón y medio de años. Como consecuencia, los procesos de divergencia poblacional que ocurrieron a distintas escalas geográficas y temporales posiblemente sean responsables de la gran diversidad de herpetozoos que se observa en la región (Vidal & Labra 2008). Algunos estudios filogeográficos en *Liolaemus* del centro-sur de Chile (~33º-45º) han identificado posibles refugios durante las glaciaciones pleistocénicas (e.g. Torres-Pérez et al. 2007; Victoriano et al. 2008; Vera-Escalona et al. 2012), los cuales han sido confirmados para otros taxa (e.g. plantas, Muellner et al. 2005; Ruiz et al. 2007; peces de agua dulce, Ruzzante et al. 2008; anuros, Nuñez et al. 2011; crustáceos, Xu et al. 2009). Las poblaciones que se vieron restringidas a refugios habrían sufrido una reducción de tamaño poblacional (cuello de botella) y mediante procesos de deriva génica o adaptación local podían haberse diferenciado, dando lugar a una alta diversidad intraespecífica, tal como se evidencia en algunas especies (e.g. *L. monticola:* Torres-Pérez et al. 2009, *L. nigroviridis:* Cianferroni et al. 2013, *L. pictus:* Vera-Escalona et al. 2012).

Liolaemus tenuis (Duméril & Bibron 1837) es una especie que se distribuye aproximadamente entre Salamanca y Valdivia, desde el nivel del mar hasta los 1800 metros de altitud, aunque también existen pequeñas poblaciones marginales en la vertiente oriental de los Andes, en la provincia de Neuquén, Argentina (Donoso-Barros 1966). *Liolaemus tenuis* está asociada a zonas con arbustos, siendo considerada semi-arborícola. Es insectívora estricta,

ovípara y presenta un marcado dimorfismo sexual, con machos más coloridos y de mayor tamaño que las hembras (Donoso-Barros 1966; Vidal et al. 2005). Se han descrito dos subespecies, principalmente en base a diferencias en los patrones de coloración: *Liolaemus tenuis tenuis* (Duméril & Bibron 1837), con localidad tipo en los alrededores de Santiago (Región Metropolitana) y *Liolaemus tenuis punctatissimus* Müller & Hellmich 1933, con localidad tipo en Lota (Región de Bíobío). Sin embargo, pocos autores reconocen las subespecies (e.g. Donoso-Barros 1966; Etheridge 1995) debido a que sus áreas de distribución se solapan entre los 37º y 39º grados (Formas 1979), lo cual entra en conflicto con el concepto de subespecie (Mayr & Ashlock 1991). Consecuentemente, se ha propuesto que la variación fenotípica que habría llevado a la descripción de las subespecies corresponde a la variación dentro de una misma especie no meritoria de una subdivisión taxonómica (e.g. Nuñez & Jaksic 1992). Sin embargo, lo anterior no ha sido evaluado formalmente. Por otra parte, estudios preliminares en *L. tenuis* estiman su origen en un millón y medio de años, y recuperan varios clados mitocondriales de divergencias profundas lo que sugiere la presencia de diversidad criptica dentro de este taxón nominal (Muñóz-Mendoza 2012). Estos linajes se encuentran geográficamente estructurados, uno al norte del Río Maipo (~30º-33ºS), otro entre el Río Maipo y el sur del Río Biobío (~33º-39ºS), y un tercero con distribución exclusiva al Sur del Biobío (~36º-39º), encontrándose por lo tanto en simpatría parcial con el clado anterior (Figura 2). Dichos patrones filogeográficos son consistentes con quiebres biogeográficos para varios taxa en la región (Sérsic et al. 2011). Adicionalmente, dichos quiebres coinciden con barreras putativas al flujo génico como lo son algunos ríos (e.g. Maipo, Biobío) y cadenas montañosas (e.g. Cantillana, Cordillera Nahuelbuta) que han promovido diversificación en otras especies de reptiles (Chesser 1999; Torres-Pérez et al. 2007; Sallaberry-Pincheira et al. 2011). Lo anterior destaca la importancia de la vicarianza como promotora de especiación al menos en esta región.

Las especies del subgénero *Liolaemus* (clado "chileno") se agrupadas en dos secciones, la sección *chiliensis* (a la cual pertenece *Liolaemus tenuis*), y la sección *nigromaculatus* (aunque no todos los autores están de acuerdo con esta clasificación). Se reconocen diez y tres grupos de especies en cada sección, respectivamente (Abdala & Quinteros 2013). Sin embargo, estudios moleculares recientes (e.g., Troncoso-Palacios et al. 2015) han demostrado que ninguna de las secciones es monofilética, y que algunas de las especies clasificadas tradicionalmente en la sección *chiliensis*, tales como *L. tenuis*, *L. fuscus*, *L. lemniscatus*, y *L. paulinae*, están más

cercanamente emparentadas a especies de la sección *nigromaculatus*. De la misma forma, muchos de estos taxa presentan problemas taxonómicos debido a altos niveles de variación en su morfología y coloración (e.g., *Liolaemus nigromaculatus*, Troncoso-Palacios & Garín 2013), lo que en algunos casos puede ser explicado por la convergencia de rasgos morfológicos en linajes divergentes (e.g., *Liolaemus monticola*, Torres-Pérez et al. 2009). Lo anterior ha llevado a que algunas subespecies hayan sido elevadas a nivel de especie (e.g., *L. nigromaculatus atacamensis*, actualmente *L. atacamensis*; Simonetti et al. 1995; Valladares 2011; Troncoso-Palacios & Garín 2013), otras especies hayan sido sinonimizadas (e.g., *L. josephorum*, ahora *L. velosoi*; Núñez et al. 2001) y varias especies candidatas hayan sido propuestas (e.g. Cianferoni et al. 2013, Troncoso-Palacios et al. 2015). Esto deja en evidencia la compleja historia evolutiva de estas lagartijas, y vuelve no solo necesaria sino urgente una reconstrucción robusta de las relaciones evolutivas de estas especies para clarificar su historia taxonómica.

Considerando entonces el escenario chileno, la historia evolutiva de *L. tenuis* y los resultados preliminares, es esperable que las poblaciones que hayan persistido en áreas históricamente estables (libres de hielo o en refugios intraglaciales) muestren una mayor estructuración, signos de estabilidad demográfica, alta diversidad y singularidad haplotípica, en contraposición con áreas históricamente inestables (e.g. áreas que estaban anteriormente glaciadas) las cuales deberían presentar poblaciones menos estructuradas, con signos de reducción poblacional y expansión reciente acompañados de una baja diversidad. Sería esperable también que quiebres filogenéticos entre clados de *L. tenuis* sean consistentes con barreras al flujo génico propuestas para otras especies de reptiles. La presencia de especies crípticas dentro de *L. tenuis* es probable cuando consideramos características tales como su larga historia evolutiva, amplia distribución y baja vagilidad. Estudios preliminares (Muñóz-Mendoza 2012) han detectado clados alopátridos de divergencias profundas, los cuales no estarían acompañados de una evidente diferenciación morfológica. Adicionalmente, a lo largo de su prolongada historia evolutiva sus poblaciones se han visto repetidamente aisladas debido a rugosidades del paisaje, los cuales en interacción con los cambios vegetacionales y climáticos habrían generado interrupciones en el flujo génico, promoviendo así la divergencia y especiación (Dépraz et al. 2009; Setiadi et al. 2011). En tal caso, se vuelve necesario evaluar la existencia de un complejo de especies dentro de *L. tenuis sensu lato.* De existir filogrupos discretos, sería

interesante explorar si los mismos pueden ser reconocidos también morfológicamente, como ha sucedido en varios reptiles europeos (Kaliontzopoulou et al. 2012b; Llorente et al. 2012; Santos et al. 2012). Sin embargo, cómo se expuso anteriormente, las especies crípticas constituyen un desafío a la hora de delimitar especies por la falta de caracteres diagnósticos que apoyen los grupos genéticos identificados. En lagartijas, la morfometría geométrica se ha utilizado exitosamente para detectar diferencias relativas a la forma de la cabeza, la cual ha mostrado ser muy informativo de un punto de vista tanto funcional como evolutivo (Fabre et al. 2014; Minoli et al. 2016) ya que se la relaciona directamente con la alimentación, competencia por hembras y cópula, uso de refugios, microhábitat y comportamiento anti-depredador (Herrel et al., 1996, 1999; Kaliontzopoulou et al. 2012a).

De corroborarse que *L. tenuis* está compuesto por más de una especie, se vuelve necesario realizar un estudio comprensivo que apunte a resolver las relaciones dentro del clado que contiene a *L. tenuis*, ya que no es poco común que las especies que componen un complejo no sean especies hermanas (Xu et al. 2003; Cunnington et al. 2005; Przyboś et al. 2015), como en el caso de las especies que forman el complejo *monticola* (Torres-Pérez et al. 2007, 2009). Adicionalmente, varias de las especies pertenecientes al "clado *tenuis*" presentan una situación taxonómica y/o posición sistemática incierta (e.g. Torres-Pérez et al. 2009) y muchas pertenecen a la Región de Atacama (*L. atacamensis, L. fuscus, L. isabelae, L. nigromaculatus, L. nitidus, L. platei, L. velosoi, L. zapallarensis*), la cual ha sido señalado como de la Región menos conocida de Chile en relación a su herpetofauna (Vidal & Labra 2008; Troncoso-Palacios 2014). Varios de estos taxa eran hasta hace poco subespecies y fueron elevadas a estatus de especie en base a diferencias en coloración (e.g. *L. nigromaculatus atacamensis*, ahora *L. atacamensis*) (Simonetti et al. 1995; Valladares 2011), mientras que estudios recientes han recuperado otras como parafiléticas (Troncoso-Palacios et al. 2015). Adicionalmente, los clados inferidos en filogenias moleculares no se corresponden con los grupos y secciones propuestas por otros autores en base a caracteres morfológicos (e.g. Lobo 2005; Lobo et al. 2010), y a los que se sigue haciendo alusión al momento de referirse a las distintas especies de *Liolaemus*. Escasos estudios han intentado resolver las relaciones filogenéticas entre especies de *Liolaemus* presentes en Chile, sobre todo si los comparamos con aquellos realizados dentro del subgénero *Eulaemus* (e.g., Camargo et al. 2012; Avila et al. 2013; Olave et al. 2011, 2014, 2015, 2016).

Lo expuesto en los párrafos anteriores justifica la importancia de estudiar la diversidad intraespecífica y evaluar rigurosamente los límites de los linajes principales (que resultados preliminares sugieren podrían ameritar status de especie) dentro de *L. tenuis.* Adicionalmente, destaca la utilidad de evaluar la validez de los grupos y secciones previamente definidos para *Liolaemus sensu stricto* en base a caracteres morfológicos, ya que parecen no reflejar relaciones de parentesco. Muchas de estas especies ocurren en una región poco explorada como lo es Atacama, con lo cual la disponibilidad de una filogenia robusta probablemente motive a otros investigadores a desarrollar estudios en estas especies que contribuyan al entendimiento de su evolución, biogeografía, y sobre todo a establecer estrategias de conservación y manejo.

El problema a estudiar. Se utilizará como modelo de estudio a *L. tenuis*, una especie que presenta una larga historia evolutiva, vagilidad media a baja y una amplia distribución que abarca áreas que históricamente han estado glaciadas así como áreas libres de hielo, con el fin de indagar los efectos de las glaciaciones y la ocurrencia de diversidad críptica dentro de este taxón nominal. Esta especie es adecuada para lograr estos objetivos ya que, además de lo mencionado anteriormente, es localmente abundante y se la encuentra en una gran variedad de ambientes dentro de un amplio rango latitudinal y altitudinal, exhibiendo además una variación considerable en patrones de coloración. Resultados preliminares muestran linajes de divergencias profundas y signos de variación fenotípica, siendo así candidata a contener linajes evolutivos independientes.

El capítulo 1 de esta tesis tiene como objetivo general evaluar la estructura filogeográfica de *Liolaemus tenuis sensu lato*, integrando indicadores demográficos y reconstrucción de las relaciones genealógicas (calculados a partir de información genética de un locus mitocondrial y dos loci nucleares) con modelaje de nicho ecológico (SDM) inferido a partir de datos de presencia de individuos (Figura 1). Considerando los atributos de *L. tenuis* expuestos en los párrafos anteriores, esperamos que esta especie haya experimentado procesos demográficos y de diversificación a nivel microevolutivo como respuesta a los cambios orogénicos y climáticos a los que ha estado

sujeto el centro-sur de Chile. Adicionalmente, teniendo en cuenta la complejidad del escenario chileno, sería esperable encontrar dentro de *Liolaemus tenuis* clados de divergencias profundas.

El capítulo 2 de esta tesis tiene como objetivo poner a prueba la existencia de especies crípticas dentro del complejo putativo *Liolaemus tenuis*, con el fin de evaluar si los linajes de divergencias profundas sugeridos por resultados preliminares corresponden a la taxonomía actual (dos subespecies), a un complejo de especies o a una única especie tal y como lo sugieren algunos autores (e.g. Nuñez & Jaksic 1992) (Figura 1). Métodos integrativos que se sirvan de aproximaciones que modelen el proceso coalescente y datos morfológicos son necesarios para determinar de forma precisa los límites de los distintos linajes evolutivos en el caso de especies crípticas. Para esto, se utilizan herramientas que se sirven de datos genéticos (seis loci nucleares y uno mitocondrial) en un marco bayesiano, morfológicos (morfometría geométrica) y ecológicos (modelaje de nicho).

El capítulo 3 de esta tesis tiene como objetivo proponer la primera hipótesis filogenómica sobre las relaciones evolutivas entre las especies pertenecientes al "clado chileno" de *Liolaemus* (subgénero *Liolaemus sensu stricto*), así como poner a prueba la monofilia de los principales grupos de especies que componen este clado. Se incluyen también representantes de los tres linajes principales de *Liolaemus tenuis* identificados en el capítulo 1. Se colectaron datos correspondientes a 585 loci (541 elementos ultraconservados y 44 genes codificantes) para inferir un árbol de especies, utilizando distintas aproximaciones (concatenación, método de cuartetos y métodos basados en árboles de genes) y conjuntos de datos (únicamente elementos ultra-conservados, únicamente genes codificantes, ambos sets de datos en conjunto). Esta gran cantidad de loci constituye una buena oportunidad para resolver las discordancias entre las filogenias moleculares y morfológicas, así como para realizar inferencias de cómo la historia evolutiva del grupo se relaciona a los principales eventos históricos que han tenido lugar en esta región de América del Sur.

BIBLIOGRAFÍA

Abdala C.S. 2007. Phylogeny of the *boulengeri* group (Iguania: Liolaemidae, *Liolaemus*) based on morphological and molecular characters. Zootaxa, 1538: 1–84.

Abdala, C. S. & A. S. Quinteros. 2013. Los últimos 30 años de estudios de la familia de lagartijas más diversa de Argentina. Actualización taxonómica y sistemática de Liolaemidae. Cuadernos de Herpetología, 28, 1-28.

Aguilar C., Wood Jr P. L., Cusi J. C., Guzmán A., Huari F., Lundberg M., Mortensen E, Ramírez C., Robles D., Suárez J., Ticona, A., Vargas V.J., Venegas P.J. & Jr. J.W. Sites. 2013. Integrative taxonomy and preliminary assessment of species limits in the *Liolaemus walkeri* complex (Squamata, Liolaemidae) with descriptions of three new species from Peru. ZooKeys, 364: 47.

Alföldi J., Di Palma F., Grabherr M., Williams C., Kong L., Mauceli E., Russell P., Lowe C.B., Glor R.E., Jaffe J.D., Ray D.A., Boissinot S., Shedlock A.M., Botka C., Castoe T.A., Colbourne J.K., Fujita M.K., Godinez-Moreno R., Hallers BF, Haussler D, Heger A, Heiman, D Janes DE, Johnson J, de Jong PJ, Koriabine MY, Lara M, Novick PA, Organ C.L., Peach S.E., Poe S., Pollock D.D., de Queiroz K., Sanger T., Searle S., Smith J.D., Smith Z., Swofford R., Turner-Maier J., Wade J., Young S., Zadissa A., Edwards S.V., Glenn T.C., Schneider C.J., Losos J.B., Lander E.S., Breen M., Ponting C.P. & K. Lindblad-Toh. 2011. The genome of the green anole lizard and a comparative analysis with birds and mammals. Nature, 477:587-591.

Araújo M.B., Nogués-Bravo D., Diniz-Filho J.A.F., Haywood A.M., Valdes P.J. & C. Rahbek. 2008. Quaternary climate changes explain diversity among reptiles and amphibians. Ecography, 31: 8–15.

Avila L. J., Olave M., Perez C. H. F., Perez D. R., & M. Morando. 2013. Molecular phylogenetic relationships of the *Liolaemus rothi* complex and a new species of lizard from Auca Mahuida Volcano (Squamata: Liolaemini). Zootaxa, 3608(4): 221-238.

Avise J.C. 1989. Gene trees and organismal histories: a phylogenetic approach to population biology. Evolution, 43:1192–1208.

Barley A. J., White J., Diesmos A. C. & R. M. Brown. 2013. The challenge of species delimitation at the extremes: diversification without morphological change in Philippine sun skinks. Evolution, 67 (12): 3556-3572.

Beheregaray L. B. 2008. Twenty years of phylogeography: the state of the field and the challenges for the Southern Hemisphere. Molecular Ecology, 17(17): 3754-3774.

Beheregaray L. & A. Caccone. 2007. Cryptic diversity in a changing world. Journal of Biology, 6: 9.

Beatty G.E. & J. Provan. 2010. Refugial persistence and postglacial recolonization of North America by the cold-tolerant herbaceous plant *Orthilia secunda*. Molecular Ecology, 19: 5009-5021.

Bejerano G., Pheasant M., Makunin I., Stephen S., Kent W. J., Mattick J. S., & D. Haussler. 2004. Ultraconserved elements in the human genome. Science, 304(5675): 1321-1325.

Bensch S., Irwin D.E., Irwin J.H., Kvist L. & S. Akesson. 2006. Conflicting patterns of mitochondrial and nuclear DNA diversity in *Phylloscopus* warblers. Molecular Ecology, 15: 161–171.

Bickford D., Lohman D.J., Sodhi N.S., Ng P.K.L., Meier R., Winkler K., Ingram K.K. & I. Das. 2007. Cryptic species as a window on diversity and conservation. Trends in Ecology and Evolution, 22: 148–155.

Blaimer B. B., Brady S. G., Schultz T. R., Lloyd M. W., Fisher B. L. & Ward P. S. 2015. Phylogenomic methods outperform traditional multi-locus approaches in resolving deep evolutionary history: a case study of formicine ants. BMC Evolutionary Biology, 15(1), 1.

Blaimer B.B., Lloyd M.W., Guillory W.X. & Brady S.G. 2016. Sequence Capture and Phylogenetic Utility of Genomic Ultraconserved Elements Obtained from Pinned Insect Specimens. PLoS ONE 11(8):e0161531

Blair C., Davy C., Ngo A., Orlov N.L., Shi H., Lu S., Gao L., Rao D. & R.W. Murphy. 2013. Genealogy and demographic history of a widespread amphibian throughout Indochina. Journal of Heredity, 104:72–85

Bock F., Fennessy J., Bidon T., Tutchings A., Marais A., Deacon F. & A. Janke. 2014. Mitochondrial sequences reveal a clear separation between Angolan and South African giraffe along a cryptic rift valley. BMC Evolutionary Biology, 14(1), 1.

Brown D.M., Brenneman R.A., Georgiadis N.J., Koepfli K., Pollinger J.P., Mila B., Louis E.L.Jr., Grether G.F., Jacobs D.K. & R.K. Wayne. 2007. Extensive population genetic structure in the giraffe. BMC Biology, 5:57.

Brown J. L., Cameron A., Yoder A. D. & M. Vences. 2014. A necessarily complex model to explain the biogeography of the amphibians and reptiles of Madagascar. Nature communications, 5.

Bryson Jr, R. W., Faircloth B. C., Tsai W. L., McCormack J. E. & J. Klicka. 2016. Target enrichment of thousands of ultraconserved elements sheds new light on early relationships within New World sparrows (Aves: Passerellidae). The Auk, 133(3), 451-458.

Buckley T.R., Marske K.A. & D. Attanayake. 2009. Identifying glacial refugia in a geographic parthenogen using palaeoclimate modelling and phylogeography: the New Zealand stick insect *Argosarchus horridus* (White). Molecular Ecology, 18: 4650-4663.

Camargo A., Sinervo B. & Jr. J.W. Sites. 2010. Lizards as model organisms for linking phylogeographic and speciation studies. Molecular Ecology, 19: 3250–3270.

Camargo A., Avila L. J., Morando M. & Jr. J.W. Sites. 2012. Accuracy and precision of species trees: effects of locus, individual, and base pair sampling on inference of species trees in lizards of the *Liolaemus darwinii* group (Squamata, Liolaemidae). Systematic Biology, 61(2): 272-288.

Carnaval A.C., Hickerson M.J., Haddad C.F.B., Rodrigues M.T. & C. Moritz. 2009. Stability predicts genetic diversity in the Brazilian Atlantic forest hotspot. Science, 323:785–89.

Cei J. M. 1986. Reptiles del centro, centro-oeste y sur de la Argentina: herpetofauna de las zonas áridas y semiáridas. Torino: Museo regionale di scienze naturali.

Cei J.M. 1979. The Patagonian Herpetofauna. En: Duellman, W.E. (Ed.), The South American Herpetofauna: Its Origin, Evolution, and Dispersal. Museum of Natural History University of Kansas, Monograph 7, p. 309-339.

Cei J. M. 1993. Reptiles del noroeste, nordeste y este de la Argentina: Herpetofauna de las selvas subtropicales, Puna y Pampas. Torino: Museo regionale di scienze naturali.

Chan L. M. & K. R. Zamudio. 2009. Population differentiation of temperate amphibians in unpredictable environments. Molecular Ecology 18:3185–3200

Chesser R. T. 1999. Molecular systematics of the rhinocryptid genus *Pteroptochos*. Condor, 439-446.

Cianferoni F., Yanez R. P., Palma R. E., Garin C. F. & F. Torres-Perez. 2013. Deep Divergences within *Liolaemus nigroviridis* (Squamata, Liolaemidae) Lineages Associated with Sky Islands in Central Chile. Zootaxa, 3619(1): 59-69.

Crawford N.G., Faircloth B.C., McCormack J.E., Brumfield R.T., Winker K. & T.C. Glenn. 2012. More than 1000 ultraconserved elements provide evidence that turtles are the sister group to archosaurs. Biology Letters, 8: 783–786.

Cunnington J. H., Lawrie A. C. & Pascoe I. G. 2005. Genetic variation within *Podosphaera tridactyla* reveals a paraphyletic species complex with biological specialization towards specific *Prunus* subgenera. Mycological research, 109(03): 357-362.

Dallman P. R., 1998. Plant Life in the World's Mediterranean Climates. California Native Plant Society-University of California Press, Berkeley.

Dépraz A., Hausser J. & M. Pfenninger. 2009. A species delimitation approach in the *Trochulus sericeus/hispidus* complex reveals two cryptic species within a sharp contact zone. BMC Evolutionary Biology, 9(1): 171.

Díaz Gómez J. M. & F. Lobo. 2006. Historical biogeography of a clade of *Liolaemus* (Iguania: Liolaemidae) based on ancestral areas and dispersal-vicariance analysis (DIVA). Papéis Avulsos de Zoologia (São Paulo), 46(24): 261-274.

Diniz-Filho J. A. F., de Campos Telles M. P., Bonatto S. L., Eizirik E., De Freitas T. R. O., De Marco Jr, P., Santos F.R., Sole-Cava A. & T.N. Soares. 2008. Mapping the evolutionary twilight zone: molecular markers, populations and geography. Journal of Biogeography, 35(5): 753-763.

Donoso-Barros R. 1966. Reptiles of Chile. Universidad de Chile, Santiago.

Duckett P. E. & A. J. Stow. 2013. Higher genetic diversity is associated with stable water refugia for a gecko with a wide distribution in arid Australia. Diversity and Distributions, 19(8): 1072-1083.

Dujardin J.P., Costa J., Bustamante D., Jaramillo. N. & S. Catalá. 2009. Deciphering morphology in Triatominae: the evolutionary signals. Acta Tropica, 110: 101–111.

Edwards D. L. & L.L. Knowles. 2014. Species detection and individual assignment in species delimitation: can integrative data increase efficacy? Proceedings of the Royal Society B: Biological Sciences, 281(1777): 20132765.

Edwards S.V., Kingan S.B., Calkins J.D., Balakrishnan C.N., Jennings W.B., Swanson W.J. & M.D. Sorenson. 2005. Speciation in birds: genes, geography, and sexual selection. Proceedings of the National Academy of Sciences of the United States of America, 102(Suppl. 1), 6550–6557.

Edwards S.V. &S. Bensch. 2009. Looking forwards or looking backwards in avian phylogeography? A comment on Zink and Barrowclough 2008. Molecular Ecology, 18: 2930–2933.

Edwards S.V., Xi Z., Janke A., Faircloth B.C., McCormack J. E., Glenn T. C. Zhong B., Wu S., Lemmon E.M., Lemmon A.R., Leaché A.D., Liu L. & C.C. Davis. 2016. Implementing and testing the multispecies coalescent model: a valuable paradigm for phylogenomics. Molecular Phylogenetics and Evolution, 94: 447-462.

Elmer K.R., Davila J.A. & S.C. Lougheed. 2007. Cryptic diversity and deep divergence in an upper Amazonian frog, *Eleutherodactylus ockendeni*. BMC Evolutionary Biology, 7:247.

Espinoza R. E., Wiens J. J. & C.R. Tracy. 2004. Recurrent evolution of herbivory in small, cold-climate lizards: breaking the ecophysiological rules of reptilian herbivory. Proceedings of the National Academy of Sciences of the United States of America, 101(48): 16819-16824.

Etheridge R. 1995. Redescription of *Ctenoblepharys adspersa* Tschudi, 1845, and the taxonomy of Liolaeminae (Reptilia, Squamata, Tropiduridae). American Museum Novitates; no. 3142.

Fabre A. C., Cornette R., Huyghe K., Andrade D. V. & A. Herrel. 2014. Linear versus geometric morphometric approaches for the analysis of head shape dimorphism in lizards. Journal of Morphology, 275(9): 1016-1026.

Faircloth, B. C., McCormack, J. E., Crawford, N. G., Harvey, M. G., Brumfield, R. T. & Glenn, T. C. 2012. Ultraconserved elements anchor thousands of genetic markers spanning multiple evolutionary timescales. Systematic biology, sys004.

Faircloth B. C., Sorenson L., Santini F. & M. E. Alfaro. 2013. A phylogenomic perspective on the radiation of ray-finned fishes based upon targeted sequencing of ultraconserved elements (UCEs). PloS one, 8(6), e65923.

Felsenstein J. 2006. Accuracy of coalescent likelihood estimates: do we need more sites, more sequences, or more loci? Molecular Biology and Evolution, 23:691–700.

Fennessy J., Bidon T., Reuss F., Kumar V., Elkan P., Nilsson M. A., Vamberger M., Fritz U.& A. Janke. 2016. Multi-locus Analyses Reveal Four Giraffe Species Instead of One. Current Biology, 26 (18): 2543–2549.

Flot J. F. 2015. Species delimitation's coming of age. Systematic Biology, 64(6): 897-899.

Fontanella F. M., Feltrin N., Avila L. J., Sites J. W. & M. Morando. 2012. Early stages of divergence: phylogeography, climate modeling, and morphological differentiation in the South American lizard *Liolaemus petrophilus* (Squamata: Liolaemidae). Ecology and Evolution, 2(4): 792-808.

Formas R. 1979. La herpetofauna de los bosques temperados de Sudamérica. Museum of Natural History University of Kansas, 341-369.

Fouquet A., Noonan B. P., Rodrigues M. T., Pech N., Gilles A. & N.J. Gemmell. 2012. Multiple quaternary refugia in the Eastern Guiana Shield revealed by comparative phylogeography of 12 frog species. Systematic Biology: syr130.

Fujita M. K. & A. D. Leaché. 2011. A coalescent perspective on delimiting and naming species: a reply to Bauer et al. Proceedings of the Royal Society B: Biological Sciences, 278:493-495.

Fujita M.K., Leaché A.D., Burbrink F.T., McGuire J.A. & C. Moritz. 2012. Coalescent-based species delimitation in an integrative taxonomy. Trends in Ecology and Evolution, 27(9): 480-488.

Funk W.C., Caminer M. & S.R. Ron. 2012. High levels of cryptic species diversity uncovered in Amazonian frogs. Proceedings of the Royal Society of B: Biological Sciences, 279: 1806–1814.

Funk D.J. & K.E. Omland. 2003. Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. Annual Review of Ecology and Systematics, 34: 397–572 423.

Gadagkar S.R., Rosenberg M.S. & S. Kumar. 2005. Inferring species phylogenies from multiple genes: concatenated sequence tree versus consensus gene tree. Journal of Experimental Zoology 304: 64–74.

García, A. 2006. Using ecological niche modelling to identify diversity hotspots for the herpetofauna of Pacific lowlands and adjacent interior valleys of Mexico. Biological Conservation, 130(1), 25-46.

Gehara M., Canedo C., Haddad C.F.B. & M. Vences. 2013. From widespread to microendemic: molecular and acoustic analyses show that *Ischnocnema guentheri* (Amphibia: Brachycephalidae) is endemic to Rio de Janeiro, Brazil. Conservation Genetics, 14: 973–982.

Gilbert P. S., Chang J., Pan C., Sobel E. M., Sinsheimer J. S., Faircloth B. C. & M.E. Alfaro. 2015. Genome-wide ultraconserved elements exhibit higher phylogenetic informativeness than traditional gene markers in percomorph fishes. Molecular Phylogenetics and Evolution, 92: 140-146.

Glaw F., Kucharzewski C., Köhler J., Vences M. & Z. T. Nagy. 2013. Resolving an enigma by integrative taxonomy: *Madagascarophis fuchsi* (Serpentes: Lamprophiidae), a new opisthoglyphous and microendemic snake from northern Madagascar. Zootaxa, 3630 (2): 317-322.

Godinho R., Crespo E. G. & N. Ferrand. 2008. The limits of mtDNA phylogeography: complex patterns of population history in a highly structured Iberian lizard are only revealed by the use of nuclear markers. Molecular Ecology, 17(21): 4670-4683.

Graham C.H., Ron S., Santos J., Schneider C. & C. Moritz. 2004. Integrating phylogenetics and environmental niche models to explore speciation mechanisms in dendrobatid frogs. Evolution, 58:1781–1793.

Haffer J. 2008. Hypotheses to explain the origin of species in Amazonia. Brazilian Journal of Biology, 68(4): 917-947.

Hebert P.D.N., Enton E.H., Burns J.M., Janzen D.H & W. Hallwachs. 2004. Ten species in one: DNA barcoding reveals cryptic species in the Neotropical skipper butterfly *Astraptes fulgerator*. Proceedings of the National Academy of Sciences U.S.A, 101: 14812–14817.

Herrel A., Van Damme R. & F. De Vree. 1996. Sexual dimorphism of head size in *Podarcis hispanica atrata*: Testing the dietary divergence hypothesis by bite force analysis. Netherlands Journal of Zoology, 46:253–262.

Herrel A., Spithoven L., Van Damme R. & F. De Vree. 1999. Sexual dimorphism of head size in *Gallotia galloti*: testing the niche divergence hypothesis by functional analyses. Functional Ecology, 13(3): 289-297.

Heusser C. J. 2003. Ice age southern Andes: a chronicle of palaeoecological events. Elsevier.

Hewitt G. M. 2004. Genetic consequences of climatic oscillations in the Quaternary. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 359(1442): 183-195.

Huey R.B. & A.F. Bennett. 1987. Phylogenetic studies of coadaptation: preferred temperatures versus optimal performance temperatures of lizards. Evolution, 41:1098–1115.

Hulton N.R.J., Purves R.S., McCulloch R.D., Sugden D.E. & M.J. Bentley. 2002. The last glacial maximum and deglaciation in southern South America. Quaternary Science Reviews, 21: 233–241.

Hoorn C., Guerrero J., Sarmiento G. A. & M.A. Lorente. 1995. Andean tectonics as a cause for changing drainage patterns in Miocene northern South America. Geology, 23(3): 237-240.

Hoorn C., Wesselingh F. P., Ter Steege H., Bermudez M. A., Mora A., Sevink J., Sanmartín I., Sanchez-Meseguer A, Anderson C.L., Figueiredo J.P., Jaramillo C, Riff D., Negri F.R., Hooghiemstra H., Lundberg J., Stadler T., Särkinen. T. & A. Antonelli. 2010. Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. Science, 330(6006): 927-931.

Kaliontzopoulou A, Adams DC, van der Meijden A, Perera A, Carretero MA. 2012a. Relationships between head morphology, bite performance and ecology in two species of *Podarcis* wall lizards. Evolutionary Ecology, 26:825–845.

Kaliontzopoulou A., Carretero M.A. & G.A. Llorente. 2012b. Morphology of the *Podarcis* wall lizards (Squamata: Lacertidae) from the Iberian Peninsula and North Africa: patterns of variation in a putative cryptic species complex. Zoological Journal of the Linnean Society, 164: 173–193.

Koniak G. & I. Noy-Meir. 2009. A hierarchical, multi-scale, management-responsive model of Mediterranean vegetation dynamics. Ecological Modelling, 220: 1148–1158.

Kubatko L. S. & J.H. Degnan. 2007. Inconsistency of phylogenetic estimates from concatenated data under coalescence. Systematic Biology, 56(1): 17-24.

Laurent R.F. 1983 Contribución al conocimiento de la estructura taxonómica del género *Liolaemus* Wiegmann (Iguanidae). Boletin de la Asociación Herpetologica Argentina, 1: 16–18.

Laurent R.F. 1985 Segunda contribución al conocimiento de la estructura taxonómica del género *Liolaemus* Wiegmann (Iguanidae). Cuadernos de Herpetología 1: 1–37.

Lawson L.P. 2010. The discordance of diversification: evolution in the tropical-montane frogs of the Eastern Arc Mountains of Tanzania. Molecular Ecology, 19: 4046-4060.

Levy E., Kennington W. J., Tomkins J. L. & N.R. LeBas. 2012. Phylogeography and population genetic structure of the Ornate Dragon Lizard, *Ctenophorus ornatus*. PloS one, 7(10), e46351.

Llorente G.A., Vidal-García M., Garriga N., Carranza S., Pleguezuelos J.M. & X. Santos. 2012. Lessons from a complex biogeographic scenario: morphological characters match mitochondrial lineages within Iberian *Coronella austriaca* (Reptilia: Colubridae). Biological Journal of the Linnean Society, 106: 210–223.

Lobo F. 2001. A phylogenetic analysis of lizards of the *Liolaemus chiliensis* group (Iguania: Tropiduridae). Herpetological Journal, 11(4):137-150.

Lobo F. 2005. Las relaciones filogenéticas dentro del grupo *chiliensis* (Iguania: Liolaemidae: *Liolaemus*): sumando nuevos caracteres y taxones. Acta zoológica llllloana, 49(1-2): 65-87.

Lobo, F., Espinoza, R. E., & Quinteros, S. 2010. A critical review and systematic discussion of recent classification proposals for liolaemid lizards. Zootaxa, 2549, 1-30.

Maddison W.P. 1997. Gene trees in species trees. Systematic Biology, 46:523–536.

Marshall J. C., Arévalo E., Benavides E., Sites J. L., & J.W. Sites. 2006. Delimiting species: comparing methods for Mendelian characters using lizards of the *Sceloporus grammicus* (Squamata: Phrynosomatidae) complex. Evolution, 60(5): 1050-1065.

Mayr E. & P. Ashlock. 1991. Principles of Systematic Zoology. 2nd edition. New York, McGraw-Hill.

McCormack J.E., Hird S.M., Zellmer A.J., Carstens B.C. & R.T. Brumfield. 2013. Applications of next-generation sequencing to phylogeography and phylogenetics. Molecular Phylogenetics and Evolution, 66: 526–538.

Minoli I., Morando M. & L.J. Avila. 2016. Sexual dimorphism and interspecific head variation in the *Liolaemus melanops* complex (Squamata: Liolaemini) based on geometric morphometrics. The Herpetological Journal, 26(3), 225-240.

Morando M., Avila L.J. Baker J. & J.W. Jr Sites. 2004. Phylogeny and phylogeography of the *Liolaemus darwinii* complex (Squamata: Liolaemidae): Evidence for introgression and incomplete lineage sorting. Evolution, 58: 842–861.

Morrone J. J. 2006. Biogeographic areas and transition zones of Latin America and the Caribbean islands based on panbiogeographic and cladistic analyses of the entomofauna. Annual Review of Entomology, 51: 467-494.

Muellner A. N., Tremetsberger K., Stuessy T. & C. M. Baeza 2005. Pleistocene refugia and recolonization routes in southern Andes: insights from *Hypochaeris palustris* (Asteraceae, Lactuceae). Molecular Ecology, 14(1): 203-212.

Muñóz-Mendoza C. 2012. Inferencias microevolutivas en *Liolaemus tenuis* (Tropiduridae) y su relación con cambios climáticos pasados. Tesis de magíster. Universidad de Concepción, Chile.

Mutanen M. & E. Pretorius. 2007. Subjective visual evaluation vs. traditional and geometric morphometrics in species delimitation: a comparison of moth genitalia. Systematic Entomology, 32(2): 371-386.

Myers N., Mittermeier R.A., Mittermeier C.G., Da Fonseca G.A. & J. Kent. 2000. Biodiversity hotspots for conservation priorities. Nature, 403(6772): 853-858.

Nichols R. 2001. Gene trees and species trees are not the same. Trends in Ecology & Evolution, 16 (7): 358-364.

Nuñez H. & F. Jaksic. 1992. Lista comentada de los reptiles terrestres de Chile continental. Boletín del Museo Nacional de Historia Natural, 43: 63-91.

Núñez, H., Schulte Ii, J. A. & C. Garín. 2001. *Liolaemus josephorum*, nueva especie de lagartija para el Norte de Chile. Boletín del Museo de Historia Natural, 50, 91-107.

Núñez J. J., Koontz N., Rabanal F., Fontanella F. & J.W.Jr. Sites. 2011. Amphibian phylogeography in the antipodes: refugia and postglacial colonization explain mitochondrial haplotype distributions in the Patagonian frog *Eupsophus calcaratus* (Cycloramphidae). Molecular Phylogenetics and Evolution, 58:343–352.

Nolte A.W. & H.D. Sheets. 2005. Shape based assignment tests suggest transgressive phenotypes in natural sculpin hybrids. Frontiers in Zoology, 2: 11.

Olave, M., Martinez, L. E., Ávila, L. J., Sites, J. W. & Morando, M. 2011. Evidence of hybridization in the Argentinean lizards *Liolaemus gracilis* and *Liolaemus bibronii* (Iguania: Liolaemini): an integrative approach based on genes and morphology. Molecular Phylogenetics and Evolution, 61: 381-391.

Olave M., Avila L. J., Sites J. W. &M. Morando. 2014. Multilocus phylogeny of the widely distributed South American lizard clade *Eulaemus* (Liolaemini, *Liolaemus*). Zoologica Scripta, 43: 323-337.

Olave M., Avila L. J., Sites J. W.& M. Morando. 2015. Model-based approach to test hard polytomies in the *Eulaemus* clade of the most diverse South American lizard genus *Liolaemus* (Liolaemini, Squamata). Zoological Journal of the Linnean Society, 174: 169-184.

Olave M., Avila L. J., Sites J. W. &M. Morando. 2016. Hidden diversity within the lizard genus *Liolaemus*: Genetic vs morphological divergence in the *L. rothi* complex (Squamata: Liolaeminae). Molecular Phylogenetics and Evolution, 107, 56-63.

Oliver P.M., Adams M., Lee M.S.Y., Hutchinson M.N. & P. Doughty. 2009. Cryptic diversity in vertebrates: molecular data double estimates of species diversity in a radiation of Australian lizards (*Diplodactylus*, Gekkota). Proceedings of the Royal Society of London Series B, Biological Sciences, 276: 2001–2007.

Palma R. E., Rivera-Milla E., Yates T. L., Marquet P. A. &A.P. Meynard. 2002. Phylogenetic and biogeographic relationships of the mouse opossum *Thylamys* (Didelphimorphia, Didelphidae) in southern South America. Molecular Phylogenetics and Evolution, 25(2), 245-253.

Pamilo P. & M. Nei. 1988. Relationships between gene trees and species trees. Molecular Biology and Evolution, 5:568–583.

Panzera, A., Guerrero, J. C., & Maneyro, R. 2017. Delimiting the Geographic Distribution of *Lygophis anomalus* (Günther, 1858) (Squamata, Dipsadidae) from Natural History and Ecological Niche Modeling. South American Journal of Herpetology, 12(1), 24-33.

Pincheira-Donoso D., Tregenza T. & D.J. Hodgson. 2007. Body size evolution in South American *Liolaemus* lizards of the *boulengeri* clade: a contrasting reassessment. Journal of Evolutionary Biology, 20(5): 2067-2071.

Pincheira-Donoso D., Bauer A.M., Meiri S. & P. Uetz. 2013. Global taxonomic diversity of living reptiles. PLoS ONE 8:e59741.

Przyboś E., Tarcz S., Rautian M. & N. Sawka. 2015. Delimiting Species Boundaries within a Paraphyletic Species Complex: Insights from Morphological, Genetic, and Molecular Data on *Paramecium sonneborni* (*Paramecium aurelia* species complex, Ciliophora, Protozoa). Protist, 166(4): 438-456.

Pons J., Barraclough T. G., Gomez-Zurita J., Cardoso A., Duran D. P., Hazell S., Kamoun S., Sumlin W.D. & Vogler, A. P. 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Systematic Biology, 55(4): 595-609.

Pyron A.R, Burbrink F.T. & J.J. Wiens. 2013. A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. BMC Evolutionary Biology, 2013: 13:93.

Ramos V.A. 1989. The birth of southern South America. American Scientist, 77: 444– 450.

Rannala B. 2015. The art and science of species delimitation. Current Zoology, 61(5): 846-853.

Rannala B. & Z. Yang. 2003. Bayes estimation of species divergence times and ancestral population sizes using DNA sequences from multiple loci. Genetics, 164(4): 1645-1656.

Rissler L. J. & J.J. Apodaca. 2007. Adding more ecology into species delimitation: ecological niche models and phylogeography help define cryptic species in the black salamander (*Aneides flavipunctatus*). Systematic Biology, 56(6): 924-942.

Rodriguez, A., Bömer, M., Pabijian, M., Gehara, M., Haddad, C.F.B. & M. Vences. 2015. Genetic divergence in tropical anurans: deeper phylogeographic structure in forest specialists and in topographically complex regions. Evolutionary Ecology 29:765–785.

Ruiz de Gamboa M. 2016. Lista actualizada de los reptiles de Chile. Boletín Chileno de Herpetología, 3: 7-12.

Ruiz E., Gonzalez F., Torres-Diaz C., Fuentes G, Mardones M. & T. Stuessy. 2007. Genetic diversity and diversification within and among Chilean populations of *Araucaria araucana* (Araucariaceae) based on allozyme variability. Taxon, 56(4): 1221–1228.

Rull V. 2011. Neotropical biodiversity: timing and potential drivers. Trends in Ecology & Evolution, 26: 508–513.

Ruzzante D. E., Walde S. J., Cussac V. E., Dalebout M. L., Seibert J., Ortubay S. & E. Habit. 2006. Phylogeography of the Percichthyidae (Pisces) in Patagonia: roles of orogeny, glaciation, and volcanism. Molecular Ecology, 15(10): 2949-2968.

Ruzzante D. E., Walde S.J., Gosse J.C., Cussac V.E., Habit E., Zemlak T.S. & M. Adams. 2008. Climate control on ancestral population dynamics: insight from Patagonian fish phylogeography. Molecular Ecology, 17:2234–2244.

Sallaberry-Pincheira N., Garin C. F., González-Acuña D., Sallaberry M. A. & J.A. Vianna. 2011. Genetic divergence of Chilean long-tailed snake (*Philodryas chamissonis*) across latitudes: conservation threats for different lineages. Diversity and Distributions, 17(1): 152-162.

Santos X., Rato C., Carranza S., Carretero M. A., de la Vega J. P. G. & J.M. Pleguezuelos. 2012. Morphology matches mtDNA lineages in the southern smooth snake (*Coronella girondica*) from Iberia. Zoology, 115(6): 398-404.

Schulte J. A. & F. Moreno-Roark. 2010. Live birth among Iguanian lizards predates Pliocene–Pleistocene glaciations. Biology Letters, 6(2): 216-218.

Schulte Ii J. A., Macey J. R., Espinoza R. E. & A. Larson. 2000. Phylogenetic relationships in the iguanid lizard genus *Liolaemus*: multiple origins of viviparous reproduction and evidence for recurring Andean vicariance and dispersal. Biological Journal of the Linnean Society, 69(1): 75-102.

Sérsic A. N., Cosacov A., Cocucci A.A., Johnson L.A., Pozner R., Avila L.J., Sites J.W.Jr. & M. Morando. 2011. Emerging phylogeographic patterns of plants and terrestrial vertebrates from Patagonia. Biological Journal of the Linnean Society, 103:475–494.

Setiadi M.I., McGuire J.A., Brown R.M., Zubairi M., Iskandar D.T, Andayani N., Supriatna J. & B. J. Evans. 2011. Adaptive Radiation and Ecological Opportunity in Sulawesi and Philippine Fanged Frog (*Limnonectes*) Communities. The American Naturalist, 178 (2): 221-240.

Simonetti J.A., Arroyo M.T.K., Spotorno A.E. & E. Lozada. 1995. Diversidad biológica en Chile. Comisión Nacional de Ciencia y Tecnología, Santiago, Chile.

Smissen P. J., Melville J., Sumner J. & T.S. Jessop. 2013. Mountain barriers and river conduits: phylogeographical structure in a large, mobile lizard (Varanidae: *Varanus varius*) from eastern Australia. Journal of Biogeography, 40 (9): 1729-1740.

Smith B.T., Harvey M.G., Faircloth B.C., Glenn T.C. & Brumfield R.T. 2014. Target capture and massively parallel sequencing of ultraconserved elements for comparative studies at shallow evolutionary time scales. Systematic Biology, 63:83–95.

Smith-Ramirez C. 2004. The Chilean coastal range: a vanishing center of biodiversity and endemism in South America temperate rainforest. Biodiversity and Conservation, 13: 373-393.

Sukumaran, J. & Knowles, L. L. 2017. Multispecies coalescent delimits structure, not species. Proceedings of the National Academy of Sciences, 114(7), 1607-1612.

Streicher J. W. & Wiens J. J. 2016. Phylogenomic analyses reveal novel relationships among snake families. Molecular Phylogenetics and Evolution, 100: 160-169.

Turchetto-Zolet A. C., Pinheiro F., Salgueiro F. & C. Palma-Silva. 2013. Phylogeographical patterns shed light on evolutionary process in South America. Molecular Ecology, 22(5): 1193-1213.

Torres-Pérez F., Lamborot M., Boric-Bargetto D., Hernández C.E., Ortiz J.C. & R.E. Palma. 2007. Phylogeography of a mountain lizard species: an ancient fragmentation process mediated by riverine barriers in the *Liolaemus monticola* complex (Sauria: Liolaemidae). Journal of Zoological Systematics and Evolutionary Research, 45: 72–81.

Torres-Pérez F., Méndez M.A., Benavides E., Moreno R.A., Lamborot M., Palma R.E. & J.C. Ortiz. 2009. Systematics and evolutionary relationships of the mountain lizard *Liolaemus monticola* (Liolaemini): how molecular evidence contributes to reveal hidden species diversity. Biological Journal of the Linnean Society, 96: 635–650.

Troncoso-Palacios J. 2014. Nueva lista actualizada de los reptiles terrestres de la Región de Atacama, Chile. Boletín Chileno de Herpetología, 1, 1-4.

Troncoso-Palacios, J. & C.F. Garin. 2013. On the identity of *Liolaemus nigromaculatus* Wiegmann, 1834 (Iguania, Liolaemidae) and correction of its type locality. ZooKeys, 294, 37.

Troncoso-Palacios J., Schulte II J. A., Marambio-Alfaro Y. & D. Hiriart. 2015. Phenotypic variation, phylogenetic position and new distributional records for the poorly known *Liolaemus silvai* Ortiz, 1989 (Iguania: Iguanidae: Liolaemini). South American Journal of Herpetology, 10(2): 71-81.

Trontelj P. & C. Fišer. 2009. Cryptic species diversity should not be trivialised. Systematics and Evolution 7: 1–3.

Uetz P. & J. Hošek. 2016. The Reptile Database, http://www.reptile-database.org, accessed May 4th 2017

Valdez L. & G. D'Elía. 2013. Differentiation in the Atlantic Forest: phylogeography of *Akodon montensis* (Rodentia, Sigmodontinae) and the Carnaval-Moritz model of Pleistocene refugia. Journal of Mammalogy, 94(4): 911-922.

Valladares Faúndez P. 2011. Análisis, síntesis y evaluación de la literatura de lagartos de la Región de Atacama, Chile. Gayana (Concepción), 75(1): 81-98.

Vera-Escalona I., D'Elía G., Gouin N., Fontanella F. M., Muñoz-Mendoza C., Sites Jr, J. W. & P.F. Victoriano. 2012. Lizards on ice: evidence for multiple refugia in *Liolaemus pictus* (Liolaemidae) during the Last Glacial Maximum in the Southern Andean Beech Forests. PloS one, 7(11), e48358.

Victoriano P.F., Ortiz J.C., Benavides E., Adams B.J. & J.W.Jr. Sites. 2008. Comparative phylogeography of codistributed species of Chilean *Liolaemus* (Squamata: Tropiduridae) from the central-southern Andean range. Molecular Ecology, 2397–2416.

Vidal M.A., Ortiz J.C., Ramírez C.C. & M. Lamborot. 2005. Intraspecific variation in morphology and sexual dimorphism in *Liolaemus tenuis* (Tropiduridae). Amphibia-Reptilia, 26: 343–351.

Vidal M. & A. Labra. 2008. Herpetología de Chile. GraficAndes, Santiago, Chile.

Villegas J., Feliciangeli M.D. & J.P. Dujardin. 2002. Wing shape divergence between *Rhodnius prolixus* from Cojedes (Venezuela) and *Rhodnius robustus* from Mérida (Venezuela). Infection, Genetics and Evolution, 2:121–128.

Wagner C. E., Keller I., Wittwer S., Selz O.M., Mwaiko S., Greuter L., Sivasundar A. & O. Seehausen. 2012. Genome-wide RAD sequence data provide unprecedented resolution of species boundaries and relationships in the Lake Victoria cichlid adaptive radiation. Molecular Ecology, 22:787–798

Wang Y. & G. Yan. 2014. Molecular Phylogeography and Population Genetic Structure of *O. longilobus* and *O. taihangensis* (Opisthopappus) on the Taihang Mountains. PloS one, 9(8), e104773.

Wiens J. J. 2007. Species delimitation: new approaches for discovering diversity. Systematic Biology, 56(6): 875-878.

Wiens J. J. & M.R. Servedio. 2000. Species delimitation in systematics: inferring diagnostic differences between species. Proceedings of the Royal Society of London. Series B: Biological Sciences, 267(1444): 631-636.

Xu G., Fang Q. Q., Keirans J. E. & L.A. Durden. 2003. Molecular phylogenetic analyses indicate that the *Ixodes ricinus* complex is a paraphyletic group. Journal of Parasitology, 89(3): 452-457.

Xu J., Pérez-Losada, M., Jara C. G. & K.A. Crandall. 2009. Pleistocene glaciation leaves deep signature on the freshwater crab *Aegla alacalufi* in Chilean Patagonia. Molecular Ecology, 18(5): 904-918.

Yang Z. & B. Rannala. 2010. Bayesian species delimitation using multilocus sequence data. Proceedings of the National Academy of Sciences, 107:9264-9269.

Zhang J., Kapli P., Pavlidis P. & Stamatakis A. 2013. A general species delimitation method with applications to phylogenetic placements. Bioinformatics, 29(22): 2869-2876.

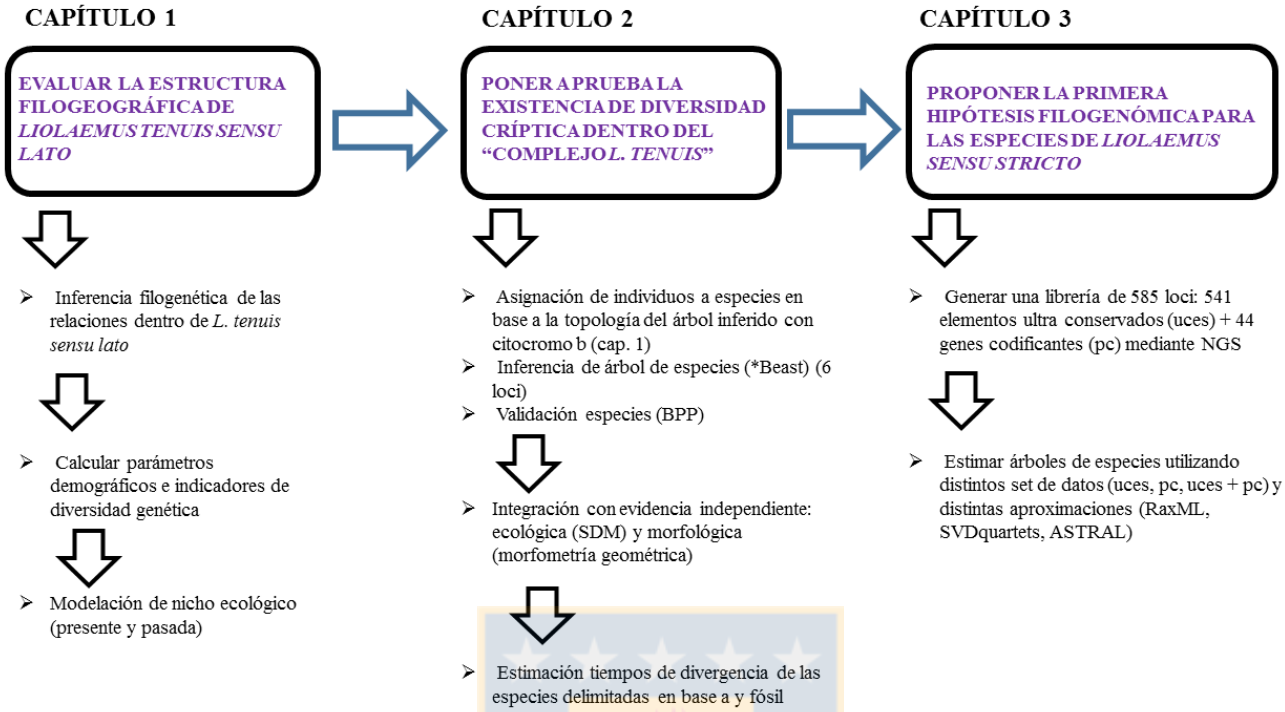

Figura 1. Diagrama de flujo que muestra los objetivos principales (dentro de los cuadros negros) de cada capítulo de esta tesis. Las flechas verticales muestran las principales etapas que se seguirán para abordar los objetivos antes mencionados.

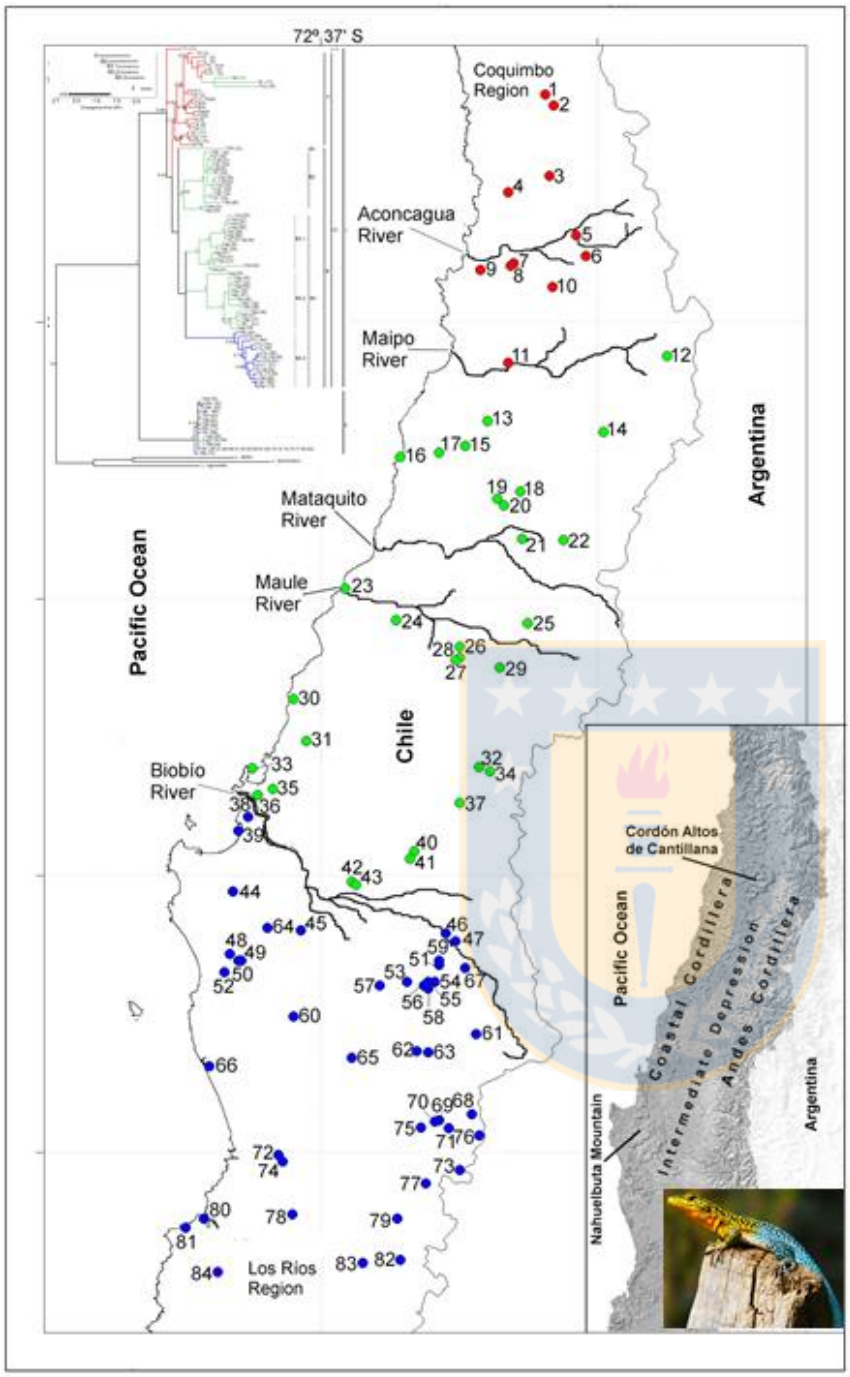

Figura 2. Distribución de *Liolaemus tenuis* en Chile, en base a las localidades muestreadas (representadas por círculos). Los colores indican procedencia geográfica: norte del Río Maipo (rojo), entre ríos Maipo y Biobío (verde), y sur del Biobío (azul). Los colores de los clados mitocondriales (figura superior izquierda) corresponden a este mismo código. Modificada de Muñoz-Mendoza et al. (en prensa).

Tabla 1. Se detalla autor, estatus de conservación de acuerdo a la IUCN, localidad tipo y región correspondiente (en el caso de las especies chilenas), para todas las especies que han sido citadas como parte del clado que incluye a *L. tenuis*. Se detallan las especies incluidas en dicho clado según trabajos basados en información genética (Schulte et al. 2000; Vidal & Labra 2008; Torres-Pérez et al. 2009, Schulte & Moreno-Roark 2010; Pyron et al. 2013) y en datos morfológicos (Lobo et al. 2005; Diaz-Gómez & Lobo 2006; en rojo). Nota: *Liolaemus bellii* figura en Diaz-Gómez & Lobo (2006) como *L. altissimus*, y *L. cyanogaster* figura en Lobo et al. (2005) y Diaz-Gómez & Lobo (2006) como *L. brattsroemi*.

ESPECIE	AUTOR	IUCN	LOCALIDAD TIPO	Región	Schulte et al. 2000	Vidal & Labra 2008	Torres-Pérez et al. 2009	Schulte & Moreno 2010	Pyron, Burbrink & Wiens 2013
Liolaemus atacamensis	Müller & Hellmich, 1933	LC	Copiapó	III				x	x
Liolaemus fuscus	Boulenger, 1885	DD	Valparaíso	V	x	x		x	x
Liolaemus hellmichi	Donoso-Barros, 1975	LC	Cerro Moreno, Antofagasta	II		x			
Liolaemus isabelae	Navarro & Núñez, 1993	NE	Salar de Pedernales	III				x	
Liolaemus lemniscatus	Gravenhorst, 1837	DD	Valparaíso	V	x	x		x	x
Liolaemus monticola	Müller & Hellmich, 1932	NE	Río San Francisco	V	x	x	x	x	x
Liolaemus nigroviridis	Müller & Hellmich, 1932	NE	Valle Río San Francisco	V	x	x	x	x	x
Liolaemus nigromaculatus	Weigmann, 1834	LC	Puerto Viejo y Copiapó	III		x	x	x	x
Liolaemus nitidus	(Wiegmann, 1834)	DD	Valparaíso	V	x	x	x	x	x
Liolaemus paulinae	Donoso-Barros, 1961	DD	Calama	II				x	x
Liolaemus platei	Werner, 1898	LC	Coquimbo	V		x	x	x	x
Liolaemus pseudolemniscatus	(Lamborot and Ortiz 1990)	NE	Las Mollacas	V		x	x		x
Liolaemus tenuis	(Duméril & Bibron, 1837)	NE	Santiago	V					
Liolaemus vellosoi	Ortiz 1987	NE	Desvio Cerro Imán, Copiapó	III		x			
Liolaemus zapallarensis	(Müller & Hellmich, 1933)	DD	Zapallar	V	x			x	x
Liolaemus bellii	**Gray 1845**	**NE**	**Rio San Francisco**	**V**					
Liolaemus chillanensis	**Müller & Hellmich, 1932**	**NE**	**Provincia de Nuble**	**VIII**					
Liolaemus coeruleus	**Cei & Ortiz-Zapata, 1983**	**NE**	**Zapala, Argentina**	**/**					
Liolaemus cyanogaster	**(Donoso-Barros, 1967)**	**NE**	**Valdivia**	**XIV**					
Liolaemus neuquensis	**Müller & Hellmich, 1939**	**NE**	**Volcán Morada, Neuquén, Argentina**	**/**					
Liolaemus pictus	**(Duméril & Bibron, 1837)**	**NE**	**Valdivia**	**XIV**					
Liolaemus valdesianus	**Hellmich 1950**	**NE**	**Baños Morales, Lo Valdes**	**V**					

CAPÍTULO 1

Geography and past climate changes have shaped the evolution of a widespread lizard from the Chilean hotspot.

Geography and past climate changes have shaped the evolution of a widespread lizard from the Chilean hotspot.

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ABSTRACT

The complex orogenic history and structure of Southern South America, coupled with Pleistocene glacial cycles, have generated paleoclimatic and environmental changes that influenced the spatial distribution and genetic composition of natural populations. Despite the increased number of phylogeographic studies in this region and given the frequent idiosyncratic phylogeographic patterns, there is still the need to focus research especially

on species that are currently distributed within a wide range of bioclimatic regimes, and that historically have been subject to contrasting scenarios. *Liolaemus tenuis* is a widely distributed lizard species inhabiting latitudinally through almost 1,000 km through central and southern Chile. Here we describe the geographical patterns of genetic variation and lineage diversification within *L. tenuis*, and their association with geography and Pleistocene glaciations, using sequences from one mitochondrial and two nuclear genes, and five microsatellite loci, and covering most of the species distributional range. Our results revealed a high diversity both within and among populations, as well as two phylogeographic breaks, which are consistent with two of the larger rivers of central Chile, the Maipo and Biobío Rivers. *Liolaemus tenuis* is characterized by several allopatric lineages, especially in its north and central range, which suggest a history of multiple vicariance processes. Conversely, populations found in the southern range, south of the Biobío River, show signatures of recent decreases in effective population sizes, coupled with recent range expansions and secondary contact. Niche "Envelope" data are consistent with patterns of genetic variation; both suggest a history of discontinuous areas of relatively stable populations throughout all of the distribution of *L. tenuis*. These data are also consistent with higher probabilities of habitat suitability north of the Maipo River (33ºS) in both coastal areas and the "Intermediate Depression" between 34º - 37ºS, as well as in the southern Coastal Cordillera between the Biobío and Araucanía regions. Interestingly, both molecular and niche envelope modelling data suggest that some populations may have persisted in fragmented refugia in Andean valleys, within the limits of the ice sheet. Finally, our results suggest that several populations of *L. tenuis* colonized glaciated regions from refugial areas in lowlands and coastal regions, and in the southern

48 distribution, historic migration events would have occurred from refugial areas within the
49 limits of the ice sheet.
50
51 **1. Introduction**
52 Geological events, coupled with past climatic changes have played a key role in shaping
53 current patterns of genetic diversity (Hewitt, 1996). Temporal and spatial environmental
54 heterogeneity are among the main drivers of intraspecific differentiation (e.g., Levy et al.,
55 2012; Wang and Yan, 2014) and ultimately of species diversification (e.g., Brown et al.,
56 2014). Consequently, a positive association between habitat discontinuity (e.g., landscape
57 roughness and/or the occurrence of barriers) and beta levels of intraspecific diversity
58 (population genetic structure and/or structured lineage distribution) is expected. For
59 example, several studies have shown the importance of large rivers acting as barriers to
60 gene flow and delimiting the distributions of closely related subspecies or species of
61 tropical birds (Capparella, 1991; Cheviron et al., 2005; Ribas et al., 2011; Maldonado-
62 Coelho et al., 2013; Voelker et al., 2013; Smith et al., 2014), lizards (Pellegrino et al.,
63 2005; Torres-Pérez et al., 2007), and mammals (Patton and da Silva, 1998; Nicolas et al.,
64 2011; Link et al., 2015). In the same context, historically restricted stable habitats, which
65 allow populations to remain in place over time, are positively correlated with high levels
66 of intrapopulation genetic variability and demographic equilibrium. The opposite situation
67 is expected for areas that have been repeatedly exposed to climatic fluctuations, as during
68 Pleistocene glaciations, which would harbor populations exhibiting signatures of recent
69 colonization and demographic expansion suggested, for example, by a lack of mutation-
70 drift equilibrium and high historical levels of gene flow (e.g., Zhang et al., 2008a, 2008b;
71 Ding et al., 2011).

One example of dynamic and heterogeneous scenario is western temperate South America, where the uplift of the Andes and Pleistocene glaciation cycles would have had important evolutionary consequences for the biota. The Andes, whose uplift started approximately 23 million years ago, have acted as a barrier between the current western slope and the Argentine Patagonia, and have shaped a high topographic heterogeneity along the western slope (Gregory-Wodzicki, 2000).This orogenic event history would have facilitated evolutionary diversification, ultimately forming much of the biota to what is now referred to as the "Chilean Biodiversity Hotspot" (Myers et al., 2000; Antonelli et al., 2010).

Along the north-south axis of the western Andean range, there is a pronounced altitudinal gradient, which is characterized by high altitudes and more heterogeneity in relief from south to north. This topographical gradient is also characterized by an increasing latitudinal gradient of fragmentation of the Chilean "lowlands" that lay between the Andes and the Pacific Coastal Cordillera (Fig. 1). The opposite topography is observed in the Andean Cordillera as it extends to the south; overall elevations are lower and intervening valleys are more connected.

Over this orogenic history, and interacting with it, four Pleistocene climatic events occurred that had important demographic consequences (Rabassa and Clapperton, 1990; McCulloch et al., 2000; Ruzzante et al., 2008). Such cycles had a greater impact along the southern Andes. During the Last Glacial Maximum (LGM) the ice sheet coverage increased in extension towards the south, also extending onto low altitude areas (Villagrán, 1991; Villagrán et al., 1995; Markgraf et al., 1995; Hulton et al., 2002). In the western slope of the Andes in Central Chile, the northern limit of the ice sheets reached

95 approximately the latitude of 33º S at high Andean regions and covering all the land up the
96 Pacific Coast south of the 42º S. According to this, the coastal mountain range, as well as
97 coastal areas north of 41° S, would have constituted stable areas where species would have
98 survived during the LGM (Armesto et al., 1994; Villagrán et al., 1995; Villagrán, 2001;
99 Sérsic et al., 2011). As such, in the western slope of the Andes the intensity of the glacial
100 effects on the biota would have increased towards the south (Amigo and Ramírez, 1998;
101 Heusser, 2003; Smith-Ramirez, 2004), reducing effective population sizes (Ne) mainly in
102 Andean and Southern populations (e.g., Victoriano et al., 2008; Lessa et al., 2010; Vera-
103 Escalona et al., 2012).

104 Another distinctive feature of Chile is the series of parallel steep, high gradient,
105 east-to-west flowing rivers (e.g., Aconcagua, Maipo, Maule, Biobío). These rivers may
106 also act as barriers to gene flow structuring intraspecific genetic diversity in Chilean
107 vertebrates and plants (Chesser, 1999; Lamborot et al., 2003; Torres-Pérez et al., 2007;
108 Unmack et al., 2009; Sallaberry-Pincheira et al., 2011; Vásquez et al., 2013, Viruel et al.,
109 2014). Nevertheless, no study has formally evaluated the combined effect of rivers,
110 topographic relief, and shifting climates (glacial advances and retreats) in structuring the
111 intraspecific genetic variation/phylogeographic history of any Chilean species. The north-
112 south axis across 39 degrees of latitude in the Chilean Andes, combined with its west-east
113 elevational gradient (sea level to above 6,000 m), coupled to multiple ice sheet glacial
114 advances and retreats in addition to simultaneous increases in river water volumes due to
115 melting ice, would have dramatically impacted terrestrial (as well as aquatic) communities
116 in this region. As such, widely distributed Chilean species constitute a good system to
117 assess the joint effects of geographical complexity, glacial cycles, and fluctuating river

118 volumes, on intraspecific population differentiation and phylogeographic structure.

119 *Liolaemus* is a highly diverse (ca. 250 species; Uetz et al., 2016) and widely distributed
120 genus of South American lizards. *Liolaemus tenuis* (Dumeril and Bibron, 1837) is a
121 broadly distributed species that encompasses a latitudinal range of 1,000 kilometers, from
122 the Chilean regions of Coquimbo (ca. 30°S) to Los Ríos (ca. 40°S; Fig. 1), with peripheral
123 populations also present on some eastern Andean slopes in the Argentinean province of
124 Neuquén. The altitude range of this species extends from sea level up to 1,800 meters
125 (Donoso-Barros, 1966; Pincheira-Donoso and Nuñez, 2005; Victoriano et al., 2008), and
126 occurs in distinct habitat types ranging from "Mediterranean" shrubland to sub-Andean
127 forests (Di Castri, 1968). As such, the species distribution encompasses both stable and
128 topographically heterogeneous areas in the north, as well as southern latitude landscapes
129 that were impacted by Pleistocene glaciations. Victoriano et al. (2008) suggested that the
130 microevolutionary history of *L. tenuis* was influenced by Pleistocene glacial cycles, but the
131 inferred demographic changes were not dated, nor were the locations of putative stable
132 areas (refugia) identified and subsequent colonization routes hypothesized.

133 The aim of this study is to evaluate the phylogeographic structure of *L. tenuis*
134 across most of its distributional range in order to test the following predictions. 1)
135 Populations that now occur in previously glaciated areas will show genetic signatures of
136 demographic expansion and lower levels of genetic structure and diversity than
137 populations that occur in areas that were free of ice during glaciations. 2). During glacial
138 advances, refugial areas would have been located near the Coastal Cordillera in the
139 Southern range of *L. tenuis*, and post-glacial population expansions would have occurred
140 in a predominantly northwest to southeast direction. 3) The east-to-west flowing rivers

141 have acted as barriers to gene flow, shaping distribution genetic structure of clades along a
142 north-south latitudinal arrangement.
143
144 **2. Materials and Methods**
145 **2.1 Sample collection**
146 The study is based on 225 specimens of *Liolaemus tenuis* collected at 84 sites (Fig.
147 1). Of these, 80 individuals were newly collected (between 2007 and 2012) at 45 localities;
148 the mitochondrial sequences of the remaining 145 specimens from 41 localities were taken
149 from the study of Victoriano et al. (2008). Specimens were captured with collection
150 permits granted by the Servicio Agrícola y Ganadero (authorizations SAG-1898 and SAG-
151 4729). All captures were carried out according to the protocols approved by the Bioethics
152 Committee of the Universidad de Concepción (Chile). Individuals newly collected were
153 deposited in the collection of the Museo de Zoología de la Universidad de Concepción
154 (MZUC).
155 Sampling sites were classified into three groups, corresponding to the main Chilean
156 bioclimatic zones, as follows: 1) North (N), including localities north of the Maipo River,
157 an area that corresponds to the dry Mediterranean bioclimate zone; 2) Central (C),
158 including localities between the Maipo and Biobío rivers, which correspond to the mesic
159 Mediterranean area; and 3) South (S), including localities south of the Biobío River,
160 covering the wet Mediterranean area, which includes the Valdivian Forests (Di Castri,
161 1968; Table S1).
162
163 Here Fig. 1
164

165 **2.2 Laboratory procedures**

166 This study is based on DNA sequences of two protein coding genes (the
167 mitochondrial cytochrome b gene: cyt *b* ; the nuclear Kinesin-like protein gene: KIF24), a
168 nuclear anonymous gene (LDB5B), and five nuclear microsatellite loci, three of which
169 tetranucleotide repeats (TET1177, TET2216, TET1501) and two dinucleotide repeats (DI
170 159, DI 7938), developed for *Liolaemus fitzingerii* (Hanna et al., 2011).

171 Genomic DNA was extracted from muscle tissue using the commercial kit Wizard
172 SV Genomic (Promega) following manufacturer's instructions. Detailed amplification and
173 sequencing methods largely follow Broadley (2006), Victoriano et al. (2008), and Portik et
174 al. (2011a and b), and are available at Appendix A.

175 Sequence edition and alignment were done using Codon Code Aligner v. 3.0.3
176 (Codon Code Corporation 2009). Coding sequences (cyt *b* and KIF24) were translated into
177 amino acids in order to corroborate the absence of stop codons. In cases where sequences
178 from nuclear markers presented heterozygous sites, haplotypes were inferred using the
179 coalescent-based Bayesian method implemented in Phase 2.1 (Stephens et al., 2001;
180 Stephens and Donnelly, 2003; Stephens and Scheet, 2005). A probability threshold was
181 first established at 0.9 but as not all haplotypes were resolved, we lowered the threshold to
182 0.6 following Garrik et al. (2010), who suggested that this value increases the number of
183 resolved haplotypes with almost no increase in false positives. In addition, recombination
184 was tested for nuclear sequences using RDP: Recombination Detection Program v3.44
185 (Martin et al., 2005; Heath et al., 2006; Martin and Rybicki, 2000). The resulting
186 heterozygous sequences were split and included separately in the matrix as paternal and

187 maternal haplotypes for subsequent analyses. The sequences obtained in this study were
188 registered in GeneBank with codes XXXXX- XXXXX and submitted to Dryad.
189 Microsatellite loci were amplified by PCR for 183 specimens collected at 32 localities
190 (Table S2). The forward primers for all loci were fluorescently labeled (Applied
191 Biosystems, Foster City, CA, USA) and each pair of primers was used individually in
192 amplification reactions. PCR products were checked on a SybrSafe stained 2.0 % agarose
193 gel and sent for direct fragment analysis to Macrogen Inc. (Korea).
194
195 **2.3 Genetic diversity and genealogical analysis**
196 For all alignments, we performed the Xia test (Xia et al., 2003) as implemented in
197 DAMBE 5.0.11 (Xia and Xie, 2001) to evaluate the degree of sequence saturation. The test
198 was done with 100 replicates using the proportion of invariant sites found by jModeltest
199 0.1.1 (Posada, 2008).
200 Standard indexes (haplotype diversity Hd, nucleotide diversity π) were calculated
201 with Arlequin 3.5 (Excoffier and Lischer, 2010) both for the total cyt *b* dataset (255
202 individuals), and for each cluster obtained from Geneland (see below). Average genetic
203 divergence values for all between-locality pairs were estimated based on p-distances using
204 Mega 6 (Tamura et al., 2011).
205 Genealogical analyses were carried out separately for each gene using non-
206 redundant matrices (i.e., including one sequence per allelic class) with Mr.Bayes 3.2.1
207 (Ronquist and Huelsenbeck, 2003) by means of two runs with four chains each. All
208 analyses ran for 10 million generations and were sampled every 1,000 steps; the first 25%
209 of the data was discarded as burn-in. The mitochondrial gene tree was rooted using

sequences of the congeners *L. nitidus*, *L. lemniscatus* and *L. nigroviridis.* The KIF24 genealogy was rooted using alleles of the species *L. pictus* and *L. chiliensis*, and that of LDB5B with sequences of *L. pictus* and *L. lemniscatus*.

The age of the most recent common ancestor (MRCA) of the main clades of *L. tenuis* was estimated with BEAST 1.8.2 (Drummond et al., 2012) using the cyt *b* matrix. A lognormal relaxed clock (not correlated) was used, with a substitution rate of 4.72 % sites per million years (Vera-Escalona. unpublished) and a TrN+I+G model, assuming a normal distribution. Input files were generated with BEAUTI 1.8.2 (included in the BEAST package).

As another way to visualize relationships among sequences, a haplotype network was inferred separately for each locus using statistical parsimony algorithms (SP; Crandall and Templeton, 1993) as implemented in TCS 1.21 (Clement et al. 2000). Ambiguities (loops) within the network were resolved following the criteria of Crandall and Templeton (1993).

2.4 Genetic structure

The cyt *b* matrix, which had the broadest and most dense geographical coverage, was used to infer the most likely number of population clusters with the Bayesian clustering method implemented in Geneland version 4.0.3 (Guillot et al., 2005) in R version 3.0.2 (R Development Core Team, 2009). We performed a preliminary run where K (the number of populations or clusters) was allowed to vary from 1 to 84 (which corresponds to the number of sampled localities), in order to determine the modal number of clusters, using 10 replicates with 5×10^5 Markov chain Monte Carlo iterations. All runs were conducted using the spatial Dirichlet model for the priors in allele frequencies. Five

234 runs with a fixed K number were performed for the spatially explicit model, and for each
235 run, the posterior probability of subpopulation membership was computed for each pixel
236 of the spatial domain (100 × 100 pixels). The Markov Monte Carlo (MCMC) chain was
237 run for 500,000 generations, thinning was set at 100, and the burn-in period was set at 200
238 iterations. Once the clusters were determined, we used Arlequin 3.5 (Excoffier and
239 Lischer, 2010) to calculate Fst values among all pairwise combinations of the inferred
240 population clusters.

241 Distinct hierarchical analyses of the distribution of genetic diversity of *L. tenuis*
242 were conducted in the form of analysis of molecular variance (AMOVA-Excoffier et al.
243 1992) using Arlequin 3.5 (Excoffier and Lischer, 2010). Hierarchical levels were defined
244 on the basis of sampling localities, results of the Geneland analyses, and river locations.
245 AMOVA groups were constructed as follows (numbers in parentheses indicate grouped
246 populations): comparison 1, north of Maipo River vs. south of Maipo River (1-11 vs. 12-
247 84); comparison 2, north of Maipo River vs. between Maipo and Biobío Rivers vs. south
248 of Biobío River (1-12 vs. 13-39 vs. 40-84); comparison 3, north of Biobío River vs. South
249 Biobío River (1-39 vs. 40-84); and comparison 4, among clusters inferred by Geneland.
250 Considering that the clusters resolved by Geneland suggest genetic structure
251 associated both to bioclimate and to the Maipo and Biobío Rivers (see below), gene flow
252 between these clusters was estimated under a Bayesian coalescent framework implemented
253 in Migrate-n version 3.6.4 (Beerli and Felsenstein, 2001; Beerli, 2006). In order to obtain
254 the posterior distribution of the number of immigrants per generation (Nm), four possible
255 models of gene flow were tested (Table S3). The marginal likelihood of each model was
256 estimated by ranking the Bayes factor (Beerli and Palczewski, 2010). A starting UPGMA

257 tree was used and the initial theta and M values were derived from the Fst calculation.
258 Static heating was applied to all four independent chains using the temperature settings of
259 1.0, 1.5, 3.0 and 1,000,000.0. A total of 500,000 steps were run and recorded every 100
260 generations, from which 12,500 were discarded as the burn-in. Stationarity was assessed
261 by examining the effective sample size (ESS) and distribution of each parameter in Tracer
262 1.6 (Rambaut et al. 2013).

263 **2.5 Historical demographic changes**

264 The demographic history of the mitochondrial clusters (as retrieved by Geneland)
265 was inferred by pairwise mismatch distribution analyses (Rogers and Harpending, 1992)
266 implemented in DnaSP. In addition, the raggedness index (Harpending, 1994) was
267 computed under the sudden expansion model. Two neutrality tests implemented in
268 Arlequin, Fu's Fs (Fu, 1997) and Tajima's D (Tajima, 1989), were used to detect
269 departures from the mutation–drift equilibrium, which would be indicative of changes in
270 historical demography or selective sweeps.

271 Population size fluctuations through time were estimated for each main clade using
272 the Bayesian Skyline Plot (BSP) method implemented in BEAST (Drummond and
273 Rambaut, 2007). These analyses were based on the mitochondrial dataset, with a
274 substitution rate of 4.72% (Vera-Escalona, unpublished). We ran 50 x 10^6 generations and
275 discarded the first 10% as "burn in", and used TrN+I+G as the best-fitting substitution
276 model. Estimated distributions were used to generate confidence intervals representing
277 phylogenetic uncertainty and coalescence (Drummond et al., 2005), and we used Tracer
278 1.6 (Rambaut et al. 2013) to monitor parameters estimates and ESS values.

279 **2.6 Microsatellite data analysis**

280 Microsatellite summary statistics, including average number of alleles per locus
281 and number of private alleles for each locus, observed (Ho) and expected (He)
282 heterozygosity, and Shannon`s index, were calculated in GenAlEx 6.5 (Peakall and
283 Smouse, 2006, 2012)

284 The Bayesian clustering algorithm implemented in Geneland was applied in order
285 to infer the most probable number of clusters. We ran Geneland 50 times allowing K to
286 vary from 1 to 32, according the total number of localities sampled, with the following
287 parameters: 20^5 MCMC iterations, maximum rate of Poisson process fixed to 100 and the
288 maximum number of nuclei in the Poisson–Voronoi tessellation fixed to 300. We
289 computed the posterior probability of subpopulation membership for each pixel of the
290 spatial domain, the modal subpopulation for each individual in all 50 runs, and we
291 examined all runs for consistency. We estimated genetic differentiation among clusters by
292 calculating pairwise Fst values (Weir and Cockerham, 1984) in Arlequin 3.5 (Excoffier
293 and Lischer 2010).

294 Considering the clusters estimated by Geneland, we implemented an analysis of
295 molecular variance (AMOVA) to determine if rivers play a significant role in the genetic
296 spatial structuring of *L. tenuis.* Four AMOVA hierarchies were constructed as for the cyt *b*
297 dataset. The microsatellites were also used to estimate gene flow under a Bayesian
298 coalescent framework using Migrate-n 3.6.4 (Beerli and Felsenstein, 2001; Beerli, 2006),
299 as the posterior distribution of the number of immigrants per generation (Nm) between the
300 clusters obtained with Geneland. We tested the same four migration models used with the
301 mitochondrial dataset (for details of geographical groups see Table S1), estimating the

302 marginal likelihoods and Bayes Factors for each model, and then ranking the models
303 accordingly (Beerli and Palczewski, 2010). Initial theta and M values were derived from
304 the Fst estimates, and static heating was applied to four independent chains using
305 temperature settings of 1.0, 1.5, 3.0 and 1,000,000. A total of 500,000 steps were run and
306 recorded every 100 generations, of which the first 12,500 were discarded as the burn-in.
307 Stationarity was assessed by examining the effective sample size (ESS) and distribution of
308 each parameter in Tracer 1.6 (Rambaut et al. 2013).
309

310 **2.7 Present and past distribution modelling**

311 We modeled *L. tenuis* ecological niche "envelopes" to identify potential refugial
312 areas during the LGM. Distribution models were generated for the present and the LGM
313 (21 kya) from a total of 146 occurrence records, based on 19 bioclimatic variables at a 2.5′
314 spatial resolution (WorldClim v1.4; Hijmans et al., 2005) in MAXENT v 3.1.0. (Phillips et
315 al., 2006). We used the default convergence threshold (10^{-5}) and maximum number of
316 iterations (500), using 25% of the localities as "burn-in" for model testing. As suggested
317 by Waltari et al. (2007), we chose a presence threshold to render each projection into a
318 binary form, and considered grid cells with a cumulative probability of more than 10 (from
319 a range of 0–100) as suitable. Model performance was evaluated using the area under the
320 curve (AUC) (receiver operating characteristic) calculated by Maxent. Values between 0.7
321 and 0.9 indicate good discrimination (Swets, 1988).
322
323

3. Results

3.1 Genetic diversity and genealogical analysis

We obtained 726 base pairs (bp) of mtDNA sequences for 225 individuals, the matrix of the nuclear gene KIF24 is composed by 158 sequences of 580 bp and we obtained 196 sequences for the nuclear gene LDB5B with 611 bp. Variable sites, mean haplotype and mean nucleotide diversity, as well as overall mean p-distance for each gene are shown in Tables 1, S4 and S5, respectively. Most of the shared cyt *b* haplotypes among localities occur in the southern range (36ºS to 40°S). In general, p-distance values were correlated with the geographical distance between localities (Table S4a).

Here Table 1

The topology of the cyt *b* gene tree generally corroborates the previous study of Victoriano et al. (2008); two well supported deep clades (Fig. 2) were obtained. Clade I includes haplotypes distributed throughout the whole distribution range of *L. tenuis* (between 31º and 40ºS), while clade II includes most haplotypes south of the Biobío River. Within clade I, main subclades are generally distributed allopatrically, and several of these are delimited by rivers. Clade I included six subclades: two of them extends from the northern limit of the distribution of *L. tenuis* to the Maipo River (I.1 and clade A). Subclade B1 is restricted to the area between the Maipo and Maule Rivers. Subclades B2.2.1, mainly distributed in Andes, and B2.2.2, mainly distributed in the Coastal range, are restricted to the area between the Maule and Biobío Rivers. Finally, subclade B2.2.3 occurs only south of the Biobío River, where it is sympatric with the main clade II. The divergence of the main clades of *L. tenuis* occurred during the Pleistocene (Fig. 2). The

348 age of the crown group *L. tenuis* is ca. 1.63 Ma, while crown ages of the main clades are:
349 Clade I ~ 1.34 Ma, and Clade II ~ 0.24 Ma. Haplotype networks were highly congruent
350 with the Bayesian genealogy. We recovered two main haplogroups corresponding to
351 mtDNA clades I and II, separated by 50 mutational steps (mp values in Fig. 3). The two
352 broadly distributed haplogroups (clades B2.2.3 and II in Fig. 2) are sympatric in the wet
353 zone in the south, one of them with a star-like shape and with a high frequency haplotype
354 (Fig. 3).
355
356 Here Fig. 2
357 Here Fig. 3
358
359 The haplotype networks for both nuclear genes were in general concordance with
360 the cyt *b* network (Figs. S1 and S2).
361
362 **3.2 Genetic structure**
363 The mtDNA based Geneland analysis identifies seven clusters (K=7; Fig. 4), with
364 smaller distributions towards the north and central parts of the species range, and with
365 cluster boundaries defined by the main rivers (e.g., N1 and N2 separated by the Aconcagua
366 River, and S6 and S7 mainly towards the south of the Biobío river). Cluster S7 extends to
367 the southern limit of the species distribution, covering the largest geographic area and
368 including the largest number of localities relative to all other clusters. This cluster also
369 includes two deeply divergent mitochondrial clades within the same range, which for
370 demographic reconstructions we analyzed separately as S7a (Clade B2.2.3 haplotypes in
371 the Bayesian tree), and S7b (Clade II haplotypes; Fig. 2).

372

373 Here Fig. 4.

374

375 Geographically contiguous clusters showed lower Fst values than comparisons
376 involving distant clusters (Table S6a). High Fst values were observed for comparisons
377 between clusters north and south of the Maipo River as well as north and south of the
378 Biobío River.

379

380 For the cyt *b* -based AMOVA, all values were significant ($p < 0.001$). The largest
381 fraction of the observed genetic variation was reached when localities are grouped
382 according to the 7 cyt *b* Geneland clusters; for the other grouping schemes, and
383 independently if Maipo is considered as a barrier or not, arrangements where the Biobío
384 River was considered a barrier showed the highest inter group values. (Table 2a).

385

386 Here Table 2

387

388 When comparing migration models, the highest support was for the Biobío River being a
389 gene flow barrier, with a log Bayes Factor difference > 10^3 units relative to the other
390 migration models (where a difference of > 10 units provide very strong support for one
391 model over another; Kass and Raferty, 1995). The historic migration scenario for our
392 samples shows a prevalence of asymmetric migration rates. Low gene flow between the
393 seven mitochondrial population clusters was estimated (Table S7a, Fig. 4). In general,
394 there is no gene flow after we contrast all clusters north of the Maipo River (N1+N2)
395 versus clusters south of it. In the northern and central areas, gene flow is lower relative to

396 rates estimated for samples in the southern range. South of the Biobío River, within cluster
397 S7 but between physiographical zones (Andes - Coast), bidirectional gene flow was
398 inferred, but it is highly asymmetrical in favor of more gene flow from the Andes towards
399 the coast (Table S7a, Fig.4).
400
401 **3.3 Historical demographic changes**
402 Fu's *Fs*, Tajima's *D,* mismatch distributions suggest that only the southern Biobío
403 clade S7b underwent a recent demographic expansion, while for other clades results
404 suggest demographic stability (Table 1a, Fig. S5). Complementary to the mismatch
405 analysis, the Raggedeness index (r) suggests demographic stability for the northern
406 clusters N1 and N2 and the central cluster C2. For central clusters C3 and C5 and the
407 southern cluster S7 the sudden-expansion model cannot be rejected (Table 1a). BSP shows
408 a reduction in effective population sizes (Ne) both in the northernmost and southern
409 groups, while central clades showed a signal of greater stability in effective population
410 sizes (Fig. 5).
411
412 Here Fig. 5.
413
414 **3.4 Microsatellite data.**
415 All five microsatellite markers tested are polymorphic, with a total of 50 alleles
416 identified, but loci vary both in terms of number of alleles and number of private alleles
417 (from 0 in DI7938 to 8 in TET1177). The TET1177 was both the most variable and the
418 most informative locus for population discrimination (I= 1.008), whereas DI7938 was the
419 least variable and least informative marker. Mean values of observed heterozygosity over

all samples were lower than expected under Hardy-Weinberg (HW) equilibrium at each locus (Table 1b, Table S8). Mean allelic richness, calculated only for populations with at least 5 individuals (rarefaction sample size=5), ranged from 4.0 in the northern locality of San Felipe (population 3 in Fig. 1) to 1.2 at Corral in the south (population 80), with an average value of 2.65. Mean allelic richness by locus, standardized by sample size, was higher in northern and central zones (0.17 and 0.15 respectively) than in the south (0.09). Private alleles were detected in 11 localities, whose means after standardization by number of analyzed localities ranged from the highest value in the northern cluster (1.0 private alleles/locality) to the lowest in the south (0.4 private alleles/locality; values not shown).

Bayesian structure analysis of microsatellite data (Fig. S4) suggested 4 clusters: one in the northernmost distribution of *L. tenuis*, two in the Central area, and one broadly distributed cluster mainly located south of the Biobío River (Table S2). Fst values reflect the geographical proximities of population pairs, with the lowest Fst scores between adjacent clusters, and the highest Fst value for the comparison between extreme clusters (Table S6b). In the multi-locus AMOVA for all comparisons, the largest percentage of variation was attributed to among individuals within localities and only a small portion of the genetic variance was attributed to differences among groups (Table 2b). The AMOVA for the Maipo River as a barrier, indicated that 6.36 % of variance could be attributed to differences between groups north and south of the Maipo River, compared to 23.16% for differences among populations within groups. For the Biobío River as a barrier, a higher and significant portion of variance was explained by differences between groups (10.36%), with a corresponding decrease in the among-population/within group variance (19.39%), compared with the first hierarchical AMOVA. Variance among groups is maximized when

443 populations are clustered into the four Geneland groups, explaining up to 12% of total
444 variance, while variance among populations within groups is minimized (16.34%).
445 Migration values based on microsatellites are shown in Table S7b and Fig. S4. A
446 stepping stone model has the highest support from the data (log Bayes Factor difference >
447 10^3 relative to the other migration models). Migration rates were asymmetrical, with larger
448 values in the number of migrants towards the south. C2 is the cluster receiving the fewest
449 migrants.
450
451 **3.5 Niche modeling**
452 The predictive ability of ecological niche modeling was significantly higher than
453 expected under a random model (AUC 0.983). During the LGM the distribution of *L.*
454 *tenuis* was considerably reduced south of 38ºS and along the Andes (Fig. 4b). Some
455 populations north of 34ºS would have remained disconnected from the rest, a pattern
456 consistent with the Maipo River basin and Cordón Altos de Cantillana mountain range
457 functioning as barriers to gene flow (Fig. 1). South of 37ºS the distribution of the species
458 would have been restricted to the Nahuelbuta region and the coastal area. At the same
459 latitude in the Andes, we identified small stable habitats around volcanic areas (38°S).
460
461 **4. Discussion**
462 **4.1 Phylogeography pattern and barriers**
463 Phylogeographic studies focused on the biota of South America and the Southern
464 Hemisphere in general are still scarce (Beheregaray, 2008; Turchetto-Zulet et al., 2012;
465 see also Sérsic et al., 2011). However, this scenario is changing, as prominent
466 phylogeographic contributions have been made for terrestrial species from the Brazilian

467 Atlantic coast (e.g., Carnaval et al., 2009; Valdez and D´Elía, 2013), Patagonia (e.g., Lessa
468 et al., 2010; Fontanella et al., 2012; Morando et al., 2004; Breitman et al., 2015), and areas
469 west of the Andes in Chile (e.g., Núñez et al., 2011; Sallaberry-Pincheira et al., 2011;
470 Vera-Escalona et al. 2012; Victoriano et al., 2015). Even when several phylogeographic
471 studies have focused on vertebrates from Central Chile, none has centered on an
472 ectothermic species distributed in more than one bioclimatic zone. This has prevented the
473 evaluation of paleoclimatic history of contrasting and topographically complex scenarios
474 within the same species. Moreover, most phylogeographic analyses base their conclusions
475 on only one genetic marker. Our study constitutes a novel contribution given its wide
476 geographic coverage, its wide character sampling (mitochondrial and nuclear loci) and an
477 integrative approach.
478 Our results suggest a complex phylogeographic history for *L. tenuis*, with contrasting
479 population behaviors according to latitude, with signals of greater stability, structure and
480 levels of genetic variability in populations from the northern and central range, compared
481 to southern populations. Evidence for co-distributed deeply divergent clades in the South
482 suggests two recent invasions to the area resulting in a large region of secondary contact,
483 where populations lack structure and show evidence of range expansion. In addition, our
484 results point to some phylogeographic breaks consistent with large Chilean rivers that
485 bisect the country from east to west. Results indicate lack of gene flow among populations
486 south and north of the Maipo River, which has been previously identified as a significant
487 barrier to dispersal in other reptiles, such as *L. monticola* (Torres-Pérez et al., 2007) and
488 *Philodryas chamissonis* (Sallaberry-Pincheira et al., 2011). The second break for *L. tenuis*
489 is located approximately between 37ºS - 38ºS, and is associated with the Biobío River

basin, one of the largest Chilean rivers. This river has been suggested to act as a geographic barrier to the distribution of other vertebrates, including low vagility birds of the genus *Pteroptochos* (Chesser, 1999). Although other rivers could have an important role limiting the gene flow in *L. tenuis*, the Maipo and Biobío seem to be those that generated the greatest impact on the observed pattern of genetic variability of this species.

4.2 Historical demography and refugia

Demographic analysis supports an historical decrease of Ne both for the southern and northern populations, in agreement with the range limits of the species, which occurred ca. 50,000 to 100,000 years before present, consistent with the Llanquihue glaciation (Rabassa and Clapperton 1990). Reductions in Ne 50,000 years ago could have also been coupled with posterior reductions during the LMG, 20,000 years ago. Historical demographic reduction in clades distributed in the northern and southern range areas, suggests stronger past changes in those environments; in particular, the decrease in Ne was of greater magnitude in populations at higher latitudes where glaciations had a larger impact in habitats, as suggested also for other congeneric lizards such as *L. pictus* (Vera-Escalona et al., 2012). For *L. tenuis*, the occurrence of broadly distributed haplogroups in the south, less genetic diversity, star-like haplogroups and redundant haplotypes found with high frequency in populations distributed in glaciated areas, is evidence of a history of recent colonization of the southern species range. In contrast, current distribution and times of divergence of haplotypes outside the LMG ice borders, suggest coastal refugia in areas with lower or without glacial effects. However, in the north end of its current distribution, although far from glacial ice sheets, this area would have been exposed to dynamic environmental conditions throughout its history, conditioned by periglacial

514 changes, such as variation in vegetation and precipitation (Villagrán 1991; Villagrán et al.
515 1995). In summary, populations distributed in both northern and southern ends of the
516 range have highly reduced their Ne, in contrast with central populations that would have
517 remained more stable.

518 On the other hand, it is interesting the likely low connectivity promoted by
519 landscape roughness and high altitude, both in the Coastal and Andean areas at northern
520 and central latitudes. According to this, overall Fst values indicated a high genetic
521 differentiation between clusters that suggest a higher structure in the northern-central
522 distribution of *L. tenuis* (Balloux and Lugon-Moulin, 2002). One example of an altitudinal
523 barrier is Cordón Altos de Cantillana (> 2000 m; cca. 33°S; Fig. 1), which could add to the
524 barrier effects of the Maipo River. Geological data from this region shows that the
525 glaciations in the Pleistocene period were important over 600 meters a.s.l. at this latitude.
526 During the LGM, glaciers in the Maipo Valley reached the Central Valley (Brüggen, 1950;
527 Vuilleumier, 1971). Although there is evidence that the western slope of the Cordillera de
528 la Costa in its lower areas remained free of ice during the Pleistocene (Formas, 1979;
529 Heusser, 1966), highlands in these mountains were affected by glaciations, probably
530 excluding lizard populations. Evidence suggests that glaciers have acted as barriers with a
531 greater magnitude towards the Andes, rather than the Cordillera de la Costa, but in Central
532 Chile, in transversal and coastal mountains with high altitudes, glaciations could affect
533 populations nonetheless. Although mtDNA data suggest a strong genetic structure for *L.*
534 *tenuis*, the microsatellite data do not corroborate that result. AMOVA of the cyt *b* dataset
535 indicated significant high variance among the main groups. In contrast, analyses of nuclear
536 microsatellite data recovered a low among-groups variance. Variance between populations

537 is usually higher in mtDNA than microsatellite data because of the high intrapopulation
538 variance in microsatellites, and because the higher effective size of the nuclear genome
539 (Naidoo et al., 2016). In addition, mitochondrial genetic structure in the presence of low
540 levels of nuclear genetic structure may indicate a higher female philopatry. Interestingly,
541 differences in genders' social behavior in *L. tenuis* have been suggested by Vidal et al.
542 (2008).
543
544 Refugia outside the ice sheet limits and close to the Pacific coast have been
545 proposed for plants (Sersic et al., 2011), which is consistent with the geographic
546 distribution of genetic variation and demographic estimations for *L. tenuis*. Interestingly,
547 in addition to the classical coastal refugia in the south, we detected probable refugial areas
548 within the boundary of the LGM ice sheet. There is evidence pointing to the occurrence of
549 small refugia at Andean areas for other species. For example, the glacial gap of Ñuble
550 (36º30'S) and the valley of Malalcahuello and Lonquimay would have been free of ice
551 during the the LGM (Heusser, 2003). As such, distinct Andean refugia have been
552 suggested for the lizard *L. pictus* (Vera-Escalona et al., 2012), crabs of the genus *Aegla*
553 (Xu et al., 2009), and for the frog *Eupsophus calcaratus* (Núñez et al., 2011). In *L. tenuis*
554 we have evidence for refugial areas south of the Biobío River, near the Valley of
555 Lonquimay in the Andes (~ 38 ° S). High haplotype diversity and suitable habitat during
556 the LGM in such area suggest that populations persisted there over time, with high and
557 stable Ne. Moreover, the results for the historical migration of *L. tenuis* suggest that in the
558 southern range, and consistent with probable Andean refugia, gene flow was lower W-E
559 than E-W. Evidence for a similar migration pattern was inferred for the lizard *L. pictus*
560 (Vera-Escalona et al., 2012). It is likely then that the Andean populations have acted as a

source of recolonization, adding variability to coastal populations in the same latitude. Although genetic variation suggests intra-Andes refugia for *L. tenuis*, we cannot exclude the possibility that in addition to refugium conditions, the Valley of Lonquimay constitute a secondary contact zone of lineages differentiated in distinct refugia (Hewitt, 1996; Petit et al., 2002; Mraz et al., 2007).

The results obtained here partially agree with previous proposals (e.g., Sérsic et al., 2011); in addition to the coastal refugia, we provide evidence for the persistence of intraglacial refugia during the LGM. *L. tenuis* has behaved as a species sensitive to climate changes associated with glacial cycles, to orographic and fluvial barriers. Demographic changes seem to have been more pronounced south of the Biobío River and at Andean areas. In the northern fraction of the distribution, populations also were negatively affected, likely due to changes in vegetation cover. Meanwhile, populations of the central range have remained more structured and stable.

4.3 Taxonomic implications

Müller and Hellmich (1933) proposed two subspecies for *L. tenuis* based on color patterns and distribution (Donoso-Barros, 1966): *L. t. tenuis*, with type locality in Santiago de Chile, distributed from the northern limit in Coquimbo south to Los Ríos (Donoso-Barros, 1966; Vidal et al., 2004); The second subspecies, *L. t. punctatisimus* whose type locality is Lota, distributed in the coastal Biobío Region. Vidal et al. (2002, 2004) integrated morphology and population genetic structure to assess the validity of these subspecies, concluding that the color pattern varies clinally within a single species with no subspecies. Nevertheless, the high levels of molecular divergence between both main lineages (I and II) of *L. tenuis*, casts doubts on the taxonomic scenario proposed by Vidal et al. (2002,

585 2004). For example, other species of the genus, such as *L. fitzingerii* and *L. chehuachekenk*
586 diverge from each other by a lower value of genetic distance (approx. 4%) than that
587 observed between the major clades of *L. tenuis* (Clades I and II show a p-distance higher
588 than 9%). These values may indicate that *L. tenuis* as currently understood is a species
589 complex; this hypothesis should be tested with an integrative taxonomic approach.
590

591 **5. Conclusions**

592 Based on thorough sampling of the species *Liolaemus tenuis*, and using both mitochondrial
593 and nuclear markers as well as microsatellite data, we conclude that both landscape
594 heterogeneity and Pleistocene glaciations have shaped this species phylogeographic
595 history. We found evidence of recent colonization of the southern range of the species, as
596 well as signs of higher stability, genetic structure and higher haplotype diversity in the
597 northern and coastal ranges, as well as in the Central Valley relative to the southern
598 populations. Our results point to phylogeographic breaks concordant with some large
599 Chilean rivers, mainly, the Maipo (~34° S) and the Biobío rivers (~37ºS-38ºS), which have
600 been previously identified as significant barriers to dispersal for other vertebrates. Finally,
601 based on p-distance and Fst values, as well as on the occurrence of co-distributed
602 divergent clades in the southern range of the species, we predict that *L. tenuis* probably
603 constitutes a species complex with cryptic diversity. Therefore, we posit that this taxon
604 merits a thorough taxonomic revision in order to determine if the evolutionary lineages
605 reported in this study merit full species status.
606

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615 2013).
616

617 **References**

618 Amigo, J., Ramírez, C., 1998.A bioclimatic classification of Chile, woodland
619 communities in the temperate zone.Plant ecology 136, 9-
620 26.doi:10.1023/A:1009714201917.
621 Antonelli, A., Quijada-Mascareñas, A., Crawford, A. J., Bates, J. M., Velazco, P. M., &
622 Wüster, W., 2010. Molecular studies and phylogeography of Amazonian tetrapods
623 and their relation to geological and climatic models, in: Hoorn, C., Wesselingh, F.P.
624 (Eds), Amazonia, landscape and species evolution: a look into the past. John Wiley
625 & Sons, pp. 386-404. doi: 10.1002/9781444306408.ch1
626 Armesto, J., Villagrán, C., Donoso, C., 1994. La historia del bosque templado chileno.
627 Ambiente y Desarrollo, 3:66–72.
628 Balloux, F., Lugon-Moulin, N., 2002.The estimation of population differentiation with
629 microsatellite markers. Mol. Ecol. 11(2), 155-165.doi: 10.1046/j.0962-
630 1083.2001.01436.x.
631 Beerli, P., 2006. Comparison of Bayesian and maximum-likelihood inference of

632 population genetic parameters. Bioinformatics 22(3), 341-345.doi:
633 10.1093/bioinformatics/bti803.
634 Beerli, P., Felsenstein, J., 2001. Maximum likelihood estimation of a migration matrix and
635 effective population sizes in n subpopulations by using a coalescent approach. Proc.
636 Natl. Acad. Sci. 98(8), 4563-4568.doi: 10.1073/pnas.081068098.
637 Beerli, P., Palczewski, M., 2010.Unified framework to evaluate panmixia and migration
638 direction among multiple sampling locations. Genetics 185(1), 313-326. doi:
639 10.1534/genetics.109.112532.
640 Beheregaray, L.B., 2008. Twenty years of phylogeography: the state of the field and the
641 challenges for the Southern Hemisphere. Mol. Ecol. 17(17), 3754-3774. doi:
642 10.1111/j.1365-294X.2008.03857.x.
643 Breitman, M.F., Bonino, M.F., Sites, Jr.J.W., Avila, L.J., Morando, M., 2015.
644 Morphological variation, niche divergence, and phylogeography of lzards of the
645 *Liolaemus lineomaculatus* section (Liolaemini) from Southern
646 Patagonia.Herpetol.Monogr. 29(1), 65-88. doi:
647 http://dx.doi.org/10.1655/HERPMONOGRAPHS-D-14-00003.
648 Broadley, D.G., Whiting, A.S., Bauer, A.M., 2006. A revision of the East African species
649 of *Melanoseps* Boulenger (Sauria: Scincidae: Feyliniinae). Afr. J. Herpetol. 55(2),
650 95-112.
651 Brown, J.L., Cameron, A., Yoder, A.D., Vences, M., 2014.A necessarily complex model
652 to explain the biogeography of the amphibians and reptiles of Madagascar.Nat.
653 commun. 5. doi:10.1038/ncomms6046.
654 Brüggen, J., 1950. Fundamentos de la Geologia de Chile. Santiago: Instituto Geográfico

655 Militar.
656 Capparella, A.P., 1991. Neotropical avian diversity and riverine barrier. Acta Congressus
657 Internationalis Ornithologici 20, 307–316.
658 Carnaval, A.C., Hickerson, M.J., Haddad, C.F.B., Rodrigues, M.T., Moritz, C., 2009.
659 Stability predicts genetic diversity in the Brazilian Atlantic forest hotspot. Science
660 323, 785–89.doi: 10.1126/science.1166955.
661 Chesser, R.T., 1999.Molecular systematics of the rhinocryptid
662 genus Pteroptochos. Condor 439-446. doi: 10.2307/1370012
663 Cheviron, Z.A., Hackett, S.J., Capparella, A.P., 2005. Complex evolutionary history of a
664 Neotropical lowland forest bird (*Lepidothrix coronata*) and its implications for
665 historical hypotheses of the origin of Neotropical avian diversity. Mol. Phylogenet.
666 Evol. 36, 338–357. doi: 10.1016/j.ympev.2005.01.015.
667 Clement, M., Posada, D., Crandall, H., 2000. TCS: a computer program to estimate gene
668 genealogies. Mol. Ecol. 9(10), 1657-1660.
669 Codoncode Corporation. 2009. Codon Code aligner v. 3.5.1.
670 Crandall, K.A., Templeton, A.R., 1993. Empirical test of some predictions from
671 coalescent theory with applications to intraspecific phylogeny
672 reconstruction.Genetics 134, 959-969.
673 Di Castri, F., 1968. Esquisse écologique du Chili, in : Delamare-Debouteville, C.,
674 Rapoport, T., (Eds), Biologie de l'Amérique austral. Edition du Centre National de
675 la Recherche Scientifique, Paris, France, pp. 7–52.
676 Ding, L. I., Gan, X.N., He, S.P., Zhao, E.M., 2011. A phylogeographic, demographic and
677 historical analysis of the short-tailed pit viper (*Gloydius brevicaudus*): evidence for

678 early divergence and late expansion during the Pleistocene. Mol. Ecol., 20(9), 1905-
679 1922.
680 Donoso-Barros, R., 1966. Reptiles de Chile. Ediciones de la Universidad de Chile,
681 Santiago, Chile.
682 Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by
683 sampling trees. BMC Evol. Biol., 7(214), 1–8. doi: 10.1186/1471-2148-7-214.
684 Drummond, A.J., Rambaut, A., Shapiro, B., Pybus, O.G., 2005. Bayesian Coalescent
685 Inference of Past Population Dynamics from Molecular Sequences. Mol. Biol. Evol.
686 22 (5), 1185-1192. doi: 10.1093/molbev/msi103
687 Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics
688 with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29, 1969-1973. doi:
689 10.1093/molbev/mss075. Epub 2012 Feb 25.
690 Duméril, A.M.C., Bibron, G., 1837. Erpétologie Genérale ou Histoire Naturelle Complète
691 des Reptiles. Volume 4. Histoire de Quarante-six Genres et de Cent Quarante-six
692 Espèces de la Famille des Iguaniens, de l'Ordre des Sauriens. Librarie
693 Enclyclopedique de Roret, Paris.
694 Excoffier, L., Lischer, H.E., 2010. Arlequin suite ver 3.5: a new series of programs to
695 perform population genetics analyses under Linux and Windows. Mol. Ecol. Res. 10
696 (3), 564-567. doi: 10.1111/j.1755-0998.2010.02847.x.
697 Fontanella, F.M., Olave, M., Avila, L.J., Sites J.W.Jr., Morando, M., 2012. Molecular
698 dating and diversification of the South American lizard
699 genus *Liolaemus* (subgenus *Eulaemus*) based on nuclear and mitochondrial DNA
700 sequences. Zool. J. Linn. Soc-Lond. 164, 825–835.doi: 10.1111/j.1096-

3642.2011.00786.x.

Formas, J.R., 1979. La herpetofauna de los bosques temperados de Sudamérica. The South American herpetofauna: its origin, evolution and dispersal. Museum of Natural History, University of Kansas, Monograph, 7, 341-369.

Fu, Y.X., 1997. Statistical test of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 131, 479-491.

Garrick, R., Sunnucks, P., Dyer, R., 2010. Nuclear gene phylogeography using PHASE: dealing with unresolved genotypes, lost alleles, and systematic bias in parameter estimation. BMC Evol.Biol. 10, 118.doi: 10.1186/1471-2148-10-118.

Gregory-Wodzicki, K.M., 2000. Uplift history of the Central and Northern Andes: a review. Geol. Soc. Am. Bull. 112 (7), 1091-1105.

Guillot, G., Mortier, F., Estoup, A., 2005. GENELAND: a computer package for landscape genetics. Mol. Ecol. notes, 5(3), 712-715. doi: 10.1111/j.1471-8286.2005.01031.x.

Hanna, N., Brown, D., Avila, L.J., Sites, J.W., Morando, M., Fontanella, F.M., 2012. Characterization of 10 polymorphic microsatellite loci in the South American lizard *Liolaemus fitzingerii* with cross-amplification in *L. chehuachekenk*.Conserv.Genet. Resour. 4(1), 105–7.doi 10.1007/s12686-011-9485-5.

Harpending, H.C., 1994. Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. Human biology, 591-600.

Heath, L., van der Walt, E., Varsani, A., Martin, D.P., 2006. Recombination patterns in aphthoviruses mirror 596 those found in other picornaviruses. J. Virol. 80, 11827–11832. doi:10.1128/JVI.01100-06.

724 Heusser, C.J., 1966. Late-Pleistocene pollen diagrams from the Province of Llanquihue,
725 southern Chile. P. Am. Philos. Soc. 110(4), 269-305.
726 Heusser, C.J., 2003. Ice age southern Andes, A cronichle of paleoecological
727 events.Developments in quaternary science, 3. Elsevier, 238 pp
728 Hewitt, G.M., 1996. Some genetic consequences of ice ages, and their role in divergence
729 and speciation.Biol. J. Linn. Soc.58, 247–276.
730 Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A., 2005. Very high
731 resolution interpolated climate surfaces for global land area. Int. J. Climatol. 25,
732 965–1978.
733 Hulton, N. R., Purves, R. S., McCulloch, R. D., Sugden, D. E., & Bentley, M. J., 2002.
734 The last glacial maximum and deglaciation in southern South America. Quaternary
735 Sci. Rev. 21(1), 233-241.doi: http://dx.doi.org/10.1016/S0277-3791(01)00103-2
736 Kass, R. E., Raftery, A.E., 1995. Bayes factors. J. Am. Stat. Assoc. 90(430), 773-795. doi:
737 10.1080/01621459.1995.10476572.
738 Lamborot, M., Eaton, L, Carrasco, B.A., 2003. The Aconcagua River as another barrier to
739 *Liolaemus monticola* (Sauria: Iguanidae) chromosomal races of central Chile. Rev.
740 Chil. Hist. Nat. 76, 23-34. doi: http://dx.doi.org/10.4067/S0716-
741 078X2003000100003.
742 Lessa, E. P., D'Elía, G., Pardinas, U.F., 2010. Genetic footprints of late Quaternary
743 climate change in the diversity of Patagonian-Fueguian rodents. Mol. Ecol. 19(15),
744 3031-3037. doi: 10.1111/j.1365-294X.2010.04734.x.
745 Levy, E., Kennington, W. J., Tomkins, J. L., LeBas, N.R., 2012.Phylogeography and
746 population genetic structure of the Ornate Dragon Lizard, *Ctenophorus ornatus*.PloS

747 one 7(10), e46351. doi: http://dx.doi.org/10.1371/journal.pone.0046351.
748 Link, A., Valencia, L.M., Cespedes, L.N., Duque, L.D., Cadena, C.D., Di Fiori, A., 2015.
749 Phylogeography of the critically endangered brown spider monkey (*Ateles*
750 *hybridus*): testing the riverine barrier hypothesis. Int. J. Primatol. 36: 530-547. doi:
751 10.1007/s10764-015-9840-6.
752 Maldonado-Coelho, M., Blake, J., Silveira, L., Batalha-Filho, H., Ricklefs, R.,
753 2013.Rivers, refuges and population divergence of fire-eye antbirds (*Pyriglena*) in
754 the Amazon Basin. J. Evolution. Biol. 26, 1090–1107.doi: 10.1111/jeb.12123
755 Markgraf, V., McGlone, M., Hope, G., 1995. Neogene paleoenvironmental and
756 paleoclimatic change in southern temperate ecosystems – a southern perspective.
757 Trends Ecol. Evol.10, 143-147.
758 Martin, D. P., Williamson, C., Posada, D., 2005. RDP2: recombination detection and
759 analysis from sequence alignments. Bioinformatics 21(2), 260-262. doi:
760 10.1093/bioinformatics/bth490.
761 Martin, D., Rybicki, E., 2000. RDP: detection of recombination amongst aligned
762 sequences. Bioinformatics 16, 562–563. doi: 10.1093/bioinformatics/16.6.562
763 McCulloch, R.D., Bentley, M, J., Purves, R.S., 2000. Climatic inferences from glacial and
764 paleoecological evidence at the last glacial termination, southern South America. J.
765 Quaternary Sci. 15, 409–417. doi: 10.1002/1099-1417(200005)15:4<409::AID-
766 JQS539>3.0.CO;2-#
767 Morando, M., Avila, L.J., Baker, J., Sites, J.W. Jr., 2004. Phylogeny and phylogeography
768 of the *Liolaemus darwinii* complex (Squamata: Liolaemidae): evidence for
769 introgression and incomplete lineage sorting. Evolution 58(4), 842-61.

770 Mráz, P., Gaudeul, M., Rioux, D., Gielly, L., Choler, P., Taberlet, P., 2007. Genetic
771 structure *of Hypochaeris uniflora* (Asteraceae) suggests vicariance in the
772 Carpathians and rapid post-glacial colonization of the Alps from an eastern Alpine
773 refugium. J. Biogeogr. 34 (12), 2100-2114.doi: 10.1111/j.1365-2699.2007.01765.x
774 Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A., Kent, J., 2000.
775 Biodiversity hotspots for conservation priorities.Nature. 403 (6772), 853-858.doi:
776 10.1038/35002501.
777 Naidoo, T., Schoeman, M. C., Goodman, S. M., Taylor, P. J., Lamb, J. M., 2016.
778 Discordance between mitochondrial and nuclear genetic structure in the bat
779 *Chaerephon pumilus* (Chiroptera: Molossidae) from southern Africa. Mamm. Biol.
780 81(2), 115-122. doi: http://dx.doi.org/10.1016/j.mambio.2015.11.002
781 Nicolas, V., Missoup, A. D., Denys, C., Kerbis Peterhans, J., Katuala, P., Couloux, A.,
782 Colyn, M., 2011.The roles of rivers and Pleistocene refugia in shaping genetic
783 diversity in *Praomys misonnei* in tropical Africa.J. Biogeogr. 38, 191–207.
784 Nuñez, J.J., Wood, N. K., Rabanal, F. E., Fontanella, F. M., Sites, J. W. Jr., 2011.
785 Amphibian phylogeography in the Antipodes, Refugia and postglacial colonization
786 explain mitochondrial haplotype distribution in the Patagonian frog *Eupsophus*
787 *calcaratus* (Cycloramphidae). Mol. Phylogenet. Evol. 58 (2), 343-352. doi:
788 10.1016/j.ympev.2010.11.026.
789 Palumbi, S.R., 1996. Nucleic acids I: the polymerase chain reaction, in: Hillis, D.M.,
790 Moritz, C., Mable, B.K. (Eds.), Molecular Systematics, 2nd Ed. Sinauer Associates,
791 Sunderland, MA, pp. 205–247.
792 Patton, J. L., da Silva, M. N. F., 1998. Rivers, refuges, and ridges: The geography of

793 speciation of Amazonian mammals, in: Howard, D.J., Berlocher, S.H. (Eds.),
794 Endless forms: Species and speciation. New York: Oxford University Press, pp 202-
795 213.
796 Peakall, R. O. D., Smouse, P. E., 2006. GENALEX 6: genetic analysis in Excel.
797 Population genetic software for teaching and research. Mol. Ecol. notes 6(1), 288-
798 295. doi: 10.1111/j.1471-8286.2005.01155.x
799 Peakall, R.O.D., Smouse, P. E., 2012. GenAlEx 6.5: genetic analysis in Excel. Population
800 genetic software for teaching and research—an update. Bioinformatics 28, 2537-
801 2539.doi: 10.1093/bioinformatics/bts460
802 Pellegrino, K. C. M., Rodriges, M. T., Waite, A. N., Morando, M., Yassuda, Y. Y., Sites,
803 J.W.J., 2005. Phylogeography and species limits in the Gymnodactylus darwinii
804 complex (Gekkonidae, Squamata): Genetic structure coincides with river systems in
805 the Brazilian Atlantic Forest. Biol. J. Linn. Soc.85, 13–26.
806 Petit, R.J., Brewer, S., Bordács, S., Burg, K.,Cheddadi, R., Coart, E., Cottrell, J., Csaikl,
807 U.M., Dam,B., Deans, J.D., Espinel,S., Fineschi,S., Finkeldey, R., Glaz, I.,
808 Goicoechea P.G., Jensen, J.S., König, A.O., Lowe, A.J., Madsen, S.F., Mátyás, G.,
809 Munro, R.C., Popescu, F., Slade, D., Tabbener, H., Vries, S.G.M., Ziegenhagen, B.,
810 Beaulieu, J., Kremer, A., 2002. Identification of refugia and post-glacial
811 colonization routes of European white oaks based on chloroplast DNA and fossil
812 pollen evidence. Forest Ecol. Manag. 59, 49–74.
813 Phillips, S.J., Anderson, R.P., Schapire, R.E., 2006. Maximum entropy modelling of
814 species geographic distributions.Ecol. Model. 190, 231–259. doi:
815 http://dx.doi.org/10.1016/j.ecolmodel.2005.03.026.

816 Pincheira-Donoso, D., Núñez, H., 2005. Las especies del genero *Liolaemus* Wiegmann,
817 1834 (Iguania, Tropiduridae, Liolaeminae). Taxonomía, sistemática y evolución.
818 Publicación Ocasional del Museo de Historia Natural. Chile 59, 7–486.
819 Portik, D.M., Bauer, A.M., Jackman, T.R., 2011a. Bridging the gap: western rock skinks
820 (*Trachylepis sulcata*) have a short history in South Africa. Mol. Ecol. 20:1744–
821 1758. doi: 10.1111/j.1365-294X.2011.05047.x
822 Portik, D.M., Wood, Jr P. L., Grismer, J. L., Stanley, E. L., Jackman, T.R., 2011b.
823 Identification of 104 rapidly-evolving nuclear protein-coding markers for
824 amplification across scaled reptiles using genomic resources.Conserv.Genet. Resour.
825 4(1), 1-10. doi: 10.1007/s12686-011-9460-1.
826 Posada, D., 2008.jModeltest: Phylogenetic Model Averaging. Mol. Biol. Evol.25, 1253-
827 1256.
828 R Development Core Team, 2009. R: A language and environment for statistical
829 computing. R Foundation for Statistical Computing, Austria.
830 Rabassa, J., Clapperton, C., 1990. Quaternary glaciations of the southern Andes.
831 Quaternary Sci. Rev. 9, 153–174. doi: 10.1016/0277-3791(90)90016-4
832 Rambaut, A., Drummond, A.J., Suchard, M., 2013.Tracer v1.6. Available from:
833 http://tree.bio.ed.ac.uk/software/tracer/
834 Ribas, C. C., Aleixo, A., Nogueira, A. C., Miyaki, C. Y., Cracraft, J., 2011. A
835 palaeobiogeographic model for biotic diversification within Amazonia over the past
836 three million years. Proc. R. Soc. Lond. B. Biol. Sci. doi:10.1098/rspb.2011.1120.
837 Rogers, A. R., Harpending, H., 1992. Population growth makes waves in the distribution
838 of pairwise genetic differences. Mol. Biol. Evol. 9(3), 552-569.

839 Ronquist, F., Huelsenbeck, J.P., 2003. MRBAYES 3: Bayesian phylogenetic inference
840 under mixed models. Bioinformatics 19, 1572-1574.
841 Ruzzante, D.E., Walde, S.J., Gosse, J.C., 2008. Climate control on ancestral population
842 dynamics: insight from Patagonian fish phylogeography. Mol. Ecol. 17, 2234–2244.
843 doi: 10.1111/j.1365-294X.2008.03738.x
844 Sallaberry-Pincheira, N., Garin, C. F., González-Acuña, D., Sallaberry, M. A., Vianna,
845 J.A., 2011. Genetic divergence of Chilean long-tailed snake (*Philodryas*
846 *chamissonis*) across latitudes: conservation threats for different
847 lineages. Divers.Distrib. 17(1), 152-162. doi: 10.1111/j.1472-4642.2010.00729.x
848 Sérsic, A., A. Cosacov, A.A. Cocucci, L. Johnson, R. Pozner, L.J. Avila, J.W. Sites,
849 Morando, M., 2011. Emerging phylogeographical patterns of plants and terrestrial
850 vertebrates from Patagonia. Biol. J. Linn. Soc. 103, 475 – 494. doi: 10.1111/j.1095-
851 8312.2011.01656.x.
852 Smith, B. T., McCormack, J. E., Cuervo, A. M., Hickerson, M. J., Aleixo, A., Cadena, C.
853 D., Pérez-Emán, J. L., Burney, C. W., Xie, X., Harvey, M. G., Faircloth, B. C.,
854 Glenn, T. C., Derryberry, E. P., Prejean, J., Fields, S., Brumfield, R. T., 2014. The
855 drivers of tropical speciation.Nature, 515,406–409.doi: 10.1038/nature13687.
856 Smith-Ramírez, C., 2004.The Chilean coastal range, a vanishing center of biodiversity
857 and endemism in South American températe rainforests.Biodivers.conserv. 13, 373-
858 393. doi: 10.1023/B:BIOC.0000006505.67560.9f
859 Stephens, M., Donnelly, P., 2003. Ancestral inference in population genetics models with
860 selection (with discussion). Aust. N. Z. J. Stat., 45(4), 395-430.
861 Stephens, M., Smith, N.J., Donnelly, P., 2001.A new statistical method for haplotype

862 reconstruction from population data. Am. J. Hum. Genet. 68, 978-989. doi:
863 10.1086/319501
864 Stephens, M., Scheet, P., 2005.Accounting for decay of linkage desequilibrium in
865 haplotype inference and missing-data imputation. Am. J. Hum. Genet. 76, 449-462.
866 doi: 10.1086/428594
867 Swets, J. A., 1988. Measuring the accuracy of diagnostic systems. Science 240(4857),
868 1285-1293.
869 Tajima, F., 1989.Statistical method for testing the neutral mutation hypothesis by DNA
870 polymorphism. Genetics 123, 585–595.
871 Tamura, K., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA 5: Molecular
872 Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance
873 and Maximum Parsimony Methods. Mol. Biol. Evol. 28, 2731-2739. doi:
874 10.1093/molbev/msr121
875 Torres-Pérez, F., Lamborot, M., Boric-Bargetto, D., Hernandez, C. E., Ortiz, J. C., Palma,
876 R. E., 2007. Phylogeography of a mountain lizard species: An ancient fragmentation
877 process mediated by riverine barriers in the *Liolaemus monticola* complex (Sauria:
878 Liolaemidae). J. Zool. Syst. Evol. Res. 45, 72–81. DOI: 10.1111/j.1439-
879 0469.2006.00392.x
880 Turchetto-Zolet.A.C., Pinheiro, F., Salgueiro, F., Palma-Silva, C., 2013.
881 Phylogeographical patterns shed light on evolutionary process in South America.
882 Mol. Ecol. 22(5), 1193-1213. doi: 10.1111/mec.12164
883 Uetz, P., Freed, P., Hošek, J., (eds.). 2016. The Reptile Database, http://www.reptile-
884 database.org, accessed [August 29 2016]

885 Unmack, P. J., Bennin, A. P., Habit, E. M., Victoriano, P. F., Johnson, J. B., 2009. Impact
886 of ocean barriers, topography, and glaciation on the phylogeography of the catfish
887 *Trichomycterus areolatus* (Teleostei: Trichomycteridae) in Chile. Biol. J. Linn. Soc.
888 97(4), 876-892. doi: 10.1111/j.1095-8312.2009.01224.x.
889 Valdez, L., D'Elía, G., 2013. Differentiation in the Atlantic Forest: phylogeography of
890 *Akodon montensis* (Rodentia, Sigmodontinae) and the Carnaval-Moritz model of
891 Pleistocene refugia. J. Mammal. 94(4), 911-922. doi: http://dx.doi.org/10.1644/12-
892 MAMM-A-227.1
893 Vásquez, D., Correa, C., Pastenes, L., Palma, R. E., Méndez, M.A., 2013. Low
894 phylogeographic structure of *Rhinella arunco* (Anura: Bufonidae), an endemic
895 amphibian from the Chilean Mediterranean hotspot. Zool. Stu. 52(1), 35. doi:
896 10.1186/1810-522X-52-35
897 Vera-Escalona, I., D'Elía, G., Gouin, N., Fontanella, F.M., Muñoz-Mendoza, C., Sites,
898 J.W., Victoriano, P.F., 2012. Lizards on ice: evidence for multiple refugia *in*
899 *Liolaemus pictus* (Liolaemidae) during the last glacial maximum in the Southern
900 Andean beech forests. PloS one 7(11), e48358. doi:10.1371/journal.pone.0048358.
901 Victoriano, P.F., Ortiz, J.C., Benavides, E., Adams, B.J., Sites, J.W. Jr., 2008.
902 Comparative phylogeography of codistributed species of Chilean *Liolaemus*
903 (Squamata, Tropiduridae) from the central-southern Andean range.Mol. Ecol. 17,
904 2397-2416. doi: 10.1111/j.1365-294X.2008.03741.x.
905 Victoriano, P. F., Muñoz-Mendoza, C., Sáez, P. A., Salinas, H. F., Muñoz-Ramírez, C.,
906 Sallaberry, M., Méndez, M. A. 2015. Evolution and Conservation on Top of the
907 World: Phylogeography of the Marbled Water Frog (*Telmatobius marmoratus*

908 Species Complex; Anura, Telmatobiidae) in Protected Areas of Chile. J.
909 Hered. 106(S1), 546-559. doi: 10.1093/jhered/esv039.
910 Vidal, M.A., 2002. Variación morfológica, cromosómica e isoenzimática en *Liolaemus*
911 *tenuis* (Duméril & Bibron, 1837) (Tropiduridae). Thesis of Science, Universidad de
912 Concepción, Concepción, Chile.
913 Vidal, M., Ortiz, JC., Astorga, M., Victoriano, P., Lamborot, M., 2004. Revision of
914 *Liolaemus tenuis* subspecies (Duméril and Bibron, 1837) by analysis of population
915 genetic structure.Amphibia-Reptilia 25, 438–445.
916 Vidal, M., Ortiz, J.C., Ramírez, C.C., Lamborot, M., 2005. Intraspecific variation in
917 morphology and sexual dimorphism in *Liolaemus tenuis* (Tropiduridae).Amphibia-
918 Reptilia 26, 343–351. doi: 10.1163/156853805774408658
919 Villagrán, C., 1991. Historia de los bosques templados del sur de Chile durante el
920 Tardiglacial y Postglacial. Rev. Chil. Hist. Nat.64, 447–460.
921 Villagrán, C., Moreno, P., Villa., 1995. Antecedentes palinologicos acerca de la historia
922 cuaternaria de los bosques chilenos, in: Armesto, J.J, Villagrán, C., Arroyo, M.T.K.
923 (Eds), Ecología de Los Bosques Nativos de Chile. Editorial Universitaria,
924 Universidad de Chile, Santiago, pp. 51-53.
925 Villagrán, C., 2001. Un modelo de la historia de la vegetación de la Cordillera de La
926 Costa de Chile central-sur: la hipótesis glacial de Darwin. Rev. Chil. Hist. Nat. 74,
927 793–803. http://dx.doi.org/10.4067/S0716-078X2001000400007.
928 Viruel, J., Catalán, P., Segarra-Moragues, J. G. 2014. Latitudinal environmental niches
929 and riverine barriers shaped the phylogeography of the central Chilean endemic
930 *Dioscorea humilis* (Dioscoreaceae). PloS one, 9(10), e110029.doi:

http://dx.doi.org/10.1371/journal.pone.0110029

Voelker, G., Marks, B. D., Kahindo, C., A'genonga, U., Bapeamoni, F., Duffie, L. E., Huntley, J. W., Mulotwa, E., Rosenbaum, S. A., Light, J. E., 2013. River barriers and cryptic biodiversity in an evolutionary museum. Ecol. Evol. 3,536–545.doi: 10.1002/ece3.482

Vuilleumier, B.S., 1971. Pleistocene changes in the fauna and flora of South America. Science 173, 771-780.

Waltari, E., Hijmans. R.J., Peterson, A. T., Nyari, A.S., Perkins, S.L., Guralnick, R.P., 2007. Locating Pleistocene Refugia: Comparing Phylogeographic and Ecological Niche Model Predictions.PLoS ONE 2 (7).doi: http://dx.doi.org/10.1371/journal.pone.0000563.

Wang Y., Yan, G., 2014. Molecular Phylogeography and Population Genetic Structure of *O. longilobus* and *O. taihangensis* (Opisthopappus) on the Taihang Mountains.PloS one 9(8), e104773.doi: 10.1371/journal.pone.0104773

Weir, B. S., Cockerham, C. C. 1984.Estimating F-statistics for the analysis of population structure. Evolution 1358-1370. doi: 10.2307/2408641.

Xia, X., Xie, Z., 2001.DAMBE.Software package for data analysis in molecular biology and evolution. J. Hered. 92 (4), 371-373. doi: 10.1093/jhered/92.4.371

Xia, X., Xie, Z., Salemi, M., Chen, L., Wang, Y., 2003.An index of substitution saturation and its application. Mol. Phylogenet. Evol. 26(1), 1-7.

Xu, J., Pérez-Losada, M., Jara, C. G., Crandall, K.A., 2009. Pleistocene glaciation leaves deep signature on the freshwater crab *Aegla alacalufi* in Chilean Patagonia. Mol. Ecol. 18(5), 904-918. doi: 10.1111/j.1365-294X.2008.04070.x

954 Zhang, W., Niu, Y., Yan, L., Cui, Z., Li, C., Mu, K., 2008a. Late Pleistocene glaciation of
955 the Changbai Mountains in northeastern China. Chinese Sci. Bull. 53 (17), 2672-
956 2684. doi: 10.1007/s11434-008-0347-9
957 Zhang, H., Yan, J., Zhang, G., Zhou, K. 2008b. Phylogeography and demographic history
958 of Chinese black-spotted frog populations (*Pelophylax nigromaculata*): evidence for
959 independent refugia expansion and secondary contact. BMC Evol. Biol. 8(1), 1. doi:
960 10.1186/1471-2148-8-21

962

Table 1. Measures of genetic diversity for mtDNA (a) and microsatellite loci (b) in different geographic groups. Cluster S7 is split in S7a and S7b corresponding to co-distributed clades B2.2.3 and II as shown in Fig. 2. Numbers in bold indicate statistical significant values. Numbers in parentheses indicate standard deviations. N: sequence numbers. Ragg: Raggedness index.

(a) Geographic group	Cluster	N	Number of haplotypes (h)	Number of polymorphic sites (S)	Haplotype diversity (Hd)	Nucleotide diversity (π)	Fs' Fu	D' Tajima	Ragg
Northern									
	N1	23	11	45	0.83 (0.07)	0.016 (0.01)	1.895	-0.226	**0.08**
	N2	21	11	82	0.90 (0.04)	0.028 (0.01)	3.822	-0.485	**0.05**
	Total Northern clusters	44	22	110	0.93 (0.02)	0.026 (0.01)	0.831	-0.915	**0.02**
Central									
	C3	27	17	115	0.95 (0.02)	0.030 (0.01)	0.854	-1.125	0.02
	C4	33	19	115	0.95 (0.02)	0.052 (0.03)	4.121	1.216	**0.03**
	C5	12	8	96	0.94 (0.06)	0.054 (0.03)	3.205	1.11	0.05
	Total Central clusters	72	44	216	0.98 (0.01)	0.065 (0.03)	-1.516	0.278	0.003
Southern									
	S6	7	1	0	---	---	**---**	---	---
	S7a	28	19	115	0.96 (0.03)	0.036 (0.02)	-0.147	-0.481	0.02
	S7b	74	10	13	0.33 (0.07)	0.001 (0.001)	**-7.254**	**-2.145**	0.31
	S7	102	29	155	0.65 (0.05)	0.048 (0.02)	10.77	0.619	0.09
	Total Southern clusters	109	30	156	0.69 (0.05))	0.046(0.02)	10.15	0.47	0.05
Total		225	96	261	0.92 (0.02)	0.072 (0.04)	-2.189	0.656	0.14

(b) Geographic group	Cluster	N	Average Observed Heterozygosity (Ho)	Average Expected Heterozygosity (He)	Average number of alleles per locus	Number of private alleles	Fixation index (F)
Northern							
	N1	28	0.210 (0.091)	0.397 (0.161)	4.8 (1.393)	4	0.358 (0.125)
Central							
	C2	39	0.278 (0.076)	0.563 (0.061)	5.6 (1.208)	3	0.538 (0.133)
	C3	38	0.265 (0.057)	0.565 (0.088)	6.2 (1.200)	5	0.510 (0.107)
	Total Central clusters	77	0.270 (0.063)	0.616 (0.080)	7.6 (1.230)	8	0.573 (0.096)
Southern							
	S4	77	0.289 (0.040)	0.562 (0.113)	7.0 (1.225)	7	0.418 (0.089)
	Total	182	0.261 (0.032)	0.522 (0.054)	5.9 (2.810)		

Table 2. AMOVA results for cyt *b* (a) and microsatellites (b). Toponymic for each group corresponds to river names. * p< 0.05; ** p < 0.001.

a	MtDNA		
Groups	**Among groups**	**Among population within groups**	**Within populations**
(North of Maipo) vs (South of Maipo)	30.44 **	58.79 **	10.77 **
(North of Maipo) vs (Maipo to Biobío) vs (South of Biobío)	43.83 **	44.68 **	11.49 **
(North of Biobío) vs (South of Biobío)	43.87 **	45.46 **	10.68 **
Among Clusters of Geneland	45.15 **	42.82 **	12.03 **

b	microsatellite		
	Among groups	**Among population within groups**	**Within populations**
(North Maipo) vs (South Maipo)	6.36 *	23.16 **	70.48 **
(North of Maipo) vs (Maipo to Biobío) vs (South of Biobío)	9.96 **	18.75 **	71.28 **
(North of Biobío) vs (South of Biobío)	10.39 **	19.39 **	70.22 **
Among Cluster of Geneland	12.18 **	16.34 **	71.47 **

Appendix A

PCR protocols. Three genes were amplified through the polymerase chain reaction (PCR): a fragment of the mitochondrial Cytochrome b (cyt *b*), an exon of the nuclear Kinesin-like protein gene (KIF24), and the anonymous nuclear gene LDB5B. Amplification and sequencing of cyt *b* was performed using primers GluDG-L (Palumbi, 1996) and WWR (Broadley, 2006) following the protocol of Victoriano et al. (2008). Nuclear genes were amplified following the protocol published by Portik et al. (2011a) using primers B5BF, B5BR, KIF24F, and KIF24R (Portik et al., 2011b). Amplifications occurred in 25uL volume reactions initiated at 95ºC for 2 min followed by 35 cycles of 95ºC for 35 s, 50ºC for 35 s and 72ºC for 1 min 35 s (with extension increasing 4 s per cycle). All PCR products were checked using a SybrSafe stained 1.5% agarose gel. Both forward and reverse strands of each amplicon were sequenced at Macrogen Inc. (Korea).

References for Appendix A

Broadley, D.G., Whiting, A.S., Bauer, A.M., 2006. A revision of the East African species of *Melanoseps* Boulenger (Sauria: Scincidae: Feyliniinae). Afr. J. Herpetol. 55(2), 95-112.

Palumbi, S.R., 1996. Nucleic acids I: the polymerase chain reaction, in: Hillis, D.M., Moritz, C., Mable, B.K. (Eds.), Molecular Systematics, 2nd Ed. Sinauer Associates, Sunderland, MA, pp. 205–247.

Portik, D.M., Bauer, A.M., Jackman, T.R., 2011a. Bridging the gap: western rock skinks (*Trachylepis sulcata*) have a short history in South Africa. Mol. Ecol. 20:1744–

1758. doi: 10.1111/j.1365-294X.2011.05047.x

Portik, D.M., Wood, Jr P. L., Grismer, J. L., Stanley, E. L., Jackman, T.R., 2011b. Identification of 104 rapidly-evolving nuclear protein-coding markers for amplification across scaled reptiles using genomic resources. Conserv.Genet. Resour. 4(1), 1-10. doi: 10.1007/s12686-011-9460-1

Victoriano, P.F., Ortiz, J.C., Benavides, E., Adams, B.J., Sites, J.W. Jr., 2008. Comparative phylogeography of codistributed species of Chilean *Liolaemus* (Squamata, Tropiduridae) from the central-southern Andean range. Mol. Ecol. 17, 2397-2416. doi: 10.1111/j.1365-294X.2008.03741.x.

Figure captions

Fig. 1. Sampling sites of *Liolaemus tenuis* across its Chilean range. Numbers correspond to those of Table S1. Colors indicate their geographical assignation: red correspond to the dry Mediterranean bioclimate zone (North); green signal the mesic Mediterranean area (Central); and blue the wet Mediterranean area (South). The dashed line corresponds to the ice sheet's limits during the LGM (adapted from Heusser 2003).

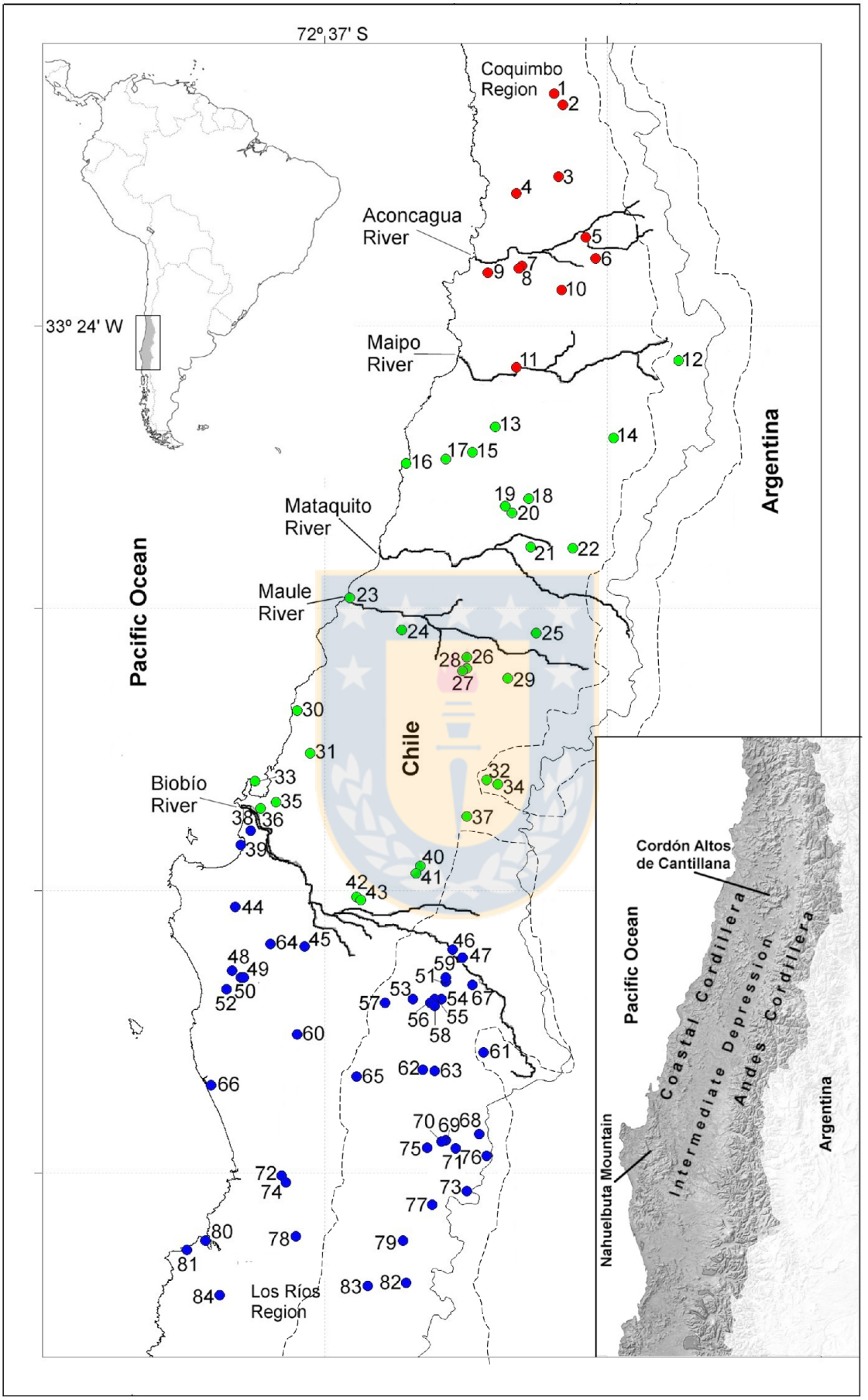
Fig. 2. Genealogical relationships of cyt *b* *Liolaemus tenuis* haplotypes as inferred by Bayesian analysis. Branch colors correspond to the colors depicted in the map of Fig. 1. Numbers on branches represent Bayesian posterior probabilities. Haplotype numbers on tips are the same as in Fig. 3. Numbers within parentheses refer to the locality numbers detailed in Table S1. Vertical black bars correspond to the main clades. Divergence times

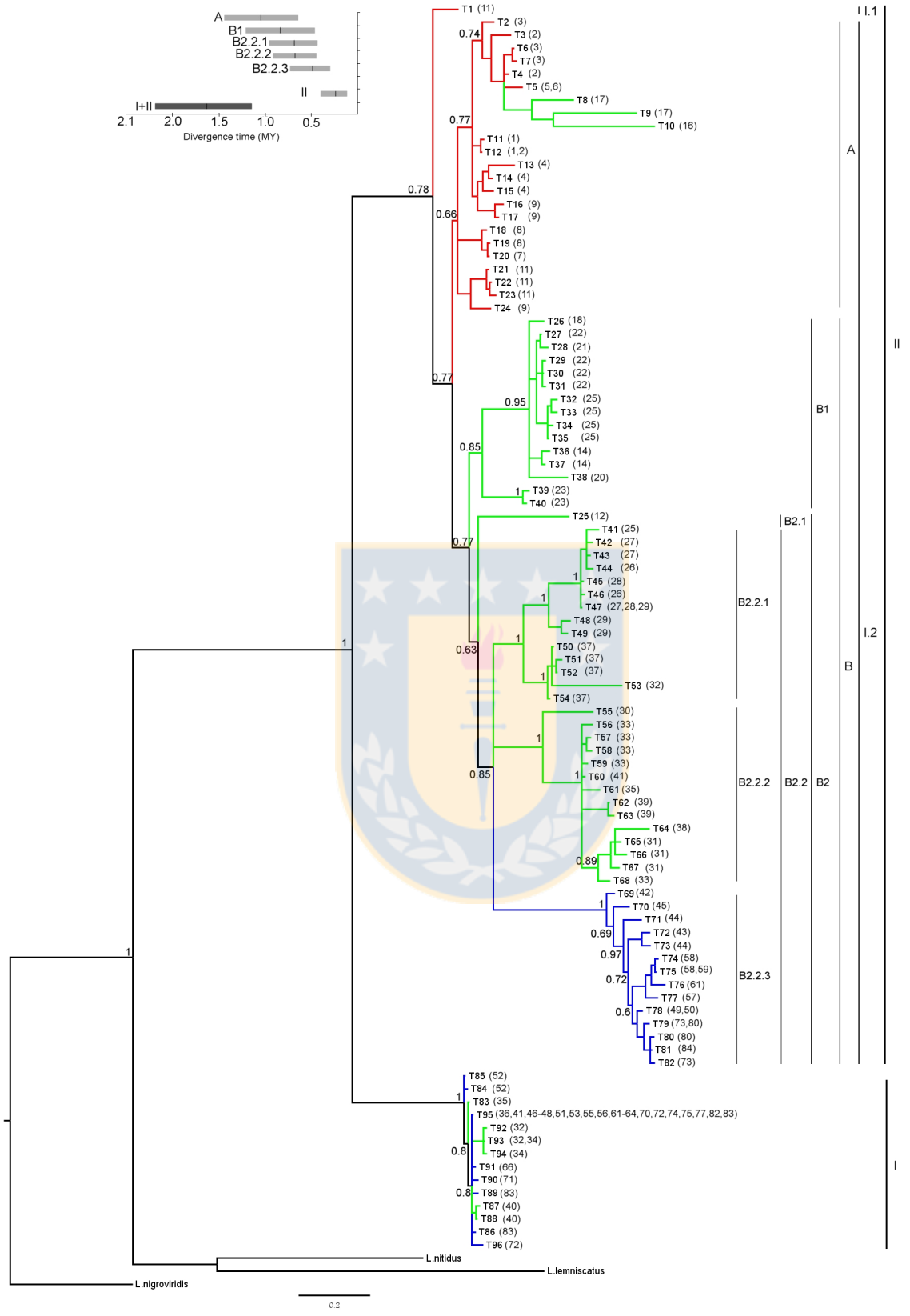
(in million years) for each clade are shown in the upper left side.

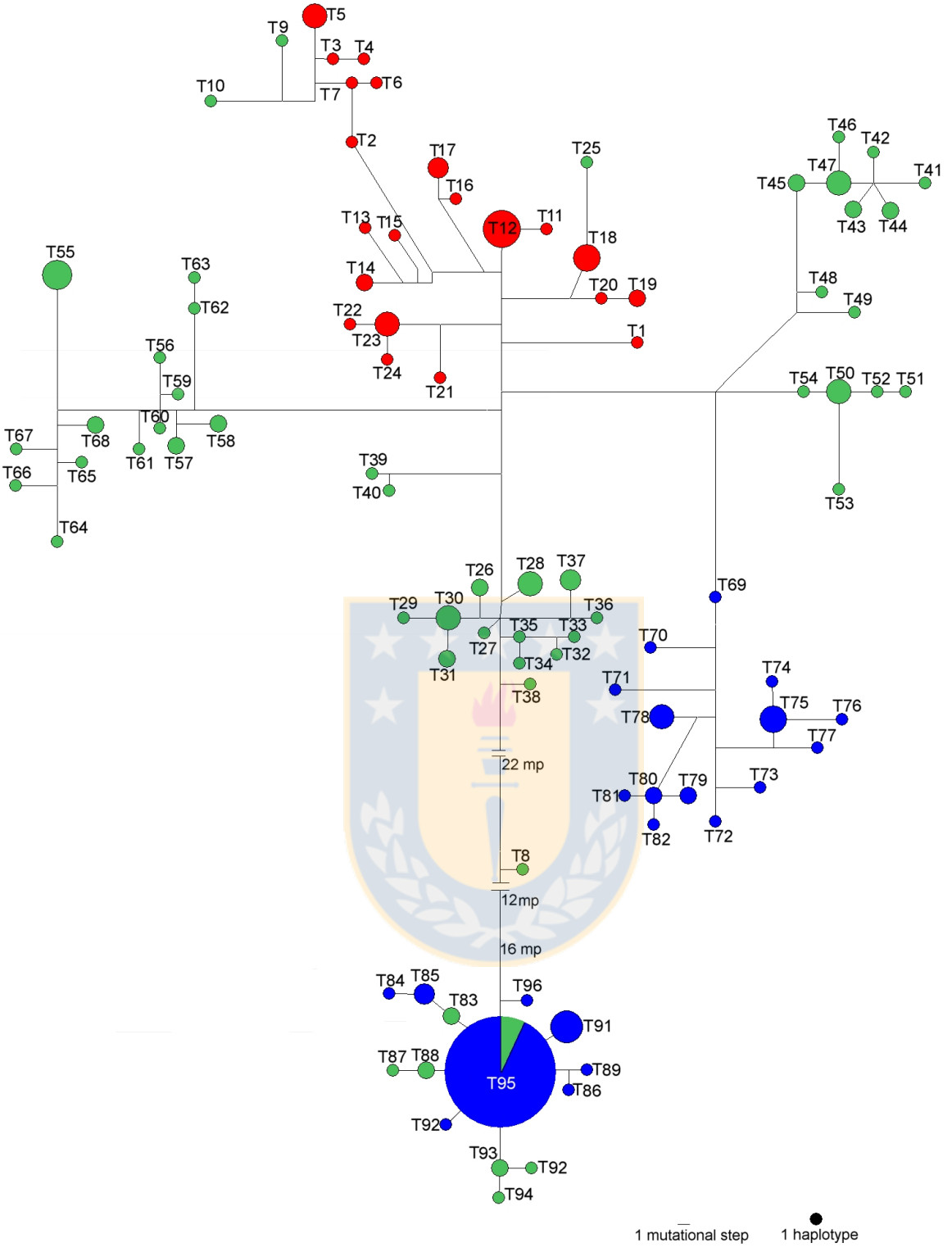
Fig. 3. Parsimony-based haplotype network of mitochondrial sequences (cyt *b*) of *Liolaemus tenuis.* Colors indicate geographic origin as depicted in Fig. 1. Table S1 gives details of collection site and group assignation for the haplotypes.

Fig. 4.A) Population clusters (polygons) of *Liolaemus tenuis* identified by Geneland, based on cyt *b* sequences. Colors of site circles correspond to those depicted in Fig. 1. Black arrows represent direction and migration rates between clusters, and grey arrows represent direction and migration rates between Andes –Coast intra Cluster S7. Migration rate values for selected cluster pairs are shown in the upper left corner. B) Habitat suitability map for *L. tenuis* during the Last Glacial Maximum (upper right) and the present (lower right).

Fig. 5. Bayesian Skyline plots representing recent demographic trends of each of the six main phylogroups of *Liolaemus tenuis* based on mitochondrial sequences (cyt *b*). Light-blue area represents 95% confidence intervals for Ne.


72º 37' S
33º 24' W
Coquimbo Region
Aconcagua River
Maipo River
Mataquito River
Maule River
Biobío River
Los Ríos Region
Pacific Ocean
Chile
Argentina
Cordón Altos de Cantillana
Coastal Cordillera
Intermediate Depression
Andes Cordillera
Nahuelbuta Mountain


A
B1
B2.2.1
B2.2.2
B2.2.3
II
I+II
2.1
2.0
1.5
1.0
0.5
Divergence time (MY)
T1 (11)
T2 (3)
T3 (2)
T6 (3)
T7 (3)
T4 (2)
T5 (5,6)
T8 (17)
T9 (17)
T10 (16)
T11 (1)
T12 (1,2)
T13 (4)
T14 (4)
T15 (4)
T16 (9)
T17 (9)
T18 (8)
T19 (8)
T20 (7)
T21 (11)
T22 (11)
T23 (11)
T24 (9)
T26 (18)
T27 (22)
T28 (21)
T29 (22)
T30 (22)
T31 (22)
T32 (25)
T33 (25)
T34 (25)
T35 (25)
T36 (14)
T37 (14)
T38 (20)
T39 (23)
T40 (23)
T25 (12)
T41 (25)
T42 (27)
T43 (27)
T44 (26)
T45 (28)
T46 (26)
T47 (27,28,29)
T48 (29)
T49 (29)
T50 (37)
T51 (37)
T52 (37)
T53 (32)
T54 (37)
T55 (30)
T56 (33)
T57 (33)
T58 (33)
T59 (33)
T60 (41)
T61 (35)
T62 (39)
T63 (39)
T64 (38)
T65 (31)
T66 (31)
T67 (31)
T68 (33)
T69 (42)
T70 (45)
T71 (44)
T72 (43)
T73 (44)
T74 (58)
T75 (58,59)
T76 (61)
T77 (57)
T78 (49,50)
T79 (73,80)
T80 (80)
T81 (84)
T82 (73)
T85 (52)
T84 (52)
T83 (35)
T95 (36,41,46-48,51,53,55,56,61-64,70,72,74,75,77,82,83)
T92 (32)
T93 (32,34)
T94 (34)
T91 (66)
T90 (71)
T89 (83)
T87 (40)
T88 (40)
T86 (83)
T96 (72)
L.nitidus
L.lemniscatus
L.nigroviridis
0.2
I I.1
A
B1
II
B
I.2
B2.1
B2.2.1
B2.2.2
B2.2
B2
B2.2.3
I


T5
T9
T3
T4
T10
T7
T6
T2
T17
T16
T25
T13
T15
T12
T11
T18
T14
T20
T19
T22
T23
T24
T21
T1
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T59
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T64
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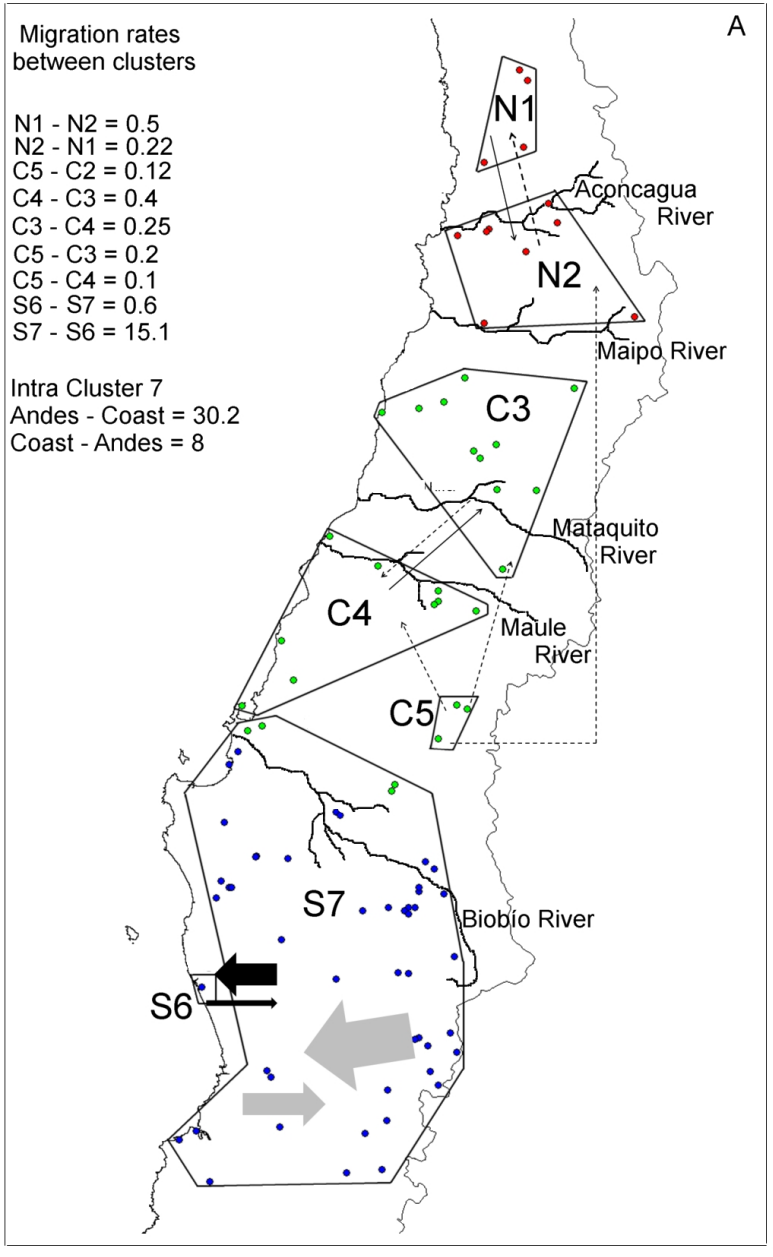
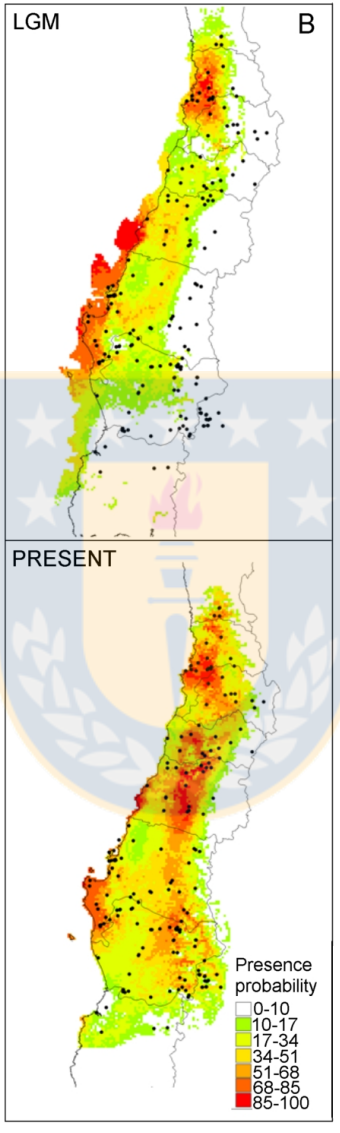
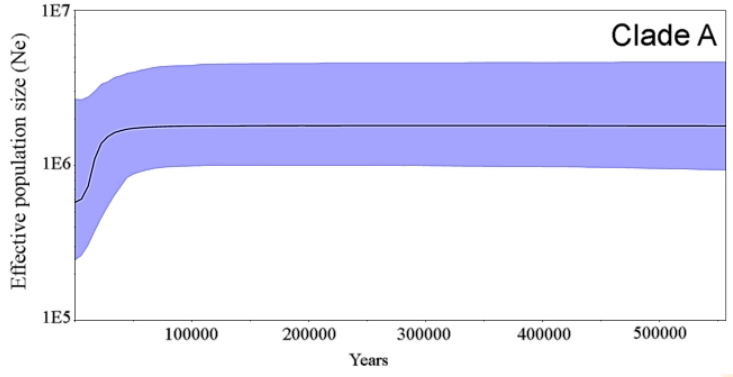
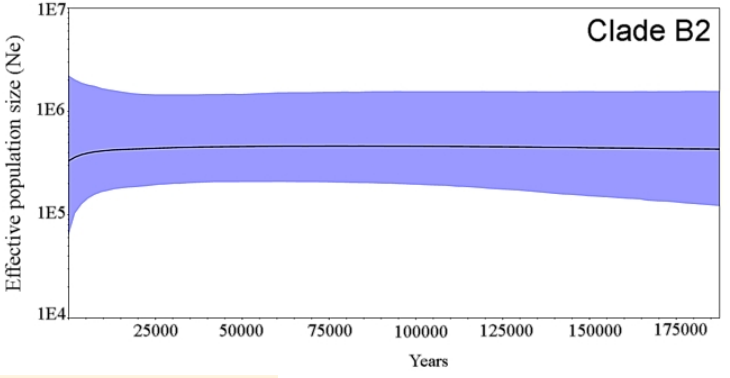
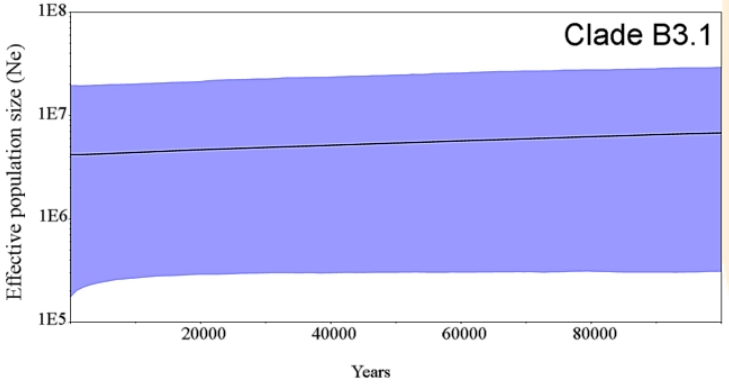
T40
T54
T50
T52
T51
T53
T26
T28
T37
T69
T29
T30
T36
T35
T33
T70
T27
T32
T34
T31
T74
T71
T38
T75
T76
T78
T77
22 mp
T80
T79
T73
T81
T82
T72
T8
12mp
16 mp
T84
T85
T96
T83
T91
T87
T88
T89
T95
T86
T92
T93
T92
T94
1 mutational step
1 haplotype

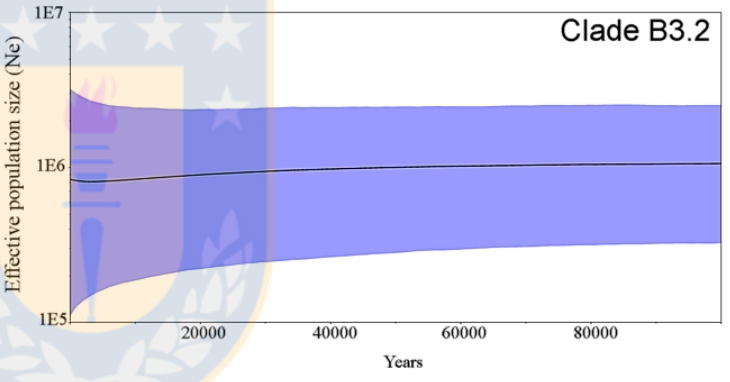

A
Migration rates
between clusters
N1 - N2 = 0.5
N2 - N1 = 0.22
C5 - C2 = 0.12
C4 - C3 = 0.4
C3 - C4 = 0.25
C5 - C3 = 0.2
C5 - C4 = 0.1
S6 - S7 = 0.6
S7 - S6 = 15.1
Intra Cluster 7
Andes - Coast = 30.2
Coast - Andes = 8
N1
N2
C3
C4
C5
S7
S6
Aconcagua River
Maipo River
Mataquito River
Maule River
Biobío River

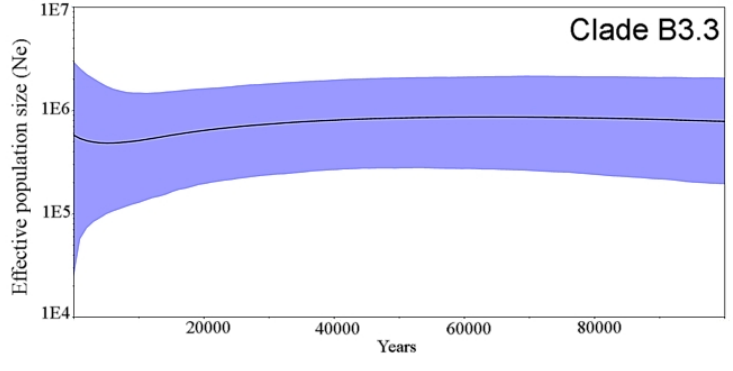

LGM
B
PRESENT
Presence probability
0-10
10-17
17-34
34-51
51-68
68-85
85-100

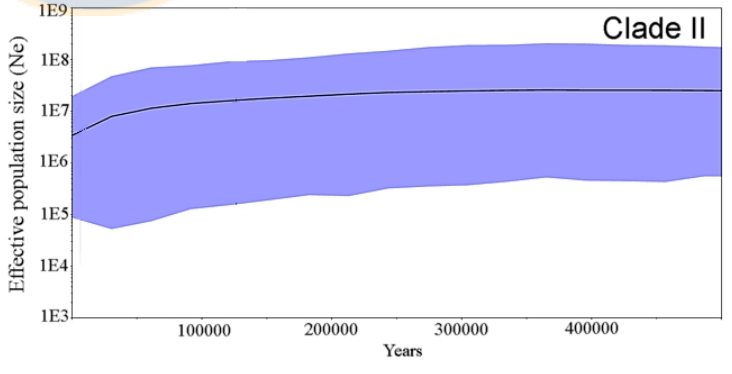

Clade A
Effective population size (Ne)
1E7
1E6
1E5
100000
200000
300000
400000
500000
Years


Clade B2
Effective population size (Ne)
1E7
1E6
1E5
1E4
25000
50000
75000
100000
125000
150000
175000
Years


Clade B3.1
Effective population size (Ne)
1E8
1E7
1E6
1E5
20000
40000
60000
80000
Years


Clade B3.2
Effective population size (Ne)
1E7
1E6
1E5
20000
40000
60000
80000
Years


Clade B3.3
Effective population size (Ne)
1E7
1E6
1E5
1E4
20000
40000
60000
80000
Years


Clade II
Effective population size (Ne)
1E9
1E8
1E7
1E6
1E5
1E4
1E3
100000
200000
300000
400000
Years

Highlights:

-We evaluate the phylogeographic structure of *L. tenuis* using 3 genes and 5 microsatellites.

-Northern and central populations are more stable, structured and diverse.

-We found evidence for co-distributed deeply divergent clades in the south

-Phylogeographic breaks are concordant with two large Chilean rivers.

-*Liolaemus tenuis* probably constitutes a species complex with cryptic diversity.

Keywords: climate change, *Liolaemus*, Chile, LGM, phylogeography

SUPPLEMENTARY MATERIAL

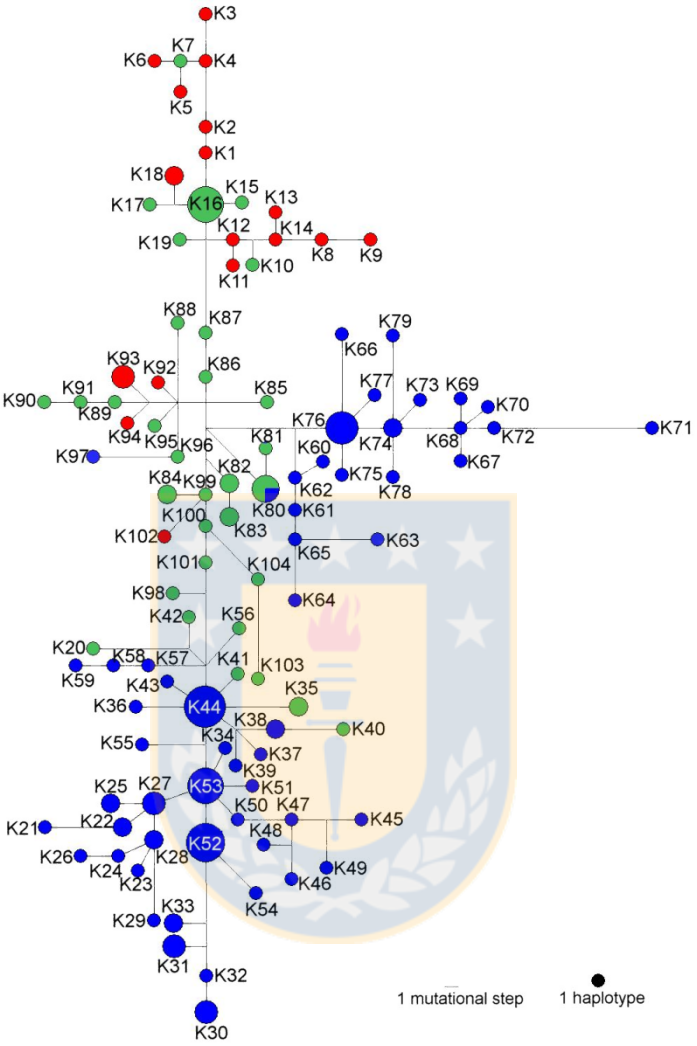


Figure S1. Parsimony-based haplotype network of nuclear sequences (KIF24) of *Liolaemus tenuis.* Colors indicate geographic origin as depicted in Fig. 1. Table S1 gives details of locality and group assignation for the haplotypes.

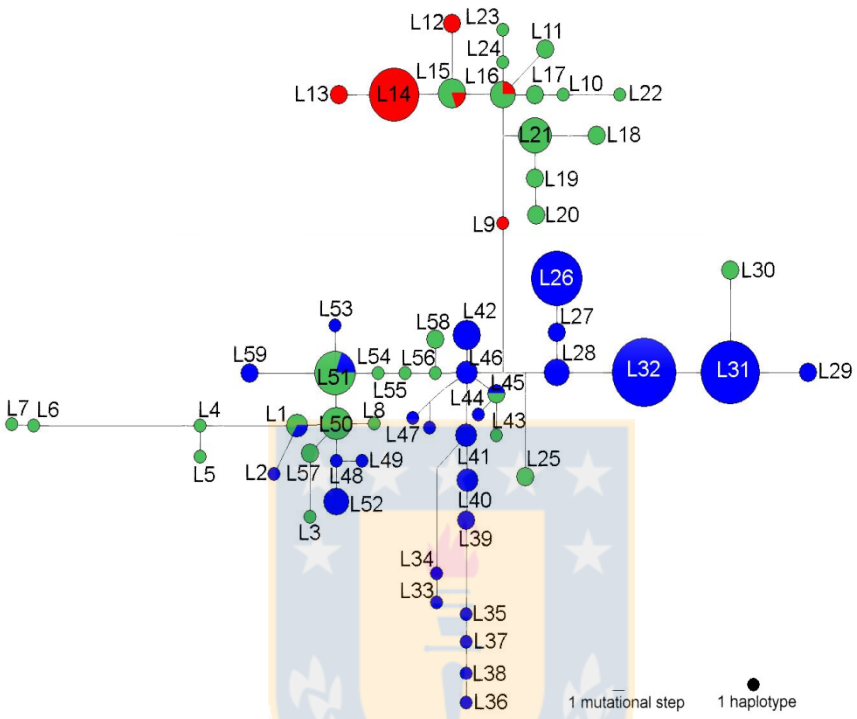


Figure S2. Parsimony-based haplotype network of nuclear sequences (LDB5B) of *Liolaemus tenuis.* Colors indicate geographic origin as depicted in Fig. 1. Table S1 gives details of locality and group assignation for the haplotypes.

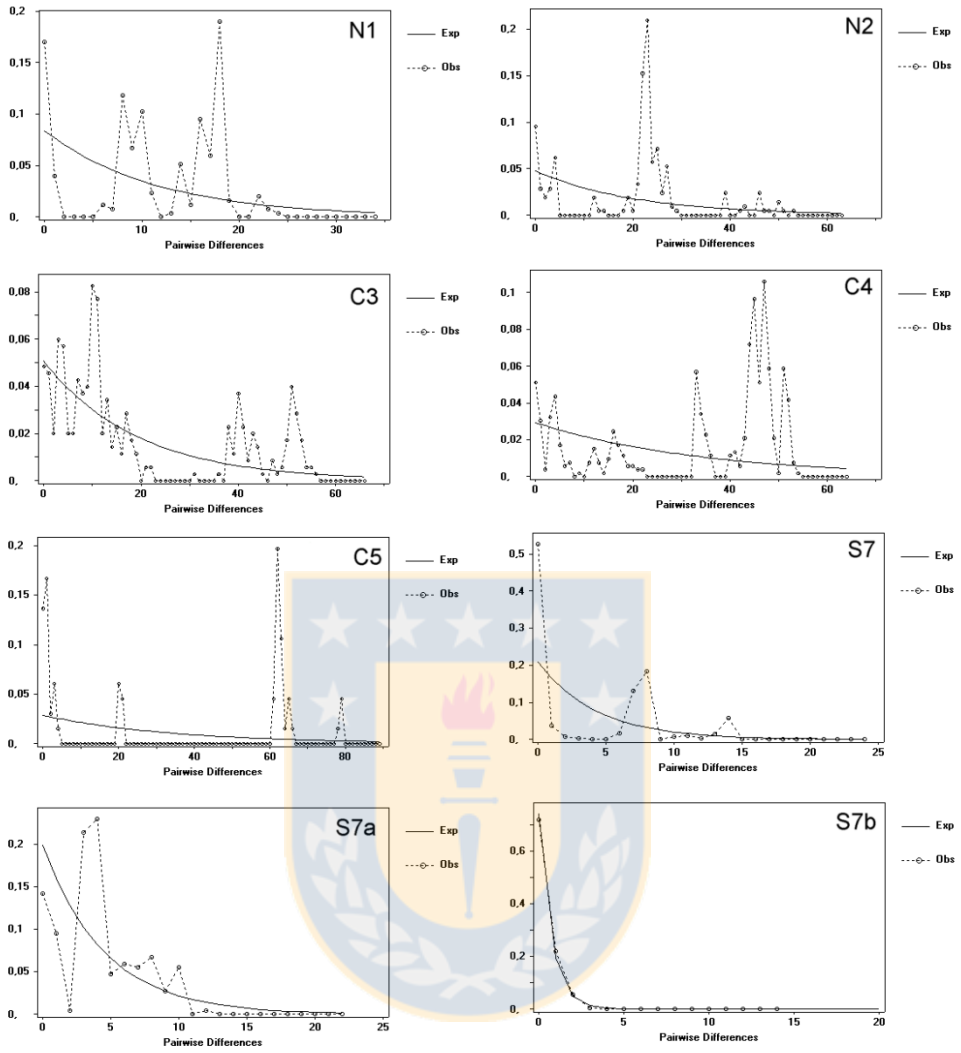


Figure S3. Pairwise nucleotide distances (mismatch distribution) for each of the clusters inferred by Geneland using cyt *b* sequences of *Liolaemus tenuis* (cluster names as in Fig. 4 A). As explained in the Results section, figures S7a and S7b correspond to the subdivision of cluster S7. Dash and continuous lines represent observed and expected pairwise nucleotide differences respectively, under models of sudden demographic expansion.

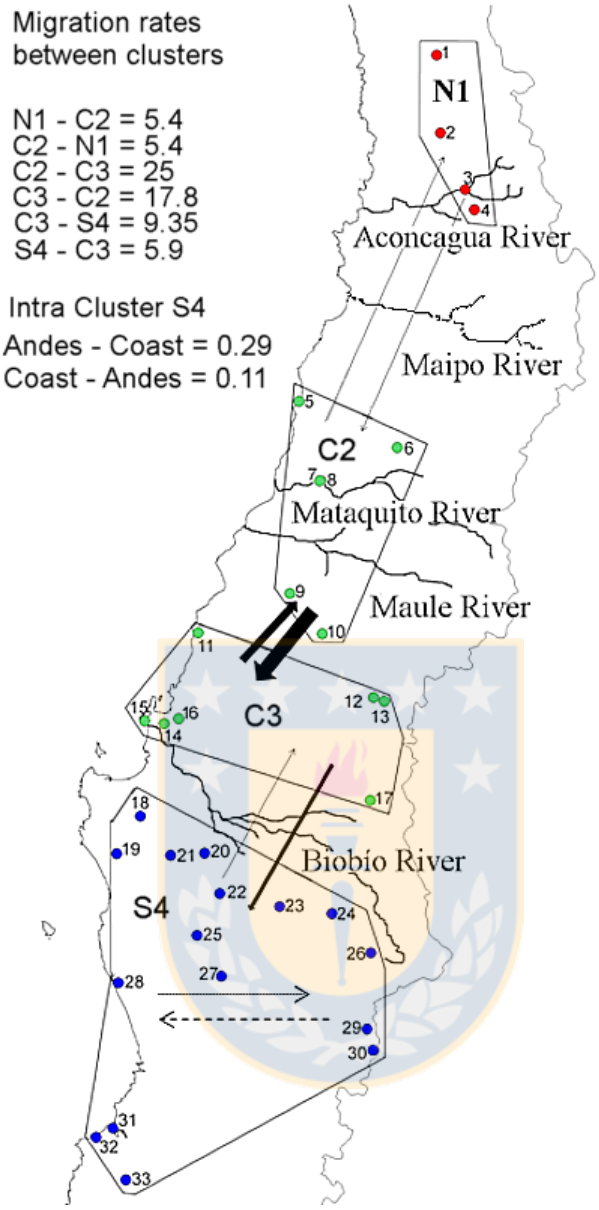


Figure S4. Map of the population clusters (polygons) identified by Geneland based on microsatellite loci. Colors of circles correspond to those depicted in Fig. 1. Arrow lines represent results of Migrate-n (see Table S7).

Table S1. Collection localities for *Liolaemus tenuis,* geographic coordinates, sample sizes and haplotype codes for cyt *b*, KIF24 and LDB5B genes. Asterisks indicate localities sampled by Victoriano et al. (2008).

					Gene			Haplotype		
	Locality	Latitude	Longitude	Geographic zone	cyt*b*	KIF24	LDB5B	cyt*b*	KIF24	LDB5B
1	Illapel - Salamanca	31º 46' S	70º 56' W	Northern	3	2	2	**T12**	K92,**K93**	**L14**
2	Cementerio Salamanca *	31° 46' S	70° 57' W	Northern	9	2	2	T3, T4, **T12**	**K93**	**L14**
3	Petorca - Cabildo	32º 14' S	70º 55' W	Northern	3	2	2	T2, T6, T7	K8,K9	L12,**L16**
4	La Ligua *	32º 28' S	71º 13' W	Northern	4	2	2	T13, T14, T15	K1,K2	**L14**
5	San Felipe	32º 44' S	70º 43' W	Northern	3	8	8	**T5**	K3,K5,K11 K12,K13,K14,K94,K102	L9,**L12**, **L14 L15**
6	North west of Los Andes	32º 47' S	70º 39' W	Northern	1			**T5**		
7	Olmué - Valparaiso *	33º 00' S	71º 12' W	Northern	1			T20		
8	Olmué *	32º 59' S	71º 11' W	Northern	7	2	2	T18, T19	K4,K6	L13
9	Quilpué *	33º 02' S	71º 25' W	Northern	4			T16, T17, T24		
10	Tiltil	33º 08' S	70º 51' W	Northern			2			**L14**,**L15**
11	Melipilla *	33º 42' S	71º 13' W	Northern	7			T1, T11, T21, T22, T23		
12	Cajón del Maipo *	33º 39' S	70º 04' W	Northern	1			T25		
13	Rapel	34º 07' S	71º 22' W	Central			2			L10,L22
14	Coya *	34º 12' S	70º 32' W	Central	4			T36, T37		
15	Fundo La Esperanza	34º 18' S	71º 32' W	Central		2	2		K87,K95	**L21**
16	Rinconada del Halcón	34º 21' S	71º 43' W	Central	1	2	2	T10	K18	**L21**
17	Pichilemu	34º 23' S	72º 0' W	Central	2	2	6	T8, T9	**K16**	**L15**,L17**,** 18**,L21,** L25
18	San Fernando - Pichilemu	34° 37' S	70° 59' W	Central	2	2	2	T26	K7,K10	**L15**,**L21**
19	Santa Cruz	34º 41' S	71º 18' W	Central			4			L11,**L16**
20	Chépica	34º 44' S	71º 15' W	Central	1		2	T38,T39		**L15**
21	Romeral *	34º 58' S	71º 07' W	Central	4	2	2	T28	K88,K89	**L16**,**L21**
22	Los Queñes *	34º 59' S	70º 49' W	Central	8			T27, T29, T30, T31		
23	Constitución *	35º 20' S	72º 24' W	Central	2			T39, T40		
24	South of San Javier	35º 34' S	72º 02' W	Central			2			L20
25	Alto Vilches *	35º 35' S	71 º05' W	Central	5	10	4	T41	K15,**K16**, K17**,**K19,K85K86	L19,L30

Table S1. Continued

					Gene			Haplotype		
	Locality	Latitude	Longitude	Geographic zone	cyt*b*	KIF24	LDB5B	cyt*b*	KIF24	LDB5B
26	Yerbas Buenas *	35º 44' S	71º 34' W	Central	4			T44,T46		
27	Linares a *	35º 50' S	71º 34'W	Central	4			T42,T43 **T47**		
28	Linares b *	35º 50' S	71º 35' W	Central	4			T45,**T47**		
29	Embalse Ancoa *	35º 54' S	71º 17' W	Central	3	2	2	**T47**,T48 T49	K90,K91	L23,L24
30	Cobquecura *	36º 07' S	72º 47' W	Central	6			T55		
31	Coelemu	36º 29' S	72º 42' W	Central	3	4	6	T65,T66 T67	K40,K84, K96	**L1**,L3,**L50** L57
32	Puquios	36º 37' S	71º 26' W	Central	3	2	2	T53,T92,**T93**	**K80**	**L51**
33	Isla Quiriquina *	36º 37' S	73º 03' W	Central	8			T56,T57 T58,T59 T68		
34	Caracol	36º 39' S	71º 21' W	Central	2	4	4	**T93**,T94	**K80**,K81 K103,K104	**L51**,L53
35	Nonguén *	36º 47' S	72º 55' W	Central	4	2	2	T60,T61 T83	**K35**,K101	L8,**L50**
36	Concepción	36º 50' S	73º 01' W	Central	2			**T95**		
37	Las Trancas *	36º 53' S	71º 34' W	Central	7	4	6	T50,T51 T52,T54	K82,K83	**L51,**L54,L55,L56,L58
38	Huepil - Trupan	37º 16' S	71º 50' W	Central	1	2	4	T64	**K35**,K100	L4,L5,L6,L7
39	Tucapel	37º 18' S	71º 18' W	Central	2	4	4	T62,T63	K41,K42, K98,K99	**L1**,**L50**,**L51**
40	Los Angeles a	37º 23' S	72º 01' W	South	3	2	2	T87,T88	K20,K56	**L50**
41	Los Angeles b *	37° 28' S	72° 19' W	South	2			**T95**		
42	Coronel	36º 59' S	73º 06' W	Southern	1	2	4	T69	K55,K97	**L1**,L2,L48, L49
43	Playa Negra *	37º 05' S	73º 10' W	Southern	1			T72		
44	Curanilahue	37º 28' S	73º 09' W	Southern	2	4	6	T71,T73	K29,K39, **K52,K53**	L33,L34,L43,**L45**
45	Angol	37º 48' S	72º 42' W	Southern	2	4	2	T70,**T95**	K21,K50, K65,K72	**L28**
46	Ralco	37° 49' S	71° 40' W	Southern	2			**T95**		
47	Ralco - Represa	37º 53' S	71° 36' W	Southern	7			**T95**		
48	Close Contulmo	37° 58' S	73° 14' W	Southern	2			**T95**		
49	N.M. Contulmo	38° 01' S	73° 09' W	Southern	3	2		**T78**	K36,K60	
50	Contulmo	38° 01' S	73° 08' W	Southern	1	2	2	**T78**	K62,K66	**L26**

Table S1. Continued

					Gene			Haplotype		
	Locality	Latitude	Longitude	Geographic zone	cyt*b*	KIF24	LDB5B	cyt*b*	KIF24	LDB5B
51	Termas de Pemehue *	38º 03' S	71º 43' W	Southern	4		4	**T95**		L35,L36,L40,L41
52	Lago Lanalhue *	38º 06' S	73º 16' W	Southern	4	2		T84,T85	**K52**,K54	
53	Niblinto - Victoria *	38º 10' S	71º 57' W	Southern	6			**T95**		
54	N.R. Malleco *	38º 10' S	71º 45' W	Southern	2		4			**L51**
55	Close N.R. Malleco *	38º 10' S	71º 48' W	Southern	1		4	**T95**		**L42**,L59
56	R.N. Malleco-Las Mentas *	38º 12' S	71º 50' W	Southern	2		6	**T95**		L37,L38
57	West P.N. Tolhuaca	38° 12' S	72° 09' W	Southern	1	4	4	T77	**K22**,K67 **K74,K76**	**L42**,L46
58	N.P. Tolhuaca	38° 13' S	71° 48' W	Southern	5		2	T74,**T75**		L39
59	N.P. Tolhuaca (Laguna Verde)	38º 12' S	71º 43' W	Southern	1		2	**T75**		**L42**
60	Galvarino	38º 25' S	72º 46' W	Southern		12	8		K24,**K25, K27,**K37, **K44,K52**, K68,K69, **K74,K76**, K79	**L32**
61	Lonquimay	38° 33' S	71° 27' W	Southern	5	2	6	T76,**T95**	K61,K78	L47,L52
62	Refugio Volcán Llaima *	38º 41' S	71º 48' W	Southern	1			**T95**		
63	Cherquenco-Volcán Llaima *	38º 40' S	71º 53' W	Southern	5			**T95**		
64	Nahuelbuta	38º 43' S	72º 34' W	Southern	1			**T95**		
65	Cerro Ñielol	38º 43' 'S	72º 35' W	Southern		6	8		K26,K34, K45,**K52, K53,**K70	**L26**
66	Puerto Saavedra	38°47' S	73° 23' W	Southern	7	4	4	T91	K23**,K33**, K48, K77	**L26**,**L32**
67	Hualalafquen	39º 4' S	71º 32' W	Southern		6	6		**K38**,K44, **K53**,K57, **K80**	**L31**
68	Reigolil	39º 8' S	71º 29' W	Southern		4	4		**K27**,K43,**K44**	**L31,L32**
69	N.P. Huerquehue *	39º 10' S	71º 43' W	Southern		6	4		**K28**,**K31**,K32,**K 38,**K49,K71	**L31,L32**,L44,**L45**
70	Close N.P. Huerquehue *	39º 11' S	71º 45' W	Southern	1			**T95**		
71	Puesco *	39º 32' S	71º 34' W	Southern	1			T90		
72	Close La Capilla *	39º 25' S	72º 53' W	Southern	5		4	**T95**		L27,**L31,L32**
73	Pucon - Curarrehue	39°26' S	71°38' W	Southern	2	2	4	**T79**,T82	**K44**,**K53**	L29,**L31,L32**
74	Calquinco-La Capilla *	39º 28' S	72º 51' W	Southern	6			**T95**,T96		
75	Lago Caburga *	39º 14' S	71º 39' W	Southern	2			**T95**		
76	Curarrehue - Reigolil	39º 17' S	71º 26' W	Southern		8	8		**K28**,**K44**,**K52**,**K 53**,K59	**L28**,**L31**
77	Neltume	39º 34' S	72º 20' W	Southern	1			**T95**		
78	Los Lagos	39º 51' S	72º 47"W	Southern		8	6		**K22**,**K25**,**K30**,K 51,**K52,K53,**K58	**L26**,**L32**
79	Choshuenco	39º 54' S	72º 8'W	Southern		6	6		**K27**,K**30**,**K31**,**K 33**	**L32**

Table S1. Continued

					Gene			Haplotype		
	Locality	Latitude	Longitude	Geographic zone	cyt*b*	KIF24	LDB5B	cyt*b*	KIF24	LDB5B
80	Corral	39º 53' S	73º 25' W	Southern	3	4	4	**T79,**T80	K46,K47,K63,K64	**L26,L32**
81	N.P. Valdivia	39º 57' S	73º 33' W	Southern		6	6		K73,K75,**K76**	**L31,L32**
82	Los Llolles *	40º 11' S	72º 00' W	Southern	1			**T95**		
83	Lago Ranco *	40º 12' S	72º 16' W	Southern	6			T86,T89		
84	La Union - Hueicolla	40º 16' S	73º 19' W	Southern	1		2	T81		**L26,L32**

Table S2. Collection localities for *Liolaemus tenuis* included in the microsatellite analyses.

	Locality	Latitude	Longitude	N	Cluster	Geographic zone
1	Salamanca	-31.7736	-70.962	11	N 1	North
2	Petorca	-32.2333	-70.9167	4	N 1	North
3	San Felipe	-32.7378	-70.7183	14	N 1	North
5	Pichilemu	-34.3847	-72.0028	13	C 2	Central
6	Chepica	-34.7376	-71.2564	8	C 2	Central
7	Iloca	-34.9686	-71.8288	3	C 2	Central
8	Hualañe	-34.9742	-71.7983	5	C 2	Central
9	Toribio	-35.8358	-72.0678	2	C 2	Central
10	Parral	-36.1389	-71.8237	8	C 2	Central
11	Cobquecura	-36.1306	-72.7903	11	C 3	Central
12	Puquios	-36.6069	-71.4561	5	C 3	Central
13	Caracol	-36.6567	-71.3656	3	C 3	Central
14	Rocoto	-36.7991	-73.1781	3	C 3	Central
15	Concepcion	-36.8173	-73.0739	6	C 3	Central
16	Nonguen	-36.8713	-72.9927	3	C 3	Central
17	Curanilahue	-37.0043	-73.0905	4	C 3	South
18	Antuco	-37.397	-71.455	3	C 3	South
19	Cañete	-37.8008	-73.3931	4	S 4	South
20	Angol	-37.8028	-72.717	7	S 4	South
21	Nahuelbuta	-37.8156	-72.9831	9	S 4	South
22	Reñico	-38.1031	-72.6042	4	S 4	South
23	Tolhuaca	-38.2211	-71.7822	10	S 4	South
24	Las Raices	-38.2574	-71.7498	11	S 4	South
25	Galvarino	-38.4201	-72.778	3	S 4	South
26	Lonquimay	-38.5592	-71.4514	5	S 4	South
27	Temuco	-38.7271	-72.5923	4	S 4	South
28	Puerto Saavedra	-38.7889	-73.385	6	S 4	South
29	Reigolil	-39.1455	-71.4969	2	S 4	South
30	Curarrehue	-39.291	-71.4337	4	S 4	South
31	Corral	-39.8881	-73.4264	4	S 4	South
32	Valdivia	-39.9504	-73.5601	4	S 4	South

Table S3. Migration models assessed with the coalescent approach implemented in Migrate-n using cyt *b* sequences and microsatellite loci.

Migration model	Description
Full migration	Gene flow between all possible pairs of population units (clusters) is permitted.
Maipo River as a barrier to gene flow	Gene flow between northern (N1-N2) and between central-southern clusters (C3-S7) is allowed, but not between northern and central-southern clusters.
Biobío River as a barrier to gene flow	Gene flow between southern clusters (S6 –S7) and between central-northern clusters (N1-C5) is allowed but not between these two groupings.
Stepping stone model	Only geographically neighboring clusters are connected by gene flow.

Table S4. Genetic divergence (p distance) between clusters estimated by Geneland for (a) cyt *b*, (b) KIF24, and (c) LDB5B DNA sequences of *Liolaemus tenuis.* Values below diagonal represent uncorrected p-distances, and values above diagonal represent Standard Error estimates. Values on the diagonal are intra cluster p-distances.

(a)

	Northern clusters		Central clusters			Southern clusters	
	Cluster N1	Cluster N2	Cluster C3	Cluster C4	Cluster C5	Cluster S6	Cluster S7
Cluster N1	**0.017 (0.003)**	0.004	0.007	0.007	0.007	0.01	0.009
Cluster N2	0.032	**0.028 (0.003)**	0.007	0.006	0.006	0.009	0.008
Cluster C3	0.057	0.059	**0.031 (0.003)**	0.007	0.007	0.01	0.009
Cluster C4	0.066	0.064	0.073	**0.052 (0.005)**	0.006	0.01	0.008
Cluster C5	0.077	0.071	0.083	0.077	**0.057 (0.006)**	0.007	0.007
Cluster S6	0.1	0.092	0.103	0.11	0.074	**0**	0.003
Cluster S7	0.096	0.09	0.101	0.102	0.079	0.035	**0.021 (0.005)**

(b)

Geographical group	1	2	3
1. Northern group	0.014 (0.003)	0.030	0.04
2. Central group	0.015	0.015 (0.003)	0.03
3. Southern group	0.019	0.017	0.012 (0.002)

(c)

Geographical group	1	2	3
1. Northern group	0.003 (0.001)	0.003	0.006
2. Central group	0.018	0.017 (0.003)	0.004
3. Southern group	0.027	0.019	0.008 (0.002)

Table S5. Summary statistics exploring the demographic history for each of the *Liolaemus tenuis* geographic groups using the nuclear markers (a) KIF24 and (b) LDB5B.

(a)

Geographic group	N	Number of haplotypes (h)	Number of polymorphic sites (S)	Haplotype diversity (Hd)	Nucleotide diversity (π)	Fs' Fu	D' Tajima	Ragg
Northern	22	20	23	0.99 (0.02)	0.014 (0.007)	**-9.6194**	1.3028	0.012
Central	42	29	39	0.95 0.02	0.014 0.008	**-6.5772**	-0.2195	0.007
Southern	94	55	43	0.97 (0.01)	0.012 (0.006)	**-25.0012**	-0.5625	0.004
Total	158	104	71	0.98 (0.003)	0.015 (0.007)	**-24.4420**	-0.8303	0.004

(b)

Geographic group	N	Number of haplotypes (h)	Number of polymorphic sites (S)	Haplotype diversity (Hd)	Nucleotide diversity (π)	Fs' Fu	D' Tajima	Ragg
Northern	25	7	11	0.70 (0.09)	0.004 (0.002)	-0.6023	-0.8999	0.046
Central	59	29	51	0.96 (0.01)	0.018 (0.009)	-5.0165	-0.4134	0.011
Southern	112	28	37	0.88 (0.02)	0.009 (0.005)	**-7.0621**	-0.2370	0.013
Total	196	59	74	0.95 (0.01)	0.02 (0.01)	**-23.7756**	-0.6091	0.005

Table S6. Measure of pairwise Fst between clusters obtained by Geneland for (a) cyt *b* and (b) microsatellites. Numbers in bold indicate statistical significant values.

(a)

	Cluster N1	Cluster N2	Cluster C3	Cluster C4	Cluster C5	Cluster S6
Cluster N2	**0.32**					
Cluster C3	**0.59**	**0.52**				
Cluster C4	**0.45**	**0.37**	**0.44**			
Cluster C5	**0.57**	**0.43**	**0.51**	**0.31**		
Cluster S6	**0.54**	**0.47**	**0.54**	**0.5**	**0.29**	
Cluster S7	**0.91**	**0.83**	**0.81**	**0.69**	**0.559**	0.1

(b)

	Cluster N1	Cluster C2	Cluster C3
Cluster C2	**0.13**		
Cluster C3	**0.19**	**0.12**	
Cluster S4	**0.24**	**0.17**	**0.11**

Table S7. Migration rate values among clusters assessed with the coalescent approach implemented in Migrate-n, using (a) cyt *b* sequences and (b) microsatellite loci. The 95% confidence interval is constructed from minimum and maximum estimates of 0.025 and 0.975 percentiles. Theta values (Θ) for each basin is given on the identity diagonal in italics.

(a)

		Donor						
		Northern clusters		Central clusters			Southern clusters	
	Receiver	Cluster N1	Cluster N2	Cluster C3	Cluster C4	Cluster C5	Cluster S6	Cluster S7
Northern	Cluster N1	***0.01 (0 - 0.18)***	0.22 (0 - 18.1)	0.001 (0 - 8.7)	0.001 (0 - 5.5)	0.001 (0 - 12.7)	---	---
	Cluster N2	0.5 (0 - 18.7)	***0.02 (0 - 0.19)***	0.002 (0 - 9.6)	0.002 (0 - 6.6)	0.12 (0 - 9.3)	---	---
Central	Cluster C3	0.004 (0 - 9.7)	0.004 (0 - 10.3)	***0.04 (0 - 0.2)***	0.4 (0 - 17)	0.2 (0 - 16.5)	---	---
	Cluster C4	0.002 (0 - 5.7)	0.002 (0 - 6.6)	0.25 (0 - 15.8)	***0.02 (0 - 0.19)***	0.11 (0 - 8.8)	---	---
	Cluster C5	0.0003 (0 - 13.6)	0.02 (0 - 9.6)	0.02 (0 - 16)	0.01 (0 - 9.1)	***0.003 (0 - 0.19)***	---	---
Southern	Cluster S6	---	---	---	---	---	***0.08 (0 - 0.79)***	15.09 (0 - 157)
	Cluster S7	---	---	---	---	---	0.59 (0 - 34.7)	***0.003 (0 - 0.17)***

(b)

		Donor			
		Northern cluster	Central clusters		Southern cluster
	Receiver	Cluster N1	Cluster C2	Cluster C3	Cluster S4
Northern	Cluster N1	***1.0 (0 - 4.4)***	5.4 (0 - 78)	---	---
Central	Cluster C2	5.4 (0 - 109)	***1.0 (0 - 6.1)***	17.8 (0 - 151)	---
	Cluster C3	---	25 (0 - 115)	***1.4 (0 - 4.6)***	5.9 (0 - 44)
Southern	Cluster S4	---	---	9.35 (0 - 54)	***2.2 (0 - 5.6)***

Table S8. Numbers of Alleles (Na), Shannon's Index (I), Observed Heterozygosity (Ho) and Expected Heterozygosity (He) for each microsatellite loci.

		TET2216	TET1501	TET1177	DI7938	DI159	Total
Na	**Mean**	2.333	2.133	3.433	1.967	3.367	2.647
	SE	0.237	0.142	0.286	0.140	0.297	0.114
I	**Mean**	0.465	0.548	1.008	0.482	0.928	0.686
	SE	0.077	0.065	0.078	0.062	0.097	0.039
Ho	**Mean**	0.212	0.331	0.269	0.184	0.323	0.264
	SE	0.038	0.048	0.044	0.045	0.043	0.020
He	**Mean**	0.261	0.346	0.563	0.313	0.510	0.399
	SE	0.043	0.041	0.037	0.040	0.049	0.021

CAPÍTULO 2

Are there cryptic lizard species in the Chilean biodiveristy hotspot? A case study using a widespread *Liolaemus* species.

ARE THERE CRYPTIC LIZARD SPECIES IN THE CHILEAN BIODIVERSITY HOTSPOT? A CASE STUDY USING A WIDESPREAD *LIOLAEMUS* SPECIES

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ABSTRACT

For many taxa, inaccuracy in species boundaries and distributions hampers inferences about diversity and evolution. This is particularly true in the Neotropics, where a prevalence of cryptic species has often been demonstrated. One such case is the lizard genus *Liolaemus*, which is suspected to include many more species than the approximately 250 currently recognized. *Liolaemus tenuis* occurs in a historically complex area due to recurring climatic and geomorphological events. This taxon shows phenotypic variation as well as high levels of genetic divergence, suggesting that it may harbor three valid species within the nominal species. In order to test species boundaries, we integrated genetic, morphological and ecological evidence. First, we used multi-locus data (seven loci, including six nuclear and one mitochondrial) to estimate a tree for the candidate species using a coalescent method that integrates over gene-tree discordance due to incomplete lineage sorting. We then validated these species using a species delimitation approach that evaluates the relative support for the fully-delimited species tree and for collapsed tree models with fewer species. Second, we used geometric morphometrics of head shape to assess if the putative species could also be differentiated on a morphological basis. Third, we generated habitat suitability maps for each of the three putative species to test for distribution and environmental

overlap among these candidate species. Lastly, we dated the divergence between our delimited species. Our integrative approach supports the existence of two valid species within the nominal *Liolaemus tenuis*. Names for these forms are already available and correspond to *L. tenuis sensu stricto*. and *L. punctatissimus*. Therefore, we give these subspecies a species status and redefine their distribution based on our genetic data.

Keywords: gene tree, species tree, Bayesian, multi-species coalescent, niche modelling, geometric morphometrics, *BEAST, BPP

Highlights

- We tested the hypothesis that *Liolaemus tenuis* is a species complex using seven genes
- Our results support the existence of two valid species within *L. tenuis sensu lato*
- The age of *Liolaemus tenuis sensu lato* crown group is ca. 1. 8 mya
- Morphology and ecological data support genetic results
- We elevate *L. t. tenuis* and *L. t. punctatissimus* to full species

1. INTRODUCTION

Establishing species boundaries can be problematic when diagnostic morphological characters are scarce; in these cases, the traditional typological approach is uninformative and it underestimates species richness (e.g. Bickford et al., 2007; Fouquet et al., 2007). Over the past decade, advances in molecular systematics have led to the discovery of many cryptic species (two or more distinct species that have erroneously been regarded as one, following Bickford et al., 2007), changing the way we study and value biodiversity. Cases of cryptic species have been reported for many groups, including mammals, birds, reptiles, frogs, fishes, echinoderms and arthropods (e.g., Colborn et al., 2011; Dasmahapatra et al., 2010; Omland et al., 2000; Smith et al., 2005; Sponer and Roy, 2002; Stuart et al. 2006; Vera-Escalona et al., 2012; Yoder et al., 2000), and can result from morphological stasis or just be the product of a recent speciation event. Many evolutionary processes including convergent evolution and niche conservatism have been proposed to explain morphological stasis (e.g. Dupuis et al., 2012). Nevertheless, the latter can also result from phylogenetic inertia, chance alone and pre-existing constraints (Losos, 2011). Despite that the traditional morphologic assessment fails to tell apart these forms, geometric morphometrics (GM) has proved to be useful in detecting cryptic species, which can be

distinguished with a robust statistical framework by variation in shape and size (Adams and Funk, 1997; Tofilski, 2008). Particularly in reptiles, its use has notoriously expanded over the last two decades, as it has helped unveil the subtle morphological diversity across extant and extinct taxa (Kaliontzopoulou, 2011). In recent years, GM have been used in concert with methods such as Species Distribution Modelling (hereafter SDM) (e.g. Leaché et al., 2009) in what is now called integrative taxonomy (Dayrat, 2005). SDM is a technique aimed to infer species abiotic preferences based on presence records. This information can be used to estimate the past, current and future potential distribution of a species (Graham and Hijmans, 2006; Warren et al., 2008). It is therefore an invaluable tool to, among others, identify potential location of past populations, characterize species environmental preferences, test for niche divergence, and evaluate alternative biogeographic hypotheses (Alvarado-Serrano and Knowles, 2014). It has been suggested that SDM could be useful for species delimitation, as geographical barriers that could impede gene flow between populations could support the hypothesis that uncovered lineages are isolated (Rissler and Apodaca, 2007).

The genus *Liolaemus* is one of the most ecologically diverse and species-rich genera of lizards worldwide, with over 250 species currently recognized (Uetz et al., 2016). *Liolaemus* species are endemic to the temperate South America, distributed over a wide geographic area (latitudinally from 14° 30’ N to ~ 52° 30’ S, and altitudinally from the ocean coasts up to 4500 meters above sea level), covering contrasting climatic regimes, from the Atacama Desert to the temperate *Nothofagus* rainforest including Patagonian landscapes (Donoso-Barros, 1966; Lobo et al., 2010). As is usually the case with diverse groups, the genus has a complex taxonomic history. Species of *Liolaemus* have been grouped into two main groups based on morphological characters (Laurent, 1983), which were later supported by phylogenetic analysis of molecular data (e.g., Abdala, 2007; Schulte et al., 2000). In general, species distributed west of the Andes belong to the subgenus *Liolaemus* (the “Chilean” group), while those occurring east of the Andes belong to the subgenus *Eulaemus* (the “Argentine” group). In Chile, the subgenus *Liolaemus* is represented by approximately 96 species (Ruiz de Gamboa, 2016), most of them being abundant, with the occurrence of sympatry for different forms at different areas (Donoso-Barros, 1966). This broad diversity of ecological, morphological and life history traits, makes *Liolaemus* an ideal group to address a wide variety of evolutionary questions.

Over the last years, cryptic diversity within *Liolaemus* has been uncovered in Chile (e.g., Torres-Pérez et al., 2009; Vera-Escalona et al., 2012), Perú (e.g., Aguilar et al., 2013), and Argentina (e.g., Avila et al., 2006). Broadly distributed species that show morphological variation and occur in areas that have experienced variable climatic and geomorphological regimes are likely to harbor cryptic diversity (e.g., Crochet et al., 2004; Kaliontzopoulou et al., 2012;

Vera-Escalona et al., 2012). *Liolaemus tenuis* (Duméril and Bibron, 1837), a member of the "Chilean group" (Laurent, 1983; Schulte et al., 2000), is an oviparous, strictly insectivorous, and semi-arboreal species associated with grasslands and shrub habitats. It presents a marked sexual dimorphism, with more colorful and larger males (Donoso-Barros, 1966; Vidal et al., 2005). The species inhabits a large area between southern Coquimbo (29° 58′ S, 71° 21′ W) and southern Los Lagos (39° 51′ S, 72° 50′ W) regions, from sea level up to 1800 meters; marginal populations also exist in the eastern slope of the Andes, in the Argentinean province of Neuquén (Donoso-Barros, 1966). This area has been deeply impacted by glacial cycles throughout the Pleistocene, which altered landscape connectivity and fragmented populations that subsequently shifted their ranges (McCulloch et al., 2000; Rabassa and Clapperton, 1990; Ruzzante et al., 2008). Müller and Hellmich (1933) recognized two subspecies on the base of color patterns, typical *Liolaemus t. tenuis* (*terra typica*: Santiago) and *L. t. punctatissimus* (*terra typica*: Lota). However, only some authors recognize their distinction (e.g. Donoso-Barros, 1966; Etheridge, 1995) because these two forms partially overlap in their distributions (Formas, 1979), which come into conflict with the subspecies definition (Mayr and Ashlock, 1991). In addition, several studies assessing patterns of morphologic variation have suggested that *L. tenuis* is a widespread species without subspecies (Nuñez and Jaksic, 1992; Vidal et al., 2005). However, a recent molecular study recovered several mitochondrial lineages within *L. tenuis* (Muñoz-Mendoza et al., in press). A northern lineage, which includes variants retrieved from specimens collected near type locality of *L. t. tenuis*, is sister to a central-southern lineage that includes variants from the type locality of *L. t. punctatissimus*; and a third lineage, sister to the northern + central-southern clade, presents a distribution that overlaps with that of the central-southern. Therefore, Muñoz-Mendoza et al. (in press) suggested that as currently understood *L. tenuis* may encompass more than one species (that does not necessarily match the two described subspecies) and expressed the need of further integrative analyses to evaluate if such lineages merit species status.

Given the molecular evidence previously presented, which suggests the existence of several independently evolving lineages within the current concept of *L. tenuis* (Muñoz-Mendoza et al., in press), as well as the wide range of variation in color patterns and bioclimatic (ecological) distribution within *L. tenuis* (Vidal et al., 2005, 2007), we aimed to test the hypothesis that *Liolaemus tenuis* is a species complex that harbors at least three distinct evolutionary lineages. With an extensive geographic sampling effort (316 individuals from 99 localities), we conducted a systematic study that integrates molecular (seven loci), morphological, and environmental data. First, we assessed species boundaries within *L.*

tenuis. Second, we estimated divergence times among species newly delimited. This approach allowed us to interpret the evolutionary patterns in the context of the recent geologic and climatic history of Central Chile.

2. MATERIALS AND METHODS

2.1. Sampling and laboratory protocols

This study was conducted using tissue samples from 316 individuals of the nominal species *Liolaemus tenuis* from 99 localities encompassing its entire distribution, including the type localities of both subspecies (Figure 1, Table S1). Animals were caught by hand or with nooses, between 2004 and 2014. For details regarding captures and collection permits, see Victoriano et al. (2008) and Muñóz-Mendoza et al. (in press).

We constructed our character sampling over that of Muñóz-Mendoza et al. (in press), which included one mitochondrial and two nuclear genes (KIF24 and LBD5B). In addition to increasing the number of specimens sequenced those two nuclear genes (Table S1), we added four more markers because it has been shown that coalescent-based species delimitation analyses perform better with more loci (Camargo et al., 2012). As such, our sampling included one mitochondrial locus (cytochrome b: cytb) and six nuclear loci, the protein-coding genes exophilin 5 (EXPH5) and kinesin family member 24 (KIF24) and the anonymous nuclear genes A12F, A9C, B1D and LDB5B (Table 1).

2.2 Sequence data

Genomic DNA was extracted from muscle using the commercial kit Wizard SV Genomic (Promega). Primers used in amplification and sequencing are shown in Table 1. In all cases, reaction conditions per sample were as follows: 1.0 µL dNTPs (10 mM), 4.0 µL buffer 5X, 1.0 µL of each primer (10 lM), 2.0. µL MgCl (25 mM) and 0.25 µ.L Taq DNA polymerase (5 µ/ µL; Promega Corp., Madison, WI; 23 µL total reaction volume). All nuclear genes were amplified using a standard touchdown PCR cycle: initial denaturation at 95ºC for 5 min, annealing 5x (60-56º for 15 sec), 25x (54º for 15 sec), with a final elongation at 72°C for 7 min. The mitochondrial marker was amplified following the procedure implemented in Victoriano et al. (2008). Amplification products were visualized on agarose gels 1x. Purification and sequencing, using amplification primers, were performed by MACROGEN Inc. (Korea). Sequences were edited, including searching for internal stop codons, and aligned using CodonCode Aligner v. 3.5.1. (CodonCode Corp. 2009). This software was also used for resolving position and length of indels occurring in some heterozygous individuals. Ambiguity codes were used to represent site polymorphisms in heterozygous individuals. To infer haplotypes for

individuals with multiple heterozygous sites, data were phased using Phase version 2.1 (Stephens and Donnelly, 2003; Stephens et al., 2001). One hundred iterations were run (which were sufficient to reach stationarity) after a burn-in of 100, and a thinning interval of 1; the default cut-off threshold was used. With these alignments, statistical parsimony networks were computed using TCS 1.21 (Clement et al., 2000) with a 95% connection limit to see which haplotypes were redundant. Redundant haplotypes were excluded from subsequent analyses. Saturation was evaluated using Xia's test (Xia and Lemey, 2009; Xia et al., 2003), as implemented in DAMBE v. 5.0 (Xia, 2013). Recombination was not tested, as it would only be a problem in species tree analyses under extremely short internodes (Lanier and Knowles, 2012), which according to previous results would not be the case (Muñóz-Mendoza et al., in press). Average genetic divergence values between and within putative species were estimated based on the proportion of shared differences (p-distance) using Mega 6 (Tamura et al., 2011).

2.3. Coalescent-based species tree estimation and species delimitation

Candidate species considered in this study correspond to clades A, B and II from the mitochondrial gene tree of a previous study (see Fig. 1 in Muñóz-Mendoza et al., in press). Hereon, we refer to clade A as *L. t. tenuis*, as this clade includes haplotypes recovered from specimens collected at Santiago, the type locality of *L. t. tenuis*; similarly, we refer to clade B as *L. t. punctatissimus*, as specimens from the type locality of this subspecies are embedded in this clade. Following Panzera et al. (in review) we refer to clade II as to *L. sp*. because this divergent clade, if validated as a separate species, does not have an available name. *Liolaemus lemniscatus* (Gravenhorst, 1838) was selected to form the outgroup following the relationships presented by Schulte et al. (2000). Sequence Matrix (Vaidya et al., 2011) was used to standardize individual ID's. After removal of some taxa, the remaining sequences were re-aligned using the online application of MAFFT v.7 (Katoh and Standley, 2013).

A species tree was estimated for the *L. tenuis* complex using the coalescent-based *BEAST approach (Heled and Drummond, 2010) in the program BEAST v1.8.3 (Drummond et al., 2012). This is a Bayesian method that co-estimates gene trees and species trees, considering cases of gene-tree discordance as a result of incomplete lineage sorting, which is highly likely when species have radiated recently or over a short interval of time. All BEAST analyses were carried out in the CIPRES science gateway (Miller et al., 2010). Assignment of individuals to the candidate species described above (*L. t. tenuis*, *L. t. punctatissimus* and *L. sp*.) was done following the mitochondrial gene-tree from Muñoz-Mendoza et al. (in press). When for a given specimen no mitochondrial gene was available, and therefore the previously defined criterion

could not be applied, we assigned specimens to a candidate species according to the closest locality present in the cytb gene tree. The site models, clock models, and gene trees were unlinked across loci, and the ploidy type for each locus was specified to account for differences in effective population sizes of the nuclear genes relative to the mitochondrial locus. The Bayesian information criteria (BIC; Schwartz, 1978) from jModeltest 2 (Darriba et al., 2012) was used to determine the most appropriate model of evolution for each locus (Table 2). Following Leaché et al. (2014) model searches were limited to models with three substitution schemes and rate variation under a gamma parameter with four rate categories. For nuclear genes, a uniform prior was established for substitution rate with a lower and upper bound of 0 and 0.01 respectively, and 0.005 as a starting value based on the results obtained by Olave et al. (2015). We assessed the adequacy of a strict clock versus an uncorrelated relaxed clock by evaluating the behavior of the ucld.stdev parameter. A Yule prior (Yule, 1925) was chosen for the species tree and the population size parameter was set to constant linear. A chain length of 300 million generations, sampled every 6000 steps was used. Convergence was monitored using Tracer 1.6 (Rambaut et al., 2013) by verifying that effective sampling size (ESS) values were > 200. A conservative cutoff of 10% was used for the burn-in. Trees were combined using LogCombiner and then summarized in a maximum credibility tree with TreeAnnotator (available in BEAST package at http://beast.bio.ed.ac.uk/). Species trees were managed with FigTree v. 1.3 (also in the BEAST package, Rambaut and Drummond, 2009). Nodes were considered strongly supported if they received posterior probability (pp) support values ≥ 0.95 (Huelsenbeck and Rannala, 2004).

Joint Bayesian species delimitation and species tree estimation was conducted using BPP 3.3 (Bayesian Phylogenetics and Phylogeography; Rannala and Yang, 2016; Yang, 2015; see also Sukumaran and Knowles, 2017). The method uses the multispecies coalescent model to compare different models of species delimitation and species phylogeny in a Bayesian framework, accounting for incomplete lineage sorting due to ancestral polymorphism and gene tree uncertainty (Rannala and Yang, 2013; Yang and Rannala, 2010, 2014). Although previous versions of the software were dependent on a fixed guide tree to delimit species, recent modifications use nearest-neighbor interchange (NNI) for branch swapping to change the topology of the guide tree in order to explicitly incorporate phylogenetic uncertainty when delimiting species (Yang and Rannala, 2014). Only nuclear genes were included in this analysis because their characterizations, including different mutation rates and effective population sizes, are different relative to mitochondrial genes (Rannala and Yang, 2016). To reduce computational time, one random allele per individual was chosen from the phased matrix to represent heterozygous individuals. Three different starting trees were used, considering all possible rearrangements within the "*tenuis* complex", including the topology recovered in the *BEAST analysis (see Results).

Priors for divergence times and theta were chosen according to the maximum sequence divergence between species and the average sequence divergence within species, respectively (Table S2, S3). Based on these empirical estimates, the population size parameters (θs) were assigned the gamma prior G (0.03, 2), with mean 0.03/2 = 0.015. The divergence time at the root of the species tree (τ_0) was assigned the gamma prior G (0.09, 2). Each analysis was run with both algorithms (0 and 1) at least twice to confirm consistency between runs. rjMCMC analyses were run for 200,000 iterations, with a sampling interval of 4. Speciation probability values > 0.95 were considered as strong support for a speciation event. Simulations have suggested that this protocol has good power in identifying distinct species in the presence of small amounts of gene flow (with ≤ 1 expected immigrant per generation). More importantly for our particular case, BPP is not misled to infer geographical populations as distinct species when the migration rate is high (Zhang et al., 2011).

2.4. Modeling of Species Distributions

SDM was used to help us understand the processes structuring the genetic variation of *L. tenuis s. l.* across landscapes (Chan et al., 2011; Knowles, 2009), aiming to identify plausible isolating barriers (e.g., current areas of low suitability). The estimation of past distributions during glaciations, which could also help explaining observed genetic breaks (e.g., Beatty and Provan, 2010; Lawson, 2010) was tackled by Muñóz-Mendoza et al. (in press). In total, 99 unique localities were included for the estimation of the ecological niche models, including five localities shared between *L. t. punctatissimus* and *L. sp.* (Table S1). As with the morphometric analysis, assignation of individuals to species was done based on the phylogenetic results as a way to implement a species validation approach with the SDM inferences. Fifteen occurrence points for *L. t. tenuis*, 51 for *L. t. punctatissimus*, and 38 for *L. sp*. were considered (Table S1). A maximum entropy algorithm implemented in MaxEnt 3.3.3k (Phillips et al., 2006) was used to estimate the current ecological niche of the putative species comprised in the *L. tenuis* complex (Phillips and Dudik, 2008; Phillips et al., 2006). MaxEnt generates distributional models (SDM) using presence-only records, contrasting them with background/pseudoabsence data sampled from the remainder of the study area. For this, 19 bioclimatic variables taken from the WorldClim database were used (Hijmans et al., 2005; http://worldclim.org/). For the final modeling, variables with no contribution to the model and those with negative values in the jackknife-based test gain were discarded. Therefore, variables 1, 3, 6, 5, 10-12, 16-17 were discarded for *L. t. tenuis*, variables 1-2, 7, 10-12 and 17 for *L. t. punctatissimus*, and variables 1, 4, 7, 10-12 for *L. sp.* Maximum number of iterations was set at 1000, number of replicates to 10, and the convergence threshold

was set at 0.00001. A fraction of 25% of the localities were used as test points. The jack-knife feature (assuming no interaction between environmental variables) was used to estimate the contribution of each variable in predicting suitability (Phillips et al., 2006). The SDM results were visualized in DIVA GIS 7.5 (Hijmans et al., 2004). The area under the receiver operating characteristic curve (AUC) was used to evaluate the model fit. AUC values ≤ 0.5 correspond to predictions that are equal or worse than random. Values ranging from 0.5 to 0.7 are considered poor predictions, from 0.7 to 0.9 reasonable predictions and values > 0.90 are considered very good predictions (Swets, 1988). The logistic output (probability values) was chosen and the distributional models were mapped, showing areas from the average minimum logistic values (threshold) to 1 as areas suitable for species.

2.5. Morphometric Analyses

As another species validation approach, landmarks and semi-landmarks (Bookstein, 1997a, 1997b) were used to analyze head shape among the genetic lineages previously identified. Analyses were based on 88 specimens (Table S4) that were sexed according to the presence or absence of pre-cloacal pores (which in *L. tenuis s. l.* are not reported for females) and enlargement of the tail at the base of the cloaca (Duméril and Bibron, 1837; Labra et al., 2002). Heads were photographed in dorsal view with a digital microscope (CHRONOS, Digi Scope 1.3 Mpx, magnification ratio 60-230X) in a fixed tripod, using a grid as a background for scaling. Nine landmarks and five semi-landmarks were selected (Figure. 2, Table 3), which were digitized on the dorsal view of the head using the TPS package (Rohlf, 2004a, b; Rohlf and Marcus, 1993).

The Shapiro-Wilk normality test (Shapiro and Wilk, 1965) and the Levene homoscedasticity test (Levene, 1960) were performed. Prior to the proper GM analyses, a One-Way ANOVA test implemented in the software package PAST v. 3.12 (Hammer et al., 2001), was conducted using centroid sizes in order to evaluate the existence of sexual dimorphism (significant differences in head size). This determined if females and males would be analyzed together or separately. Species-specific consensus configurations and centroid sizes were calculated with TPSRelw v. 1.6 (Rohlf, 2015). Subsequent analyses of the landmark data were conducted using MorphoJ v. 1.06 software package (Klingenberg, 2011). The generalized least square Procrustes superimposition algorithm (GPA; Rohlf, 1996) was performed in order to remove all non-shape component of the variation. An outliers test (Viscosi and Cardini, 2011) was used to control and exclude any individual specimen that deviated strongly from the average; an allometry test was done to evaluate the potential for strong bias due correlation between size and shape (Claude, 2008; Zelditch et al., 2004). First, shape diversity variation was analyzed with a Principal Component Analysis (PCA), a method for ordinate specimens along the major axes of

shape variation (Chatfield and Collins, 1980; Lawing and Polly, 2009; Morrison, 1990). Secondly, a Canonical Variate Analysis (CVA) was used to find features that best differentiate proposed groups (Campbell and Atchley, 1981; Mardia et al., 1979). For the CVA, specimens were grouped according to the three candidate species proposed in this study. The degree of differentiation among groups was measured by the Mahalanobis distance (Mahalanobis, 1936). To quantify relative amounts of variation among groups, a Procrustes ANOVA was performed using the candidate species (*L. t. tenuis*, *L. t. punctatissimus* and *L. sp.*) as a factor (Klingenberg, 2015; Klingenberg and McIntyre, 1998; Klingenberg et al., 2002).

The significance of differences (at 0.05 level of significance) in the shape variations within males and females was determined using the Kruskal-Wallis test (Kruskal and Wallis, 1952) with the Bonferroni correction in RStudio (RStudio Team, 2015). We compared means among the groups that were statistically different according to the CVA. Finally, we conducted a One-Way ANOVA separately for each species identified by the BPP analyses in order to determine if the delimited species presented sexual dimorphism.

2.6. Divergence times

Divergence times were calculated using *BEAST (Heled and Drummond, 2010) using BEAST v. 1.8.3 (Drummond et al., 2012) with a matrix that included one sequence of each of the delimited species as retrieved by BPP (see results), along one sequence of *L. pictus*, *L. chiliensis* and *L. lemniscatus* and two species belonging to the *Eulaemus* subgenus, namely *Liolaemus darwinii* and *L. uspallatensis* (Tables 2, S5, and S6). When possible, individuals that had sequences corresponding to all seven loci were used. The option of "species tree ancestral reconstruction" was chosen in order to account for gene-tree discordance and ploidy type of each marker. Substitution (determined by the BIC criterion in jmodeltest were used; Table 2) and models clock models as well as trees were unlinked across loci. For nuclear genes, a uniform prior was established, with a lower and upper bound of 0 and 0.01 respectively, and 0.005 as a starting value. The same tree prior as for the species tree analyses were used. The best fit for molecular clock was also evaluated.

Following Fontanella et al. (2012) and Ho (2007), we used a lognormal prior with a mean of 20 Mya and a standard deviation of 0.13 on the node at the base of the *Liolaemus* clade in order to calibrate de tree. This age corresponds to the Early Miocene sub-epoch from which the fossil was collected (Albino, 2008).We applied a lognormal prior as it is thought to be the most appropriate to reflect the uncertainty in the fossil calibration and that the divergence

event actually occurred sometime before the appearance of the fossil (Hedges and Kumar, 2004). The .xml file was manually modified to "MeanInRealSpace = true" for the prior of the calibrated node height. This analysis also estimates the mean substitution rate for each locus. Finally, analyses were run for 100 million generations with the parameters logged every 1000th iteration. Results for the *BEAST analyses were obtained by combining posterior samples from two independent runs of a chain-length of 100 million each. Convergence and effective sample sizes (ESS) were monitored with Tracer 1.6 (Rambaut et al., 2013).

3. RESULTS

All markers were consistent with a neutral model of evolution ($p>0.10$) and showed little saturation. Heterozygotes individuals were found for all nuclear markers. A brief description of sequence matrices for each marker can be found in Table 4.

3.1. Coalescent-based species tree estimation and species delimitation

The complete aligned matrix, including the outgroup, has a length of 5393 base-pairs and consisted of 198 specimens (34 corresponded to *L. t. tenuis*, 110 to *L. t. punctatissimus*, 53 to *L. sp*. and one to *Liolaemus lemniscatus* (the outgroup). After assessing the adequacy of the molecular clock, final analyses were run using an uncorrelated relaxed clock, as for most genes the ucld.stdev parameter deviated from zero.

Sequences of *Liolaemus tenuis sensu lato* fall in three divergent and well-supported clades. There is a strong support for the monophyly of the three candidate species of *L. tenuis* (posterior probability = pp = 1). *Liolaemus sp.* and *L. t. punctatissimus* are strongly supported as sister taxa (pp = 1, Figure S1), and the clade they form is sister to *L. t. tenuis* with high posterior probability (pp = 1). Individual gene trees are available as supplementary material (Figures S2-S8).

Our final BPP matrix contained sequences from 18 individuals assigned to *L. t tenuis*, from 70 individuals assigned to *L. t. punctatissimus* and from 43 individuals assigned to *L. sp*. All BPP analyses, regardless of the starting species tree and the algorithm used were consistent, supporting the same species tree and the same delimited species. All analyses supported with high posterior probability values (pp > 0.95) that *L. tenuis s. l.* consisted of two and not three species, as originally hypothesized: candidate species *Liolaemus t. punctatissimus* and *L. sp.* would constitute a single species, while *L. t. tenuis* represents the other one (Table 5). This two-species model was strongly supported (pp > 0.95),

306 consisting of *L. t. punctatissimus–L. sp.* (pp > 0.95) and *L. t. tenuis* (pp = 1) (Table 6) On the contrary, there was no
307 support for considering *L. t. punctatissimus* and *L. sp.* as distinct species (pp < 0.05) (Table 7).
308
309 **3.2. Species Distribution's Modeling**
310 The distributional models for each species showed stability and good support (AUC = 0.991 for *L. t. tenuis*, AUC = 0.988
311 for *L. t. punctatissimus*, and AUC = 0.989 for *L. sp.*). The variables that contributed the most (accounting for 78.1% of the
312 total variable contribution) for the SDM of *L. t. tenuis* were Precipitation of Warmest Quarter (bio18, 30.4%), Mean
313 Temperature of Driest Quarter (bio9, 26.6%) and Precipitation Seasonality (Coefficient of Variation) (bio15, 21.1%);
314 while for the SDM of *L. t. punctatissimus* and *L. sp.* variables that contributed the most (94.7% and 93.4%, respectively)
315 were Precipitation of Coldest Quarter (bio19, 58% and 64.7% respectively) and Precipitation Seasonality (Coefficient of
316 Variation) (bio15, 36.8% and 28.7%, respectively).
317 For *Liolaemus t. tenuis*, habitat suitability was high (> 0.7, in red) around its type locality in the Metropolitan Region,
318 in the central part of the Valparaíso Region, and in central and coastal O'Higgins Region (Figure 3A). Therefore, the
319 distribution of this taxon is comprised within the Mediterranean Blioclimatic Zone. (Amigo and Ramírez, 1998). Both *L.*
320 *t. punctatissimus* and *L. sp.* have a high probability of occurrence in the Andean area of southern Biobío Region and
321 northern Araucanía Region (Figure 3B, C). This area lies within the temperate bioclimatic region according to Amigo and
322 Ramírez (1998). *Liolaemus t. punctatissimus* has also a high probability of occurrence in the coastal area of the Biobío
323 Region, which includes type locality (Lota) of the subspecies, and in central O'Higgins Region, adjacent (but not
324 overlapping) to the southernmost distribution of *L. t. tenuis* (Figure 3A, B). Finally, the probability of occurrence of *L. sp.*
325 is moderate (> 0.52, in orange) in a narrow area of the intermediate depression, from Maule to Los Rios Regions (Figure
326 3C).
327
328 **3.3. Morphometric Analyses**
329 Out of the 88 analyzed specimens, 35 were females and 53 were males (Table S4). Normality test (Shapiro-Wilk)
330 indicated our samples had a normal distribution (p-males = 0.2544, p-females = 0.4801), while the Levene test indicated
331 that variances among samples are equal (p = 0.027). ANOVA results showed that the male and female samples were
332 significantly different in head shape (p = 9.469e-6), so we treated them separately in subsequent analyses.

For females, the regression results showed that size and shape were not correlated ($p = 0.3556$), meaning that this species does not have allometric growth. No outliers were detected. The first three PCs of size-corrected shape variation for females account for almost 70% of the total variance in the data set; PC1 explained 38.742% of the variation. Landmark 7, showed the highest contribution to PC1. PC2 explains 17.085 % of the variation and the contributing landmarks are landmarks 4, 5 and 10, which lay in the snout, and could therefore be considered to describe variation in snout shape. Additionally, landmarks 6, 12 and 13, which lay in the jaw, contribute to this PC. PC3 explained 14.054% of the variation in heads shape, with landmarks 2-6, 8-12 and 14 contributing to it. In the Canonical Variate Analyses, p-values for Malahanobis distance indicate that all comparisons (among the three putative species) are significantly different ($p < 0.0001$ in all cases). Case distribution in the two Canonical variates plot resulted in three discrete and non-overlapping groups. Mahalanobis distances in all three pair comparisons were highly similar: *L. t. tenuis* vs. *L. t. punctatissimus* (3.7184), *L. t. tenuis* vs *L. sp.* (3.8186), and *L. t. punctatissimus* vs *L. sp.* (3.4121). For the first and second canonical variates, the three candidate species formed distinct clusters with the CVA (Figure 4A). The first canonical variate (CV1) accounted for 57.76 % of the variance. Shape between candidate species was statistically different ($p = 0.0147$).

In males, the regression results showed that size and shape were not correlated ($p = 0.7113$). No outliers were detected. The first three PCs account for 70% of the total variation. PC1, PC2 and PC3 explain the 41.8%, 16.3% and 10.1% of the variance, respectively. Landmarks 5-7 and 13 contribute to PC1, while 3-6, 9-11 and 14 contribute to PC2. In PC3, landmarks showing contributions are 2-4, 6, 9, and 12. In the Canonical Variate Analyses, p-values for Malahanobis distance indicate differences between *L. t. tenuis* and *L. sp.* and between *L. t. tenuis* and *L. t. punctatissimus* are significantly different ($p < 0.0001$); however, differences between *L. t. punctatissimus* and *L. sp.* are not significant ($p = 0.7253$). For the first and second canonical variates for males, the three groups formed overlapping clusters (Figure 4B). The first canonical variate (CV1) accounted for 73.638 % of the variance. Mahalanobis distances in all comparisons were highly similar but lower than in female comparisons: *L. t. tenuis* vs. *L. t. punctatissimus* (2.5573), *L. t. tenuis* vs *L. sp.* (2.7554), and *L. t. punctatissimus* vs *L. sp.* (1.5193).

The Kruskal-Wallis test reported significant differences ($p < 0.05$) between some landmark means in females and males (Table S7). Our results suggest that females of *L. t. punctatissimus* and *L. t. tenuis* differ in their snout shape (y2, x4, x10), and that females of *L. t. punctatissimus* and *L. sp.* could differ in their eye's shape (x8) (Figure S9). Males of *L. t.*

punctatissimus and *L. t. tenuis* seem to present differences in the position of the pineal eye (y3) and in their eye shape (y9, x12, y14) (Figure S10) (Table S7).

Within the species retrieved by BPP, our AMOVA results found that there are significant differences between sample means, implying there is possibly sexual dimorphism (*L. t. tenuis*, p=0.002451; *L. t. punctatissimus* + *L. sp.*, p=8.696e-8).

3.4. Divergence times.

An uncorrelated relaxed clock was used for all genes following preliminary runs because the ucld.stdev parameter for most genes deviated from zero. The final data matrix used for analyses contained seven terminals and 5604 base-pairs. The time-calibrated species tree shows that *Liolaemus tenuis sensu lato* shares a common ancestor with *L. lemniscatus* at ca. 8.97 mya (node height 95% HDP interval: 4.34-14.84). On the other hand, *L. tenuis* and *L. punctatissimus* share their most recent common ancestor at ca. 1.82 mya (node height 95% HDP interval: 0-3.85). (Figure 5). The estimated mean substitution rate for each locus (in site per million years) were as follows: A9C = 0.0014738 (st. dev. = 0.00068539); B1D = 0.0025871 (st. dev. = 0.0011956); cytochrome b = 0.0138 (st. dev. = 0.0042098); EXPH5 = 0.00075607 (st. dev. = 0.00023863); KIF24 = 0.0013411 (st. dev. = 0.00040624), and LDB5B = 0.0066335 (st. dev. = 0.0021287).

4. DISCUSSION

It has long been recognized that relying solely on morphological characters to delimit species is problematic (Mayr, 1942). There are cases, such as in cryptic species, in which morphology provides little information to assess species boundaries (Bickford et al., 2007). New approaches have been developed that while accounting for the discordance between gene trees and species trees, provide a natural framework for estimating species trees (Heled and Drummond, 2008; Kubatko et al., 2009; Liu et al., 2009) while simultaneously delimiting species (Yang and Rannala, 2010, 2014). Herein, we use multilocus coalescent methods to assess species limits and to elucidate the timing of lineage diversification within *Liolaemus tenuis sensu lato*. Additionally, as recommended by Sukumaran and Knowles (2017) we use independent lines of evidence to empirically delimit species.

389 **4.1. Taxonomic implications**

390 Based on our results, we identify two separate evolving lineages that can be recognized as two distinct species genetically
391 as well as morphologically. GM results also suggest that there is sexual dimorphism in both delimited species.
392 One species includes individuals allocated here to *L. t. tenuis*, and the other species includes individuals from the lineages
393 referred here as *L. t. punctatissimus* and *L. sp*. Therefore, we elevate *Liolaemus tenuis tenuis* and *Liolaemus tenuis*
394 *punctatissimus* to full species, as follows:
395 *Liolaemus* (*Liolaemus*) *tenuis* (Duméril and Bibron, 1837). Type locality: Outskirts of Santiago. Distribution. Endemic
396 from Chile, occurring approximately from 31.76°S (Salamanca) to 33.70°S (Melipilla) (Regions of Coquimbo,
397 Valparaíso, Metropolitana) (Figure 6).
398 *Liolaemus* (*Liolaemus*) *punctatissimus* (Müller and Hellmich, 1933). Type locality: Lota. Distribution: Approximately
399 from 33.72°S South (Rio Clarillo) to 40.26°S (La Unión-Hueicolla) (encompassing the Chilean regions of O'Higgins,
400 Maule, Biobío, Araucanía and Los Ríos), including marginal populations in the Neuquén Province (Argentina) (Figure 6).
401 Interestingly, the subspecies geographic distributions as originally described, in which they overlapped in their southern
402 range, do not match that of the lineages described above as these two entities distributions do not overlap (Figure 6).
403
404 It is worth mentioning at this point that even though we have included some individuals from populations occurring south
405 to the Maipo River (haplotypes from localities 18 and 19, Table S1) as potentially belonging to candidate species *L. t.*
406 *tenuis*, this was done following a cytochrome b phylogeny, which was only our starting hypothesis considering that gene
407 trees are unlikely to represent the true, underlying species tree. Given the results obtained by Muñóz-Mendoza et al. (in
408 press), who found evidence supporting no gene flow between individuals of *L. tenuis sensu lato* north and south of the
409 Maipo River, we consider these individuals to belong to *Liolaemus punctatissimus*. The fact that appear within the same
410 mitochondrial clade as individuals north of the Maipo River (here considered as *L. tenuis sensu stricto*), could be
411 explained by the inherent stochasticity of the coalescent process; despite the fact that mtDNA coalesces four times faster
412 than nuclear DNA, that does not assure that its alleles have reached reciprocal monophyly. Therefore, and also
413 considering that the Maipo River has impeded gene flow for other taxa (including other *Liolaemus* species, e.g., Lamborot
414 and Eaton, 1997), is more conservative to assume that these few individuals from south of the Maipo River, which appear
415 in clade A (Fig. 1; Muñóz-Mendoza et al., in press) belong to *Liolaemus punctatissimus.*
416

4.2. Historic factors promoting speciation in Central-Southern Chile

Glaciations

The large topographic variation of the Chilean territory, coupled with a complex climatic history, have promoted a fragmented distribution of the different environmental regimes through space and time, which in turn largely impacted the genetic composition and differentiation of its populations (e.g. Torres-Pérez et al., 2007; Victoriano et al., 2008; Vidal et al., 2012). Central-Southern Chile is known to have suffered important climatic fluctuations caused by the advance and retreat of an ice sheet during glaciations, from approximately 6 million years ago (mya) until the Last Glaciation, which ended ca. 11,700 years ago (Kaiser et al., 2005; Rabassa et al., 2011). The Pleistocene climatic shifts have often been invoked to explain diversification of Neotropical biota, but evidence in favor of this hypothesis remains controversial to this day (Hewitt, 2000; Klicka and Zink, 1997; Lessa et al. 2003); as such, some studies have proposed that Neotropical speciation events of lowland taxa occurred in the Pliocene (Blair et al., 2015; Weir, 2006; Weir and Schluter, 2004). Both Pliocene and Pleistocene glaciations were frequent in Central-Southern Chile. Our results suggest that the diversification between *L. tenuis* and *L. punctatissimus* occurred in the mid-Pleistocene, around 1.8 mya (Gradstein et al., 2012). It is known that glaciations started well before the Great Patagonian Glaciation (GPG) (~1 mya), and are referred to as Pre-GPG Glaciations; several (as much as seven glaciations) took place between 2 and 1 mya (Mercer and Sutter, 1982). Specifically, a thick till of more than 40 m is reported for 2.05-1.86 mya (Mercer, 1976), suggesting glaciations were taking place around the time of diversification of these species. Additionally, from 1.8 mya onwards, glaciations were more durable, so that ice sheets over 2500 kilometers long extended at least from 36° to 56° S, covering all Patagonian Andes (Rabassa, 2008). Speciation could have been initiated during glacial periods, by displacements into multiple refugia (allopatric speciation), or during interglacials, by the founding of new populations when individuals expanded from ancestral, glacial-source populations (peripatric speciation). Given that the estimated timing of divergence between *L. tenuis* and *L. punctatissimus* predates the most recent glaciations (e.g., GPG, LGM), in the absence of a new, permanent vicariant barrier, reproductive isolation must have evolved very rapidly for incipient species divergence to be maintained across subsequent shifts in species distributions.

Rivers

In some cases, rivers promote species divergence through allopatric speciation (Giarla and Jansa, 2014), which has been termed the "riverine hypothesis" (Wallace, 1852). There is both evidence against (e.g., Gascon et al., 2000; Patton et al.,

1994; Roratto et al., 2014) and in favor of this hypothesis (e.g., Aleixo, 2004; Ayres and Clutton-Brock, 1992; Boubli et al., 2015; Moraes et al., 2016). Chile has a series of parallel steep, high gradient, east-to-west flowing rivers; some major rivers have originated around 4-5 mya, such as the Maipo River (Quezada Romegialli, 2014), which is known to impede gene flow among populations (e.g., *Liolaemus monticola*: Torres-Pérez et al., 2007; *Philodryas chamissonis*: Sallaberry-Pincheira et al., 2011). *Liolaemus tenuis* seems to be restricted to the north of the Maipo River (Figure 6), area which corresponds to the arid and semi-arid Mediterranean bioclimatic zones (di Castri, 1968). On the other hand, populations allocated to *L. punctatissimus* are restricted to the south of the Maipo River, encompassing sub-humid, humid, per-humid Mediterranean and Ocean with Mediterranean influence bioclimatic areas (di Castri, 1968). This is in agreement with findings by Muñóz-Mendoza et al. (in press), who reported that there was no gene flow between *L. tenuis* populations at both sides of the Maipo River, as well as high *Fst* values for comparisons between clusters at both sides of the river. Therefore, the Maipo River sets the northern (hard) limit to *L. punctatissimus* distribution and the southern (hard) limit to *L. tenuis* distribution. This riverine barrier could have been further enhanced by the adjacent Cantillana Mountain Range (~33º58' S, 70º58' W), which is part of the Coastal Mountain range, and has an extension of approximately 70 kilometers wide, reaching up to 2281 meters (Romero-Gárate and Tellier, 2014). Moreover, in the Maipo valley, glaciers are known to have occurred during Pleistocene glaciations (Caviedes and Paskoff, 1975), reaching also into the Central Valley, which could have enhanced the barrier effect. Climate as well as landscape features, such as mountains and rivers, could be have jointly prompted the differentiation of *L. tenuis* and *L. punctatissimus*.

4.3. Phenotypic variation

In Reptiles, GM has proven to be a useful approach in capturing variation in head shape between sexes and subspecies (e.g., Gentilli et al., 2009; Vincent et al., 2004). Head shape is linked to feeding ecology and sexual selection (e.g., Baeckens et al., 2016; Donihue et al., 2015; Segall et al., 2016), and is therefore considered as a good starting point for determining if morphological differences exist among previously identified lineages, therefore functioning as a way of assessing species delimitation. Our results suggest that there is sexual dimorphism and variation in head morphology within the *Liolaemus tenuis* complex, as well as within the delimited *L. tenuis* and *L. punctatissimus*. The fact that the differences we found were previously undetected (either by the approach or data used) suggests that these are subtle morphological changes accompanying genetic divergence, and implies that *L. tenuis* and *L. punctatissimus* have probably diverged rapidly. Medel and collaborators (1988) provided evidence of a habitat shift between northern populations of *L.*

tenuis, which would be rock and trunk dwellers (preferring perches between 0-30 centimeters), relative to those from the south (now *L. punctatissimus*), which would restrict its microhabitat use to trees only, but to higher perches (30-60 centimeters). Perhaps a difference in the type of prey encountered by these species in the ground relative to prey available on tree trunks could explain the differences reported here on head shape between *L. tenuis* and *L. punctatissimus*.

On the other hand, *Liolaemus tenuis sensu lato* is known to have both sexual and geographic color variation (Vidal et al., 2007). Females have a melanistic (dark) coloration, with brown scales increasing in number towards the north. Males, on the other hand, are very colorful, with predominance of yellow in the anterior part of the body and bluegreen in the posterior part, and these scales increase towards the south of the species' distribution (Donoso-Barros, 1966; Vidal et al., 2007). It is sexual selection what usually determines the occurrence of colorful males, particularly during the reproductive season (Cooper, 1988; Cooper et al., 1983; Watkins, 1997), as colors are supposed to reflect male quality (Hamilton and Zuk, 1982; Folstad and Karter, 1992). Female mate choice has been shown to be based on both coloration and patterns (Lancaster et al., 2007, 2009). Particularly, studies of bicoloured lizards (as it is the case with *Liolaemus tenuis sensu lato*) have shown that the proportion of the two different colors in the individuals yielded differential male dominance (Thompson et al., 1991). Both colour and colour patterns are heritable and in some cases, lizard that are polymorphic in coloration show strong differences in genetically encoded reproductive tactics (Olsson et al., 2013). Despite that one gene has been identified as candidate for reptile pigmentation (the melanocortin-1 receptor gene, Mc1r), the genetic basis for coloration in reptiles is not well studied (Corso et al., 2012; Nunes et al., 2011; Rosenblum et al., 2004), and evidence indicates that different mutations in this gene can account for similar (Rosenblum et al., 2004) and different phenotypes (Nunes et al., 2011). *Liolaemus tenuis* subspecies were originally described mainly on colour patterns, a trait that is often found to be variable within species and thus not considered as a reliable indicator of evolutionary relationships (Burbrink et al., 2000). Nevertheless, variation in coloration is only biologically meaningful if it can be perceived by conspecifics, predators, or prey (Olsson et al., 2013); lizards in particular appear to have an acute vision so this trait may be particularly relevant for intraspecific communication (Fleishman, 1992; New et al., 2011). Considering this, and the fact that *L. tenuis* and *L. punctatissimus* seem to be sister species, it is possible that there are strong selective pressures due to sexual selection acting on body size coloration. This process could be maintained due to the fact that both species seem to be allopatric, and they might have developed other forms of reproductive isolation. Many examples in nature show that a strong phylogeographic structure can be coupled with a lack of phenotypic differentiation, pattern that can the consequence of various processes such as retention of ancestral polymorphisms,

balancing selection and phenotypic plasticity (Zamudio et al., 2016). Nevertheless, some studies suggest that southern populations (which would correspond to *L. punctatissimus*), which are associated to dense forests are more colorful, with intense blue and yellow, than northern populations (*L. tenuis*), which inhabit open scrublands and are more brownish (Donoso-Barros, 1966; Hellmich, 1950; Müller and Hellmich, 1933; Vidal et al., 2007). A thorough study would be needed in order to determine if *L. tenuis* and *L. punctatissimus* can be differentiated based on coloration patterns, and if so, if mating systems and reproductive tactics are similar in both taxa.

4. 4. Conclusions

We show that integrative approaches yield more robust results compared to delimiting species with a single line of evidence (Schlick-Steiner et al., 2010), as the incorporation of morphology helps visualizing the phenotypic novelties that characterize molecular groupings (Lee and Palci, 2015) and SDM techniques contribute to the correct delimitation of species geographic distributions. We highlight a case in which the current taxonomy under represents the number of species in a *Liolaemus* lizard from Central Chile. The two described subspecies of *L. tenuis*, which were defined based on coloration patterns (Müller and Hellmich, 1933), do not form natural entities. The discrepancy between the current classification of subspecies and observed genetic differentiation is problematic because subspecies are used as conservation units by conservation organizations such as the International Union for Conservation of Nature (IUCN). Therefore, we elevate *Liolaemus tenuis tenuis* and *L. t. punctatissimus* to full species, and re-describe their distribution. Geographic range data for each of these species indicate that these genetic clades are likely isolated from each other by the Maipo River. These taxonomic changes carry important consequences as *L. tenuis* distribution is considerably reduced, rendering this species endemic to Chile (as the populations that occur in the Argentine province of Neuquén are allocated to *L. punctatissimus*), consequently making it a valuable conservation target. These results are in agreement with several studies that suggest that many *Liolaemus* species remain to be described. The underestimation of species diversity in this group of lizards is particularly important because it occurs in a biodiversity conservation hotspot (Myers et al., 2000). We hope that the species proposed here can be treated as management units in the context of major conservation planning and actions in the future, as failure to do so could lead to the loss of potentially vital genetic diversity that is critical for the survival of the whole species.

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REFERENCES

Abdala, C. S., 2007. Phylogeny of the *boulengeri* group (Iguania: Liolaemidae, *Liolaemus*) based on morphological and molecular characters. Zootaxa, (1538), 1-84.

Adams, D.C., Funk, D.J., 1997. Morphometric inferences on sibling species and sexual dimorphism in *Neochlamisus bebbianae* leaf beetles: multivariate applications of thin plate spline. Syst. Biol. 46, 180–194. DOI: https://doi.org/10.1093/sysbio/46.1.180

Aguilar, C., Wood, P.L.Jr., Cusi, J.C., Guzmán, A., Huari, F., Lundberg, M., Mortensen, E., Ramírez, C., Robles, D., Suárez, J., Ticona, A., Vargas, V.J., Venegas, P.J., Sites, J.W. Jr., 2013. Integrative taxonomy and preliminary assessment of species limits in the *Liolaemus walkeri* complex (Squamata, Liolaemidae) with descriptions of three new species from Peru. Zookeys 18(364), 47-91. DOI: http://dx.doi.org/10.5061/dryad.0q7pc

Albino, A.M., 2008. Lagartos iguanios del Colhuehuapense (Mioceno Temprano) de Gaiman (provincia del Chubut, Argentina). Ameghiniana 45, 775–782.

Aleixo, A., 2004. Historical diversification of a terra-firme forest bird superspecies: a phylogeographic perspective on the role of different hypotheses of Amazonian diversification. Evolution 58, 1303–1317. doi: http://dx.doi.org/10.1554/03-158

556 Alvarado-Serrano, D. F., Knowles, L. L., 2014. Ecological niche models in phylogeographic studies: applications,
557 advances and precautions. Mol. Ecol. Res. 14(2), 233-248. DOI: 10.1111/1755-0998.12184
558 Amigo, J., Ramírez, C., 1998. A bioclimatic classification of Chile: woodland communities in the temperate zone. Plant
559 Ecol., 136(1), 9-26. doi:10.1023/A:1009714201917
560 Avila, L.J., Morando, M., Sites, J.W.Jr., 2006. Congeneric phylogeography: hypothesizing species limits and evolutionary
561 processes in Patagonian lizards of the *Liolaemus boulengeri* group (Squamata: Liolaemini). Biol. J. Linn. Soc. 89,
562 241–275. DOI: 10.1111/j.1095-8312.2006.00666.x
563 Ayres, J.M., Clutton-Brock, T.H., 1992, River boundaries and species range size in Amazonian primates. Am. Nat. 140,
564 531–537.
565 Baeckens, S., García-Roa, R., Martín, J., Ortega, J., Huyghe, K., Van Damme, R., 2016. Fossorial and durophagous:
566 implications of molluscivory for head size and bite capacity in a burrowing worm lizard. J. Zool. 1-13. DOI:
567 10.1111/jzo.12412
568 Beatty, G.E., Provan, J., 2010. Refugial persistence and postglacial recolonization of North America by the cold-tolerant
569 herbaceous plant *Orthilia secunda*. Mol. Ecol. 19, 5009–5021. DOI: 10.1111/j.1365-294X.2010.04859.x
570 Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K.L., Meier, R., Winker, K., Ingram, K.K., Das I., 2007. Cryptic species
571 as a window on diversity and conservation. Trends Ecol. Evol. 22, 148–155.
572 http://dx.doi.org/10.1016/j.tree.2006.11.004
573 Blair, C., de la Cruz, F. R. M., Law, C., Murphy, R. W., 2015. Molecular phylogenetics and species delimitation of leaf-
574 toed geckos (Phyllodactylidae: *Phyllodactylus*) throughout the Mexican tropical dry forest. Mol. Phylogenet.
575 Evol. 84, 254-265. http://dx.doi.org/10.1016/j.ympev.2015.01.003
576 Bookstein, F. L., 1997a. Landmark methods for forms without landmarks: morphometrics of group differences in outline
577 shape. Med. Image Anal. 1(3), 225-243. http://dx.doi.org/10.1016/S1361-8415(97)85012-8
578 Bookstein, F. L. 1997b. Morphometric tools for landmark data: geometry and biology. Cambridge University Press.
579 Boubli, J. P., Ribas, C., Alfaro, J. W. L., Alfaro, M. E., da Silva, M. N. F., Pinho, G. M., Farias, I. P., 2015. Spatial and
580 temporal patterns of diversification on the Amazon: A test of the riverine hypothesis for all diurnal primates of
581 Rio Negro and Rio Branco in Brazil. Mol. Phylogenet. Evol. 82, 400-412.
582 http://dx.doi.org/10.1016/j.ympev.2014.09.005

583 Burbrink, F.T., Lawson, R., Slowinski, J.B., 2000. Mitochondrial DNA phylogeography of the polytypic North American
584 rat snake (*Elaphe obsoleta*): a critique of the subspecies concept. Evolution 54, 2107–2118. doi:
585 http://dx.doi.org/10.1554/0014-3820(2000)054[2107:MDPOTP]2.0.CO;2
586 Camargo, A., Avila, L. J., Morando, M., Sites, J.W., 2012. Accuracy and precision of species trees: effects of locus,
587 individual, and base pair sampling on inference of species trees in lizards of the *Liolaemus darwinii* group
588 (Squamata, Liolaemidae). Syst. Biol. 61(2), 272-288. DOI: https://doi.org/10.1093/sysbio/syr105
589 Camargo, A., Werneck, F. P., Morando, M., Sites, J. W., Avila, L. J., 2013. Quaternary range and demographic expansion
590 of *Liolaemus darwinii* (Squamata: Liolaemidae) in the Monte Desert of Central Argentina using Bayesian
591 phylogeography and ecological niche modelling. Mol. Ecol., 22(15), 4038-4054. DOI: 10.1111/mec.12369
592 Campbell, N. A., Atchley, W. R., 1981. The geometry of canonical variate analysis. Syst. Biol. 30(3), 268-280. DOI:
593 https://doi.org/10.1093/sysbio/30.3.268
594 Caviedes, C. N., Paskoff, R., 1975. Quaternary glaciations in the Andes of north-central Chile. J. Glaciol. 14(70), 155-
595 170.
596 Chan, L.M., Brown, J.L., Yoder, A.D., 2011. Integrating statistical genetic and geospatial methods brings new power to
597 phylogeography. Mol. Phylogenet. Evol. 59, 523–537. http://dx.doi.org/10.1016/j.ympev.2011.01.020
598 Chatfield, C., Collins, A.J., 1980. Introduction to multivariate analysis. London & New York: Chapman & Hall.
599 Claude, J., 2008. Morphometrics with R. XVIII. New York: 235. Springer.
600 Clement M., Posada D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9 (10),
601 1657-1660.
602 Codoncode Corporation. 2009. Codon Code aligner v. 3.5.1.
603 Colborn, J., Crabtree, R.E., Shaklee, J.B., Pfeiler, E., Bowen, B.W., 2001. The evolutionary enigma of bonefishes (*Albula*
604 spp.): Cryptic species and ancient separations in a globally distributed shorefish. Evolution 55(4), 807–820. doi:
605 http://dx.doi.org/10.1554/0014-3820(2001)055[0807:TEEOBA]2.0.CO;2
606 Cooper, W.E., 1988. *Sceloporus undulates consobrinus* (southern prairie lizard). Coloration. Herpetol. Rev. 19-34.
607 Cooper, W.E., Adams, C.S., Dobie, J.L., 1983. Female color change in the keeled earless lizard, *Holbrookia propinqua*:
608 Relationship to the reproductive cycle. Southwestern Nat. 28, 275-280. DOI: 10.2307/3670787

609 Corso, J., Goncalves, G.L., De Freitas, T.R.O., 2012. Sequence variation in the melanocortin-1 receptor (MC1R)
610 pigmentation gene and its role in the cryptic coloration of two South American sand lizards. Genet. Mol. Biol. 35,
611 81–7. http://dx.doi.org/10.1590/S1415-47572012005000015
612 Crochet, P.A., Chaline, O., Surget-Groba, Y., Debain, C., Ceylan, M., 2004. Speciation in mountains: phylogeography
613 and phylogeny of the rock lizards genus *Iberolacerta* (Reptilia: Lacertidae). Mol. Phylogenet. Evol. 30, 860–866.
614 Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel
615 computing. Nat. Methods 9(8), 772. doi: 10.1038/nmeth.2109.
616 Dasmahapatra, K.K., Elias, M., Hill, R.I., Hoffman, J.I., Mallet, J., 2010. DNA barcoding. Mitochondrial DNA barcoding
617 detects some species that are real, and some that are not. Mol. Ecol. Res. 10, 264–273. DOI: 10.1111/j.1755-
618 0998.2009.02763.x
619 Dayrat, B., 2005. Towards integrative taxonomy. Biol. J. Linn. Soc. 85(3), 407-415. DOI: 10.1111/j.1095-
620 8312.2005.00503.x
621 di Castri, F., 1968. Esquisse écologique du Chili, in: Delamare Debouteville, C., Rapoport, T. (Eds.), Biologie de
622 l'Amérique Austral. Edition du Centre National de la Recherche Scientifique, Paris, pp. 7-52.
623 Donihue, C. M., Brock, K. M., Foufopoulos, J., Herrel, A., 2015. Feed or fight: testing the impact of food availability and
624 intraspecific aggression on the functional ecology of an island lizard. Funct. Ecol. 30, 566-575. doi:
625 10.1111/1365-2435.12550
626 Donoso-Barros, R., 1966. Reptiles de Chile. Universidad de Chile.
627 Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7.
628 Mol. Biol. Evol. 29, 1969-1973. DOI: https://doi.org/10.1093/molbev/mss075
629 Duméril, A.M.C., Bibron, G., 1837. Erpétologie Générale ou Histoire Naturelle Complète des Reptiles. Volume 4.
630 Histoire de Quarante-six Genres et de Cent Quarante-six Espèces de la Famille des Iguaniens, de l'Ordre des
631 Sauriens. Librarie Enclyclopedique de Roret, Paris.
632 Dupuis, J.R., Doe, A.D., Sperling, F.A.H., 2012. Multi-locus species delimitation in closely related animals and fungi: one
633 marker is not enough. Mol. Ecol. 21, 4422–4436. DOI: 10.1111/j.1365-294X.2012.05642.x
634 Etheridge, R., 1995. Redescription of *Ctenoblepharys adspersa* Tschudi 1845, and the taxonomy of Liolaeminae
635 (Reptilia: Squamata: Tropiduridae). Amer. Mus. Nov. 3142, 1-34.

Fleishman, L.J., 1992. The influence of the sensory system and the environment on motion patterns in the visual displays of anoline lizards and other vertebrates. Am. Nat. 139, S36–61. DOI: 10.1086/285304

Folstad, I., Karter, A.K., 1992. Parasites, bright males and the immune-competence handicap. Am. Nat. 139, 603–22. DOI: 10.1086/285346

Fontanella, F.M., Olave, M., Avila, L.J., Sites, J.W.Jr., Morando, M., 2012. Molecular dating and diversification of the South American lizard genus *Liolaemus* (subgenus *Eulaemus*) based on nuclear and mitochondrial DNA sequences. Zool. J. Linn. Soc-Lond. 164, 825–835.doi: 10.1111/j.1096-701 3642.2011.00786.x.

Formas, R., 1979. La herpetofauna de los bosques temperados de Sudamérica. Mus. Nat. Hist. Univ. Kansas 7, 341-369.

Fouquet, A., Gilles, A., Vences, M., Marty, C., Blanc, M., Gemmell, N. J., 2007. Underestimation of species richness in Neotropical frogs revealed by mtDNA analyses. PLoS one, 2(10), e1109. http://dx.doi.org/10.1371/journal.pone.0001109

Gascon, C., Malcolm, J. R., Patton, J. L., da Silva, M. N., Bogart, J. P., Lougheed, S. C., Peres, C.A., Nechel, S. Boag, P. T., 2000. Riverine barriers and the geographic distribution of Amazonian species. Proc. Natl. Acad. Sci. USA 97(25), 13672-13677. doi: 10.1073/pnas.230136397

Gentilli, A., Cardini, A., Fontanetto, D., Zuffi, M.A.L., 2009. The phylogenetic signal in cranial morphology of *Vipera aspis*: a contribution from geometric morphometrics. Herpetol. J. 19, 69–77.

Giarla, T. C., Jansa, S. A., 2014. The role of physical geography and habitat type in shaping the biogeographical history of a recent radiation of Neotropical marsupials (*Thylamys*: Didelphidae). J. Biogeogr. 41(8), 1547-1558. https://doi.org/10.1016/j.ympev.2013.09.019

Gradstein, F. M., Ogg, J. G., Schmitz, M., Ogg, G., 2012. The geologic time scale 2012. elsevier.

Graham, C. H., Hijmans, R. J., 2006. A comparison of methods for mapping species ranges and species richness. Global Ecol. Biogeogr. 15, 578–587. DOI: 10.1111/j.1466-8238.2006.00257.x

Hamilton, W.D., Zuk, M., 1982. Heritable true fitness and bright birds: a role for parasites? Science 218, 384–7. DOI: 10.1126/science.7123238

Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontol. Electron. 4(1), 9.

Hedges, S.B., Kumar, S., 2004. Precision of molecular time estimates. Trends Genet. 20, 242–247. http://dx.doi.org/10.1016/j.tig.2004.03.004

Heled, J., Drummond, A., 2008. Bayesian inference of population size history from multiple loci. BMC Evol. Biol. 8, 289. DOI: 10.1186/1471-2148-8-289

Heled, J., Drummond, A., 2010. Bayesian inference of species trees from multilocus data. Mol. Biol. Evol. 27, 570-580. DOI: https://doi.org/10.1093/molbev/msp274

Hellmich, W., 1950. Die Eidechsen der Ausbeute Schröder (Gattung *Liolaemus*, Iguan.) (Beiträge zur Kenntnis der Herpetofauna Chiles XIII). Veröffentlichungen der Zoologischen Staatssammlung München 1.

Hewitt, G., 2000. The genetic legacy of the Quaternary ice ages. Nature 405, 907– 913. doi:10.1038/35016000

Hijmans, R. J., Guarino, L., Mathur, P., 2004. DIVA-GIS. Version 7.5. A geographic information system for the analysis of species distribution data for global land areas. Int. J. Climatol. 25, 1965–1978.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A., 2005. Very high resolution interpolated climate surfaces for global land area. Int. J. Climatol. 25, 732 965–1978. DOI: 10.1002/joc.1276

Ho, S.Y.M., 2007. Calibrating molecular estimates of substitution rates and divergence times in birds. J. Avian Biol. 38, 409–414. DOI: 10.1111/j.0908-8857.2007.04168.x

Huelsenbeck, J. P., Rannala, B., 2004. Frequentist properties of Bayesian posterior probabilities of phylogenetic trees under simple and complex substitution models. Syst. Biol. 53(6), 904-913. DOI: https://doi.org/10.1080/10635150490522629

Kaiser, J., Lamy F., Hebbeln, D., 2005. A 70-kyr sea surface temperature record off southern Chile (Ocean Drilling Program Site 1233). Paleoceanogr. 20, 1–15. DOI: 10.1029/2005PA001146

Kaliontzopoulou A., 2011. Geometric morphometrics in herpetology: modern tools for enhancing the study of morphological variation in amphibians and reptiles. Basic Appl. Herpet. 25, 5–32.

Kaliontzopoulou, A., Carretero, M. A., Llorente, G.A., 2012. Morphology of the *Podarcis* wall lizards (Squamata: Lacertidae) from the Iberian Peninsula and North Africa: patterns of variation in a putative cryptic species complex. Zool. J. Linn. Soc-Lond. 164(1), 173-193. DOI: http://dx.doi.org/10.11160/bah.11016

Katoh, K., Standley, D. M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30(4), 772-780. DOI: https://doi.org/10.1093/molbev/mst010

Klicka, J., Zink, R.M., 1997. The importance of recent ice ages in speciation: a failed paradigm. Science 277, 1666–1669. DOI: 10.1126/science.277.5332.1666

691 Klingenberg, C.P., 2011. MorphoJ: an integrated software package for geometric morphometrics. Mol. Ecol. Res. 11, 353-
692 357. DOI: 10.1111/j.1755-0998.2010.02924.x
693 Klingenberg, C. P., 2015. Analyzing fluctuating asymmetry with geometric morphometrics: concepts, methods, and
694 applications. Symmetry 7(2), 843-934. Doi: 10.3390/sym7020843
695 Klingenberg, C. P., McIntyre, G. S., 1998. Geometric morphometrics of developmental instability: analyzing patterns of
696 fluctuating asymmetry with Procrustes methods. Evolution 1363-1375. DOI: 10.2307/2411306
697 Klingenberg, C. P., Barluenga, M., Meyer, A., 2002. Shape analysis of symmetric structures: quantifying variation among
698 individuals and asymmetry. Evolution 56(10), 1909-1920. doi: http://dx.doi.org/10.1554/0014-
699 3820(2002)056[1909:SAOSSQ]2.0.CO;2
700 Knowles, L.L., 2009. Statistical phylogeography. Ann. Rev. Ecol. Evol. Syst. 40, 593–612. DOI:
701 10.1146/annurev.ecolsys.38.091206.095702
702 Kruskal, W. H., Wallis, W. A., 1952. Use of ranks in one-criterion variance analysis. J Am Stat Assoc. 47(260), 583-621.
703 DOI: 10.2307/2280779
704 Kubatko, L.S., Carstens, B.C., Knowles, L.L., 2009. STEM: species tree estimation using maximum likelihood for gene
705 trees under coalescence. Bioinformatics 25, 971– 973. DOI: https://doi.org/10.1093/bioinformatics/btp079
706 Labra, A. Escobar, C. A., Aguilar, P. M., Niemeyer, H. M., 2002. Sources of pheromones in the lizard *Liolaemus tenuis*.
707 Rev. Chil. Hist. Nat. 75, 141-147.
708 Lamborot, M., Eaton, L., 1997. The Maipo River as a biogeographical barrier to *Liolaemus monticola* (Tropiduridae) in
709 the mountain ranges of central Chile. J. Zoolog. Syst. Evol. Res. 35(3), 105-111.
710 Lancaster, L.T., McAdam, A.G., Wingfield, J.C., Sinervo, B.R., 2007. Adaptive social and maternal; induction of
711 antipredator dorsal patterns in a lizard with alternative social strategies. Ecol. Lett. 10, 798–808. DOI:
712 10.1111/j.1461-0248.2007.01069.x
713 Lancaster, L.T., Hipsley, C.A., Sinervo, B., 2009. Female choice for optimal combinations of multiple male display traits
714 increases offspring survival. Behav. Ecol. 20(5), 993–9. DOI: https://doi.org/10.1093/beheco/arp088
715 Lanier, H. C., Knowles, L. L., 2012. Is recombination a problem for species-tree analyses? Syst. Biol. 61(4), 691-701.
716 DOI: https://doi.org/10.1093/sysbio/syr128
717 Laurent, R.F., 1983. Contribución al conocimiento de la estructura taxonómica del género *Liolaemus* Wiegmann
718 (Iguanidae). Boletín de la Asociación Herpetológica Argentina 1, 16–18.

719 Lawson, L.P., 2010. The discordance of diversification: evolution in the tropical-montane frogs of the Eastern Arc
720 Mountains of Tanzania. Mol. Ecol. 19, 4046–4060. DOI: 10.1111/j.1365-294X.2010.04788.x
721 Lawing, A. M., Polly, P. D., 2010. Geometric morphometrics: recent applications to the study of evolution and
722 development. J. Zool. 280(1), 1-7. DOI: 10.1111/j.1469-7998.2009.00620.x
723 Leaché, A. D., Koo, M. S., Spencer, C. L., Papenfuss, T. J., Fisher, R. N., McGuire, J. A., 2009. Quantifying ecological,
724 morphological, and genetic variation to delimit species in the coast horned lizard species complex (*Phrynosoma*).
725 Proc. Natl. Acad. Sci. 106, 12418–12423. doi: 10.1073/pnas.0906380106
726 Leaché, A. D., Wagner, P., Linkem, C. W., Böhme, W., Papenfuss, T. J., Chong, R. A., Lavin, B.R, Bauer, A.M., Nielsen,
727 S.V., Greenbaum, E., Rödel, M, Schmitz, A., LeBreton, M., Ineich, I., Chirio, L., Ofori-Boateng, C., Eniang,
728 E.A., Dinn, S.B.E., Lemmon, A.R, Burbrink, F.T., 2014. A hybrid phylogenetic–phylogenomic approach for
729 species tree estimation in African Agama lizards with applications to biogeography, character evolution, and
730 diversification. Mol. Phylogenet. Evol. 79, 215-230. http://dx.doi.org/10.1016/j.ympev.2014.06.013
731 Lee, M. S., Palci, A., 2015. Morphological phylogenetics in the genomic age. Curr. Biol. 25(19), R922-R929.
732 http://dx.doi.org/10.1016/j.cub.2015.07.009
733 Lessa, E. P., Cook, J. A., Patton, J. L., 2003. Genetic footprints of demographic expansion in North America, but not
734 Amazonia, during the Late Quaternary. Proc. Natl. Acad. Sci. 100(18), 10331-10334. doi:
735 10.1073/pnas.1730921100
736 Levene, H., 1960. Robust tests for equality of variance. In: Olkin, I. (Ed.), Contributions to probability and statistics:
737 Essays in honor of Howard Hotelling. Palo Alto, CA: Stanford University Press, pp. 278–292.
738 Liu, L., Yu, L., Kubatko, L., Pearl, D.K., Edwards, S.V., 2009. Coalescent methods for estimating phylogenetic trees.
739 Mol. Phylogenet. Evol. 53, 320–328. http://dx.doi.org/10.1016/j.ympev.2009.05.033
740 Lobo, F., Espinoza, R.E., Quinteros, S., 2010. A critical review and systematic discussion of recent classification
741 proposals for liolaemid lizards. Zootaxa 2549, 1–30.
742 Losos J B., 2011. Convergence, adaptation, and constraint. Evolution 65, 1827–1840. DOI: 10.1111/j.1558-
743 5646.2011.01289.x
744 Mahalanobis, P.C., 1936. On the generalized distance in statistics. Proc. Natl Inst. Sci. India 2, 49–55.
745 Medel, R. G., Marquet, P. A., Jaksić, F. M., 1988. Microhabitat shifts of lizards under different contexts of sympatry: a
746 case study with South American *Liolaemus*. Oecologia 76(4), 567-569. doi:10.1007/BF00397871

747 Mayr, E., 1942. Systematics and the origin of species, from the viewpoint of a zoologist, Harvard University Press,
748 Cambridge.
749 Mayr, E., Ashlock, P., 1991. Principles of Systematic Zoology. 2nd edition. New York, McGraw-Hill.
750 McCulloch, R.D., Bentley, M. J, Purves, R.S., 2000. Climatic inferences from glacial and paleoecological evidence at the
751 last glacial termination, southern South America. J. Quaternary Sci. 15, 409–417. doi: 10.1002/1099-
752 1417(200005)15:4<409::AID-JQS539>3.0.CO;2-#
753 Mardia, K.V., Kent, J.T., Bibby, J.M., 1979. Multivariate analysis. London: Academic Press. DOI:
754 10.1002/zamm.19810610315
755 Mercer, J. H., 1976. Glacial history of southernmost South America. Quatern. Res. 6(2), 125-166.
756 http://dx.doi.org/10.1016/0033-5894(76)90047-8
757 Mercer, J.H., Sutter, J.F., 1982. Late Miocene-earliest Pliocene Glaciation in southern Argentina: implications for global
758 ice~heet history. Palaeogeogr. Palaeocl. 38 (1982): 185-206.
759 Miller, M. A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic
760 trees. In Gateway Computing Environments Workshop (GCE), 2010 (pp. 1-8). DOI: 10.1109/GCE.2010.5676129
761 Moraes, L. J., Pavan, D., Barros, M. C., Ribas, C. C., 2016. The combined influence of riverine barriers and flooding
762 gradients on biogeographical patterns for amphibians and squamates in south-eastern Amazonia. J. Biogeogr.
763 43(11), 2113-2124. DOI: 10.1111/jbi.12756
764 Morando, M., Avila, L. J., Baker, J., Sites Jr, J. W., 2004. Phylogeny and phylogeography of the *Liolaemus darwinii*
765 complex (Squamata: Liolaemidae): evidence for introgression and incomplete lineage sorting. Evolution, 58(4),
766 842-861. doi: http://dx.doi.org/10.1554/03-009
767 Morrison, D.F., 1990. Multivariate statistical methods, 3rd edn. New York: McGraw-Hill.
768 Müller, L.; Hellmich, W., 1933. Beiträge zur Kenntnis der Herpetofauna Chiles. VIII. Bemerkungen über *Liolaemus*
769 *tenuis* (Duméril et Bibron). Zool. Anz. 104 (11/12), 305-310.
770 Muñóz-Mendoza, C., D´Elia, G., Panzera, A., Mendez, M., Villalobos-Leiva, A., Sites, J.W.Jr., Victoriano, P. F. in press.
771 Geography and past climate changes have shaped the evolution of a widespread lizard from the Chilean hotspot.
772 Molecular Phylogenetics and Evolution.
773 Myers, N., Mittermeier R. A., Mittermeier C. G., da Fonseca G. A. B., Kent, J., 2000 Biodiversity hotspots for
774 conservation priorities. Nature 403, 853–858. doi:10.1038/35002501

New, S.T.D., Bull, C.M., 2011. Retinal ganglion cell topography and visual acuity of the sleepy lizard (*Tiliqua rugosa*). J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol. 197, 703–9. DOI: 10.1007/s00359-011-0635-8

Nuñez, H., Jaksic, F., 1992. Lista comentada de los reptiles terrestres de Chile continental. Boletín del Museo Nacional de Historia Natural 43, 63-91.

Nunes, V.L., Miraldo, A., Beaumont, M.A., Butlin, R.K., Paulo, O.S., 2011. Association of Mc1r variants with ecologically relevant phenotypes in the European ocellated lizard, *Lacerta lepida*. J. Evol. Biol. 24, 2289–98. DOI: 10.1111/j.1420-9101.2011.02359.x

Olave, M., Avila, L. J., Sites, J. W., Morando, M., 2015. Model-based approach to test hard polytomies in the Eulaemus clade of the most diverse South American lizard genus *Liolaemus* (Liolaemini, Squamata). Zool. J. Linn. Soc. 174(1), 169-184. DOI: 10.1111/zoj.12231

Olsson, M., Stuart-Fox, D., Ballen, C., 2013. Genetics and evolution of colour patterns in reptiles. Semin. Cell Dev. Biol. 24 (6), 529-541. http://dx.doi.org/10.1016/j.semcdb.2013.04.001

Omland, K.E., Tarr, C.L., Boarma, W.I., Marzluff, J.M., Fleischer, R.C., 2000. Cryptic genetic variation and paraphyly in ravens. Proc Royal Soc Lond B Biol Sci. 267, 2475–2482. DOI: 10.1098/rspb.2000.1308

Patton, J. L., Da Silva, M. N. F., Malcolm, J. R., 1994. Gene genealogy and differentiation among arboreal spiny rats (Rodentia: Echimyidae) of the Amazon basin: a test of the riverine barrier hypothesis. Evolution 1314-1323. DOI: 10.2307/2410388

Phillips, S. J., Dudík, M., 2008. Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. Ecography, 31(2), 161-175. DOI: 10.1111/j.0906-7590.2008.5203.x

Phillips, S.J., Anderson, R.P., Schapire, R.E., 2006. Maximum entropy modeling of species geographic distributions. Ecol. Model. 190, 231–259. http://dx.doi.org/10.1016/j.ecolmodel.2005.03.026

Portik, D. M., Wood, Jr. P. L., Grismer, J. L., Stanley, E. L., Jackman, T.R., 2011. Identification of 104 rapidly-evolving nuclear protein-coding markers for amplification across scaled reptiles using genomic resources. Conserv. Genet. Resour. 4(1), 1-10. doi: 10.1007/s12686-011-9460-1.

Quezada Romegialli, C.A., 2014. Ecología de ríos mediterráneos: Historia, estructuración y funcionamiento. PhD Dissertation. Universidad de Chile, Chile.

Rabassa, J., 2008. Late cenozoic glaciations in Patagonia and Tierra del Fuego. Dev. Quat. Sci. 11, 151-204. http://dx.doi.org/10.1016/S1571-0866(07)10008-7

Rabassa, J., Clapperton, C., 1990. Quaternary glaciations of the southern Andes. Quaternary Sci. Rev. 9, 153–174. doi: 10.1016/0277-3791(90)90016-4.

Rabassa, J, Coronato, A., Martínez, O., 2011. Late Cenozoic Glaciations in Patagonia and Tierra del Fuego: an updated review. Biol. J. Linn. Soc. 103, 316-335. DOI: 10.1111/j.1095-8312.2011.01681.x

Rambaut, A., Drummond, A., 2009. FigTree v1. 3.1. Available from: http://tree.bio.ed.ac.uk/software/figtree/

Rambaut, A., Drummond, A.J., Suchard M., 2013. Tracer v1.6. Available from: http://tree.bio.ed.ac.uk/software/tracer/

Rannala, B., Yang, Z., 2013. Improved reversible jump algorithms for Bayesian species delimitation. Genetics, 194(1), 245-253. DOI: 10.1534/genetics.112.149039.

Rannala, B., Yang, Z., 2016. Efficient Bayesian species tree inference under the multispecies coalescent. Systematic Biology, syw119. DOI: https://doi.org/10.1093/sysbio/syw119

Rissler, L. J., Apodaca, J. J., 2007. Adding more ecology into species delimitation: ecological niche models and phylogeography help define cryptic species in the black salamander (*Aneides flavipunctatus*). Syst. Biol. 56, 924–942. DOI: https://doi.org/10.1080/10635150701703063

Rohlf, F.J., 1996. Morphometric spaces, shape components and the effects of linear transformations. In: Marcus, L.F., Corti, M., Loy, A., Naylor, G.J.P., Slice, D. (Eds), Advances in Morphometrics, NATO ASI, Series A, Life Sciences. Plenum Publishing, New York, NY, pp, 117-129.

Rohlf, F.J., 2004a. *TpsDig*. Stony Brook, NY: Department of Ecology and Evolution, State University of New York.

Rohlf, F.J., 2004b. *TpsUtil*. Stony Brook, NY: Department of Ecology and Evolution, State University of New York.

Rohlf, F.J., 2015. The tps series of software. Hystrix 26, 1–4. DOI: http://dx.doi.org/10.4404/hystrix-26.1-11264

Rohlf, F.J., Marcus, L.F., 1993. A revolution in morphometrics. Trends Ecol. Evol. 8, 129–132. http://dx.doi.org/10.1016/0169-5347(93)90024-J

Romero-Gárate. F., Teillier, S., 2014. Flora vascular de los Altos del Cantillana, Región Metropolitana, Chile: pisos de vegetación subandino y andino. Chloris Chilensis 17 (1).

Roratto, P. A., Fernandes, F. A., Freitas, T. R., 2015. Phylogeography of the subterranean rodent *Ctenomys torquatus*: an evaluation of the riverine barrier hypothesis. J. Biogeogr. 42(4), 694-705. DOI: 10.1111/jbi.12460

RStudio Team, 2015. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/.

Ruiz de Gamboa, M., 2016. Lista actualizada de los reptiles de Chile. Boletín Chileno de Herpetología, 3, 7-12.

Ruzzante, D.E., Walde, S.J., Gosse, J.C., Cussac, V.E., Habit, E., Zemlak, T.S., Adams, E.D.M., 2008. Climate control on ancestral population dynamics: insight from Patagonian fish phylogeography. Mol. Ecol. 17, 2234–2244. DOI: 10.1111/j.1365-294X.2008.03738.x

Rosenblum, E.B., Hoekstra, H.E., Nachman, M.W., 2004. Adaptive reptile color variation and the evolution of the Mc1r gene. Evolution 58, 1794–808. doi: http://dx.doi.org/10.1554/03-741

Sallaberry-Pincheira, N., Garin, C. F., González-Acuña, D., Sallaberry, M. A., Vianna, J. A., 2011. Genetic divergence of Chilean long-tailed snake (*Philodryas chamissonis*) across latitudes: conservation threats for different lineages. Divers. Distrib. 17(1), 152-162. DOI: 10.1111/j.1472-4642.2010.00729.x

Schlick-Steiner, B. C., Steiner, F. M., Seifert, B., Stauffer, C., Christian, E., Crozier, R. H., 2010. Integrative taxonomy: a multisource approach to exploring biodiversity. Annu. Rev. Entomol. 55, 421-438. DOI: 10.1146/annurev-ento-112408-085432

Schulte, J.A., Macey, J.R., Espinoza, R.E., Larson, A., 2000. Phylogenetic relationships in the iguanid lizard genus *Liolaemus*: multiple origins of viviparous reproduction and evidence for recurring Andean vicariance and dispersal. Biol. J. Linn. Soc. 69, 75–102. http://dx.doi.org/10.1006/bijl.1999.0346

Schwartz, G., 1979. Estimating the dimension of a model. Ann. Stat. 6(2), 461–464, 1979.

Segall, M., Cornette, R., Fabre, A. C., Godoy-Diana, R., Herrel, A., 2016. Does aquatic foraging impact head shape evolution in snakes? Proc. R. Soc. B. 283, 20161645. DOI: 10.1098/rspb.2016.1645

Shapiro, S. S., Wilk, M. B., 1965. An analysis of variance test for normality (complete samples). Biometrika 52(3/4), 591-611.

Smith, M.A., Fisher, B.L., Hebert, P.D.N., 2005. DNA barcoding for effective biodiversity assessment of a hyperdiverse arthropod group: the ants of Madagascar. Phil. Trans. R Soc. of London 360, 1825–1834. DOI: 10.1098/rstb.2005.1714

Sponer, R., Roy, M.S., 2002. Phylogeographic analysis of the brooding brittle star *Amphipholis squamata* (Echinodermata) along the coast of New Zealand reveals high cryptic variation and cryptic dispersal potential. Evolution 56, 1954–1967. doi: http://dx.doi.org/10.1554/0014-3820(2002)056[1954:PAOTBB]2.0.CO;2

Stephens, M., Donnelly, P., 2003. Ancestral inference in population genetics models with selection (with discussion). Aust. N. Z. J. Stat. 45(4), 395-430. DOI: 10.1111/1467-842X.00295

Stephens, M., Smith, N.J., Donnelly, P., 2001. A new statistical method for haplotype reconstruction from population data. Am. J. Hum. Genet. 68, 978-989. doi: 863 10.1086/319501

Stuart, B.L., Inger, R.F., Voris, H.K., 2006. High level of cryptic species diversity revealed by sympatric lineages of Southeast Asian forest frogs. Biol. Lett. 2(3): 470-474. DOI: 10.1098/rsbl.2006.0505

Sukumaran, J., Knowles, L. L., 2017. Multispecies coalescent delimits structure, not species. Proc. Natl. Acad. Sci. 201607921. doi: 10.1073/pnas.1607921114

Swets, J. A., 1988. Measuring the accuracy of diagnostic systems. Science 240(4857), 1285-1293.

Tamura, K., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2.011. MEGA 5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance and Maximum Parsimony Methods. Mol. Biol. Evol. 28, 2731-2739. DOI: https://doi.org/10.1093/molbev/msr121

Tofilski, A., 2008. Using geometric morphometrics and standard morphometry to discriminate three honeybee subspecies. Apidologie 39, 558–563. DOI: 10.1051/apido:2008037

Thompson, C.W., Moore, M.C. 1992. Throat colour reliably signals status in male tree lizards, *Urosaurus ornatus*. Anim. Behav. 42, 745–53. http://dx.doi.org/10.1016/S0003-3472(05)80120-4

Torres-Pérez, F., Lamborot, M., Boric-Bargetto, D., Hernández, C. E., Ortiz, J. C., Palma, R. E., 2007. Phylogeography of a mountain lizard species: an ancient fragmentation process mediated by riverine barriers in the *Liolaemus monticola* complex (Sauria: Liolaemidae). J. Zoolog. Syst. Evol. Res. 45(1), 72-81. DOI: 10.1111/j.1439-0469.2006.00392.x

Uetz, P., Freed, P., Hošek, J., (eds.). 2016. The Reptile Database, http://www.reptile- database.org, accessed [August 29 2016]

Vaidya G., Lohman D.J., Meier, R., 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. Cladistics, 27 (2), 171–180 DOI: 10.1111/j.1096-0031.2010.00329.x

Vera-Escalona, I., D'Elía, G., Gouin, N., Fontanella, F.M., Muñoz-Mendoza, C., Sites, J.W.Jr., Victoriano, P.F., 2012. Lizards on ice: evidence for multiple refugia in *Liolaemus pictus* (Liolaemidae) during the Last Glacial Maximum in the southern Andean beech forests. PLoS ONE 7(11), e48358. 798. http://dx.doi.org/10.1371/journal.pone.0048358

884 Victoriano, P. F., Ortiz, J. C., Benavides, E., Adams, B.J., Sites, J.W.Jr., 2008. Comparative phylogeography of
885 codistributed species of Chilean *Liolaemus* (Squamata: Tropiduridae) from the central-southern Andean range.
886 Mol. Ecol. 17(10), 2397-2416. DOI: 10.1111/j.1365-294X.2008.03741.x
887 Vidal, M.A., Ortiz, J.C., Astorga, M., Victoriano, P., Lamborot, M., 2004. Revision of *Liolaemus tenuis* subspecies
888 (Duméril & Bibron, 1837) by analysis of population genetic structure. Amphibia-Reptilia 25(4), 438-445.
889 Vidal, M.A., Ortiz, J.C., Ramírez, C.C., Lamborot, M., 2005. Intraspecific variation in morphology and sexual
890 dimorphism in *Liolaemus tenuis* (Tropiduridae). Amphibia-Reptilia 26, 343–351.
891 Vidal, M., Ortiz, J.C., Labra, A., 2007. Sexual and geographic variation of color patterns in *Liolaemus tenuis* (Squamata,
892 Liolaeminae). Gayana 71(1), 27-33.
893 Vidal, M.A., Ortiz, J.C., Marín, J.C., Poulin, E., Moreno, P.I., 2012. Comparative phylogeography of two co-distributed
894 species of lizards of the genus *Liolaemus* (Squamata: Tropiduridae) from Southern Chile Amphibia-Reptilia 33,
895 55-67.
896 Vincent, S.E., Herrel, A., Irschik, D.J., 2004. Ontogeny of intersexual head shape and prey selection in the pitviper
897 *Agkistrodon piscivorus*. Biol. J. Linn. Soc. 81, 151–159. DOI: 10.1111/j.1095-8312.2004.00282.x
898 Viscosi, V., Cardini, A., 2011. Leaf morphology, taxonomy and geometric morphometrics: a simplified protocol for
899 beginners. PLoS ONE 6, e25630. http://dx.doi.org/10.1371/journal.pone.0025630
900 Wallace, A.R., 1852. On the monkeys of the Amazon. Proc. Zool. Soc. Lond. 20, 107–110
901 Warren, D. L., Glor, R. E., Turelli, M., 2008. Environmental niche equivalency versus conservatism: quantitative
902 approaches to niche evolution. Evolution, 62, 2868–2883. DOI: 10.1111/j.1558-5646.2008.00482.x
903 Watkins, G. G., 1997. Intersexual signally and the functions of females coloration in the tropidurid lizard *Microlophus*
904 *accipitalis*. Anim. Behav. 53, 843-852.
905 Weir, J.T., 2006. Divergent timing and patterns of species accumulation in lowland and highland neotropical birds.
906 Evolution 60, 842–855. doi: http://dx.doi.org/10.1554/05-272.1
907 Weir, J.T., Schluter, D., 2004. Ice sheets promote speciation in boreal birds. Proc. Roy. Soc. London. Ser. B: Biol. Sci.
908 271, 1881–1887. DOI: 10.1098/rspb.2004.2803
909 Xia, X., Lemey, P., 2009. Assessing substitution saturation with DAMBE. In: Lemey, P., Salemi, M., Vandamme, A.M.
910 (Eds), The Phylogenetic Handbook: A Practical Approach to DNA and Protein Phylogeny, 2nd edition.
911 Cambridge University Press, Pp. 615-630.

912 Xia, X., Xie, Z., Salemi, M., Chen, L., Wang, Y., 2003. An index of substitution saturation and its application. Mol.
913 Phylogenet. Evol. 26(1), 1-7. http://dx.doi.org/10.1016/S1055-7903(02)00326-3
914 Xia, X., 2013. DAMBE5: A comprehensive software package for data analysis in molecular biology and evolution. Mol.
915 Biol. Evol. 30, 1720-1728. 819. DOI: https://doi.org/10.1093/molbev/mst064
916 Yang, Z., 2015. The BPP program for species tree estimation and species delimitation. Curr. Zool. 61(5), 854-865. DOI:
917 https://doi.org/10.1093/czoolo/61.5.854
918 Yang, Z., Rannala, B., 2010. Bayesian species delimitation using multilocus sequence data. Proc. Natl. Acad. Sci.
919 107(20), 9264-9269. doi: 10.1073/pnas.0913022107
920 Yang, Z., Rannala, B., 2014. Unguided species delimitation using DNA sequence data from multiple loci. Mol. Biol.
921 Evol. 31, 3125–3135. DOI: https://doi.org/10.1093/molbev/msu279
922 Yoder, A.D., Rasoloarison, R.M., Goodman, S.M., Irwin, J.A., Atsalis, S., Ravosa, M.J., Ganzjorn, J.U., 2000.
923 Remarkable species diversity in Malagasy mouse lemurs (Primates, *Microcebus*). Proc. Natl. Acad. Sci. USA 97,
924 1325–1330. doi: 10.1073/pnas.200121897
925 Yule, G.U., 1925. A mathematical theory of evolution, based on the conclusions of Dr. J.C. Willis, F.R.S. Philos Trans R
926 Soc Lond Biol 213, 21–87.
927 Zelditch, M.L., Swiderski, D.L., Sheets, H.D., Fink, W.L., 2004. Geometric morphometrics for biologists a primer.
928 Amsterdam; Boston: Elsevier Academic Press.
929 Zamudio, K. R., Bell, R. C., Mason, N. A., 2016. Phenotypes in phylogeography: Species' traits, environmental variation,
930 and vertebrate diversification. Proc. Natl. Acad. Sci. 113(29), 8041-8048. doi/10.1073/pnas.1602237113
931 Zhang, C., Zhang, D. X., Zhu, T., Yang, Z., 2011. Evaluation of a Bayesian coalescent method of species delimitation.
932 Syst. Biol. 60, 6, 747-761. DOI: https://doi.org/10.1093/sysbio/syr071
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940 **TABLES AND FIGURES**

941 Table 1. List of the seven molecular markers and primers used for PCR and Sanger sequencing.

Locus	Marker type	Primers	Reference
CYTB	mitDNA	5'-TGACTTGAARAACCAYCGTTG-3 (Glu DGL)	Morando et al., 2004
		5'-GGCAAATAGGAARTATCATTC-3' (cyt-b-3)	
		5'- TGGCCRATGATRATAAATGGGT-3' (WWR)	
		5'-TGAGGACARATATCHTTYTGRGG-3' (F1)	
LDB5B	ANL	F 5'-GCAGAGCCAAAGCCATGT -3'	Camargo et al., 2012
		R 5'- GGTAGCTTGGTGGTAGGTCA-3'	
EXPH5	Coding	F 5'-AATAAACTKGCAGCTATGTACAAAACAAGTC-3'	Portik et al., 2011
		R 5'-AAYCGCCCTTCTGTGAGTGACCTCT-3	
KIF24	Coding	F 5'-SAAACGTRTCTCCMAAACGCATCC -3'	Portik et al., 2011
		R 5'-WGGCTGCTGRAAYTGCTGGTG -3'	
A9C	ANL	F 5'-GTGCCCAGTTCTGGTCTTGT-3'	Camargo et al., 2012
		R 5'-AGCCTGAGCCAAACATAGGA-3'	
A12F	ANL	F 5'-CAGAGGCCCTGAAGGTTACA-3'	Camargo et al., 2013
		R 5'-AGATGCACACCAACTGTGGA-3'	
B1D	ANL	F 5'-GATATCGAGGGATTTCAGTTTCC-3'	Camargo et al., 2012
		R 5'-CCAGTGTTTATGAGCAACTGAGTA-3'	

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Table 2. Markers used for species tree estimation in *BEAST and species validation in BPP are shown. The mitochondrial marker was not used for BPP. N=number of sequences. Length is expressed in base-pairs. Substitution model as retrieved by jmodeltest.

Marker	Species Tree & BPP			Divergence Time Estimation		
	N	Length	Substitution model	N	Length	Substitution model
A12F	64	595	GTR+G	7	724	HKY+G
A9C	38	798	JC+G	7	852	K80
B1D	75	1011	HKY+G	7	966	HKY
CYTB	121	726	GTR+G	7	726	HKY+G
EXPH5	69	932	GTR+G	7	959	HKY
KIF24	64	582	HKY+G	7	582	HKY+G
LDB5B	70	749	HKY+G	7	796	HKY

963 Table 3. Description of dorsal landmarks and semilandmarks.

Landmark number	Description
1	Limit between head and body scales
2	Distal point of suture between parietal scales
3	Pineal eye
4	Anterior most point of the rostral scale
5	Anterior most point of circumorbital scales
6	Jaw width
7	Narrow neck
8	Point of maximum curvature of circumorbital scales
9	Posterior point of maximum curvature of circumorbital scales
10	Medial point between landmarks 4 and 5
11	Intersection between landmark 5 and middle head line
12	Medial point between landmarks 5 and 6
13	Medial point between landmarks 6 and 7
14	Medial point between landmark 8 and 9

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Table 4. Properties of the sequence matrices of each marker used in this study for species tree estimation.

	Sequences	Sites	Nucleotide diversity	Sites with gaps or missing data	Polymorphic sites	Parsimony informative sites	Indel events
A12F	64	595	0.02422	60	135	48	14
A9C	38	798	0.01323	22	92	45	7
B1D	75	1011	0.01205	46	154	71	16
CYTB	121	726	0.14209	721	2	2	0
EXPH5	69	932	0.00840	104	69	28	7
KIF24	64	582	0.01223	27	59	22	1
LDB5B	70	749	0.02878	323	196	33	48

995 Table 5. Posterior probabilities for the species tree recovered by BPP v.3.3 given the tree different starting species tree.

996 For every starting species tree, two runs per algorithm were made, but we show the results of one random run.

Inferred species tree	Algorithm 0	Algorithm 1
(*L. lemniscatus*, (*L. t. tenuis*, (*L. sp_L. t. punctatissimus*));	0.96982	0.99912
	0.94920	0.98488
	0.95612	0.98428

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Table 6. BPP posterior probabilities for different number of species (P[1]=Probability of one species; P[2]; Probability of two species; P[3]: Probability of three species and P[4]: Probability of four species). Strong support (> 0.95) is indicated in bold. Values between parentheses correspond to second runs. In these analyses, the three species model includes the outgroup, *L. t. tenuis*, and *L. t. punctatissimus* + *L. sp.*

Algorithm	P[1]	P[2]	P[3]	P[4]
0	0.0 (0.0)	0.0 (0.0)	**0.9698 (0.9595)**	0.0302 (0.0405)
1	0.0 (0.0)	0.0 (0.0)	**0.9991 (0.9555)**	0.0009 (0.0445)

Table 7. Delimited species and their posterior probabilities, as estimated by BPP v. 3.3. Numbers in bold indicate strong support.

	Starting species tree 1		Starting species tree 2		Starting species tree 3	
Delimited species	Alg 0	Alg 1	Alg 0	Alg 1	Alg 0	Alg 1
L .lemniscatus	**1,000**	**1,000**	**1,000**	**1,000**	**1,000**	**1,000**
L. t. tenuis	**1,000**	**1,000**	**1,000**	**1,000**	**0,999**	**1,000**
L .t. punctatissimus_L. sp.	**0,970**	**0,999**	**0,949**	**0,985**	**0,956**	**0,984**
L. t. punctatissimus	0,030	0,001	0,051	0,015	0,044	0,016
L. sp.	0,030	0,001	0,051	0,015	0,043	0,016
L. t. tenuis_L. t. punctatissimus					0,001	

FIGURES

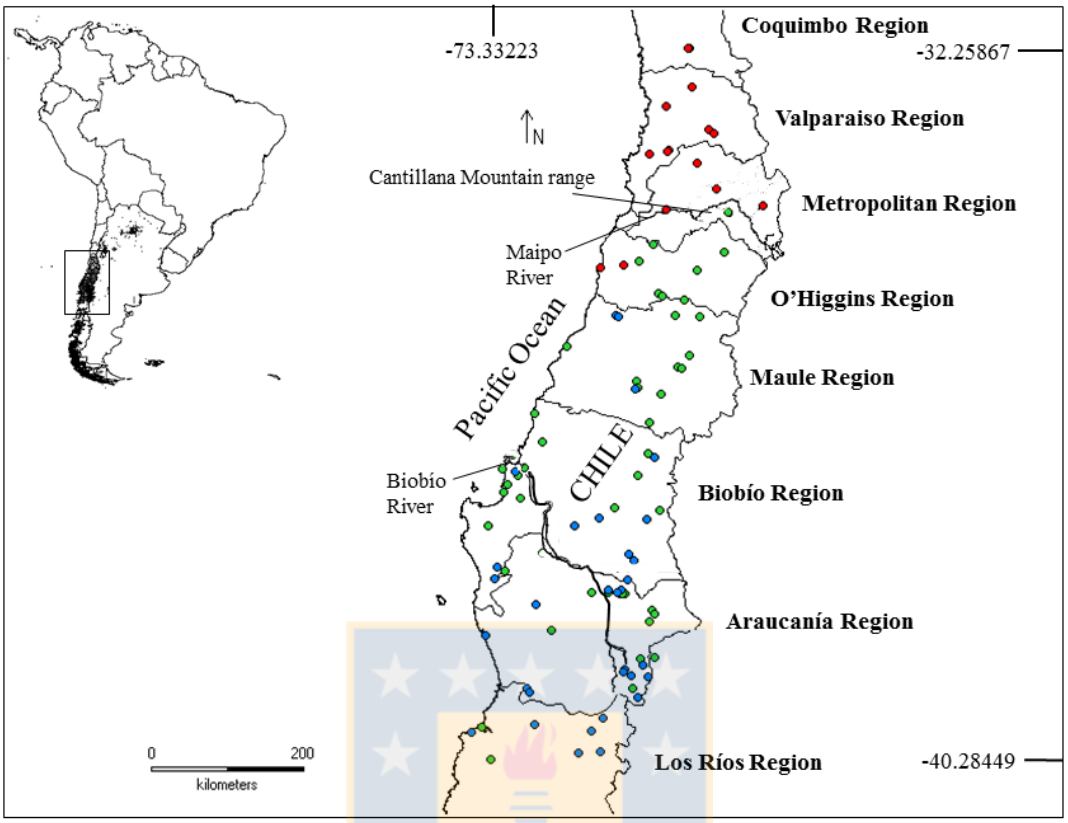

Figure 1. Geographic origin of the samples used for *Liolaemus tenuis sensu lato*. Red, green and blue circles indicate samples assigned to candidate species *L. t. tenuis*, *L. t. punctatissimus* and *L. sp.*, respectively. Together, all these samples provide a rough idea about *Liolaemus tenuis sensu lato* distribution. Maipo and Biobío rivers, as well as the approximate location of the Cantilla Mountain-range are indicated.

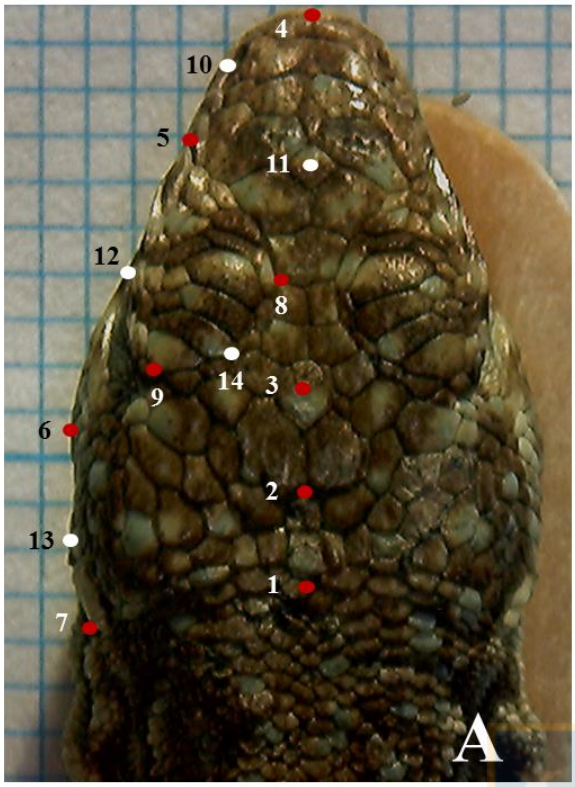

Figure 2. Landmarks (red circles) and semi-landmarks (white circles) used in the geometric morphometric analysis of *L. tenuis sensu lato.*

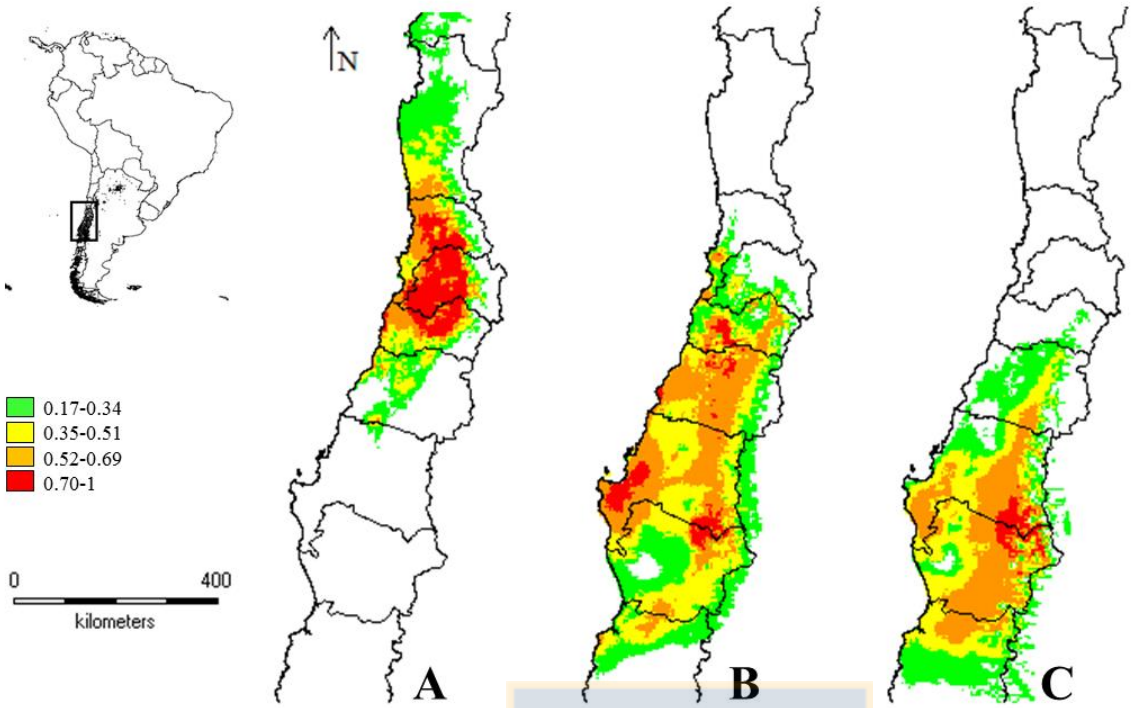

Figure 3. Ecological Niche Modelling for *L. t. tenuis* (A), *L. t. punctatissimus* (B) and *L. sp.* (C). Distances and probability value scales are shown.

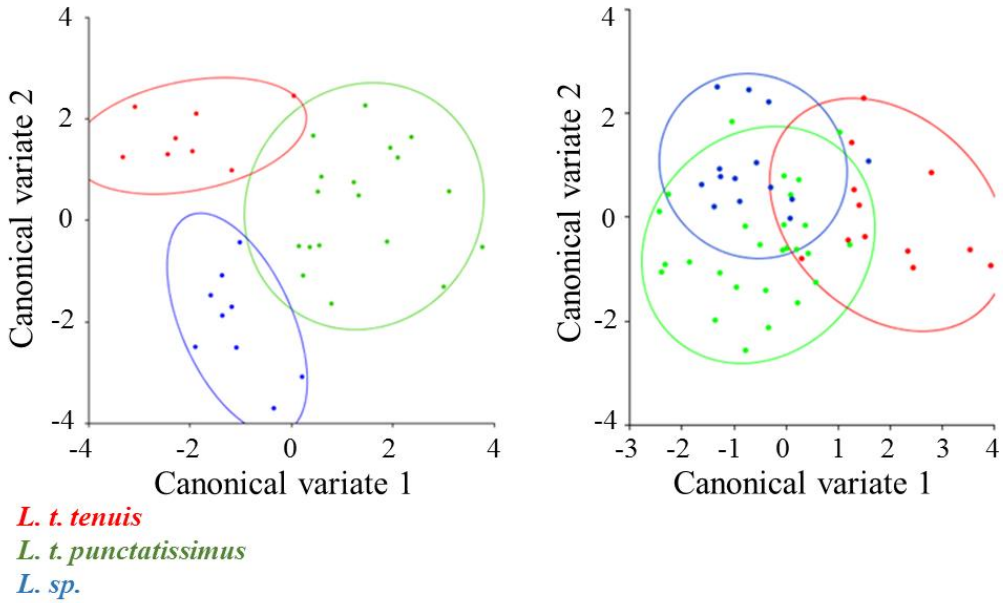

Figure 4. Scatter plot of first versus second canonical variate axes (CV1 and CV2) based on head morphometry for individuals belonging to the three putative species. Females are shown on the left size and males are shown on the right side.

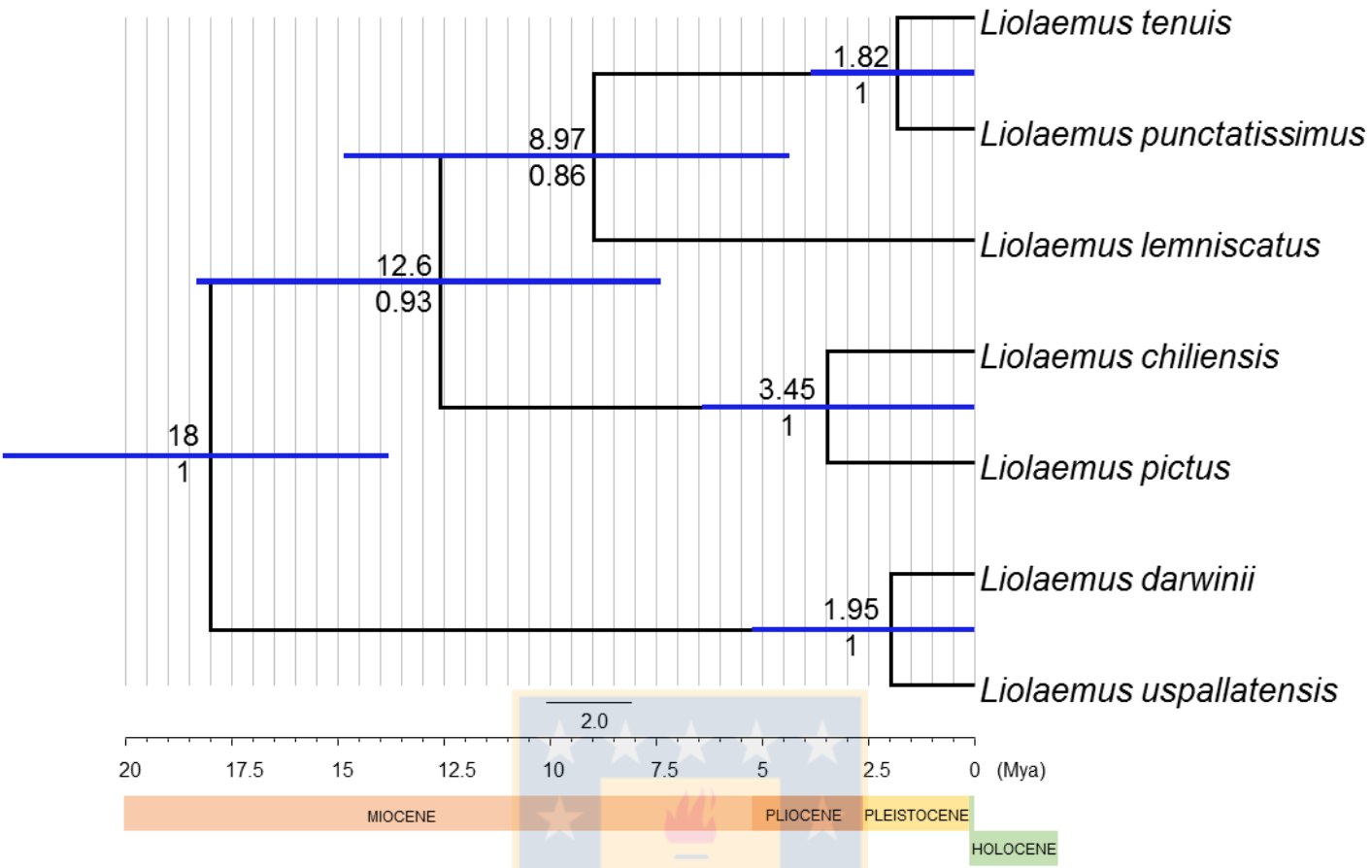

Figure. 5. Time-calibrated Bayesian species tree for *Liolaemus tenuis sensu lato* estimated using *BEAST (mtDNA + six nuclear genes). Numbers above branches indicate divergence times in million years, and numbers below branches indicate Bayesian posterior probability values. The 95% HPD bar is also shown.

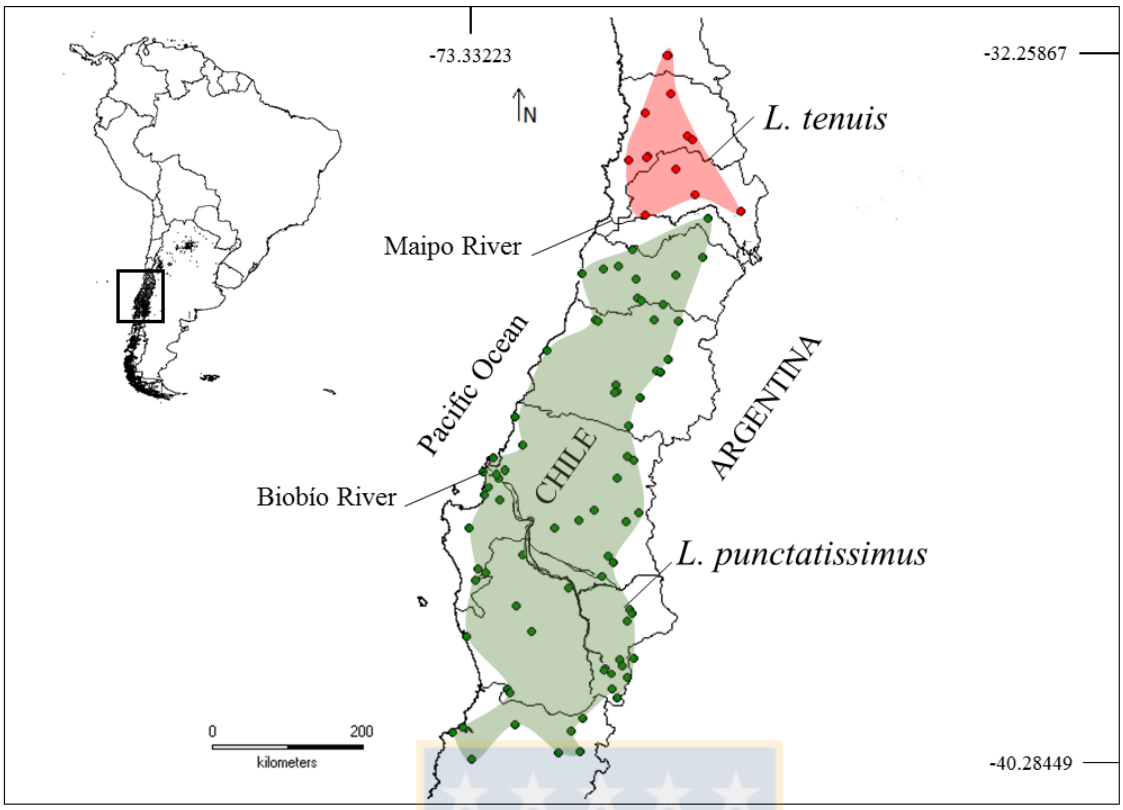

Figure 6. Map showing the distribution in Chile of the two species proposed in this study. Circles indicate locality records.

SUPPLEMENTARY INFORMATION

Table S1. Geographic coordinates (in degrees decimals) and locality details for the specimens used for species tree inference and ecological niche modelling. Haplotype codes for each marker and locality, as in Figures S2-S8, are shown. Species to which each specimen was assigned (based on the cytb phylogeny in Muñóz-Mendoza et al., in press) to is also indicated.

	Locality	Country	Latitude	Longitude	A12F	A9C	B1D	CYTB	EXPH5	KIF24	LDB5B	Putative species
1	Salamanca a	Chile	-31,7667	-70,9500				CYTB27				*L. t. tenuis*
2	Salamanca b	Chile	-31,7736	-70,9619	A12F15	A9C17	B1D16	CYTB26, CYTB33, CYTB36, CYTB38, CYTB40	EXP14, EXP20	KIF10	LDB8	*L. t. tenuis*
3	Petorca - Cabildo	Chile	-32,2333	-70,9167	A12F11		B1D24	CYTB37	EXP13	KIF40	LDB10	*L. t. tenuis*
4	La Ligua	Chile	-32,4667	-71,2167	A12F20			CYTB28, CYTB29, CYTB30		KIF35		*L. t. tenuis*
5	Hacienda Quilpué	Chile	-32,7378	-70,7183	A12F12, A12F18	A9C7	B1D25, B1D26, B1D27	CYTB39	EXP19, EXP22, EXP53, EXP55	KIF11, KIF34, KIF41, KIF42	LDB6, LDB25	*L. t. tenuis*
6	North-West of the Andes	Chile	-32,7833	-70,6500								*L. t. tenuis*
7	Olmue	Chile	-32,9833	-71,1833	A12F19		B1D1	CYTB18, CYTB20	EXP12	KIF33	LDB7	*L. t. tenuis*
8	Olmué – Valparaíso	Chile	-33,0000	-71,2000				CYTB19				*L. t. tenuis*
9	Quilpue	Chile	-33,0333	-71,4167				CYTB22, CYTB31, CYTB32				*L. t. tenuis*
10	Tiltil	Chile	-33,1353	-70,8553				CYTB81			LDB5	*L. t. tenuis*
11	Santiago – Ñuñoa	Chile	-33,4544	-70,6120				CYTB21				*L. t. tenuis*
12	Cajón del Maipo	Chile	-33,6500	-70,0667				CYTB83			LDB54	*L. t. tenuis*
13	Melipilla	Chile	-33,7000	-71,2167				CYTB23, CYTB24, CYTB25, CYTB41		KIF1, KIF43	LDB14	*L. t. tenuis*
14	National Reservation Rio Clarillo	Chile	-33,7253	-70,4874		A9C1		CYTB82	EXP18		LDB17	*L. t. punctatissimus*
15	Rapel	Chile	-34,1189	-71,3781							LDB23	*L. t. punctatissimus*

16	Coya	Chile	-34,2000	-70,5333				CYTB65, CYTB66				*L. t. punctatissimus*
17	Fundo La Esperanza	Chile	-34,3162	-71,5397	A12F10		B1D37		EXP15	KIF2	LDB19	*L. t. punctatissimus*
18	Chépica a	Chile	-34,3587	-71,7289	A12F8		B1D18	CYTB80	EXP24	KIF44		*L. t. tenuis*
19	Pichilemu	Chile	-34,3847	-72,0028	A12F16	A9C2	B1D5	CYTB34, CYTB35	EXP21		LDB21, LDB22, LDB52	*L. t. tenuis*
20	Rengo	Chile	-34,4163	-70,8549		A9C3, A9C4	B1D4, B1D66, B1D74	CYTB62	EXP28, EXP29	KIF8, KIF29	LDB18, LDB69	*L. t. punctatissimus*
21	Santa Cruz	Chile	-34,6953	-71,3158							LDB2	*L. t. punctatissimus*
22	Chépica b	Chile	-34,7376	-71,2647				CYTB64			LDB4	*L. t. punctatissimus*
23	San Fernando – Pichilemu	Chile	-34,7846	-70,9975			B1D2	CYTB63		KIF39		*L. t. punctatissimus*
24	Romeral	Chile	-34,9667	-71,1167		A9C23	B1D19	CYTB74	EXP50	KIF6	LDB20	*L. t. punctatissimus*
25	Hualañé a	Chile	-34,9667	-71,8167						KIF5	LDB24	*L. sp.*
26	Hualañé – Illoca	Chile	-34,9686	-71,8288			B1D17, B1D58		EXP27, EXP31	KIF36, KIF45	LDB1, LDB13	*L. sp.*
27	Hualañé b	Chile	-34,9742	-71,7983			B1D14, B1D21, B1D22,B1D38	CYTB58, CYTB60	EXP16, EXP26, EXP30, EXP49	KIF3	LDB3, LDB9, LDB51	*L. sp.*
28	Los Queñes	Chile	-34,9833	-70,8167				CYTB75, CYTB76, CYTB77, CYTB78				*L. t. punctatissimus*
29	Constitución	Chile	-35,3390	-72,4139				CYTB60, CYTB61		KIF38		*L. t. punctatissimus*
30	National Park Radal Siete Tazas	Chile	-35,4471	-70,9428			B1D31, B1D73	CYTB67	EXP23, EXP25, EXP58	KIF37, KIF46	LDB53, LDB15	*L. t. punctatissimus*
31	Alto Vilches	Chile	-35,5833	-71,0833				CYTB17, CYTB68, CYTB69, CYTB70, CYTB72				*L. t. punctatissimus*
32	National Reservation Vilches	Chile	-35,5995	-71,0371	A12F7, A12F9, A12F13, A12F14	A9C5, A9C15, A9C16	B1D3, B1D13, B1D15, B1D32	CYTB71, CYTB73	EXP39, EXP54	KIF4	LDB16	*L. t. punctatissimus*
33	Yerbas Buenas	Chile	-35,7465	-71,5817				CYTB13, CYTB14				*L. t. punctatissimus*
34	Linares a	Chile	-35,8333	-71,5667				CYTB11, CYTB12, CYTB15				*L. t. punctatissimus*
35	Linares b	Chile	-35,8490	-71,5959				CYTB16				*L. sp.*
36	Embalse Ancoa	Chile	-35,9000	-71,2833	A12F17			CYTB9, CYTB10	EXP52	KIF7	LDB11	*L. t. punctatissimus*
37	Cobquecura	Chile	-36,1306	-72,7903				CYTB84				*L. t. punctatissimus*

38	Embalse Bullileo	Chile	-36,2485	-71,4274		A9C12	B1D11, B1D20, B1D23	CYTB1, CYTB2, CYTB3	EXP51, EXP60	KIF9, KIF19, KIF27	LTEB1, LDB12, LDB48	*L. t. punctatissimus*
39	Coelemu	Chile	-36,4833	-72,7000	A12F1, A12F2	A9C25	B1D65, B1D67	CYTB96, CYTB98, CYTB99	EXP42, EXP59	KIF12, KIF16	LDB41, LDB42	*L. t. punctatissimus*
40	Puquios	Chile	-36,6183	-71,4375	A12F59		B1D9	CYTB4, CYTB55	EXP41			*L. t. punctatissimus* (A12F59, B1D9, CYTB4, EXP41); *L. sp.* (CYTB55)
41	Isla Quiriquina	Chile	-36,6320	-73,0610				CYTB87, CYTB88, CYTB89, CYTB90, CYTB95				*L. t. punctatissimus*
42	Caracol	Chile	-36,6567	-71,3656	A12F46, A12F62	A9C14	B1D8, B1D12	CYTB56, CYTB57		KIF28, KIF31	LDB45	*L. sp.*
43	Nonguén	Chile	-36,7833	-72,9167	A12F3	A9C13	B1D70	CYTB54, CYTB85, CYTB86	EXP40	KIF32		*L. t. punctatissimus* (A12F3, A9C13, B1D70, CYTB85, CYTB86, EXP40, KIF32); *L. sp.* (CYTB54)
44	Rocoto	Chile	-36,7991	-73,1781			B1D7, B1D35, B1D41	CYTB97	EXP17	KIF18, KIF25, KIF26	LDB43	*L. t. punctatissimus*
45	Concepción	Chile	-36,8342	-73,0311								*L. sp.*
46	National Reservation Nonguén	Chile	-36,8713	-72,9927		A9C8	B1D6, B1D68, B1D72	CYTB93, CYTB94			LDB36, LDB38	*L. t. punctatissimus*
47	Las Trancas	Chile	-36,8833	-71,5667	A12F5, A12F58	A9C9, A9C22	B1D33, B1D34	CYTB5, CYTB6, CYTB7, CYTB8	EXP37, EXP38	KIF14	LDB50, LDB56	*L. t. punctatissimus*
48	Coronel	Chile	-36,9928	-73,1158	A12F28	A9C31	B1D28	CYTB103	EXP66	KIF15	LDB37	*L. t. punctatissimus*
49	Playa Negra	Chile	-37,0833	-73,1667				CYTB120				*L. t. punctatissimus*
50	Close Santa Juana	Chile	-37,1426	-72,9803								*L. t. punctatissimus*
51	Huepil – Trupan	Chile	-37,2669	-71,8333	A12F63	A9C11		CYTB100	EXP48	KIF48	LDB40	*L. t. punctatissimus*
52	Tucapel	Chile	-37,3011	-71,3011	A12F4, A12F6		B1D69, B1D71	CYTB91, CYTB92	EXP36, EXP57	KIF30		*L. t. punctatissimus*
53	Los Angeles a	Chile	-37,3833	-72,0167	A12F47	A9C10	B1D64	CYTB45, CYTB46	EXP35	KIF13		*L. sp.*
54	Antuco	Chile	-37,3970	-71,4550			B1D36, B1D39	CYTB47		KIF17, KIF47	LDB44, LDB39	*L. sp.*
55	Los Angeles b	Chile	-37,4719	-72,3236								*L. sp.*
56	Curanilahue	Chile	-37,4795	-73,3574	A12F39	A9C6, A9C29		CYTB101, CYTB102	EXP32, EXP65	KIF57, KIF60	LDB30, LDB34	*L. t. punctatissimus*
57	Angol	Chile	-37,8028	-72,7017	A12F33, A12F40	A9C26	B1D29, B1D30	CYTB119	EXP47, EXP64	KIF23, KIF52	LDB55	*L. t. punctatissimus* (A12F33; B1D29, CYTB119, EXP64, KIF52, LDB55); *L. sp.* (A12F40,

												A9C26, B1D30, EXP47, KIF23)
58	Ralco	Chile	-37,8181	-71,6758								*L. sp.*
59	Ralco reservoir	Chile	-37,8961	-71,6144								*L. sp.*
60	Close Contulmo	Chile	-37,9783	-73,2486								*L. sp.*
61	Contulmo National Monument	Chile	-38,0200	-73,1500	A12F31, A12F51		B1D49, B1D50	CYTB117	EXP62	KIF49		*L. t. punctatissimus*
62	Termas de Pemehue	Chile	-38,0500	-71,7167							LDB29	*L. t. punctatissimus*
63	Lake Lanalhue	Chile	-38,1147	-73,2700			B1D43	CYTB52		KIF54		*L. sp.*
64	Close National Reservation Malleco	Chile	-38,1667	-71,8000							LDB33	*L. t. punctatissimus*
65	Cherquenco - Llaima Volcano	Chile	-38,1667	-71,9500								*L. sp.*
66	West National Park Tolhuaca	Chile	-38,2000	-72,1500	A12F30, A12F41		B1D10	CYTB104	EXP45		LDB32	*L. t. punctatissimus*
67	National Reservation Malleco	Chile	-38,2000	-71,8333								*L. sp.*
68	National Reservation Malleco - Las Mentas	Chile	-38,2000	-71,9500							LDB28, LDB47	*L. t. punctatissimus*
69	National Park Tolhuaca	Chile	-38,2180	-71,8095	A12F42			CYTB109	EXP3		LDB26	*L. t. punctatissimus*
70	National Park Tolhuaca (Laguna Verde)	Chile	-38,2206	-71,7442	A12F32			CYTB106	EXP56			*L. t. punctatissimus*
71	Close Tolhuaca	Chile	-38,2211	-71,7822				CYTB108				*L. t. punctatissimus*
72	Tolhuaca – Chucao	Chile	-38,2218	-71,7783		A9C19, A9C31	B1D46, B1D62	CYTB105	EXP67		LDB27	*L. t. punctatissimus* (A9C19, B1D62, CYTB105, LDB27); *L. sp.* (A9C31, B1D46, EXP67)
73	Galvarino	Chile	-38,4201	-72,7780	A12F48, A12F49, A12F56, A12F57, A12F60	A9C18, A9C27, A9C28, A9C37	B1D52, B1D53, B1D57	CYTB49	EXP2, EXP9, EXP10	KIF53	LDB60, LDB61, LDB62	*L. sp.*
74	Las Raíces	Chile	-38,4235	-71,4260		A9C36		CYTB110				*L. t. punctatissimus*
75	Lonquimay – Alaska	Chile	-38,4727	-71,3978		A9C34	B1D45	CYTB107	EXP44	KIF50	LDB49	*L. t. punctatissimus*
76	Lonquimay	Chile	-38,5592	-71,4514	A12F43		B1D60	CYTB111	EXP7, EXP61	KIF22, KIF24	LDB31, LDB46	*L. t. punctatissimus* (CYTB111, EXP61, LDB46); *L. sp.* (A12F43,

												B1D60, EXP7, KIF22, KIF24, LDB31)
77	Cerro Ñielol	Chile	-38,7271	-72,5923	A12F44, A12F55	A9C20, A9C30	B1D48, B1D59	CYTB112, CYTB115	EXP4, EXP8, EXP68	KIF20, KIF51	LDB68	*L. t. punctatissimus*
78	Puerto Saavedra	Chile	-38,7889	-73,3850	A12F22,A12F61		B1D47	CYTB51	EXP11	KIF61, KIF63		*L. sp.*
79	Ruta Provincial 11, W Remecó Stream	Argentina	-39,0505	-71,3585								*L. t. punctatissimus*
80	Hualalafquen	Chile	-39,0689	-71,5366	A12F23, A12F38, A12F54		B1D61		EXP5		LDB64	*L. t. punctatissimus*
81	Reigolil	Chile	-39,1455	-71,4969	A12F37, A12F52			CYTB59	EXP33	KIF55		*L. sp.*
82	National Park Huerquehue Close National Park	Chile	-39,1685	-71,7272	A12F34, A12F36		B1D40, B1D44		EXP6, EXP43	KIF59, KIF62	LDB35, LDB58	*L. sp.*
83	Huerquehue	Chile	-39,1856	-71,7514								*L. sp.*
84	Lake Caburgua	Chile	-39,2333	-71,6511								*L. sp.*
85	Camino – Reigolil	Chile	-39,2910	-71,4337	A12F35, A12F45, A12F53		B1D51		EXP34		LDB63	*L. sp.*
86	Close La Capilla	Chile	-39,4183	-72,8858							LDB66	*L. sp.*
87	Pucon – Curarrehue	Chile	-39,4300	-71,6300				CYTB118	EXP46		LDB65	*L. t. punctatissimus*
88	Calquinco - La Capilla	Chile	-39,4692	-72,8525				CYTB48				*L. sp.*
89	Puesco	Chile	-39,5333	-71,5667				CYTB44				*L. sp.*
90	Niblinto – Victoria	Chile	-39,5333	-71,5667								*L. sp.*
91	Neltume	Chile	-39,7834	-71,9844				CYTB50				*L. sp.*
92	Los Lagos	Chile	-39,8614	-72,7935	A12F24, A12F27	A9C35				KIF56		*L. sp.*
93	Choshuenco a	Chile	-39,8617	-72,7932	A12F29		B1D42, B1D55					*L. sp.*
94	Corral	Chile	-39,8881	-73,4264		A9C24, A9C32	B1D63	CYTB14, CYTB16	EXP1	KIF58	LDB57	*L. t. punctatissimus*
95	Choshuenco b	Chile	-39,9386	-72,1242	A12F26		B1D56					*L. sp.*
96	National Park Valdivia	Chile	-39,9504	-73,5601	A12F21, A12F50		B1D54		EXP63	KIF21	LDB59	*L. sp.*
97	Los Llolles	Chile	-40,1861	-72,0050								*L. sp.*
98	Lago Ranco	Chile	-40,2022	-72,2683				CYTB42, CYTB43				*L. sp.*
99	La Unión - Hueicolla	Chile	-40,2683	-73,3286	A12F25	A9C21		CYTB113				*L. t. punctatissimus*

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Table S2. Specimens used for the divergence time estimation. LDG=Laboratorio de Diversidad Genómica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción. There were no sequences available for the following markers and species: A12F for *L. chiliensis*, *L. pictus* and *L. uspallatensis*, A9C for *L. chiliensis*, and EXPH5 for *L. chiliensis*.

Species	Specimen ID	Locality data	Source
L. t. punctatissimus	CO67	-36.4833, -72.7000	LDG
L. t. tenuis	CSLT21	-31.7736, -70.9619	LDG
L. chiliensis	PV220	-41.9191, -73.9136	LDG
L. lemniscatus	LDLL21 (CALL7 for EXPH5)	-33.1390, -71.7035	LDG
L. pictus	LTCNE-1	-38.7271, -72.5923	LDG

Table S3. Sequences from the *Eulaemus* subgenus used for the divergence time estimation taken from GenBank. Specimens ID and accession number (in parenthesis) are shown.

	L. darwinii	*L. uspallatensis*	Reference
A12F	LJAMM-CNP 8333 (KC150728.1)	-	Camargo et al., 2013
A9C	LJAMM-CNP 8333 (KC150632.1)	LJAMM-CNP 12506 (JN682719.1)	Camargo et al., 2013
B1D	LJAMM-CNP 5104 (JN682850.1)	LJAMM-CNP 12630 (JN682876.1)	Camargo et al., 2012
CYTB	LJAMM-CNP 8333 (KCI50404.1)	LJAMM-CNP 12630 (JN683167.1)	Camargo et al., 2012, 2013
EXPH5	LJAMM-CNP 5104 (JN683189.1)	LJAMM-CNP 12630 (JN683214.1)	Camargo et al., 2012
KIF24	LJAMM-CNP 5104 (JN683232.1)	LJAMM-CNP 12630 (JN683261.1)	Camargo et al., 2012
LDB5B	LJAMM-CNP 8333 (KC150752.1)	LJAMM-CNP 12630 (JN682964.1)	Camargo et al., 2012, 2013

Table S4. Within group p-distance by marker (pairwise deletion) as estimated by MEGA v. 6. As we had only one sequence for *L. lemniscatus*, nothing is shown in the first row.

	A12F	A9C	B1D	EXPH5	KIF24	LDB5B
L. lemniscatus	-	-	-	-	-	-
L. sp.	0.006	0.016	0.008	0.010	0.014	0.016
L. t. punctatissimus	0.013	0.026	0.013	0.011	0.011	0.016
L. t. tenuis	0.020	0.008	0.009	0.009	0.010	0.013

Table S5. Between groups mean p-distance (pairwise deletion) by marker (in alphabetical order: A12F, A9C, B1D, cytb, EXPH5, KIF24 and LDB5B) as estimated by MEGA v. 6.

	L. lemniscatus	*L. sp.*	*L. t. punctatissimus*	*L. t. tenuis*
L. lemniscatus				
L. sp.	0.034			
	0.092			
	0.030			
	0.015			
	0.033			
	0.469			
L. t. punctatissimus	0.037	0.010		
	0.095	0.023		
	0.032	0.011		
	0.015	0.010		
	0.033	0.013		
	0.019	0.0473		
L. t. tenuis	0.043	0.014	0.016	
	0.097	0.032	0.028	
	0.030	0.010	0.012	
	0.016	0.010	0.011	
	0.033	0.016	0.014	
	0.019	0.475	0.017	

Table S6. Data for individuals used for the geometric morphometrics analyses. For individuals that were sequenced, haplotype codes according to Table S1 are indicated. When individuals were not sequenced, the locality number (according to Table S1) for the closest sequenced individual is indicated. Gender is denoted as F=female; M=male. Geographic coordinates in degrees decimals are shown.

Putative species	Gender	Latitude	Longitude	Haplotype	Closest sequenced locality
L. t. tenuis	F	-31,7736	-70,9619	CYTB33	
L. t. tenuis	F	-31,7736	-70,9619		2
L. t. tenuis	F	-31,7736	-70,9619	CYTB36	
L. t. tenuis	F	-32,2333	-70,9167	A12F11, B1D24, EXP13, KIF40, LDB10	
L. t. tenuis	M	-32,2333	-70,9167	CYTB37	
L. t. tenuis	F	-32,7378	-70,7183	A12F18, CYTB39, EXP53, KIF34, LDB6	
L. t. tenuis	M	-32,7378	-70,7183		5
L. t. tenuis	M	-32,7378	-70,7183		5
L. t. tenuis	F	-32,7378	-70,7183		5
L. t. tenuis	M	-32,7378	-70,7183		5
L. t. tenuis	M	-32,7378	-70,7183	B1D25, EXP19, KIF42	
L. t. tenuis	M	-32,7378	-70,7183	A12F12, A9C7, B1D27, EXP55, KIF41	
L. t. tenuis	M	-32,7833	-70,65		5
L. t. tenuis	F	-33,1353	-70,8553	LDB5	
L. t. tenuis	M	-33,1353	-70,8553	CYTB81	
L. t. tenuis	F	-33,5938	-71,6055		13
L. t. punctatissimus	F	-33,7253	-70,4874	A9C1, EXP18, LDB17	
L. t. punctatissimus	M	-33,7253	-70,4874	CYTB82	
L. t. punctatissimus	M	-34,1189	-71,3781		20
L. t. punctatissimus	M	-34,3162	-71,5397	A12F10, B1D37, EXP15, KIF2, LDB19	
L. t. tenuis	M	-34,3587	-71,7289		18
L. t. tenuis	M	-34,3587	-71,7289	A12F8, B1D18, CYTB80, EXP24, KIF44	
L. t. tenuis	M	-34,3847	-72,0028		19
L. t. tenuis	M	-34,3847	-72,0028	LDB52	
L. t. punctatissimus	F	-34,4163	-70,8549	B1D66, KIF29, LDB69	
L. t. punctatissimus	F	-34,4792	-70,7403		20
L. t. punctatissimus	F	-34,5856	-70,9803		23
L. t. punctatissimus	F	-34,6953	-71,3158		22
L. t. punctatissimus	F	-34,7467	-71,2422		22
L. t. punctatissimus	M	-34,7467	-71,2422		22
L. sp.	F	-34,9686	-71,8288	KIF45, LDB13	
L. sp.	M	-34,9686	-71,8288	B1D58, EXP31	
L. sp.	M	-34,9742	-71,7983	B1D22, EXP30	
L. t. punctatissimus	F	-35,0661	-71,2975		30
L. t. punctatissimus	F	-35,3408	-71,6381		33
L. t. punctatissimus	M	-35,4471	-70,9428	CYTB68, EXP25, KIF46, LDB53	
L. t. punctatissimus	M	-35,5686	-72,0475		33
L. t. punctatissimus	F	-35,5995	-71,0371	A12F14, EXP39,	

L. t. punctatissimus	M	-35,5995	-71,0371	A9C16, B1D3, CYTB64, LDB16	
L. t. punctatissimus	M	-35,5995	-71,0371	A12F13, A9C15, B1D15, EXP54	
L. t. punctatissimus	M	-35,5995	-71,0371	A9C36	
L. t. punctatissimus	M	-35,8358	-72,0678		36
L. t. punctatissimus	M	-36,2485	-71,4274	B1D20, CYTB2, KIF27, LDB12	
L. t. punctatissimus	M	-36,6011	-72,1247		39
L. sp.	F	-36,6183	-71,4375	CTB55	
L. sp.	F	-36,6567	-71,3656	A12F46, B1D8, CYTB56, KIF28	
L. t. punctatissimus	F	-36,7991	-73,1781	B1D41, KIF26	
L. t. punctatissimus	M	-36,7991	-73,1781	B1D7, EXP17, LDB43	
L. t. punctatissimus	M	-36,7991	-73,1781	CYTB97	
L. t. punctatissimus	M	-36,8342	-73,0311		46
L. t. punctatissimus	F	-36,8713	-72,9927	CYTB93	
L. t. punctatissimus	M	-36,8713	-72,9927	A9C8, B1D6	
L. t. punctatissimus	M	-36,9928	-73,1158	A12F28, A9C31, B1D28, CYTB103, EXP66, KIF15, LDB37	
L. t. punctatissimus	F	-37,0786	-73,1478		49
L. t. punctatissimus	F	-37,0786	-73,1478		49
L. t. punctatissimus	F	-37,1219	-72,0131		52
L. sp.	F	-37,397	-71,455		54
L. sp.	M	-37,397	-71,455	B1D36, LDB39	
L. sp.	M	-37,397	-71,455		54
L. sp.	M	-37,397	-71,455		54
L. t. punctatissimus	M	-37,4795	-73,3574	A12F39, A9C29, CYTB101, EXP65, KIF60, LDB34	
L. t. punctatissimus	M	-37,4795	-73,3574	A9C6, CYTB102, EXP32, KIF57, LDB30	
L. t. punctatissimus	M	-37,8028	-72,7017	A12F40, A9C26, B1D30, EXP47, KIF23	
L. t. punctatissimus	M	-37,8028	-72,7017	B1D29, CYTB119, EXP64, KIF52, LDB55	
L. sp.	M	-37,8181	-71,6758		58
L. sp.	M	-37,8961	-71,6144		59
L. t. punctatissimus	M	-38,01	-73,15	A12F31, B1D49	
L. t. punctatissimus	M	-38,1353	-73,3617		61
L. t. punctatissimus	M	-38,2	-72,15	A12F30, CYTB104, EXP45	
L. t. punctatissimus	F	-38,2211	-71,7822	CYTB108	
L. t. punctatissimus	F	-38,2218	-71,7783	A9C19, B1D62, LDB27	
L. t. punctatissimus	M	-38,4156	-72,0697		69
L. sp.	F	-38,4201	-72,778	A12F60, A9C27, B1D52, EXP10	
L. sp.	F	-38,4201	-72,778	A12F56, A9C28, KIF23, LDB62	
L. t. punctatissimus	F	-38,4727	-71,3978	CYTB107, EXP44, KIF50, LDB49	
L. t. punctatissimus	M	-38,5592	-71,4514	A12F43, B1D60, EXP7, KIF22, LDB31	
L. t. punctatissimus	M	-38,5592	-71,4514	CYTB111, EXP61, LDB46	
L. t. punctatissimus	F	-38,7271	-72,5923	A12F55, CYTB112, EXP68, KIF51	
L. sp.	F	-38,7889	-73,385	A12F22, B1D47, EXP11, KIF61	
L. sp.	M	-38,7889	-73,3853		78
L. sp.	M	-39,1455	-71,4969	A12F37, CYTB59, EXP33, KIF55	
L. sp.	M	-39,1685	-71,7272	A12F36, B1D40, EXP43, KIF62, LDB35	
L. sp.	M	-39,291	-71,4337	A12F35, B1D51	
L. sp.	F	-39,8614	-72,7935		92

L. sp.	M	-39,8617	-72,7932	
L. t. punctatissimus	M	-39,8881	-73,4264	A9C32, B1D63, EXP1, LDB57
L. sp.	F	-39,9504	-73,5601	A12F21
L. t. punctatissimus	M	-40,2683	-73,3286	A12F25, A9C21, CYTB113, LDB67

Table S7. Landmarks (x, y) for which a significant difference in the mean (in parenthesis) was found by the Kruskal-Wallis test.

	Landmark	P value	Species (mean)
Females	y2	0.0371	*L. t. tenuis* (1.824244e+13) vs. *L. t. punctatissimus* (1.952727e+13)
	x4	0.00670	*L. t. tenuis* (1.445357e+13) vs. *L. t. punctatissimus* (1.351612e+13)
	x8	0.0028	*L. t. punctatissimus* (-9.384179e+12) vs. *L. sp.* (-1.008529e+13)
	x10	0.0059	*L. t. tenuis* (-2.565152e+12) vs. *L. t. punctatissimus* (-1.708551e+12)
	y13	0.0403	*L. t. tenuis* (2.547886e+13) vs. *L. t. punctatissimus* (2.429745e+13)
	x14	0.0069	*L. t. tenuis* (-3.345036e+12) vs. *L. t. punctatissimus* (-4.265037e+12)
		0.0064	*L. t. tenuis* (-3.345036e+12) vs. *L. sp*. (-4.412076e+12)
Males	y3	0.0240	*L. t. tenuis* (6.405833e+12) vs. *L. t. punctatissimus* (7.205366e+12)
	y9	0.0008	*L. t. tenuis* (6.156667e+12) vs. *L. t. punctatissimus* (7.666341e+12)
	x12	0.0190	*L. t. tenuis* (1.201667e+13) vs. *L. t. punctatissimus* (1.274390e+13)
	y14	0.0115	*L. t. tenuis* (-3.787500e+12) vs. *L. t. punctatissimus* (-4.235122e+12)

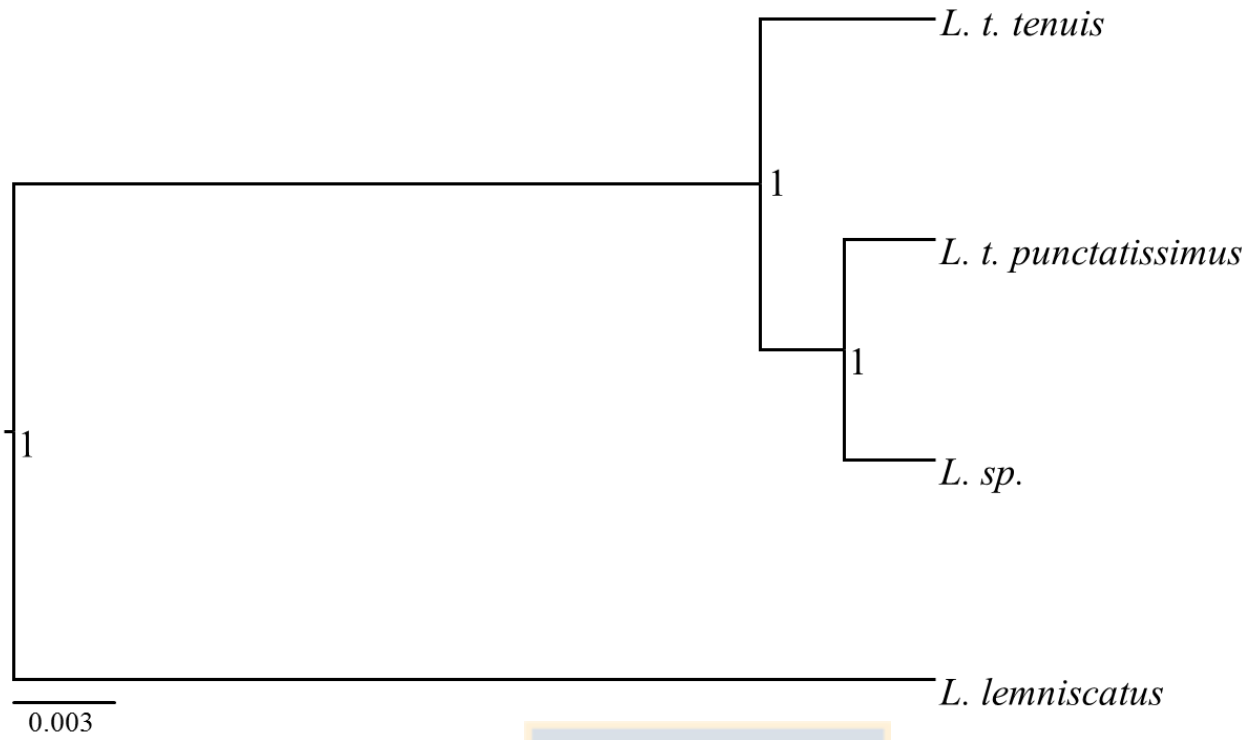


Figure S1. Species Tree reconstruction using *BEAST (mtDNA + six nuclear genes). Numbers next to nodes indicate Bayesian posterior probability values.

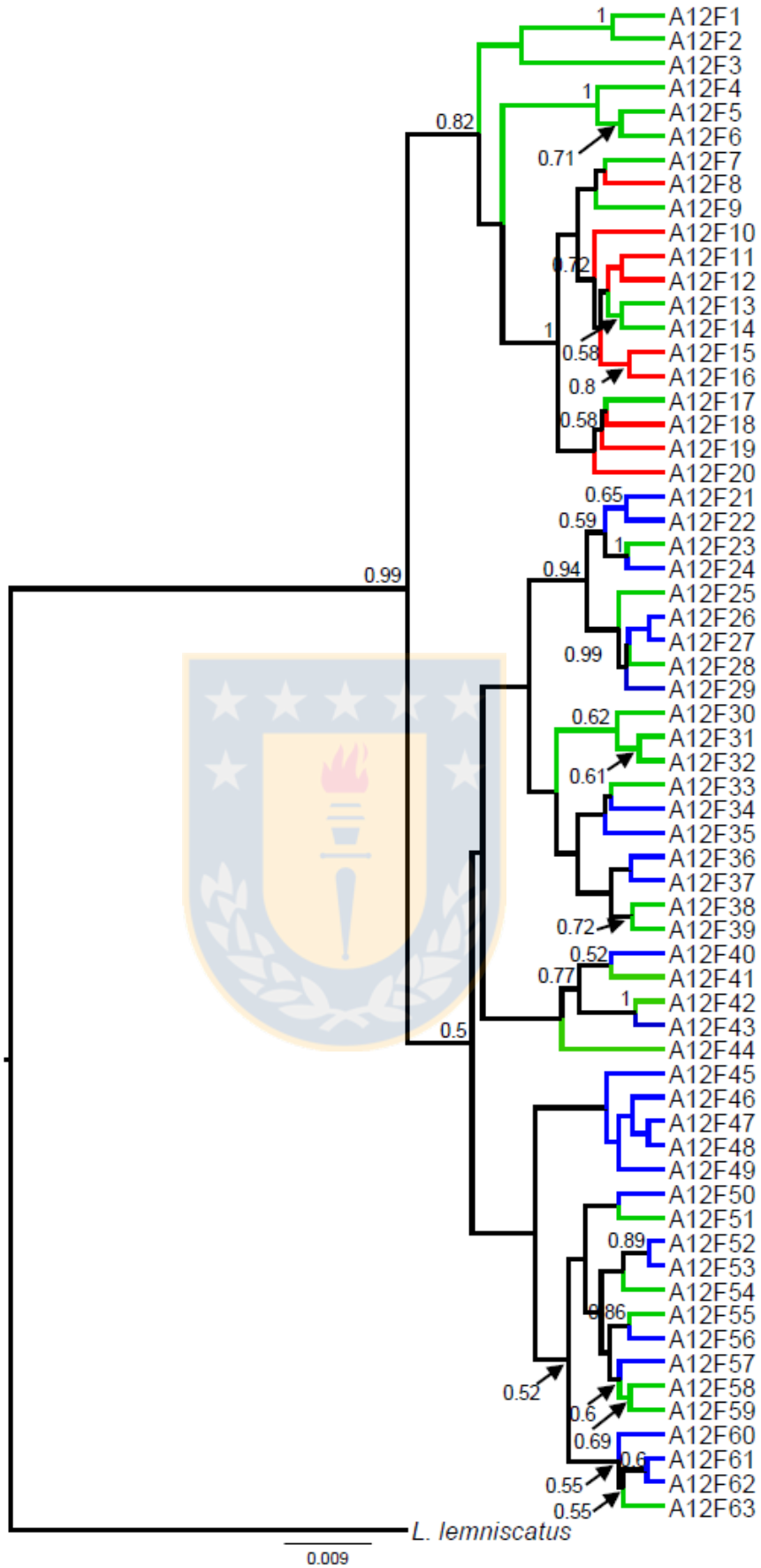


Figure S2. Gene tree inferred for sequences of nuclear anonymous gene A12F during species tree estimation using *BEAST. Only posterior probability values above 0.5 are shown. Haplotype codes follow Table S1.

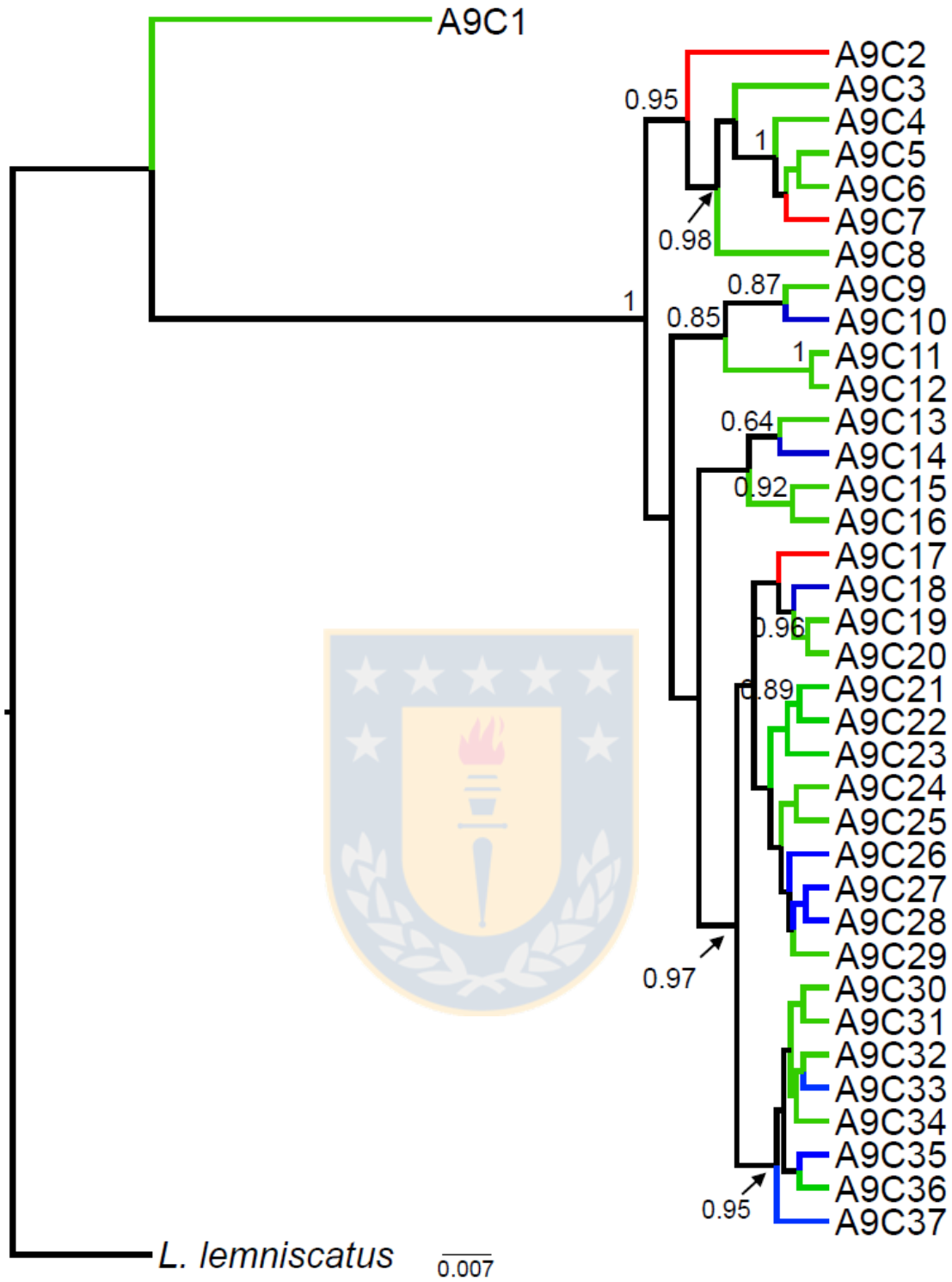
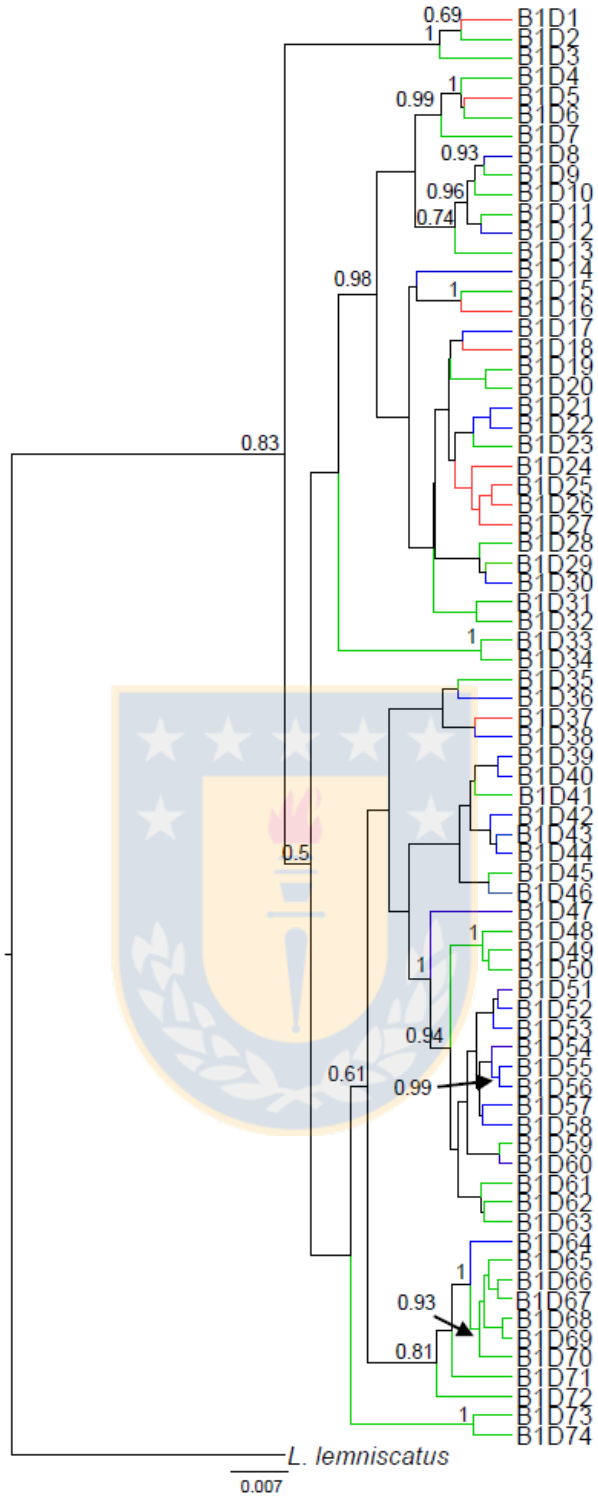


Figure S3. Gene tree inferred for sequences of nuclear anonymous gene A9C during species tree estimation using *BEAST. Only posterior probability values above 0.5 are shown. Haplotype codes follow Table S1.



290

291 Figure S4. Gene tree inferred for sequences of nuclear anonymous gene B1D during species tree estimation using

292 *BEAST. Only posterior probability values above 0.5 are shown. Haplotype codes follow Table S1.

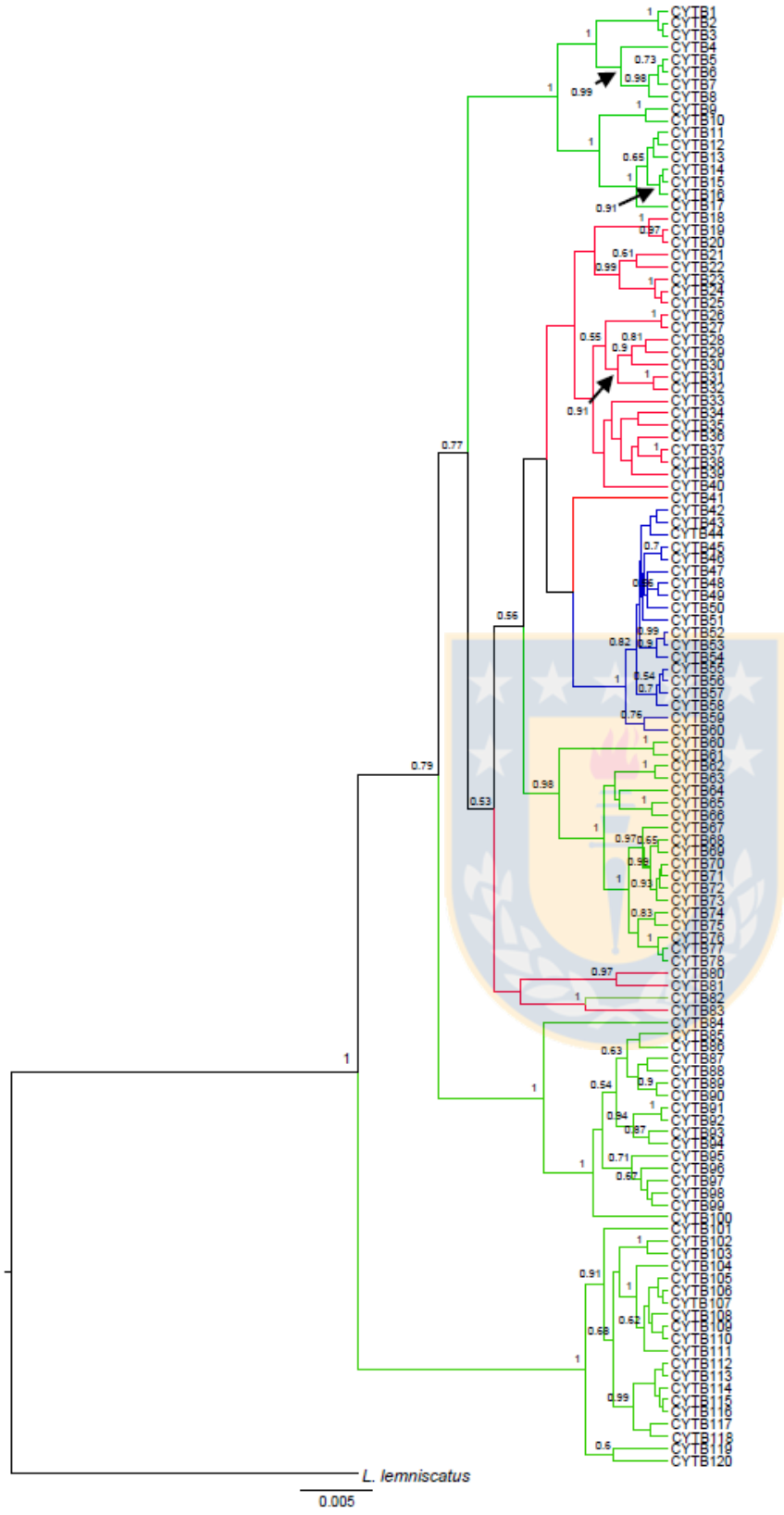


Figure S5. Gene tree inferred for sequences of mitochondrial gene cytochrome b during species tree estimation using *BEAST. Only posterior probability values above 0.5 are shown. Haplotype codes follow Table S1.

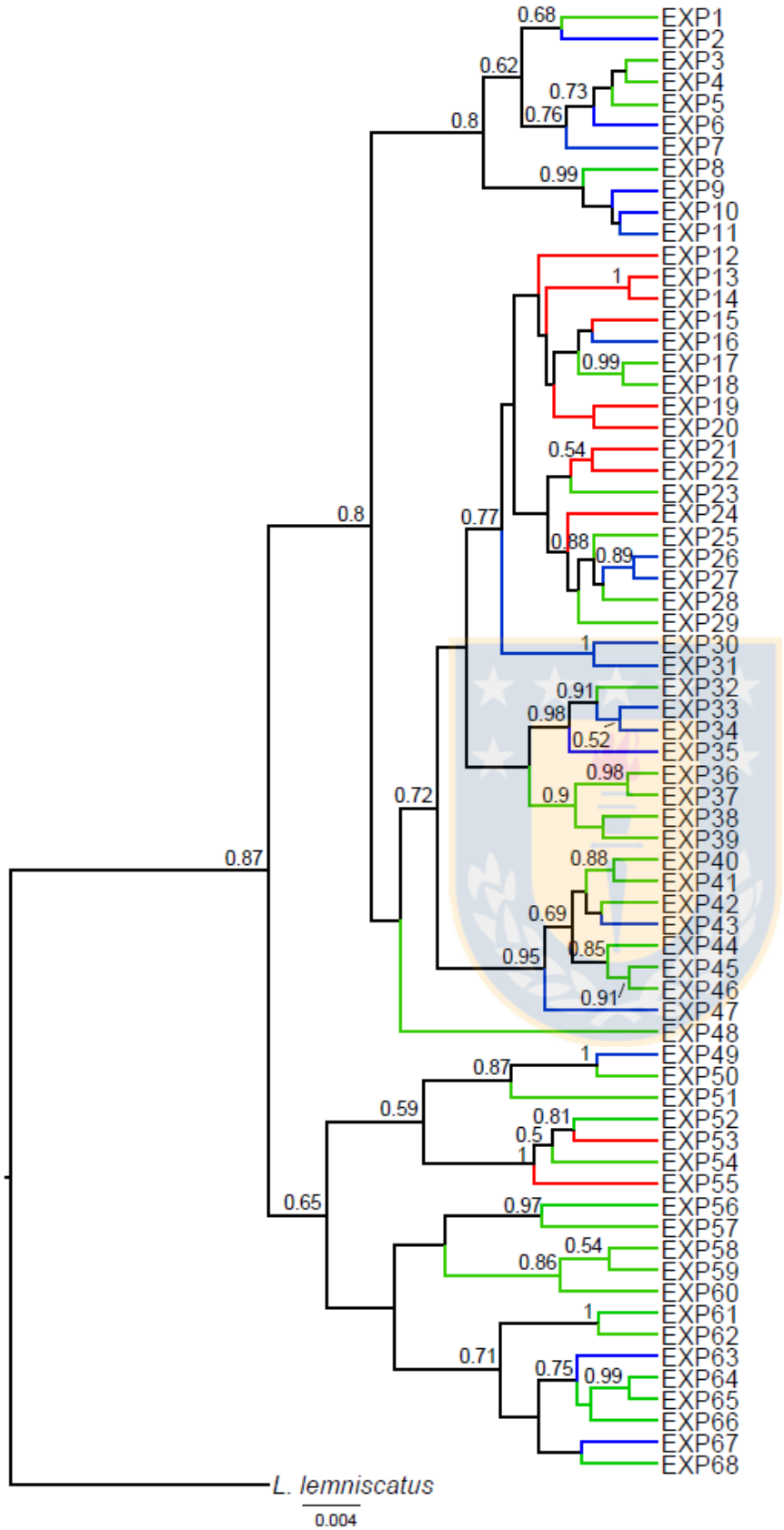


Figure S6. Gene tree inferred for sequences of nuclear protein-coding gene EXPH5 during species tree estimation using *BEAST. Only posterior probability values above 0.5 are shown. Haplotype codes follow Table S1.

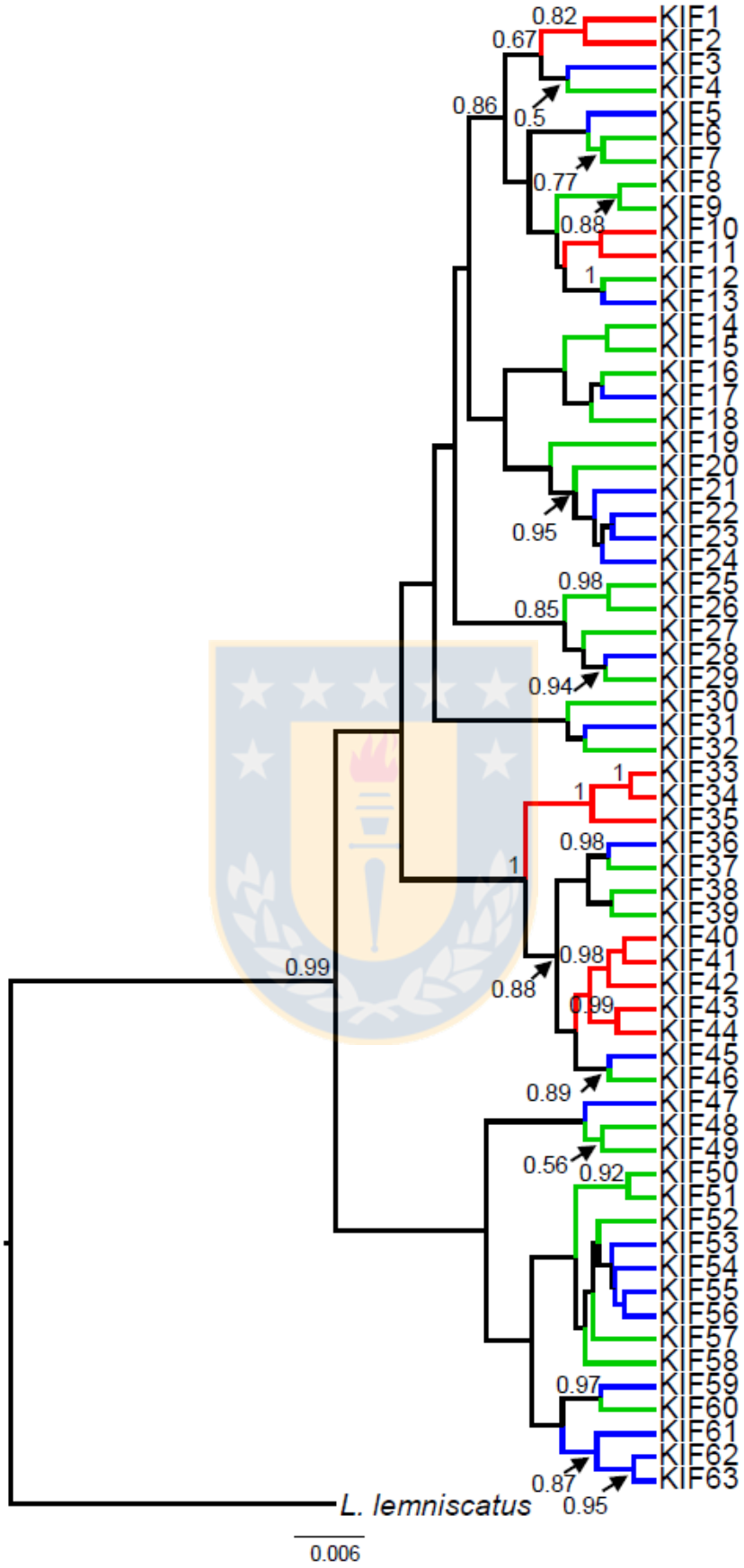


Figure S7. Gene tree inferred for sequences of nuclear protein-coding gene KIF24 during species tree estimation using *BEAST. Only posterior probability values above 0.5 are shown. Haplotype codes follow Table S1.

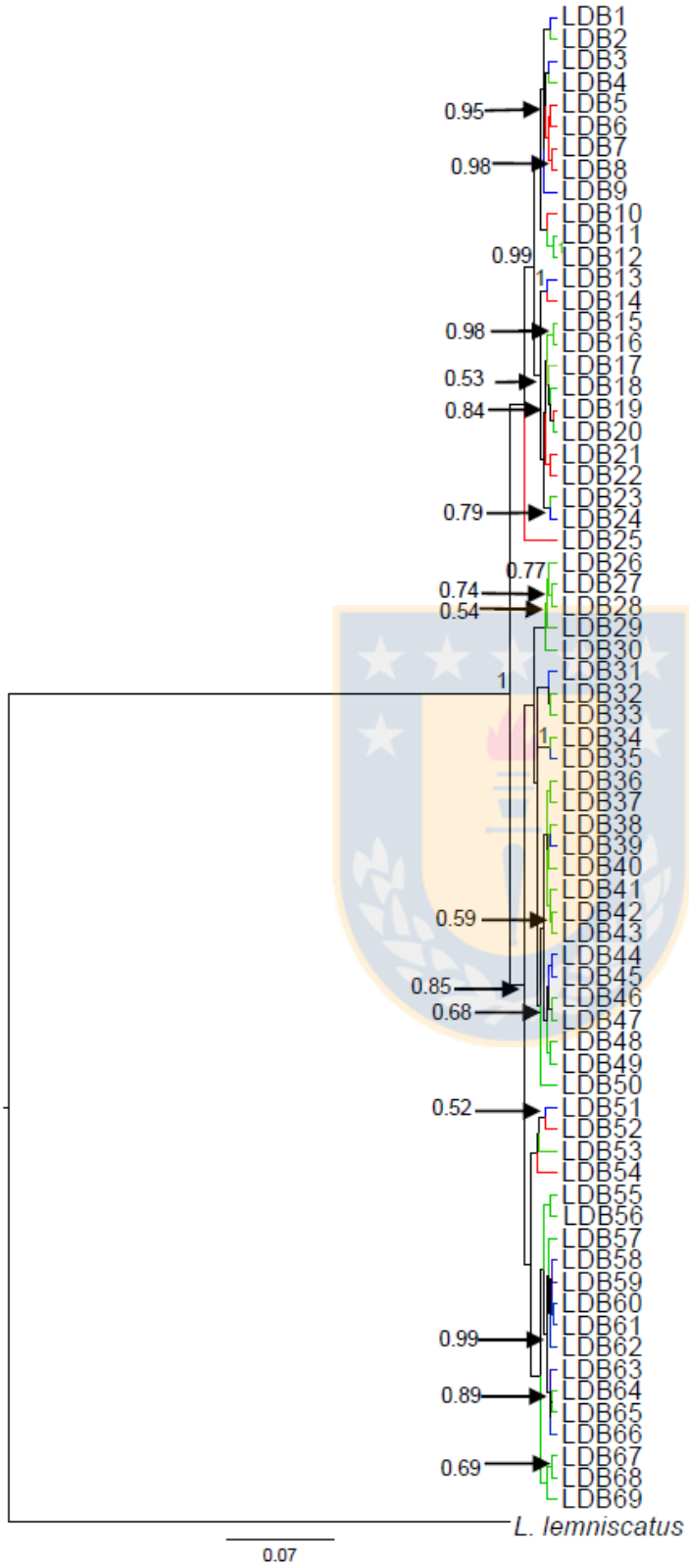


Figure S8. Gene tree inferred for sequences of nuclear anonymous gene LDB5B during species tree estimation using *BEAST. Only posterior probability values above 0.5 are shown. Haplotype codes follow Table S1.

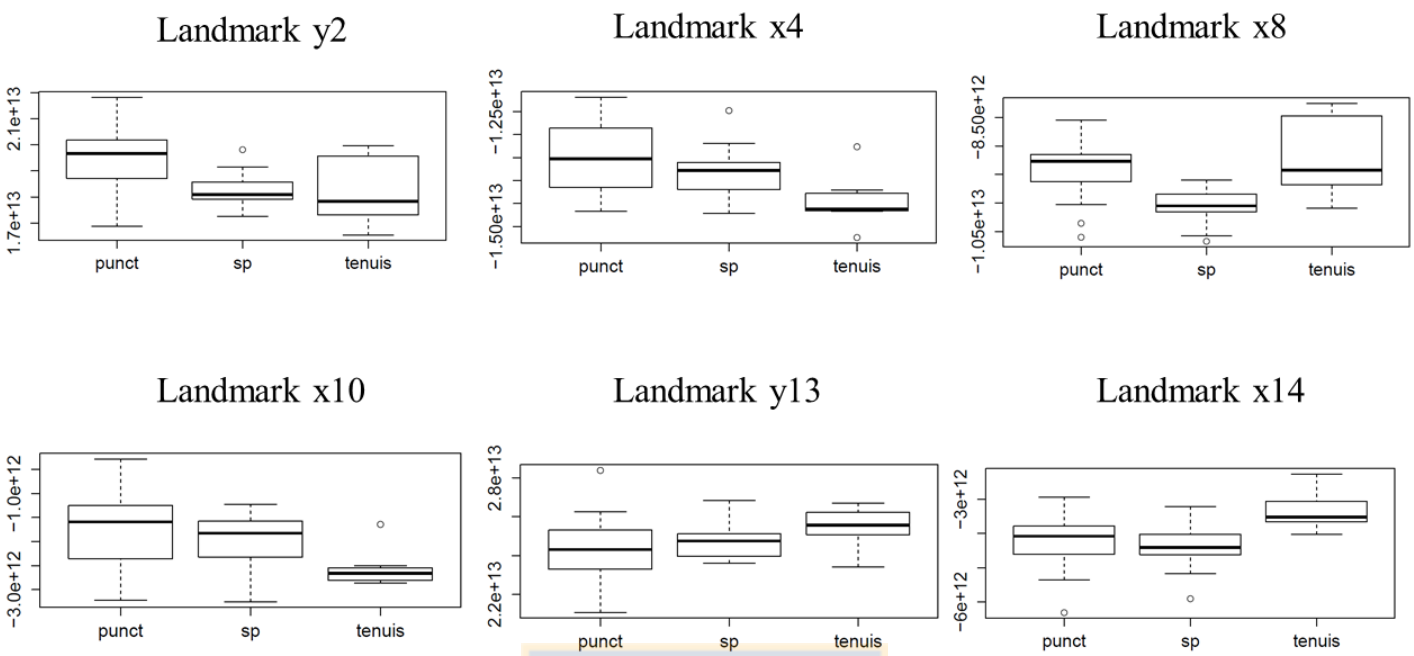
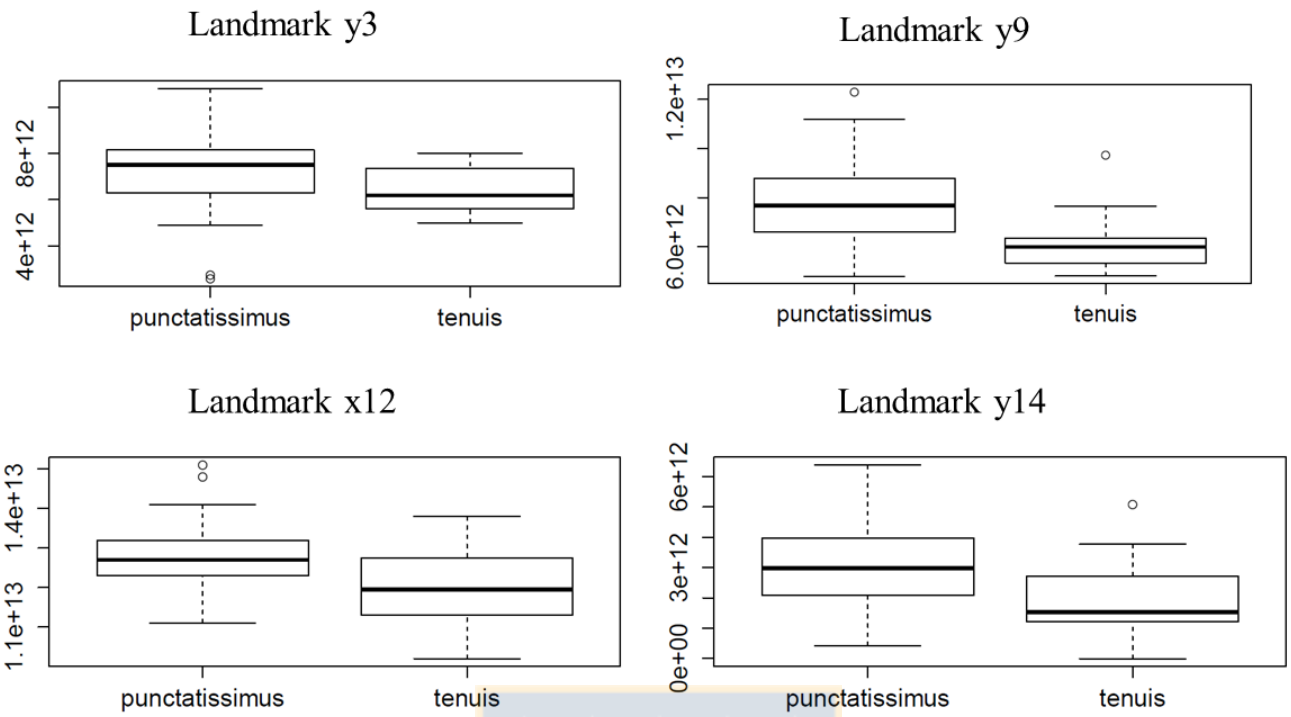


Figure S9. Boxplots showing the value distribution and means for those landmarks for which significant diferences ($p<0.05$) were found in females.



311

312 Figure S10. Boxplots showing the value distribution and means for those landmarks for which significant differences

313 ($p<0.05$) were found in males.

CAPÍTULO 3

Phylogenomic analysis of the Chilean clade of *Liolaemus* lizards (Squamata: Liolaemidae) based on sequence capture data.

Phylogenomic analysis of the Chilean clade of *Liolaemus* lizards (Squamata: Liolaemidae) based on sequence capture data

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ABSTRACT

The genus *Liolaemus* is one of the most ecologically diverse and species-rich genera of lizards worldwide. It currently includes more than 250 recognized species, which have being subject to many ecological and evolutionary studies. Nevertheless, *Liolaemus* lizards have a complex taxonomic history, mainly due to the incongruence between morphological and genetic data, incomplete taxon sampling, incomplete lineage sorting and hybridization. In addition, as many species have restricted and remote distributions, this has hampered their examination and inclusion in molecular systematic studies. The aims of this study are to infer a robust phylogeny for a subsample of lizards representing the Chilean clade (subgenus *Liolaemus* sensu stricto), and to test the monophyly of several of the major species groups. We use a phylogenomic approach, targeting 541 ultra-conserved elements (UCEs) and 44 protein-coding genes for 16 taxa. We conduct a comparison of phylogenetic analyses using concatenation and several species tree inference methods. The UCEs provide stronger support for phylogenetic relationships compared to the protein-coding genes; however the UCEs outnumber the protein-coding genes by 10-fold. On average, the protein-coding genes contain over twice the number of informative sites. Based on our phylogenomic analyses, all the groups sampled are polyphyletic. *Liolaemus tenuis tenuis* is difficult to place in the phylogeny, because only a few loci (nine) were recovered for this species. The phylogenomic analyses provide strong support for sister group relationships between *L. pictus* and *L. paulinae,* and *L. platei* and *L. velosoi*. Despite our limited taxon sampling, we have provided a robust starting hypothesis for the relationships among many major groups of the Chilean clade of *Liolaemus* that will help future work aimed at resolving the *Liolaemus* phylogeny.

Introduction

Lizards of the genus *Liolaemus* Wiegmann 1834 are widely distributed throughout temperate South America, occurring mainly in Argentina and Chile, but also in Bolivia, Brazil, Paraguay, Peru and Uruguay (Abdala & Quinteros, 2013). They cover a wide range of climatic regimes, from the Atacama Desert to the Valdivian rainforest, and from Mediterranean shrub lands to Patagonian steppes (Donoso-Barros, 1966; Cei, 1986; Cei, 1993). *Liolaemus* has a long evolutionary history dating back to 18-22 million years ago (Schulte et al., 2000; Fontanella et al., 2012). The genus is highly variable, exhibiting a wide range of reproductive modes, habitats, life histories, coloration patterns and body sizes (e.g., Koslowsky, 1989; Carothers et al., 1997; Schulte et al., 2000; Ibargüengoytía & Cussac, 2002; Espinoza, Wiens & Tracy, 2004). Not surprisingly, *Liolaemus* has been subject to many ecological and evolutionary studies (e.g., Harmon et al., 2003; Camargo, Sinervo & Sites, 2010; Cianferoni et al., 2013; Sheldon, Leaché & Cruz, 2015). Nevertheless, phylogenetic relationships among species of *Liolaemus* have been difficult to resolve, due in part to the incomplete taxon sampling of most studies (but see Olave et al., 2014), the discordance between inferences based on molecular and morphological data (Schulte et al., 2000; Lobo, 2001; Lobo, 2005; Vidal & Labra, 2008), and to the incongruences between nuclear and mitochondrial gene trees caused by, likely, incomplete lineage sorting and hybridization (e.g., Olave et al., 2011).

Liolaemus currently includes more than 250 species (Uetz et al., 2016), although this is thought to be a rough underestimate (Morando, Avila & Sites, 2003). Since 2010, more than 40 new species have been described (Uetz et al., 2016), and several phylogeographic studies have reported new candidate species (e.g., Cianferoni et al., 2013; Troncoso-Palacios et al., 2015; Olave et al., 2016), which suggests that the number of species will continue to increase. The genus is subdivided into two subgenera, *Liolaemus* (sensu stricto) and *Eulaemus* (Laurent, 1985), distributed mainly on the western and eastern sides of the Andes, respectively, although some species of the subgenus *Liolaemus* are found in the eastern slope of the Andes (e.g., in the *elongatus* group; Abdala & Quinteros, 2013). The monophyly of each subgenus has been strongly supported by both morphological and molecular data (Young-Downey, 1998; Schulte et al., 2000; Espinoza, Wiens & Tracy, 2004; Morando et al., 2004). Each subgenus is also divided into several sections, species groups and species complexes (e.g., Pincheira-Donoso & Núñez,

2005; Lobo, Espinoza & Quinteros, 2010; Abdala & Quinteros, 2013). In Chile, the genus is represented by approximately 96 species (Ruiz de Gamboa, 2016). Studies attempting to resolve the phylogenetic relationships among Chilean species of *Liolaemus* are scarce compared to studies focused on Argentinean species (e.g., Camargo et al., 2012; Avila et al., 2013; Olave et al., 2011, 2014, 2015, 2016). In Chile, the low number of phylogenetic studies relative to the high diversity of *Liolaemus* has hampered the development of comprehensive biogeographical studies that in turn, may help to improve conservation strategies (Vidal & Diaz-Paez, 2012).

Chilean species of *Liolaemus* are grouped into two sections, the *chiliensis* and the *nigromaculatus* sections (although not all authors agree on this classification). Ten and three species groups are recognized within each section, respectively (Abdala & Quinteros, 2013) (Table 1). However, recent molecular based studies (e.g., Troncoso-Palacios et al., 2015) have shown that neither section is monophyletic, as species traditionally allocated to the *chiliensis* section, such as *L. tenuis, L. fuscus, L. lemniscatus*, and *L. paulinae* are more closely related to species of the *nigromaculatus* section. Similarly, most species present taxonomic problems due to high level of within species morphological and coloration variation (e.g., *Liolaemus nigromaculatus*, Troncoso-Palacios & Garín, 2013), or to the presence of convergence of morphological traits coupled with genetically divergent lineages (e.g., *Liolaemus monticola*, Tórres-Pérez et al., 2009). This scenario has led to some subspecies being recently elevated to species (e.g., *L. nigromaculatus atacamensis*, now *L. atacamensis*; Simonetti et al., 1995; Valladares, 2011; Troncoso-Palacios & Garín, 2013), others recently synonimized (e.g., *L. velosoi* under *L. josephorum*, Núñez, Schulte & Garín, 2001; *L. lonquimayensis* under *L. elongatus*, Troncoso-Palacios et al., 2016b), and several candidate species proposed (e.g., Cianferoni et al., 2013, Troncoso-Palacios et al., 2015). Therefore, it is necessary to infer a robust phylogeny to clarify the taxonomy of the group.

In this study, we aim to develop a robust phylogenetic tree for lizards within the *Liolaemus* subgenus using a sample of species from the major groups. Classification should reflect natural groups, therefore our main aim is to provide a back bone tree of the subgenus *Liolaemus* useful to future phylogenetic studies; in addition we aim to test the monophyly of some of the species groups currently recognized. This study differs from previous studies focused on the subgenus *Liolaemus*, which have mainly used mitochondrial genes (e.g., Schulte

et al., 2000; Tórres-Pérez et al., 2009; Guerrero et al., 2013; Troncoso-Palacios et al., 2016a; Troncoso-Palacios et al., 2016b), in its large character sampling that has a genomic scale and analytical tools. Nevertheless, we are aware that incomplete taxon sampling has drawbacks, but the new phylogenomic tree presented here should be considered a reliable starting hypothesis based on a high volume of data (Degnan & Salter, 2005; Maddison & Knowles, 2006). We use a sequence capture method, which employs short probes (60–120 base pairs) to hybridize to specific genomic regions, which are then isolated and sequenced using next-generation sequencing (Gnirke et al., 2009; McCormack et al., 2012), a method successfully used to resolve relationships among mammals (McCormack et al., 2012), birds (McCormack et al., 2013), turtles (Crawford et al., 2012) and squamates (Pyron et al., 2014; Leaché et al., 2015), among others. We use phylogenetic methods that have been developed to estimate species trees using the multispecies coalescent model to accommodate the stochastic segregation of multiple independent loci (Rannala & Yang, 2003). Coalescent approaches are advantageous for phylogenomics, because their accuracy increases as more loci are sampled (Edwards, Liu & Pearl, 2007; Leaché & Rannala, 2011).

Materials and Methods

Sampling

We followed the classification of *Liolaemus* advanced by Abdala & Quinteros (2013) (Table 1), but updated it with recent species descriptions and taxonomic rearrangements (see Table S1 for details). We selected 16 taxa from the *alticolor-bibronii*, *gravenhorstii*, *monticola*, *nigromaculatus*, *nigroviridis, pictus* and *robertmertensi* species groups of the subgenus *Liolaemus* (Table 2). For the *nigromaculatus* section, we generated sequences of specimens of *L. atacamensis*, *L. nigromaculatus*, *L. platei, L. velosoi* and *L. zapallarensis* of the *nigromaculatus* species group, of *L. nigroviridis* and *L. isabelae* of the *nigroviridi*s species group and of *L. monticola* of the *monticola* species group. Within the *chiliensis* section, we sampled specimens of *L. fuscus* and *L. paulinae* of the *alticolor-bibronii* species group, *L. cyanogaster* of the *gravenhorstii* species group (but placed within the *chiliensis sensu stricto* group by Pincheira-Donoso & Núñez, 2005)*, L. tenuis* and *L. pictus* of the *pictus* species group and *L. nitidus* of the *robertmertensi* species group. Within *Liolaemus tenuis*, two subspecies had been described

(Müller & Hellmich, 1933) based primarily on color patterns: *Liolaemus t. tenuis* (*terra typica*: Santiago) and *L. t. punctatissimus* (*terra typica*: Lota). Nevertheless, few authors recognize these two taxa as their overlap in part of their distribution (Formas, 1979) conflicting with the subspecies concept (Mayr & Ashlock, 1991). A recent phylogeographic study found three deeply divergent clades within *Liolaemus tenuis sensu lato*, suggesting these could constitute distinct species (Muñóz-Mendoza et al., submitted). Therefore, we have included representatives of these three lineages in this study, representing the two subspecies of *Liolaemus tenuis* (*L. t. tenuis* and *L. t. punctatissimus*), as well as the third divergent lineage found within the nominal *Liolaemus tenuis*, which is here referred to as *L. sp.* When possible, we used specimens collected at type localities (Table 2); detailed information regarding collection localities, including GPS coordinates, are found in Table S2. Specimens included in this study are deposited in the collection of Museo de Zoología de la Universidad de Concepción (MZUC), in the Monte L. Bean Life Science Museum at Brigham Young University (BYU), in the Laboratory of Zoology, Epidemiology and Evolution of the Pontificia Universidad Católica de Valparaíso (PUCV) and in the Colección Patricio Sánchez Reyes de la Pontificia Universidad Católica de Chile (SSUC) (Table 2). Those specimens housed at MZUC were collected by us under collection permits N° 9487/2014, 1898 and 4729 (SAG) and 04/2013 IX (CONAF) and were sacrificed by a pericardiac injection of sodium tiopenthal (Abbot®/Pentovet®).

Library preparation, target enrichment, and sequencing

We extracted DNA using the DNeasy extraction kit (Qiagen, Inc.). We quantified DNA concentrations for each sample using a Qubit fluorometer (Life Technologies, Inc.). We randomly sheared 300 ng of DNA to a target size of approximately 400-600 bp by sonication with 7 cycles using Bioruptor Pico (Diagenode BioRuptor); fragment size distributions was verified using Agilient Tape Station 2200 (Agilient Tech.).

We prepared sequencing libraries using TruSeq Nano DNA Sample Prep Kit (http://www.illumina.com/products/truseq-nano-dna-sample-prep-kit.ilmn) following, with some modifications, protocols available at http://ultraconserved.org/#protocols. We attached adapters from MyBaits custom probe kit (http://www.mycroarray.com/mybaits/mybaits-custom.html) to each library. We used probes designed by Leaché et al. (2015) specific for iguanian lizards,

targeting 541 ultra-conserved elements (herein UCEs) (which are a subset of the 5472 UCEs published by Faircloth et al., 2012) and 44 genes from the Squamate Tree of Life project (Wiens et al., 2012). UCEs are highly conserved regions (>60 base pairs) with flanks that exhibit increasing variation as the distance from the conserved core increases (Bejerano et al., 2004; Faircloth et al., 2012). After library amplification, we quantified 2 μL of each library using fluorometry (Qubit, Life Technologies), and prepared two pools each containing eight libraries totaling 500 ng per pool (62.5 ng each library). We concentrated library pools using a Vacufuge Plus vacuum concentrator (Eppendorf) and rehydrated each library in 3.4 μL of ddH2O. We enriched pooled libraries using a synthesis of 1170 RNA probes (two 120 bp probes per locus) (Mycroarray, Inc.). Libraries were incubated with RNA probes for 24 hours at 65°C. Post-hybridized libraries were enriched using TruSeq adapter primers with Phusion High-Fidelity DNA polymerase (New England BioLabs Inc) in order to minimize errors during PCR (Brelsford et al., 2012), for 17 cycles and cleaned by bead purification. We quantified enrichment by comparing non-enriched libraries versus enriched libraries in a qPCR (Applied Biosystems Inc.) with primers targeting five loci mapping to five chromosomes (based on the genome of *Anolis carolinensis,* Alföldi et al., 2011).

We sequenced the enrichment products on a single PE100 Illumina HiSeq2500 lane. Sequencing was performed at the QB3 Vincent J. Coates Sequencing Laboratory at the University of California, Berkeley.

Bioinformatics

Particular sequences were associated with individual specimens using strict matching of their unique bar codes in CASAVA (Illumina, Inc.). Demultiplexed sequence reads were subjected to quality control in Trimmomatic (Bolger, Lohse & Usadel, 2014), which removes contaminating adapter sequences and low quality bases, using the parallel wrapper script Illumiprocessor (http://dx.doi.org/10.6079/J9ILL). We assembled the reads using IDBA (Peng et al., 2012). We repeated our IDBA assemblies iteratively over k-mer values from 50 to 90, and selected the k-mer value that produced the largest N50 value for each species.

One important step during NGS data analysis is the successful alignment of short reads to a reference genome (Flicek & Birney, 2009; Zhang et al., 2011). We used phyluce (Faircloth, 2015) to assemble loci across species and to produce alignments for phylogenetic analysis. We

started by aligning species-specific contigs to the set of probes (match contigs to probes.py) with LASTZ (Harris, 2007). We aligned FASTA sequences of each locus using MAFFT (Katoh & Standley, 2013).

Phylogenetic analyses

Because it has been suggested that the use of gene trees based on relatively low variation markers could be problematic for species tree analyses (Lanier, Huang & Knowles, 2014; Xi, Liu & Davis, 2015; Xu & Yang, 2016), and some empirical studies using UCE data have supported this claim (e. g. McCormack et al., 2013), we conducted phylogenetic analyses using three different data sets: (1) ultra-conserved elements (538 loci); (2) protein-coding genes (41 loci), and (3) a combined data set containing all UCEs and protein-coding genes (581 loci). Our comparison of species trees estimated with UCEs and protein-coding genes allowed us to explore the phylogenetic signal in the different types of markers and make inferences about their utility for phylogenetic inference. We estimated phylogenetic trees using three different approaches: (1) concatenation, (2) quartet inference, and (3) a gene-tree summary method. Based on the phylogenies inferred by Schulte et al. (2000) and Pyron, Burbrink & Wiens (2013), we used sequences of *Liolaemus cyanogaster* to root the trees, as this species appears in a clade (along with *L. pictus*) that is sister to a clade containing the rest of the species included in this analyses.

Before phylogenetic inference, summaries of the sequence capture loci were generated using scripts available from https://github.com/dportik/Alignment_Assessment (Portik, Smith & Bi, 2016). These, in addition to visual inspection of individual gene trees allowed us to detect outliers that could result from poor alignments. These loci were manually edited by eye and checked again using the same pipeline.

Concatenation. Concatenated matrices were subjected to maximum likelihood (ML) analyses with RAxML v8.2 (Stamatakis, Hoover & Rougemont, 2008; Stamatakis, 2014) using 1000 bootstrap (BS) replicates. For each data set we conducted unpartitioned analyses using the GTR+Γ model of evolution. Input files for analyses were created using the command phyluce_align_format_nexus_files_for_raxml available in the phyluce package (Faircloth, 2015).

Quartet inference. We used SVD quartets (Chifman & Kubatko, 2014) as implemented in PAUP* (Swofford, 2002) to infer a species tree. This method, unlike gene tree summary statistics methods, uses sequence data directly and therefore incorporates more sources of variability in the species tree estimation process (Chifman & Kubatko, 2014). We evaluated all possible quartets and treated ambiguities as missing data. We conducted 1,000 bootstrap (BS) replicates.

Gene tree-based analyses. These methods use rooted (e.g., MP-EST, Liu, Yu & Edwards, 2010) or unrooted gene trees (e.g., ASTRAL, Mirarab et al., 2014) as an input to generate a species tree. To estimate the appropriate substitution model for each locus, we used modeltest_runner.py (available at https://github.com/cwlinkem/linkuce), a wrapper script to run jmodeltest (Posada, 2008), and the Bayesian information criterion (BIC; Schwarz, 1978). Next, we estimated ML trees using RAxML v8.2. ML analyses used the models selected from jmodeltest, and implemented an auto-bootstrap procedure (Pattengale et al., 2010). To infer the species tree we used ASTRAL (Mirarab et al., 2014), which can outperform other methods when levels of incomplete lineage sorting are high (Chou et al., 2012). One advantage of ASTRAL is that it does not need rooted trees (the outgroup was not amplified for all loci used). In addition, ASTRAL has been shown to perform well using UCEs in difficult phylogenetic scenarios (Meiklejohn et al., 2016). To generate the species tree, we inputted ML bootstrap trees of all loci into ASTRAL and performed 1,000 bootstrap (BS) replications, by sampling with replacement from the ML bootstrap gene tree files.

Results

Sequence capture data

The number of loci (UCEs and protein-coding genes) recovered per species is shown in Table 3. Although the majority of loci were sequenced across species, a significant proportion of loci were not recovered in *Liolaemus tenuis tenuis* (with only nine of the 585 loci captured). Therefore, instead of choosing the k-mer with the highest N50 value for *L. t. tenuis*, we chose the k-mer that maximized the number of loci recovered (nine loci, k-mer=50; Table S2). As a consequence, only one loci was shared across all 16 terminals. If *L. t. tenuis* is removed, the total number of shared loci across the remaining 15 terminals rises to 20. Our final incomplete dataset

yielded a total of 581 loci, which consisted in 538 UCEs and 43 protein-coding genes. The four loci that were excluded from the final dataset were three UCEs (corresponding to chromosomes z_3515 and z_5971, and chromosome 9_7188) and one protein-coding gene (UBN1: ubinuclein1).

Summaries of the sequence capture loci are available in Figures 1 and 2. The average number of base-pairs across alignments was 396, and both average percent of gaps per alignment and average percent of missing data across alignments were 0.4%. In average, protein-coding genes were more informative than UCEs, with 2.1% and 0.7% of informative sites, respectively (Table 4). The substitution models inferred for all loci are available at Figure 3. The best-fitting model most often identified by jmodeltest was F81 (46% of markers), followed by HKY (21%) and JC (13%).

Phylogenetic analyses

Most analyses support the same topology in which *L. fuscus*, *L. monticola*, *L. nigroviridis,* and *L. nitidus* always formed a highly supported clade, which in turn is sister to another well-supported clade formed by *L. nigromaculatus*, *L. platei*, *L. velosoi* and *L. zapallarensis*. In most cases, this group of species is sister to a clade form by *L. t. punctatissimus* and *L. sp.* This whole clade is sister to a clade formed by *L. pictus* and *L. paulinae*. Sister to this grouping is a clade containing *L. atacamensis* and *L. isabelae*. Although most analyses inferred these relationships, the only ones that received strong support across all analyses (BS ≥ 70%) are the following clades: *L. pictus* sister to *L. paulinae*, and *L. platei* sister to *L. velosoi* (Table 5). Other relationships received high support in all analyses except when phylogenies were inferred using only protein-coding genes in SVD quartets (*L. atacamensis* sister to *L. isabelae*, BS=51; *L. t. punctatissimus* sister to *L. sp.*, BS=44; *L. zapallarensis* sister to *L. nigromaculatus*, BS=54; and clade formed by *L. nigromaculatus*, *L. zapallarensis*, *L. platei* and *L. velosoi*, BS=49) and RAxML (*L. t. punctatissimus* sister to *L. sp.*, BS=55) (Table 5). Moreover, some notable differences were observed in some of the analyses (described below). Table 5 provides a summary of support values for the estimated relationships from across all of the analyses. Remarkably, all species groups from which more than one species were sampled are polyphyletic (Figure 4).

289 *Concatenation*. The ML analysis of the combined data (Figure 4A) supports a clade (BS=84)
290 containing *L. isabelae*, *L. atacamensis* and *L. t. tenuis* as sister to a clade formed by the
291 remaining species (BS=91); within the former clade *L. atacamensis* and *L. t. tenuis* are sister to
292 each other (BS=68). Meanwhile, within the second main clade of *Liolaemus*, *L. paulinae* and *L.*
293 *pictus* are sister species (BS=100), and this clade is sister to the remaining species of the clade
294 (BS=91). *Liolaemus t. punctatissimus* and *L. sp.* are sister to each other (BS=97) and together
295 sister to a clade (BS=95) containing two smaller clades, each composed of two subclades: one
296 (BS=95) contains (*L. fuscus* + *L. nigroviridis*) and (*L. monticola* + *L. nitidus*), while the other
297 (BS=100) contains (*L. zapallarensis* + *L. nigromaculatus*) and (*L. velosoi* + *L. platei*).
298 There are several key differences between the protein-coding vs. UCE based topologies
299 (Table 5). First, the protein-coding tree fails to support the *L. fuscus* + *L. nigroviridis* clade.
300 Second, the protein-coding tree places *L. t. tenuis* as sister to the clade composed by the
301 remaining species. Finally, the UCE tree fails to support the monophyly of the *L. monticola* + *L.*
302 *nitidus* clade.
303
304 *Quartet inference.* In SVD quartets, 1820 quartets were analyzed, and the ratio of incompatible
305 to compatible quartets was 240/1104 for the UCEs (21.7%; N = 1344 informative quartets),
306 157/649 for the protein-coding genes (2.3%; N = 806), and 227/1132 for the combined data
307 (20.0%; N =1359). Overall, SVD quartets inferred the same relationships as concatenation
308 (Figure 4A, B). Again, the main differences relative to the tree resulting from the concatenated
309 full data set (Figure 4A) are the relationships between *L. monticola. L. nitidus, L. fuscus* and *L.*
310 *nigroviridis*, as well as the placement of *L. t. tenuis.* As opposed to its placement in Figure 4A, in
311 SVD quartets *L. t. tenuis* is not sister to *L. atacamensis* but diverges right after *L. atacamensis* +
312 *L. isabelae* (Figure 4C). In the combined tree (Figure 4B) the topology shows the following
313 arrangement (*L. fuscus* (*L. nigroviridis* (*L. monticola*, *L. nitidus*))) (BS=99.4), while in the
314 protein-coding tree (Figure S4) relationships are (*L. nigroviridis* (*L. fuscus* (*L. monticola*, *L.*
315 *nitidus*))) (BS=82.9). In the combined tree as well as in the UCE tree (Figure 4B, S3), *L.*
316 *atacamensis* and *L. isabelae* form a clade that is sister to that formed by all other species; within
317 the later *L. t. tenuis* is sister to the other species. Meanwhile, in the protein coding tree *L.*
318 *atacamensis* and *L. isabelae* are also sister to all other species (BS=50), but the clade formed by
319 *L. paulinae* + *L. pictus* is sister to the remaining species.

320

321 *Gene-tree summary method.* Overall, ASTRAL inferred the same relationships as the
322 concatenated full data set (Figure 4A, C). The key difference is the placement of *L. t. tenuis,*
323 which is sister to *L. monticola* in the UCE (BS=56) (Figure S5) and combined trees (BS=41)
324 (Figure 4C, S5), while the protein-coding tree (Figure S6) supports *L. t. tenuis* as sister to *L. t.*
325 *punctatissimus* (BS=67). The protein-coding tree also supports a (*L. fuscus* (*L. nitidus* (*L.*
326 *monticola*, *L. nigroviridis*))) clade (BS=88). The protein-coding tree does not support the clade
327 of *L. atacamensis* and *L. isabelae.*

328

329 **Discussion**

330 Studies of reptile systematics are starting to make the transition to a phylogenomic approach
331 (Leaché et al., 2015; Grismer et al., 2016; Streicher & Wiens, 2016). Until now, phylogenetic
332 studies of liolaemid lizards have been based on a single locus, or a few nuclear loci (Schulte et
333 al., 2000; Troncoso-Palacios et al., 2015; but see Avila et al., 2015; Olave et al., 2015). Including
334 multiple loci in phylogenetics is advantageous, because it helps attain the statistical power that is
335 typically necessary for resolving discordances that arise among independent loci (Edwards,
336 2009). This is the first study applying phylogenomic data to generate a genome-scale phylogeny
337 for *Liolaemus*.

338

339 The relatively poor understanding of the evolutionary history of *Liolaemus* has its root in many
340 factors, including phylogenetic analyses with incomplete taxon sampling, the use of few markers
341 in molecular-based studies, and the discordance between morphological and DNA-based
342 groupings (e.g. Lobo, 2005 vs. Schulte et al., 2000). In this study, we focused on the subgenus
343 *Liolaemus* by studying a sample of species allocated to its two sections, the *nigromaculatus* and
344 *chiliensis* sections, and to seven species groups (*sensu* Abdala & Quinteros, 2013; Troncoso-
345 Palacios et al., 2015; see Table 1). Despite the fact that our sample of species is modest relative
346 to the real species richness of the subgenus, our sampling is still adequate for demonstrating that
347 several sections or species groups are not monophyletic.

348 We conducted a comparison of the UCEs and protein-coding genes to determine the
349 utility of different types of markers on phylogenetic inference. Studies that have assessed the
350 efficacy of UCEs have suggested that they can provide high levels of phylogenetic

351 informativeness (Blaimer et al., 2015). Overall, our results indicate that bootstrap values are
352 generally higher when using 540 UCEs versus ~40 protein-coding genes. However, on average,
353 the protein-coding genes contained more variation (2.1% versus 0.7% variation) compared to
354 UCE's (Table 4). Many of the UCEs had few or no informative sites (Figure 1). This is not
355 surprising as UCEs are not expected to exhibit high variation at the core region, but variation
356 increases based on distance from the center of the UCE (Faircloth et al., 2012). As our UCE
357 reads were relatively short (<400 bp; Table 4), they do not show much variation. However, the
358 UCEs outnumbered the protein-coding genes by 10-fold. Therefore, this problem of low-
359 information content in individual loci can be partially alleviated by sampling more loci (Xi, Liu
360 & Davis, 2015), which illustrates a key advantage of using UCE for phylogenetic inference
361 (Blaimer et al., 2015; Gilbert et al., 2015).
362
363 Overall, the subsets of the data that we analyzed (UCEs and protein-coding genes, and the
364 combined data) supported the same phylogeny regardless of the analytical approach
365 (concatenation, quartet-based or gene-tree based methods). The only inconsistency in topology
366 appeared in the protein-coding tree inferred with ASTRAL, where the clade containing *L. platei*,
367 *L. velosoi*, *L. nigromaculatus*, *L. zapallarensis*, *L. monticola*, *L. nitidus*, *L. fuscus* and *L.*
368 *nigroviridis* appeared more closely related to *L. pictus* + *L. paulinae* than to *L. t. punctatissimus*
369 + *L. sp.* (Figure S6); nevertheless, this relationship shows low support (BS=37). The fact that we
370 obtain consistent results across a variety of datasets and methodological approaches provides
371 evidence that phylogenomic data can effectively be used to provide robust phylogenies in
372 *Liolaemus* lizards. Nevertheless, some aspects of the phylogeny remain unresolved; for example,
373 the relationships between *L. fuscus, L. monticola, L. nitidus and L. nigroviridis*. These four
374 species form a well-supported clade (Table 5), but relationships among them are unclear. It was
375 earlier suggested that *Liolaemus* underwent a rapid radiation (Olave et al., 2015; Pincheira-
376 Donoso, Harvey & Ruta, 2015) and this could cause some relationships to be difficult to resolve;
377 further studies with an even denser taxon or character sampling may prove useful to fully resolve
378 these relationships, or perhaps they represent a hard polytomy (Suh, 2016; Olave et al., 2016).
379
380 Below, we briefly describe our findings within each of the sampled species groups that contained
381 at least two sampled species. It should be kept in mind that these inferences are based on a small

number of species, although representative of several groups within *Liolaemus*, they are thus a starting point from which to keep building a robust phylogeny and at the end a natural classification of *Liolaemus*.

The *chiliensis* section: the *alticolor-bibronii* species group (Espinoza, Wiens & Tracy, 2004; Lobo 2005; Lobo, Espinoza & Quinteros, 2010; Quinteros, 2012). This group is represented by species occurring mainly along the Andes in Argentina, Bolivia, Chile, and Peru (Quinteros, 2012). There have been frequent changes in the taxonomic composition of this group; initially, it contained 10 species (Espinoza, Wiens & Tracy, 2004), a number that has grown ever since (Lobo, 2005: 12 species; Lobo, Espinoza & Quinteros, 2010: 22 species; Quinteros, 2012: 24 species) to currently reach 26 species (Table S1). Our results showed that the *alticolor-bibronii* species group is polyphyletic, as *L. paulinae* and *L. fuscus* are not sister to each other. *Liolaemus paulinae* is supported as sister of *L. pictus* (see Figure 4 and Supplementary Material). It needs to be considered that many species of this group were not included in the analyses, and as such the clade formed by these geographically distant species, *L. paulinae* from desert areas of northern Chile, and *L. pictus* from austral *Notophagus* forests (Pincheira-Donoso & Nuñez 2005), may be the result of this incomplete taxonomic coverage. On the other hand, *L. fuscus* is consistently recovered in a clade containing also *L. monticola*, *L. nigroviridis* and *L. nitidus*, a relationship that has been previously suggested in earlier studies with mitochondrial markers (e.g. Schulte et al., 2000) and is further confirmed here.

The *chiliensis* section: the *pictus* species group (Cei, 1986). This group, as currently defined, includes species from Argentina and Chile. Of its five species, we have included two, *L. pictus* and *L. tenuis*; the latter was represented by three forms that could represent distinct species (Muñóz-Mendoza et al., submitted). As mentioned before for the *alticolor-bibronii* group, *L. pictus* is consistently placed and with strong support as sister to *L. paulinae*. Therefore, *L. pictus*, *L. t. tenuis*, *L. t. punctatissimus* and *L. sp.* fail to form a monophyletic group; the *pictus* group is then retrieved as polyphyletic in our analyses (Figure 4). The position of *Liolaemus tenuis s. s.* within the radiation of *Liolaemus* remains unresolved, as it changes according to the data set and approach used (see Figure 4 and Supplementary Material). Only once, in the species tree inferred

using ASTRAL with protein-coding genes, *L. t. tenuis* was recovered as related to its two presumably closely related forms, *L. t. punctatissimus* and *L. sp.* (Figure S6), which in turn are retrieved as well- supported sister species in every analysis. However, it should be noted that the data generated for *Liolaemus tenuis s. s.* is minimal consisting of only two protein-coding genes and seven UCEs). As such, the phylogenetic position of *L. t. tenuis* remains unresolved, awaiting the generation and analysis of additional data. In addition, species delimitation analyses such as BPP (Yang & Rannala, 2010) on the base of multiple loci and multiple individuals from each lineage are been undertaken (Panzera et al., unpublished data) to test if *L. t. punctatissimus* and *L. sp.* constitute one single entity or two sister species.

The *nigromaculatus* section: the *nigromaculatus* species group. This species group has a complex taxonomic history. To our knowledge, there is only one DNA-based phylogenetic study focused on the group, which is based on mitochondrial DNA (Troncoso-Palacios et al., 2015). Most of the species from the *nigromaculatus* species group included in our study occur in sympatry. *Liolaemus nigromaculatus* and *L. velosoi* are found in the Atacama Region (Troncoso-Palacios & Garín, 2013; Troncoso-Palacios, 2014), while *L. platei* and *L. atacamensis* are present in the Atacama and Coquimbo Regions (Pincheira-Donoso & Núñez, 2005), and *L. zapallarensis* occurs in the Valparaíso and Coquimbo Regions (Pincheira-Donoso & Núñez, 2005). *Liolaemus atacamensis* and *L. zapallarensis* were first described as subspecies of *L. nigromaculatus* (Müller & Hellmich, 1933). In our analyses, four out of the five species from the *nigromaculatus* group analyzed always form a well-supported clade (Figure 4). *L. atacamensis*, which does not group with either *L. zapallarensis* or *L. nigromaculatus*, appears as sister to *L. isabelae*, far from the other species of the *nigromaculatus* species group. Therefore, although we recovered most of the species of this group forming a clade, the *nigromaculatus* species group is polyphyletic. This result is unexpected since previous analyses based on mitochondrial DNA have always found that *L. atacamensis* and *L. nigromaculatus* are sister species, and morphologically, these species are highly similar (Troncoso-Palacios & Garín, 2013). Nevertheless, is not unlikely that inferences based on a single marker of mitochondrial nature versus >500 nuclear markers support a different tree; the central tendency of multiple gene trees underlying the species tree cannot be known before having sampled multiple genes (Maddison, 1997). Troncoso-Palacios et al. (2015) recovered a *nigromaculatus* species group with strong

support (posterior probability = 0.99) but they have not considered as part of this group some species considered by Pincheira-Donoso and Nuñez (2005) as part of the *platei* group and that were included in our study. In the same study, authors found that *L. atacamensis* is sister to *L. nigromaculatus*, and this group is sister to *L. zapallarensis* and two species not included here.. This clade is sister to a clade comprising *L. isabelae*, *L. pseudolemniscatus* (not included in this study) and species from the "*platei* group" (*L. velosoi* + *L. platei*). In our study, *L. zapallarensis* and *L. nigromaculatus* are sister species, which are in turn sister of the aforementioned grouping (which is consistent with previous studies; e.g., Troncoso-Palacios & Garín, 2013). Nevertheless, and as previously mentioned, *L. atacamensis* is supported as sister to *L. isabelae* and not closely related to the "*platei*" group. In summary, our results support the *nigromaculatus* group if *L. atacamensis* is excluded from this group.

The *nigromaculatus* section: the *nigroviridis* species group. Species of the *nigroviridis* group are distributed in the highlands of central and northern Chile (Pincheira-Donoso & Núñez, 2005). Most species have a complex taxonomic history (a detailed summary can be found in Troncoso-Palacios et al., 2016), which has been difficult to untangle due to the lack of genetic information for most of them. Our trees show that this species group is polyphyletic, as *L. isabelae* and *L. nigroviridis* are not sister species; *L. isabelae* is in the majority of the topologies sister to *L. atacamensis*, while *L. nigroviridis* is recovered consistently in a clade comprising *L. monticola*, *L. fuscus* and *L. nitidus.* These four species overlap in their distributions in the Chilean Central Valley. The most widespread species are *L. fuscus* (*alticolor-bibronii* group) and *L. nitidus* (*robertmertensi* group), which can be found from the Atacama Region (~27º S) south to the Biobío Region (~36º S) (Troncoso-Palacios 2014). *Liolaemus nigroviridis* and *L. monticola* (*monticola* group) have a more restricted distribution, as the former species occurs in central Chile between latitudes ca. 30° to 34° S and above 1,100 meters, in both the Andean and Coastal mountains (Cianferoni et al., 2013 and references therein), while the latter is found in the San Francisco valley (32° 22′ S, 70° 25′ W) at 1,700 m and also in coastal and transversal mountain ranges (33° S) between 600 and 1800 m in central Chile (Torres-Pérez et al., 2009 and references therein). The relationships among these four species are not fully resolved; in most cases, *L. monticola* and *L. nitidus* appear to be sister species, while, as suggested by previous studies (Schulte et al., 2000; Schulte & Moreno-Roark, 2010; Guerrero et al., 2013; Pyron, Burbrink &

Wiens, 2013; Schulte, 2013; Troncoso-Palacios et al., 2015), *L. fuscus* and *L. nigroviridis* are sister to each other. Nevertheless, as the position among these four species varies with the dataset and method used (see Supplementary Material), we cannot draw strong conclusions from these results.

Many authors, based in morphological data, have placed *L. isabelae* in the *nigroviridis* group (Lobo, 2005; Pincheira-Donoso & Núñez, 2005; Lobo, Espinoza & Quinteros, 2010), while previous DNA-based analyses do not support this placement, questioning the affiliation of *L. isabelae* to the *nigroviridis* group (Schulte & Moreno-Roark, 2010; Troncoso-Palacios et al., 2016). Therefore, our phylogenomic study supports previous DNA based studies, suggesting a large amount of convergence in morphological character states between *L. isabelae* and *L. nigroviridis*. It is of interest to see if the suggested convergence is due to chance or promoted by living in similar highland habitats.

Regarding relationships among species groups, results also supports a sister relationship between the *monticola* and *nigroviridis* groups, and of this clade with the *nigromaculatus* group. Nevertheless, these results could change as species from other groups are included in the analysis.

Conclusions

As mentioned before, among the main challenges of *Liolaemus* systematics is that all studies have partial taxonomic coverage. Our phylogenomic scale study failed to support the monophyly of most species groups, suggesting that morphological convergence has hampered an adequate classification in liolaemid lizards and that a reappraisal of groupings is necessary. A DNA-based study with broader taxonomic coverage should help redefine species groups in order for them to be natural. Important will be to include species for which there are no DNA sequences available, such as *L. juanortizi*, *L. lorenzmuelleri* and *L. melaniceps*. This could be an important step in resolving the still unclear systematics of *Liolaemus*.

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References

Abdala, C. S. & Quinteros, A. S. (2013). Los últimos 30 años de estudios de la familia de lagartijas más diversa de Argentina. Actualización taxonómica y sistemática de Liolaemidae. *Cuadernos de Herpetología*, 28, 1-28.

Alföldi, J., Di Palma, F., Grabherr, M., Williams, C., Kong, L., Mauceli, E., Russell, P., Lowe, C.B., Glor, R.E., Jaffe, J.D., Ray, D.A., Boissinot, S., Shedlock, A.M., Botka, C., Castoe, T.A., Colbourne, J.K., Fujita, M.K., Godinez-Moreno, R., Hallers, BF, Haussler, D, Heger, A, Heiman, D., Janes, D.E., Johnson, J., de Jong, P.J., Koriabine, M.Y., Lara, M., Novick, P.A., Organ, C.L., Peach, S.E., Poe, S., Pollock, D.D., de Queiroz, K., Sanger, T., Searle, S., Smith, J.D., Smith, Z., Swofford, R., Turner-Maier, J., Wade, J., Young, S., Zadissa, A., Edwards, S.V., Glenn, T.C., Schneider, C.J., Losos, J.B., Lander ,E.S., Breen, M., Ponting, C.P. & K. Lindblad-Toh. (2011). The genome of the green anole lizard and a comparative analysis with birds and mammals. *Nature,* 477, 587-591.

Avila, L. J., Olave, M., Perez, C. H. F., Perez, D. R. & Morando, M. (2013). Molecular phylogenetic relationships of the *Liolaemus rothi* complex and a new species of lizard from Auca Mahuida Volcano (Squamata: Liolaemini).*Zootaxa*, 3608, 221-238.

Avila, L. J., Medina, C. D., Pérez, C. H. F., Sites Jr, J. W.& Morando, M. (2015). Molecular phylogenetic relationships of the lizard clade *Liolaemus elongatus* (Iguania: Liolaemini) with the description of a new species from an isolated volcanic peak in northern Patagonia. *Zootaxa*, 3947, 067-084.

533 Bejerano, G., Pheasant, M., Makunin, I., Stephen, S., Kent, W. J., Mattick, J. S. & Haussler, D.
534 (2004). Ultraconserved elements in the human genome. *Science*, 304, 1321-1325.

535 Blaimer, B. B., Brady, S. G., Schultz, T. R., Lloyd, M. W., Fisher, B. L.& Ward, P. S. (2015).
536 Phylogenomic methods outperform traditional multi-locus approaches in resolving deep
537 evolutionary history: a case study of formicine ants. *BMC evolutionary biology*, 15, 1.

538 Bolger, A.M., Lohse, M. & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina
539 sequence data. *Bioinformatics*, 30, 2114–20.

540 Brelsford, A., Collin, H., Perrin, N. & Fumagalli, L. (2012). Nonspecific PCR amplification by
541 high-fidelity polymerases: implications for next-generation sequencing of AFLP markers.
542 *Molecular Ecology Resources*, 12, 123-127.

543 Camargo, A., Sinervo, B. & Sites, Jr. J. W. (2010). Lizards as model organisms for linking
544 phylogeographic and 498 speciation studies. *Molecular Ecology*, 19, 3250–3270.

545 Camargo, A., Avila, L.J., Morando M. & Sites, Jr. J. W. (2012). Accuracy and precision in
546 species tree estimation: an empirical evaluation of performance in lizards of the
547 *Liolaemus darwinii* group (Squamata: Liolaemidae) under varying sub-sampling designs.
548 *Systematic Biology*, 61,272-288.

549 Carothers, J.H., Fox, S.F, Marquet, P.A. & Jaksic, F.M. (1997). Thermal characteristics of ten
550 Andean lizards of the Chilean genus *Liolaemus.Revista Chilena de Historia Natural*, 70,
551 297–309.

552 Cei, J. M. (1986). *Reptiles del centro, centro-oeste y sur de la Argentina: herpetofauna de las*
553 *zonas áridas y semiáridas*. Torino: Museo Regionale di Scienze Naturali.

554 Cei, J. M. (1993). *Reptiles del noroeste, nordeste y este de la Argentina: Herpetofauna de las*
555 *selvas subtropicales, Puna y Pampas*.Torino: Museo Regionale di Scienze Naturali.

556 Chifman, J. & Kubatko, L. (2014). Quartet inference from SNP data under the coalescent model.
557 *Bioinformatics*, 30, 3317-3324.

558 Chou, J., Gupta, A., Yaduvanshi, S., Davidson, R., Nute, M., Mirarab, S. & Warnow, T. (2015).
559 A comparative study of SVDquartets and other coalescent-based species tree estimation
560 methods. *BMC genomics*, 16, 1.

561 Cianferoni, F., Yanez, R. P., Palma, R. E., Garin, C. F. & Torres-Perez, F. (2013). Deep
562 divergences within *Liolaemus nigroviridis* (Squamata, Liolaemidae) lineages associated
563 with sky islands in central Chile. *Zootaxa*, 3619, 59-69.

564 Crawford, N.G., Faircloth, B.C., McCormack, J.E., Brumfield, R.T., Winker, K. & Glenn, T.C.
565 (2012). More than 1000 ultraconserved elements provide evidence that turtles are the
566 sister group to archosaurs. *Biology Letters*, 8,783–786.

567 Degnan, J. H., & Salter, L. A. (2005). Gene tree distributions under the coalescent process.
568 *Evolution*, 59(1), 24-37.

569 Díaz Gómez, J. M. & Lobo, F. (2006). Historical biogeography of a clade of *Liolaemus* (Iguania:
570 Liolaemidae) based on ancestral areas and dispersal-vicariance analysis (DIVA). *Papéis*
571 *Avulsos de Zoologia (São Paulo),* 46, 261-274.

572 Donoso-Barros, R. (1966). *Reptiles of Chile*. Santiago: Editorial Universitaria.

573 Edwards, S. V., Liu, L. & Pearl, D. K. (2007). High-resolution species trees without
574 concatenation. *Proceedings of the National Academy of Sciences*, 104, 5936-5941.

575 Edwards, S.V. (2009). Is a new and general theory of molecular systematics emerging?
576 *Evolution*, 63, 1–19.

577 Espinoza, R. E., Wiens, J. J. & Tracy, C. R. (2004). Recurrent evolution of herbivory in small,
578 cold-climate lizards: breaking the ecophysiological rules of reptilian herbivory.
579 *Proceedings of the National Academy of Sciences of the United States of America*, 101,
580 16819-16824.

581 Faircloth, B. C. (2015). PHYLUCE is a software package for the analysis of conserved genomic
582 loci. *Bioinformatics*, btv646.

583 Faircloth, B.C., McCormack, J.E., Crawford, N.G., Harvey, M.G., Brumfield, R.T. & Glenn,
584 T.C. (2012). Ultraconserved elements anchor thousands of genetic markers spanning
585 multiple evolutionary timescales. *Systematic Biology*, 61,717–726.

586 Flicek, P. & Birney, E. (2009). Sense from sequence reads: methods for alignment and assembly.
587 *Nature Methods*, 6, S6–S12.

588 Fontanella, F. M., Olave, M., Avila, L. J. & Morando, M. (2012). Molecular dating and
589 diversification of the South American lizard genus *Liolaemus* (subgenus *Eulaemus*) based
590 on nuclear and mitochondrial DNA sequences. *Zoological Journal of the Linnean*
591 *Society*, 164, 825-835.

592 Formas, J. R. (1979). La herpetofauna de los bosques temperados de Sudamérica. In W.E.
593 Duellmann (Ed) *The South American herpetofauna: its origin, evolution and dispersal*
594 (pp. 341-369). Kansas: Museum of Natural History, University of Kansas.

595 Gilbert, P.S., Chang, J., Pan, C., Sobel, E.M., Sinsheimer, J.S., Faircloth, B.C. & Alfaro, M.E.
596 (2015). Genome-wide ultraconserved elements exhibit higher phylogenetic
597 informativeness than traditional gene markers in percomorph fishes. *Molecular*
598 *Phylogenetics and Evolution*; 92, 140–146.

599 Gnirke, A., Melnikov, A., Maguire, J., Rogov, P., LeProust, E. M., Brockman, W., Fennell, T.,
600 Giannoukos, G., Fisher, S., Russ, C., Gabriel, S., Jaffe, D.B., Lander, E.S. & Nusbaum,
601 C. (2009). Solution hybrid selection with ultra-long oligonucleotides for massively
602 parallel targeted sequencing. *Nature biotechnology*, 27, 182-189.

603 Grismer, J. L., Schulte, J. A., Alexander, A., Wagner, P., Travers, S. L., Buehler, M. D., Welton
604 L.J. & Brown, R. M. (2016). The Eurasian invasion: phylogenomic data reveal multiple
605 Southeast Asian origins for Indian Dragon Lizards. *BMC evolutionary biology*, 16, 1.

606 Guerrero, P. C., Rosas, M., Arroyo, M. T. & Wiens, J. J. (2013). Evolutionary lag times and
607 recent origin of the biota of an ancient desert (Atacama–Sechura). *Proceedings of the*
608 *National Academy of Sciences*, 110, 11469-11474.

609 Harris, R.S. (2007). Improved pairwise alignment of genomic data. University of Pennsylvania,
610 PhD Dissertation.

611 Harmon, L. J., Schulte, J. A., Larson, A. & Losos, J. B. (2003). Tempo and mode of evolutionary
612 radiation in iguanian lizards. *Science*, 301(5635), 961-964.

613 Ibargüengoytía, N.R. & Cussac, V.E. (2002). Body temperatures of two viviparous *Liolaemus*
614 lizard species, in Patagonian rain forest and steppe. *Herpetological Journal*, 12, 131–134.

615 Katoh, K. & Standley, D.M. (2013). MAFFT multiple sequence alignment software version 7:
616 improvements in performance and usability. *Molecular Biology and Evolution*, 30,772–
617 780.

618 Koslowsky, J. (1898). Enumeración sistemática y distribución geográfica de los reptiles
619 argentinos. *Revista del Museo de La Plata*, 8, 161–200.

620 Lanier, H.C., Huang, H.T. & Knowles, L.L. (2014). How low can you go? The effects of
621 mutation rate on the accuracy of species-tree estimation. *Molecular Phylogenetics and*
622 *Evolution*, 70, 112-119.

623 Laurent, R.F. (1985). Segunda contribución al conocimiento de la estructura taxonómica del
624 género *Liolaemus* Wiegmann (Iguanidae). *Cuadernos de Herpetología*, 1, 1–37.

625 Leaché, A. D. & Rannala, B. (2011). The accuracy of species tree estimation under simulation: a
626 comparison of methods. *Systematic biology*, syq073.

627 Leaché, A. D., Chavez, A. S., Jones, L. N., Grummer, J. A., Gottscho, A. D. & Linkem, C. W.
628 (2015). Phylogenomics of phrynosomatid lizards: conflicting signals from sequence
629 capture versus restriction site associated DNA sequencing. *Genome Biology and*
630 *Evolution*, 7, 706-719.

631 Liu, L., Yu, L. & Edwards, S. V. (2010). A maximum pseudo-likelihood approach for estimating
632 species trees under the coalescent model. *BMC evolutionary biology*, 10, 302.

633 Lobo, F. (2001). A phylogenetic analysis of lizards of the *Liolaemus chiliensis* group (Iguania:
634 Tropiduridae). *Herpetological Journal*, 11,137-150.

635 Lobo, F. (2005). Las relaciones filogenéticas dentro del grupo *chiliensis* (Iguania: Liolaemidae:
636 *Liolaemus*): sumando nuevos caracteres y taxones. *Acta zoológica lllloana*, 49, 65-87.

637 Lobo, F., Espinoza, R. E. & Quinteros, S. (2010). A critical review and systematic discussion of
638 recent classification proposals for liolaemid lizards. *Zootaxa*, 2549, 1-30.

639 Maddison, W. P. (1997). Gene trees in species trees. *Systematic Biology*, 46(3), 523-536.

640 Maddison, W. P., & Knowles, L. L. (2006). Inferring phylogeny despite incomplete lineage
641 sorting. *Systematic Biology*, 55(1), 21-30.

642 Mayr, E. & Ashlock, P. (1991). *Principles of Systematic Zoology*. New York: McGraw-Hill.

643 McCormack, J. E., Faircloth, B. C., Crawford, N. G., Gowaty, P. A., Brumfield, R. T. & Glenn,
644 T. C. (2012). Ultraconserved elements are novel phylogenomic markers that resolve
645 placental mammal phylogeny when combined with species-tree analysis. *Genome*
646 *Research*, 22, 746-754.

647 McCormack J.E., Harvey M.G., Faircloth B.C., Crawford N.G., Glenn T.C. & Brumfield R.T. A.
648 (2013). Phylogeny of birds based on over 1,500 loci collected by target enrichment and
649 highthroughput sequencing. *PLoS One*, 8, e54848.

650 Meiklejohn, K. A., Faircloth, B. C., Glenn, T. C., Kimball, R. T. & Braun, E. L. (2016). Analysis
651 of a Rapid Evolutionary Radiation Using Ultraconserved Elements: Evidence for a Bias
652 in Some Multispecies Coalescent Methods. *Systematic biology*, syw014.

653 Mirarab, S., Reaz, R., Bayzid, M.S., Zimmermann, T., Swenson, M.S. & Warnow, T. (2014).
654 ASTRAL: Genome-scale coalescent-based species tree estimation. *Bioinformatics*, 30,
655 i541- i548.

656 Morando, M. (2004). Sistemática y filogenia de grupos de especies de los géneros *Phymaturus* y
657 *Liolaemus* (Squamata: Tropiduridae: Liolaeminae) del oeste y sur de Argentina.
658 Unpublished D. Phil. Thesis, Universidad Nacional de Tucumán, Argentina.

659 Morando M., Avila, L. J. & Sites, J. W. (2003). Sampling strategies for delimiting species:
660 genes, individuals, and populations in the *Liolaemus elongatus-kriegi* complex
661 (Squamata: Liolaemidae) in Andean–Patagonian South America. *Systematic Biology*, 52,
662 159-185.

663 Morando, M., Avila, L. J., Baker, J. & Sites, J. W. (2004). Phylogeny and phylogeography of the
664 *Liolaemus darwinii* complex (Squamata: Liolaemidae): evidence for introgression and
665 incomplete lineage sorting. *Evolution*, 58, 842-859.

666 Morando, M., Avila, L. J., Turner, C. R., & Sites, J. W. (2007). Molecular evidence for a species
667 complex in the patagonian lizard *Liolaemus bibronii* and phylogeography of the closely
668 related *Liolaemus gracilis* (Squamata: Liolaemini). *Molecular Phylogenetics and*
669 *Evolution*, 43(3), 952-973.

670 Núñez, H., Schulte Ii, J. A. & Garín, C. (2001). *Liolaemus josephorum*, nueva especie de
671 lagartija para el *Norte de Chile. Boletín del Museo de Historia Natural*, 50, 91-107.

672 Olave, M., Martinez, L. E., Ávila, L. J., Sites, J. W. & Morando, M. (2011). Evidence of
673 hybridization in the Argentinean lizards *Liolaemus gracilis* and *Liolaemus bibronii*
674 (Iguania: Liolaemini): an integrative approach based on genes and
675 morphology. *Molecular Phylogenetics and Evolution*, 61, 381-391.

676 Olave, M., Avila, L. J., Sites, J. W. & Morando, M. (2014). Multilocus phylogeny of the widely
677 distributed South American lizard clade *Eulaemus* (Liolaemini, *Liolaemus*). *Zoologica*
678 *Scripta*,43, 323-337.

679 Olave, M., Avila, L. J., Sites, J. W.& Morando, M. (2015). Model-based approach to test hard
680 polytomies in the *Eulaemus* clade of the most diverse South American lizard genus
681 *Liolaemus* (Liolaemini, Squamata). *Zoological Journal of the Linnean Society*, 174, 169-
682 184.

683 Olave, M., Avila, L. J., Sites, J. W. & Morando, M. (2016). Hidden diversity within the lizard
684 genus *Liolaemus*: Genetic vs morphological divergence in the *L. rothi* complex
685 (Squamata: Liolaeminae). *Molecular Phylogenetics and Evolution*, 107, 56-63.

686 Ortiz, J.C. (1981). Estudio multivariado de las especies de *Liolaemus* del grupo *nigromaculatus*
687 (Squamata, Iguanidae). *Anales del Museo de Historia Natural de Valparaíso*, 14, 247-
688 265.

689 Pattengale, N. D., Alipour, M., Bininda-Emonds, O.R., Moret, B.M. & Stamatakis, A. (2010).
690 How many bootstrap replicates are necessary? *Journal of Computational Biology,* 17,
691 337–354.

692 Peng, Y., Leung, H. C., Yiu, S. M. & Chin, F. Y. (2012). IDBA-UD: a de novo assembler for
693 single-cell and metagenomic sequencing data with highly uneven depth. *Bioinformatics*,
694 28, 1420-1428.

695 Pincheira-Donoso, D. & Núñez, H. (2005). Las especies chilenas del género *Liolaemus*
696 Wiegmann, 1834 (Iguania Tropiduridae: Liolaeminae). Taxonomía, sistemática y
697 evolución. *Publicación ocasional del Museo Nacional de Historia Natural,* 59, 7-486.

698 Pincheira-Donoso, D., Harvey, L. P., & Ruta, M. (2015). What defines an adaptive radiation?
699 Macroevolutionary diversification dynamics of an exceptionally species-rich continental
700 lizard radiation. *BMC evolutionary biology*, 15, 1.

701 Portik, D. M., Smith, L. L. & Bi, K. (2016). An evaluation of transcriptome-based exon capture
702 for frog phylogenomics across multiple scales of divergence (Class: Amphibia, Order:
703 Anura). *Molecular Ecology Resources*, 16, 1069-1083.

704 Posada, D. (2008). jModelTest: phylogenetic model averaging. *Molecular Biology and*
705 *Evolution*, 25, 1253-1256.

706 Pyron, A.R, Burbrink, F.T. & Wiens, J.J. (2013). A phylogeny and revised classification of
707 Squamata, including 4161 species of lizards and snakes. *BMC Evolutionary Biology*, 13,
708 93.

709 Pyron, R. A., Hendry, C. R., Chou, V. M., Lemmon, E. M., Lemmon, A. R., & Burbrink, F. T.
710 (2014). Effectiveness of phylogenomic data and coalescent species-tree methods for
711 resolving difficult nodes in the phylogeny of advanced snakes (Serpentes: Caenophidia).
712 *Molecular Phylogenetics and Evolution*, 81, 221-231.

713 Quinteros, A. S. (2012). Taxonomy of the *Liolaemus alticolor-bibronii* group (Iguania:
714 Liolaemidae), with descriptions of two new species. *Herpetologica*, 68(1), 100-120.

715 Quinteros, A. S. (2013). A morphology-based phylogeny of the *Liolaemus alticolor–bibronii*
716 group (Iguania: Liolaemidae). *Zootaxa*, 3670, 001-032.

717 Rannala, B. & Yang, Z. (2003). Bayes estimation of species divergence times and ancestral
718 population sizes using DNA sequences from multiple loci. *Genetics*, 164(4), 1645-1656.

719 Ruiz de Gamboa, M. (2016). Lista actualizada de los reptiles de Chile. *Boletín Chileno de*
720 *Herpetología,* 3,7-12.

721 Schulte, J. A. & Moreno-Roark, F. (2010). Live birth among Iguanian lizards predates Pliocene–
722 Pleistocene glaciations. *Biology letters*, 6, 216-218.

723 Schulte, Ii J. A., Macey, J. R., Espinoza, R. E. & Larson, A. (2000). Phylogenetic relationships in
724 the iguanid lizard genus *Liolaemus*: multiple origins of viviparous reproduction and
725 evidence for recurring Andean vicariance and dispersal. *Biological Journal of the*
726 *Linnean Society*, 69, 75-102.

727 Schulte, J. A. (2013). Undersampling taxa will underestimate molecular divergence dates: an
728 example from the South American lizard clade Liolaemini. *International journal of*
729 *evolutionary biology*, 2013, 1-12.

730 Schwarz, G. (1978). Estimating the dimension of a model. The Annals of Statistics, 6, 461-464.

731 Sheldon, K. S., Leaché, A. D. & Cruz, F. B. (2015). The influence of temperature seasonality on
732 elevational range size across latitude: a test using *Liolaemus* lizards *Global Ecology and*
733 *Biogeography*, *24*, 632-641.

734 Suh, A. (2016). The phylogenomic forest of bird trees contains a hard polytomy at the root of
735 Neoaves. *Zoologica Scripta*, *45*(S1), 50-62.

736 Simonetti, J.A., Arroyo, M.T.K., Spotorno, A.E. & Lozada, E. (1995). *Diversidad biológica en*
737 *Chile*. Santiago: Comisión Nacional de Ciencia y Tecnología.

738 Stamatakis, A., Hoover, P. & Rougemont, J. (2008). A rapid bootstrap algorithm for the RAxML
739 web servers. *Systematic Biology*, 57(5), 758-771.

740 Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of
741 large phylogenies. *Bioinformatics*, 30, 1312–1313.

742 Streicher, J. W. & Wiens, J. J. (2016). Phylogenomic analyses reveal novel relationships among
743 snake families. *Molecular Phylogenetics and Evolution*, 100, 160-169.

744 Swofford, D. L. (2002). *PAUP* version 4.0. Phylogenetic analysis using parsimony (and other*
745 *methods).*Sunderland: Sinauer.

746 Torres-Pérez F., Méndez M.A., Benavides E., Moreno R.A., Lamborot M., Palma R.E. & J.C.
747 Ortiz. (2009). Systematics and evolutionary relationships of the mountain lizard
748 *Liolaemus monticola* (Liolaemini): how morphological and molecular evidence
749 contributes to reveal hidden species diversity. *Biological Journal of the Linnean Society,*
750 96, 635–650.

751 Troncoso-Palacios, J. & Garin, C. F. (2013). On the identity of *Liolaemus nigromaculatus*
752 Wiegmann, 1834 (Iguania, Liolaemidae) and correction of its type locality. *ZooKeys*, 294,
753 37.

754 Troncoso-Palacios, J. (2014). Nueva lista actualizada de los reptiles terrestres de la Región de
755 Atacama, Chile. *Boletín Chileno de Herpetología*, 1, 1-4.

756 Troncoso-Palacios, J., Schulte, J. A., Marambio-Alfaro, Y. & Hiriart, D. (2015). Phenotypic
757 variation, phylogenetic position and new distributional records for the poorly known
758 *Liolaemus silvai* Ortiz, 1989 (Iguania: Iguanidae: Liolaemini).*South American Journal of*
759 *Herpetology*, 10, 71-81.

760 Troncoso-Palacios, J., Elorza, A. A., Puas, G. I. & Alfaro-Pardo, E. (2016). A new species of
761 *Liolaemus* related to *L. nigroviridis* from the Andean highlands of Central Chile (Iguania,
762 Liolaemidae). *ZooKeys*, 555, 91.

763 Troncoso-Palacios, J., Diaz, H. A., Puas, G. I., Riveros-Riffo, E., & Elorza, A. A. (2016b). Two
764 new *Liolaemus* lizards from the Andean highlands of Southern Chile (Squamata, Iguania,
765 Liolaemidae). *ZooKeys*, (632), 121.

766 Uetz, P., Freed, P. & Jirí Hošek (2016, October). The Reptile Database. Available via
767 http://www.reptile-database.org.

768 Valladares, P. (2011). Análisis, síntesis y evaluación de la literatura de lagartos de la Región de
769 Atacama, Chile. *Gayana,* 75, 81-98.

770 Vidal, M. A. & Díaz-Páez, H. (2012). Biogeography of Chilean Herpetofauna: Biodiversity
771 Hotspot and Extinction Risk. In L. Stevens (Ed.) *Global Advances in Biogeography* (pp.
772 137-154). INTECH Open Access Publisher.

773 Vidal, M. & Labra, A. (2008). *Herpetología de Chile*. Santiago: GraficAndes.

774 Wiens, J.J., Hutter, C.R., Mulcahy, D.G., Noonan, B.P., Townsend, T.M., Sites, J.W. &
775 Reederet, T.W. (2012). Resolving the phylogeny of lizards and snakes (Squamata) with
776 extensive sampling of genes and species. *Biology Letters*, 8, 1043–1046.

777 Xi Z., Liu L. & Davis, C.C. (2015). Genes with minimal phylogenetic information are
778 problematic for coalescent analyses when gene tree estimation is biased. *Molecular*
779 *Phylogenetics and Evolution*, 92, 63-71.

780 Xu B., & Yang Z. (2016). Challenges in species tree estimation under the multispecies
781 coalescent model. *Genetics* 204, 1353-1368.
782 Yang, Z. & Rannala, B. (2010). Bayesian species delimitation using multilocus sequence data.
783 *Proceedings of the National Academy of Sciences*, 107(20), 9264-9269.
784 Young-Downey, A. R. (1998). Phylogenetic studies on *Liolaemus* (Sauria: Tropiduridae): an
785 interpretation based on molecular data and a biochemical test of a biogeographic
786 hypothesis. University of Miami, PhD Dissertation.
787 Zhang, J., Chiodini, R., Badr, A. & Zhang, G. (2011). The impact of next-generation sequencing
788 on genomics. *Journal of Genetics and Genomics*, 38(3), 95-109.

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Figure 1(on next page)

Properties of the UCEs (panel A) and protein-coding genes (panel B) data set for the 16 taxa used in this study.

Properties of the UCEs data set for the 16 taxa used in this study. Frequency distributions show the (a) number of taxa across alignments; (b) number of informative sites per locus; and (c) alignment length distributions. Informative sites per alignment length (adjusted R^2=0.005577; p=0.04569) (d) are also shown.

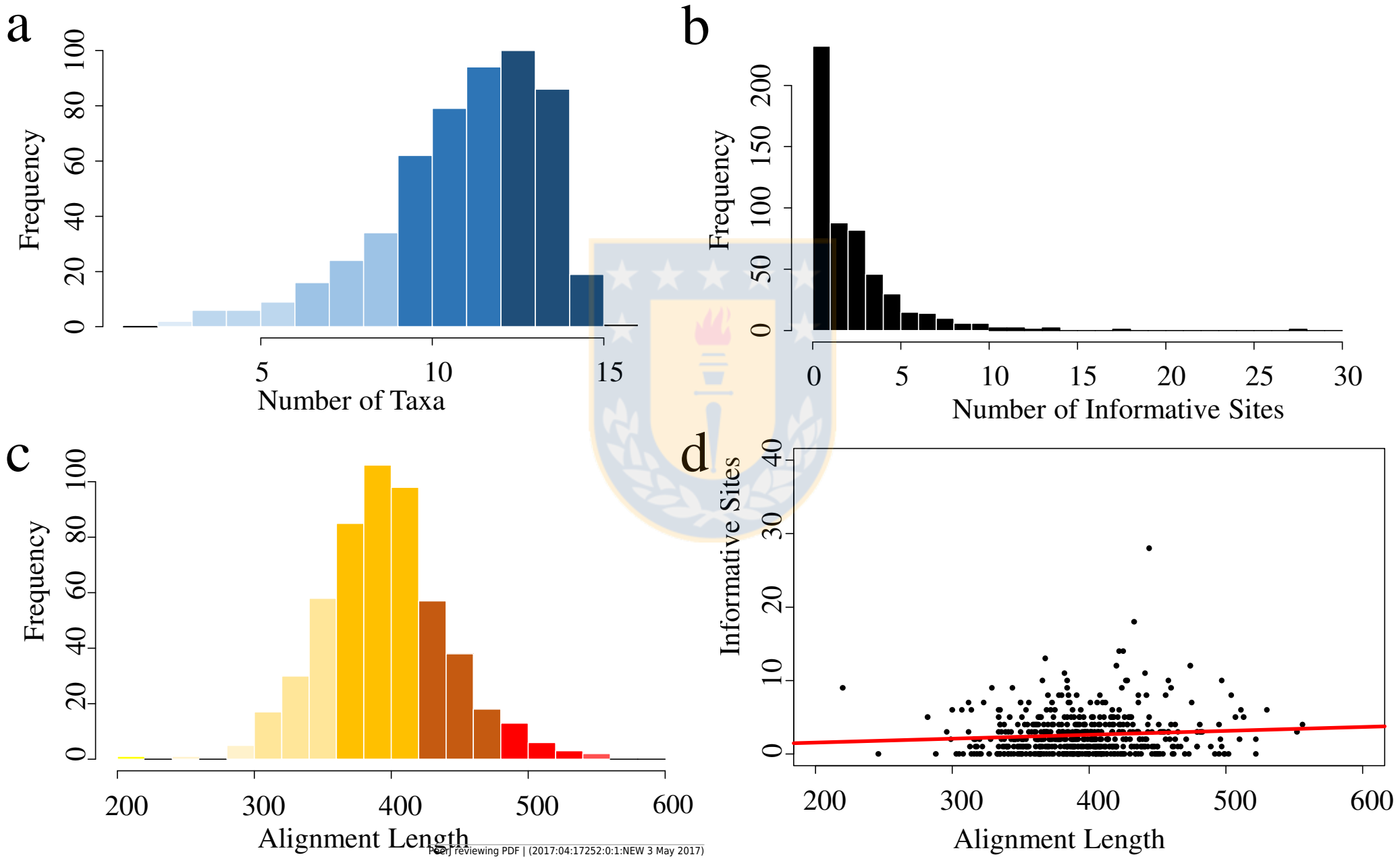
a
Frequency
0
20
40
60
80
100
5
10
15
Number of Taxa
b
Frequency
0
50
100
150
200
0
5
10
15
20
25
30
Number of Informative Sites
c
Frequency
0
20
40
60
80
100
200
300
400
500
600
Alignment Length
d
Informative Sites
0
10
20
30
40
200
300
400
500
600
Alignment Length

Figure 2(on next page)

Properties of the protein-coding gene data set for the 16 taxa used in this study.

Properties of the protein-coding gene data set for the 16 taxa used in this study. Frequency distributions show the (a) number of taxa across alignments; (b) number of informative sites per locus; and (c) alignment length distributions. Informative sites per alignment length (adjusted R^2=-0.02026; p=0.6857) (d) are also shown.

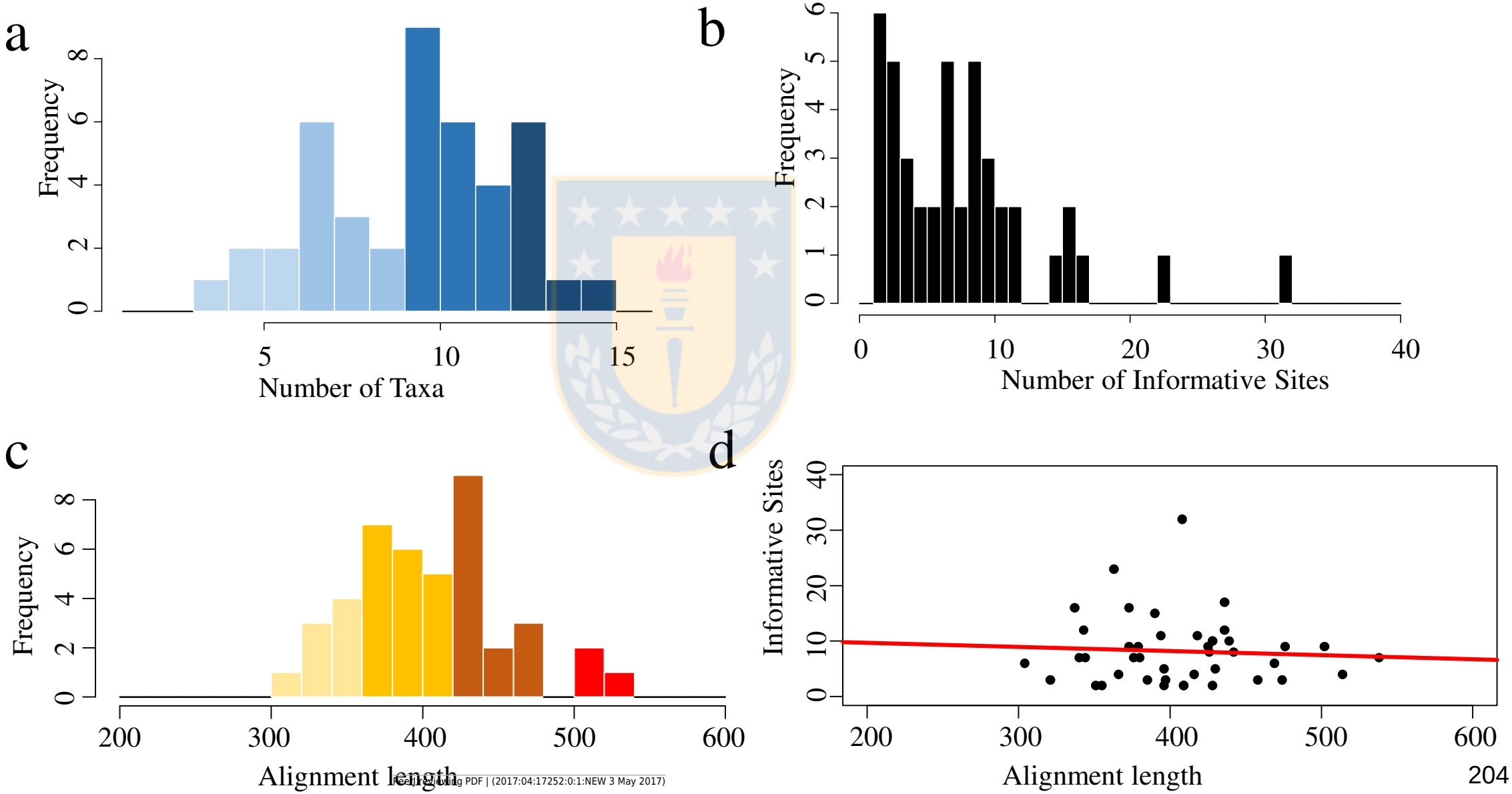
a
Frequency
0
2
4
6
8
5
10
15
Number of Taxa
b
Frequency
0
1
2
3
4
5
6
0
10
20
30
40
Number of Informative Sites
c
Frequency
0
2
4
6
8
200
300
400
500
600
Alignment length
d
Informative Sites
0
10
20
30
40
200
300
400
500
600
Alignment length

Figure 3(on next page)

Frequency of models selected by jmodeltest using the BIC criterion for protein-coding genes (grey) and UCEs (black).

Frequency of models selected by jmodeltest using the BIC criterion for protein-coding genes (grey) and UCEs (black).

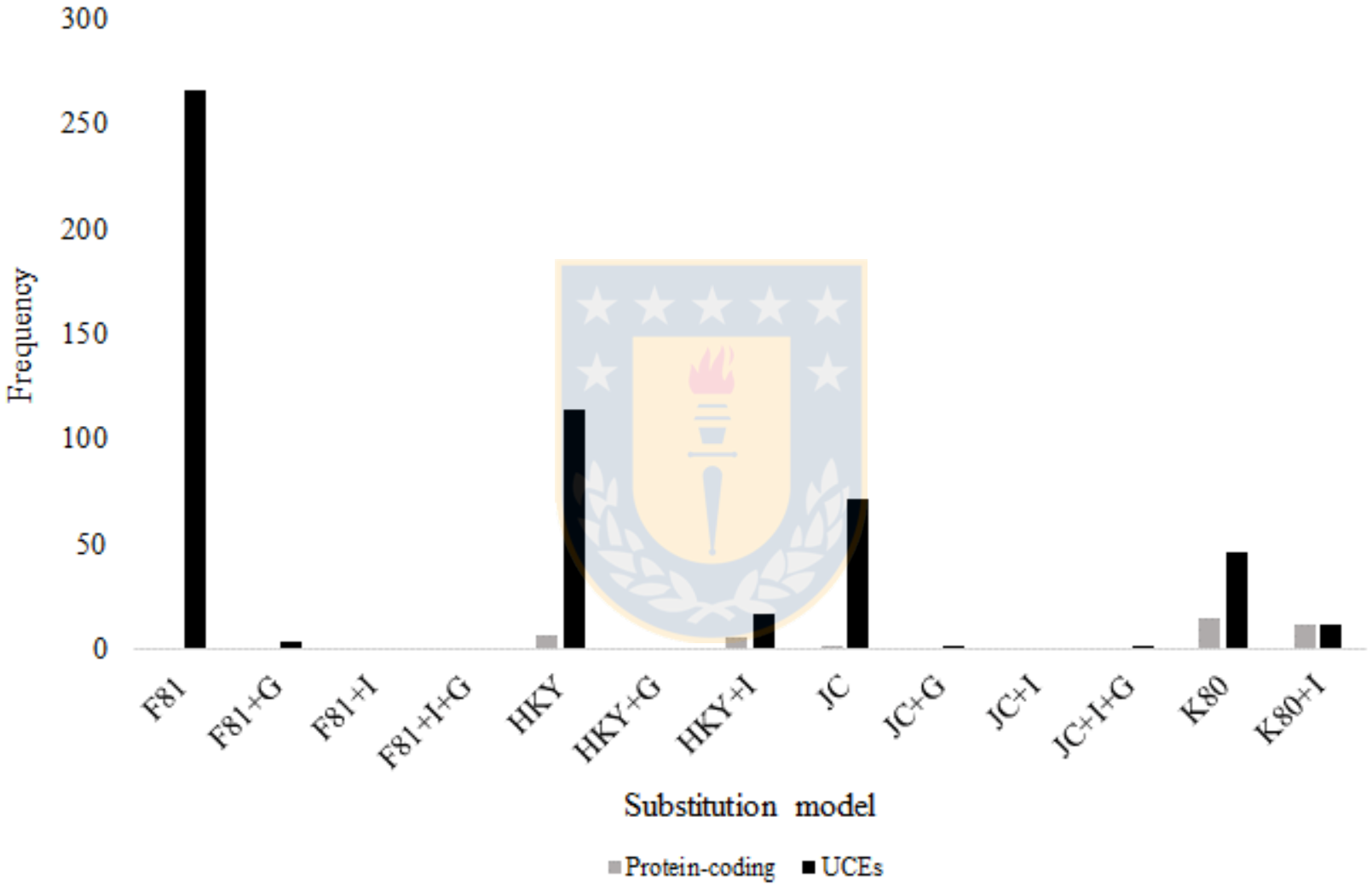
300
250
200
150
100
50
0
Frequency
F81
F81+G
F81+I
F81+I+G
HKY
HKY+G
HKY+I
JC
JC+G
JC+I
JC+I+G
K80
K80+I
Substitution model
Protein-coding
UCEs

Figure 4(on next page)

Phylogenomic relationships among *Liolaemus* lizards from the Chilean groups.

Phylogenomic relationships among *Liolaemus* lizards from the Chilean groups, estimated with sequence capture data using concatenation (A), quartet based (B) and gene-tree based methods (C). The assignation of taxa to species group was done following Abdala & Quinteros (2013).

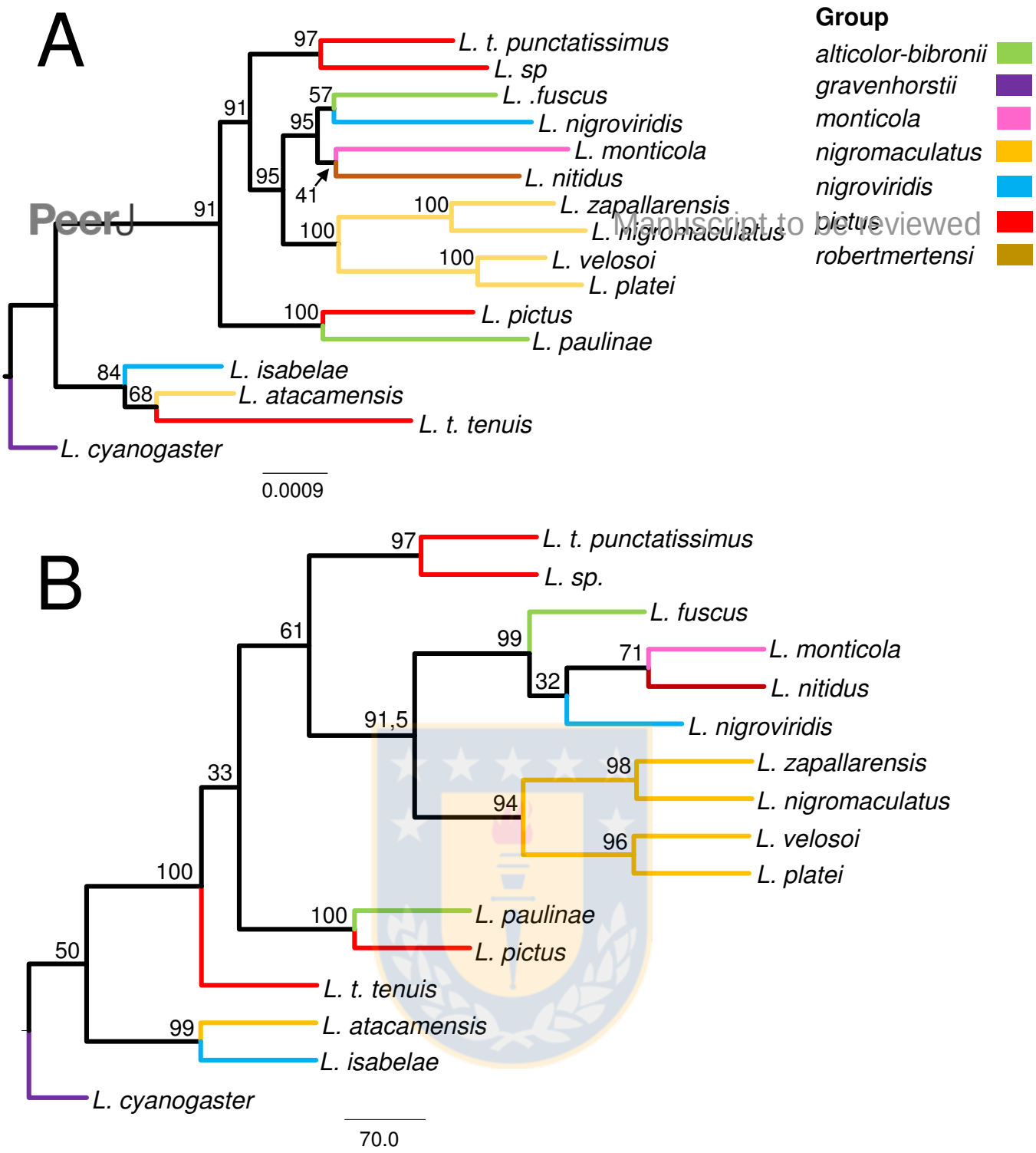

Table 1(on next page)

Species groups recognized within the Chilean clade.

Species groups recognized within the subgenus *Liolaemus* of *Liolaemus* indicating their species richness and number of species sampled. Taxonomy is based and updated from Abdala & Quinteros (2013). See Table S1 for a detailed account of taxonomic rearrangements.

Section	Group	N	Sampled
chiliensis	*alticolor–bibronii*	26	2
	bellii	4	0
	capillitas	6	0
	chillanensis	3	0
	elongatus	17	0
	gravenhorstii	3	1
	kriegi	4	0
	leopardinus	5	0
	pictus	5	2
	robertmentensi	4	1
nigromaculatus	*monticola*	2	1
	nigroviridis	8	2
	nigromaculatus	11	5

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Table 2(on next page)

Species included in the study.

Specimens of *Liolaemus* included in the study; for each specimen the species to which it belongs, museum catalog number and collection locality is provided. In addition the type locality of each species and the distance between it and collection localities are given (straight line) in kilometers. Voucher abbreviations are as follows: BYU = Brigham Young University, Monte L. Bean Life Science Museum, Provo, Utah, United States; SSUC = Colección Patricio Sánchez Reyes de la Pontificia Universidad Católica de Chile, Santiago, Chile; MZUC = Museo de Zoología de la Universidad de Concepción, Concepción, Chile. Asterisks indicate individual deposited in the Zoology, Epidemiology and Evolution Laboratory at the Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile.

Species	Voucher	Collection locality	Type locality	Distance to type locality
L. atacamensis	MZUC-45084	Finca de Chañaral	Atacama, north of Copiapó	60
L. cyanogaster	MZUC-45092	San Pedro Station	Valdivia	45
L. fuscus	MZUC-45085	La Herradura, Coquimbo	Valparaíso	335
L. isabelae	MZUC-45086	near Salar de Pedernales	near Salar de Pedernales	20
L. monticola	LMON619*	El Yeso	San Francisco river	150
L. nigromaculatus	SSUC 643	Las Terrazas Beach, Paposo	Between Puerto Viejo and Copiapó	236
L. nigroviridis	LNIG614*	El Yeso	San Francisco river	150
L. nitidus	MZUC-45087	Algarrobo	Valparaíso	34
L. paulinae	MZUC-45088	Calama	Calama on the Loa River	0
L. pictus	MZUC-45094	Valdivia National Park	Valdivia	30
L. platei	MZUC-45089	Quebrada Buenos Aires	Coquimbo	43
L. sp.	BYU 49951	Caracol	–	–
L. t. punctatissimus	BYU 48375	Lota	Lota	0
L. t. tenuis	MZUC-45093	Til-Til	Santiago	32
L. velosoi	MZUC-45090	Nantoco	Detour Cerro Imán, close to Copiapó	35
L. zapallarensis	MZUC-45091	Quebrada Buenos Aires	Zapallar	330

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Table 3(on next page)

De novo assembly results from IDBA for *Liolaemus* sequence capture data.

De novo assembly results from IDBA for *Liolaemus* sequence capture data. For each species, we report the selected k-mer value, number of contigs, N50 value, and number of sequenced loci (*N*) for protein-coding genes and UCEs in the final alignment.

Species	K-mer	Contigs	N50	*N*	Protein-coding	UCEs
L. atacamensis	90	949	388	549	37	511
L. cyanogaster	80	1,780	357	543	25	517
L. fuscus	90	1,044	326	558	37	520
L. isabelae	80	2,150	257	547	30	516
L. monticola	90	2,803	331	464	34	429
L. nigromaculatus	90	428	286	216	16	199
L. nigroviridis	90	253,864	111	424	29	394
L. nitidus	90	272,920	112	496	37	458
L. paulinae	80	111,191	320	503	33	468
L. pictus	90	574	331	360	22	337
L. platei	80	2,160	257	465	29	435
L. sp.	90	144	327	138	8	130
L. t. punctatissimus	90	517	307	328	24	304
L. t. tenuis	50	235	133	9	2	7
L. velosoi	80	57,514	293	480	25	453
L. zapallarensis	80	27,903	239	502	33	468

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Table 4(on next page)

Features of the UCEs and protein-coding genes.

Features of the UCEs and protein-coding genes.

Marker type	*N*	Average length	Average # taxa	Average % informative sites	Average % gaps and missing data
UCE	538	395.5	11.4	0.7	0.5
Protein-coding	43	406.3	9.8	2.1	0.2

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Table 5(on next page)

Bootstrap support values for the main clades inferred with three different approaches using the three data-sets.

Bootstrap support values for the main clades of *Liolaemus* inferred with three different approaches using the three data-sets. PC=protein-coding; UCEs=ultra-conserved elements; PC+UCEs=protein-coding genes and ultra-conserved elements (full data-set). Asterisks indicate that the clade includes *L. t. tenuis.*

	RAxML			SVD quartets			ASTRAL		
	PC	UCE	UCE+PC	PC	UCE	UCE+PC	PC	UCE	UCE+PC
L. atacamensis + L. isabelae	74	90*	84*	51	99	99	-	93	92
L. pictus +L. paulinae	95	100	100	85	100	100	75	100	100
L. t. punctatissimus + L. sp.	55	87	97	44	93.5	97	89*	95	99
L. platei + L. velosoi	100	99	100	99.5	100	96	100	100	100
L. zapallarensis + L. nigromaculatus	97	100	100	54	99	98	98	100	100
L. platei + L. velosoi + L. zapallarensis + L. nigromaculatus	99	99	100	49	91	94	100	87	98
L. monticola + L. nitidus	97	-	41	55	60	71	-	-	-
L. nigroviridis + L. fuscus	-	58	57	-	47	-	-	-	-
L. monticola + L. nitidus + L. nigroviridis + L. fuscus	83	93	95	83	99	99	88	97*	99*
L. platei + L. velosoi + L. zapallarensis + L. nigromaculatus + L. monticola + L. nitidus + L. nigroviridis + L. fuscus	96	88	95	50	84	91.5	97	56*	63*
L. platei + L. velosoi + L. zapallarensis + L. nigromaculatus + L. monticola + L. nitidus + L. nigroviridis + L. fuscus + L. t. punctatissimus + L. sp.	50	79	91	20	58	61	-	85*	88*
L. platei + L. velosoi + L. zapallarensis + L. nigromaculatus+ L. monticola+L. nitidus + L. nigroviridis + L. fuscus + L. t. punctatissimus + L. sp. + L. pictus + L. paulinae	46	92	91	86*	74	33	99*	100*	100*
L. platei + L. velosoi + L. zapallarensis + L. nigromaculatus + L. monticola + L. nitidus + L. nigroviridis + L. fuscus + L. t. punctatissimus + L. sp. + L. pictus + L. paulinae + L. atacamensis + L. isabelae	-	-	-	50*	50*	50*	-	-	-

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SUPPLEMENTARY INFORMATION

TABLE LEGENDS

Table S1. Diversity of the Chilean clade of *Liolaemus* (species and groups) adapted and updated from Abdala & Quinteros (2013). Synonyms and additions relative to the classification proposed by Abdala & Quinteros (2013) are shown. The recently described *Liolaemus scorialis* Troncoso-Palacios et al., (2015) is *incertae sedis* in the *elongatus-kriegi* complex (Troncoso-Palacios et al., 2015). Species included in this study are marked in bold.

Table S2. Detailed type locality information, including coordinates (in decimal degrees) when available, as well as bibliographic references are given

Table S3. Comparison of de novo assembly results from IDBA across different k-mer values. The k-mer value with the highest N50 was chosen for each species (shown in bold). One exception is *L. t. tenuis*, which had too few loci.

FIGURE LEGENDS

Figure S1. Phylogenomic relationships among *Liolaemus* lizards from the Chilean group, estimated with ultraconserved elements using concatenation (RAxML).

Figure S2. Phylogenomic relationships among *Liolaemus* lizards from the Chilean group, estimated with protein-coding using concatenation (RAxML).

Figure S3. Phylogenomic relationships among *Liolaemus* lizards from the Chilean group, estimated with ultraconserved elements using a quartet inference method (SVD quartets).

Figure S4. Phylogenomic relationships among *Liolaemus* lizards from the Chilean group, estimated with protein-coding genes using a quartet inference method (SVD quartets).

Figure S5. Phylogenomic relationships among *Liolaemus* lizards from the Chilean group, estimated with ultraconserved elements using a gene-tree summary based method (ASTRAL).

Figure S6. Phylogenomic relationships among *Liolaemus* lizards from the Chilean group, estimated with protein-coding genes using a gene-tree summary based method (ASTRAL).

	***sensu* Abdala & Quinteros, 2013**	**Synonym**	**Additions**
alticolor–bibronii group	*L. alticolor*		
	L. abdalai		
	L. aparicioi		
	L. araucaniensis		
	L. bibronii		
	L. bitaeniatus		
	L. chaltin		
	L. curicensis		
	L. cyaneinotatus		
	L. exploratorum		
	L. fuscus		
	L. gracilis		
	L. incaicus		
	L. lativitattus	Synonimized with *L. alticolor sensu* Müller & Hellmich (1938), not accepted by Lobo, Espinoza & Quinteros (2010) and Quinteros (2012).	
	L. lemniscatus		
	L. pagaburoi		
	L. paulinae		
	L. puna		
	L. pyriphlogos		
	L. ramirezae		
	L. saxatilis		
	L. tacnae		
	L. tandiliensis		
	L. variegatus		

	L. walkeri		
	L. yanalcu		
			L. yalguaraz Abdala et al., 2015
bellii group	*L. bellii*		
	L. curis		
	L. fitzgeraldi		
	L. modestus	Is now *Stenocercus modestus* (Tschudi, 1845) (Laurent, 1984; Torres-Carvajal, 2007).	
	L. moradoensis		
capillitas group	*L. capillitas*		
	L. dicktracyi		
	L. heliodermis		
	L. talampaya		
	L. tulkas		
	L. umbrifer		
chillanensis group	*L. chillanensis*		
	L. villaricensis		
			L. leftrarui Troncoso-Palacios et al., (2016a)
elongatus group	*L. austromendocinus*		
	L. antumalguen		
	L. burmeisteri		
	L. carlosgarini		
	L. choique		
	L. elongatus		

	L. flavipiceus	
	L. gununakuna	
	L. parvus	
	L. petrophilus	
	L. punmahuida	
	L. riodamas	
	L. shitan	
	L. smaug	
	L. thermarum	
	L. tregenzai	
		L. janequeoae Troncoso-Palacios et al., (2016a)
gravenhorstii group	***L. cyanogaster***	
	L. gravenhorsti	
	L. schroederi	
kriegi group	*L. buergeri*	
	L. ceii	Synonymized with *L. kriegi* by Morando et al., (2003) and Troncoso-Palacios et al., (2015)
	L. cristiani	
	L. kriegi	
		L. zabalai Troncoso-Palacios et al., (2015)
leopardinus group	*L. frassinettii*	
	L. leopardinus	
	L. ramonensis	
	L. valdesianus	

		L. ubaghsi Esquerré et al., 2014
monticola group	***L. monticola***	
	L. confusus	
nigromaculatus group	***L. atacamensis***	
	L. ater	*L. ater* is currently considered subspecies of *L. zapallarensis* (Ortiz, 1981).
	L. bisignatus	*Liolaemus bisignatus is* a *nomen nudum*, and populations attributed to *L. bisignatus* should be referred to *L. nigromaculatus* (Troncoso-Palacios & Garín, 2013).
	L. copiapensis	*L. copiapensis* is a synonym of *L.bisignatus* (Pincheira-Donoso & Núñez, 2005; Troncoso-Palacios & Garín, 2013), which is in turn synonym to *L. nigromaculatus* (Troncoso-Palacios & Garín, 2013).
	L. donosoi	*L. donosoi* is currently considered synonym of *L. constanzae* (Veloso et al., 1982; Troncoso-Palacios, 2013).
	L. hellmichi	
	L. kuhlmanni	Pincheira-Donoso et al., (2008) considered *L. kuhlmanni* as a synonym of *L. zapallarensis*, a decision that is rejected by Lobo, Espinoza & Quinteros (2010) and Ruiz de Gamboa (2016).
	L. melaniceps	
	L. nigromaculatus	
	L. platei	
	L. pseudolemniscatus	
	L. sieversi	*L. sieversi* is currently considered subspecies of *L. zapallarensis* (Ortiz, 1981).
	L. silvai	
	L. velosoi	
	L. zapallarensis	

			L. nigrocoeruleus Marambio-Alfaro & Troncoso-Palacios (2014)
nigroviridis group	*L. constanzae*		
	L. isabelae		
	L. juanortizi		
	L. lorenzmuelleri		
	L. maldonadae		
	L. melanopleurus		
	L. nigroviridis		
			L. uniformis Troncoso-Palacios et al., (2016b)
pictus group	*L. brattstroemi*	Pincheira-Donoso & Núñez (2005) elevate *L. cyanogaster brattstroemi* to full species. Lobo, Espinoza & Quinteros (2010) support this decision while Vidal et al., (2012) & Ruiz de Gamboa (2016) disagree.	
	L. argentinus	Is a subspecies of *L. pictus* (Ruiz de Gamboa, 2016)	
	L. chiloensis	It was formerly a subspecies of *L. pictus* although Vera-Escalona et al., (2012) synonymized it to *L. pictus.*	
	L. codoceae	Is a subspecies of *L. pictus* (Pincheira-Donoso & Núñez, 2005)	
	L. major	Synonimized with *L. pictus codoceae* by Pincheira-Donoso & Núñez (2005) and supported by Lobo, Espinoza & Quinteros (2010)	
	L. pictus		

	L. septentrionalis	Pincheira-Donoso et al., (2008) elevated *L. pictus septentrionalis* to full species, which has been supported by Lobo, Espinoza & Quinteros (2010) and Vera-Escalona et al., (2012).
	L. talcanensis	Is a subspecies of *L. pictus* (Pincheira-Donoso & Núñez, 2005)
	L. tenuis	
	L. coeruleus	
	L. neuquensis	
robertmertensi group	*L. chiliensis*	
	L. nitidus	
	L. robertmertensi	
	L. sanjuanensis	

REFERENCES

Abdala, C. S. & Quinteros, A. S. (2013). Los últimos 30 años de estudios de la familia de lagartijas más diversa de Argentina. Actualización taxonómica y sistemática de Liolaemidae. *Cuadernos de Herpetología*, 28, 1-28.

Abdala, C. S., Quinteros, A. S. & Semham, R. V. (2015). A new species of *Liolaemus* of the *Liolaemus alticolor-bibronii* group (Iguania: Liolaemidae) from Mendoza, Argentina. *South American Journal of Herpetology*, 10(2), 104-115.

Esquerre, D., Troncoso-Palacios, J., Garin, C. F. & Nunez, H. (2014). The missing leopard lizard: *Liolaemus ubaghsi* sp. nov., a new species of the *leopardinus* clade (Reptilia: Squamata: Liolaemidae) from the Andes of the O'Higgins Region in Chile. *Zootaxa*, 3815(4), 507-525.

Laurent, R. F. (1984). On some iguanid genera related to or previously confused with *Liolaemus* Wiegmann. *Journal of Herpetology*, 357-373.

Lobo, F., Espinoza, R. E. & Quinteros, S. (2010). A critical review and systematic discussion of recent classification proposals for liolaemid lizards. *Zootaxa*, 2549, 1-30.

Marambio-Alfaro, Y. & Troncoso-Palacios, J. (2014). A new species of *Liolaemus* of the *L. nigromaculatus* group (Iguania: Liolaemidae) from Atacama Region, Chile. *Basic and Applied Herpetology*, 28, 65-77.

Morando, M., Avila, L. J. & Sites, J. W. (2003). Sampling strategies for delimiting species: genes, individuals, and populations in the *Liolaemus elongatus-kriegi* complex (Squamata: Liolaemidae) in Andean–Patagonian South America. *Systematic Biology*, 52(2), 159-185.

Müller, L., & Hellmich, W. (1938). Liolaemus-Arten aus dem westlichen Argentinien (ergebnisse der argentinienreise von Prof. Dr. W. Goetsch, Breslau). I. *Liolaemus darwini* (Bell) und *Liolaemus goetschi* n. sp. *Zoologischer Anzeiger*, 123(5-6), 130-142.

Ortiz, J.C. (1981). Estudio multivariado de las especies de *Liolaemus* del grupo *nigromaculatus* (Squamata, Iguanidae). *Anales del Museo de Historia Natural de Valparaíso*, 14, 247-265.

Pincheira-Donoso, D. & Núñez, H. (2005). Las especies chilenas del género *Liolaemus* Wiegmann, 1834 (Iguania Tropiduridae: Liolaeminae). Taxonomía, sistemática y evolución. *Publicación ocasional del Museo Nacional de Historia Natural*, 59, 7-486.

Pincheira-Donoso, D., Scolaro, J. A. & Sura, P. (2008). A monographic catalogue on the systematics and phylogeny of the South American iguanian lizard family Liolaemidae (Squamata, Iguania). *Zootaxa*, 1800, 1–85.

Quinteros, A. S. (2012). Taxonomy of the *Liolaemus alticolor-bibronii* group (Iguania: Liolaemidae), with descriptions of two new species. *Herpetologica*, 68(1), 100-120.

Ruiz de Gamboa, M. (2016). Lista actualizada de los reptiles de Chile. *Boletín Chileno de Herpetología*, 3, 7-12.

Torres-Carvajal, O. (2007). A taxonomic revision of South American *Stenocercus* (Squamata: iguania) lizards. *Herpetological monographs*, 21(1), 76-178.

Troncoso-Palacios, J. (2013). Revisión del estatus taxonómico *de Liolaemus donosoi* Ortiz, 1975 (Iguania: Liolaemidae). *Boletín del Museo Nacional de Historia Natural de Chile*, 62, 119-127.

Troncoso-Palacios, J. & Garin, C. F. (2013). On the identity of *Liolaemus nigromaculatus* Wiegmann, 1834 (Iguania, Liolaemidae) and correction of its type locality. *ZooKeys*, 294, 37.

Troncoso-Palacios, J., Díaz, H. A., Esquerré, D. & Urra, F. A. (2015). Two new species of the *Liolaemus elongatus-kriegi* complex (Iguania, Liolaemidae) from Andean highlands of southern Chile. *ZooKeys*, 500, 83.

Troncoso-Palacios, J., Diaz, H. A., Puas, G. I., Riveros-Riffo, E. & Elorza, A. A. (2016a). Two new *Liolaemus* lizards from the Andean highlands of Southern Chile (Squamata, Iguania, Liolaemidae). *ZooKeys*, 632, 121.

Troncoso-Palacios, J., Elorza, A. A., Puas, G. I. & Alfaro-Pardo, E. (2016b). A new species of *Liolaemus* related to *L. nigroviridis* from the Andean highlands of Central Chile (Iguania, Liolaemidae). *ZooKeys*, (555), 91.

Veloso, A., Sallaberry, M., Navarro, J., Iturra, P., Valencia, J., Penna, M. & Díaz, Y.N. (1982) Contribución sistemática al conocimiento de la herpetofauna del extremo norte de Chile. In Veloso, A. y E. Bustos (Eds.). El hombre y los ecosistemas de montaña. Oficina Regional de Ciencias y Tecnología de la UNESCO para América Latina y el Caribe. (Montevideo, Uruguay). I, 135-268

Vera-Escalona, I., D'Elía, G., Gouin, N., Fontanella, F. M., Muñoz-Mendoza, C., Sites Jr, J. W. & Victoriano, P. F. (2012). Lizards on ice: evidence for multiple refugia in *Liolaemus pictus* (Liolaemidae) during the last glacial maximum in the southern Andean beech forests. *PloS one*, 7(11), e48358.

Vidal, M. A., Ortiz, J. C., Marín, J. C., Poulin, E. & Moreno, P. I. (2012). Comparative phylogeography of two co-distributed species of lizards of the genus *Liolaemus* (Squamata: Tropiduridae) from Southern Chile. *Amphibia-Reptilia*, 33(1), 55-67.

Species	Voucher	Locality	Latitude	Longitude	Type locality	Source
L. atacamensis	MZUC-45084	Entre Finca de Chañaral e Inca de Oro, Km 10, ruta c-253	-26,6784	-69,8441	Atacama, north of Copiapó	Müller & Hellmich, 1933
L. cyanogaster	MZUC-45092	Estación San Pedro	-39,7582	-72,6728	Chile, and restricted to Valdivia afterwards	Hellmich, 1934
L. fuscus	MZUC-45085	La Herradura, Coquimbo	-29,9792	-71,3713	Valparaíso	Boulenger, 1885
L. isabelae	MZUC-45086	Ruta c-189, pasando Salar de Pedernales hacia Diego de Almagro	-26,4001	-69,2808	El Cerrito, 12 km North-West from campamento La Ola, near Salar de Pedernales (26°27' S, 69°03 W/26°12' S, 69°08 W)	Navarro & Núñez, 1993
L. monticola	LMON619	El Yeso	-33,7232	-70,6169	San Francisco river, 1700 m. altitude (32°22′S, 70°25′W)	Torres-Pérez et al., 2009
L. nigromaculatus	SSUC 643	Las Terrazas Beach, Paposo	-25,14	-70,4613	Between Puerto Viejo and Copiapó	Troncoso-Palacios & Garín, 2013
L. nigroviridis	LNIG614	El Yeso	-33,7232	-70,6169	San Francisco river (32°22' S, 70°25' W)	Cianferoni et al., 2013
L. nitidus	MZUC-45087	2 km north of Algarrobo	-33,3601	-71,6463	Chile, and restricted to Valparaíso afterwards	Hellmich, 1934
L. paulinae	MZUC-45088	Rio Loa, Calama	-22.4784	-68.9156	Calama on the Loa River, 2600 m. altitude.	Donoso-Barros, 1961
L. pictus	MZUC-45094	Valdivia National Park (Chaiguin)	-39,9504	-73,5601	Chile, and restricted to Valdivia afterwards	Hellmich, 1934
L. platei	MZUC-45089	Quebrada Buenos Aires	-29,57036	-71,24391	Coquimbo	Werner, 1898
L. sp.	BYU 49951	Caracol	-36,6567	-71,3656	-	-
L. t. punctatissimus	BYU 48375	Playa Negra, Lota-Coronel	-37,0833	-73,1667	Lota (37°04 S, 73°10 W)	Donoso-Barros, 1966
L. t. tenuis	MZUC-45093	Tiltil	-33,1355	-70,8552	Chile, and restricted to Santiago afterwards	Hellmich, 1934
L. velosoi	MZUC-45090	Nantoco	-27,57215	-70,25961	Desvio Cerro Imán près de Copiapó (27°20’ S 70°30 W)	Ortiz, 1987
L. zapallarensis	MZUC-45091	Quebrada Buenos Aires	-29,57036	-71,24391	Zapallar	Müller & Hellmich, 1933

REFERENCES

Boulenger, G.A. (1885). *Catalogue of the lizards in the British Museum (Natural History*). Second edition. London.

Cianferoni, F., Yanez, R. P., Palma, R. E., Garin, C. F. & Torres-Perez, F. (2013). Deep divergences within *Liolaemus nigroviridis* (Squamata, Liolaemidae) lineages associated with sky islands in central Chile. *Zootaxa*, 3619, 59-69

Donoso-Barros, R. (1961). Three new lizards of the genus *Liolaemus* from the highest Andes of Chile and Argentina. *Copeia*, 1961(4), 387-391.

Donoso-Barros, R. (1966). *Reptiles of Chile*. Santiago: Editorial Universitaria.

Hellmich, W. (1934). Die Eidechsen Chiles insbesonders die Gattung *Liolaemus*. Nach den Sammlungen Goetsch-Hellmich. Abhandlungen. Bayerischen Akademie der issenschaften. *Mathematisch-Naturwissenschaftliche Klasse,* 24, 1–140

Müller, L. & Hellmich, W. (1933). Beiträge zur Kenntnis der Herpetofauna Chiles. VII. Der Rassenkreis *der Liolaemus nigromaculatus*. *Zoologischer Anzeiger*, 103, 128-142.

Navarro, J. & Núñez, H. (1993). *Liolaemus patriciaiturrae* y *Liolaemus isabelae*, dos nuevas especies de lagartijas para el norte de Chile. Aspectos biogeográficos y citotaxonómicos (Squamata, Tropiduridae). *Boletín del Museo Nacional de Historia Natural de Chile*, 44, 99-113.

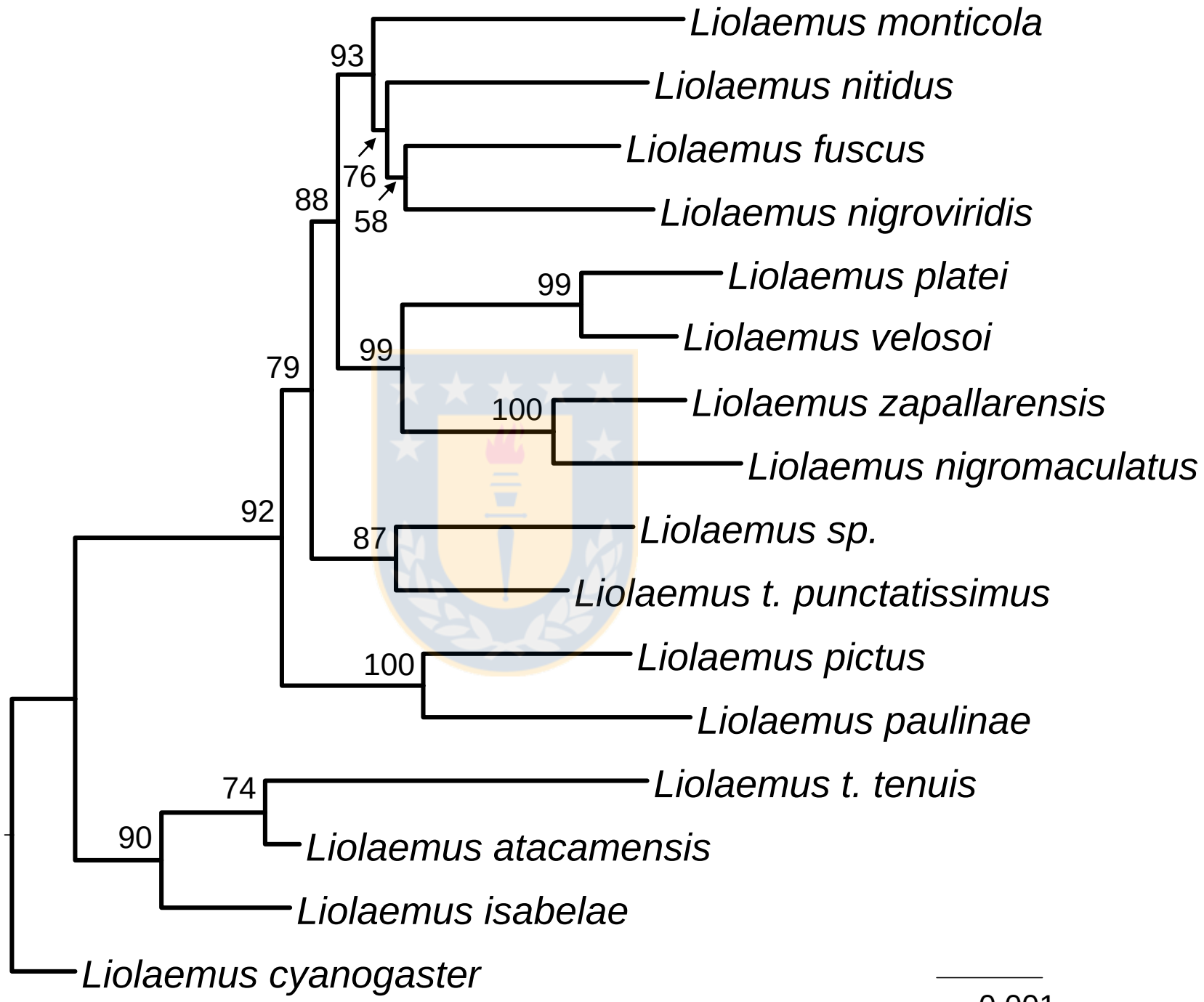
Ortiz, J.C. (1987). Une nouvelle espèce de *Liolaemus* (Sauria, Iguanidae) du Chili. *Bulletin Museum National d'Histoire Naturelle, Paris*, 265-270.

Torres-Pérez F., Méndez M.A., Benavides E., Moreno R.A., Lamborot M., Palma R.E. & J.C. Ortiz. (2009). Systematics and evolutionary relationships of the mountain lizard *Liolaemus monticola* (Liolaemini): how morphological and molecular evidence contributes to reveal hidden species diversity. *Biological Journal of the Linnean Society*, 96, 635–650.

Troncoso-Palacios, J. & Garin, C. F. (2013). On the identity of *Liolaemus nigromaculatus* Wiegmann, 1834 (Iguania, Liolaemidae) and correction of its type locality. *ZooKeys*, 294, 37.

Werner, F. (1898). Die Reptilien und Batrachier der Sammlung Plate. *Zoologische Jahrbuecher Abteilung fuer Systematik Oekologie und Geographie der Tiere*, 4, 244-278.

	K-mer value									
	50		60		70		80		90	
Species	**Contigs**	**N50**	**Contigs**	**N50**	**Contigs**	**N50**	**Contigs**	**N50**	**Contigs**	**N50**
L. atacamensis	8,020	158	2,857	213	1,651	283	1,085	358	**949**	**388**
L. cyanogaster	12,715	150	4,162	216	2,464	294	**1,780**	**357**	1,813	346
L. fuscus	6,055	131	3,181	160	1,846	225	1,240	293	**1,044**	**326**
L. isabelae	20,852	102	9,477	121	4,306	178	**2,150**	**257**	2,707	221
L. monticola	26,585	119	10,697	145	5,034	205	2,906	263	**2,803**	**331**
L. nigromaculatus	2,640	148	1,115	187	625	249	478	281	**428**	**286**
L. nigroviridis	1,746,196	58	1,246,148	69	741,647	83	399,483	97	253,864	111
L. nitidus	1,169,208	62	862,813	72	522,466	86	276,282	103	**272,920**	**112**
L. paulinae	333,832	204	192,398	255	141,917	292	**111,191**	**320**	101,712	317
L. pictus	6,463	140	1,408	217	898	273	641	322	**574**	**331**
L. platei	16,473	131	7,379	151	3,650	205	**2,160**	**257**	2,026	253
L. t. punctatissimus	11,397	97	3,523	130	1,238	204	661	277	**517**	**307**
L. sp.	605	189	250	279	202	304	171	319	**144**	**327**
L. t. tenuis	**235**	**133**	62	157	33	167	11	213	3	232
L. velosoi	271,652	160	131,141	204	82,958	252	**57,514**	**293**	51,813	290
L. zapallarensis	167,794	120	92,148	138	45,614	192	**27,903**	**239**	26,430	235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Liolaemus nigromaculatus
Liolaemus sp.
Liolaemus t. punctatissimus
Liolaemus pictus
Liolaemus paulinae
Liolaemus t. tenuis
Liolaemus atacamensis
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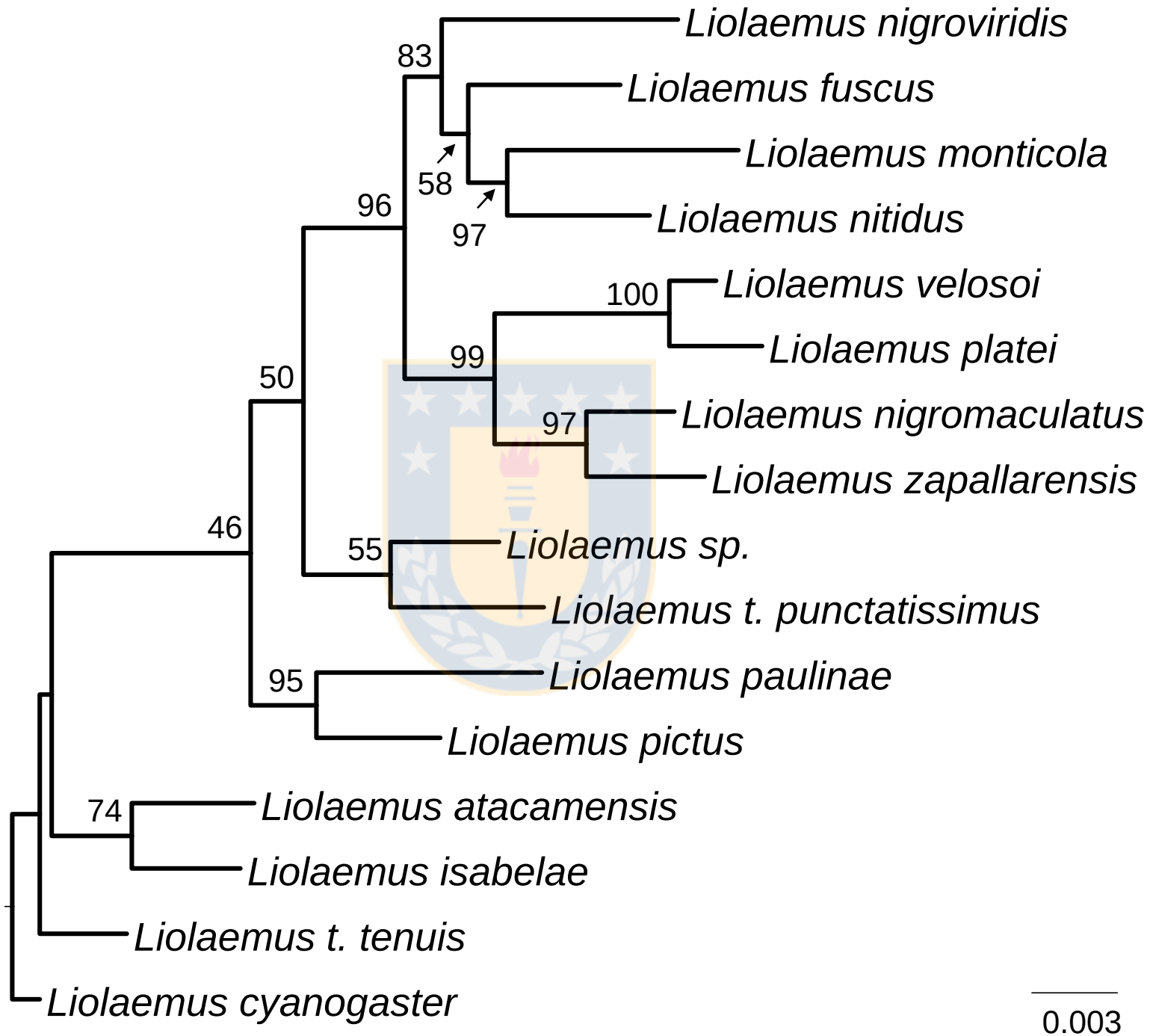
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Liolaemus zapallarensis
Liolaemus sp.
Liolaemus t. punctatissimus
Liolaemus paulinae
Liolaemus pictus
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Liolaemus isabelae
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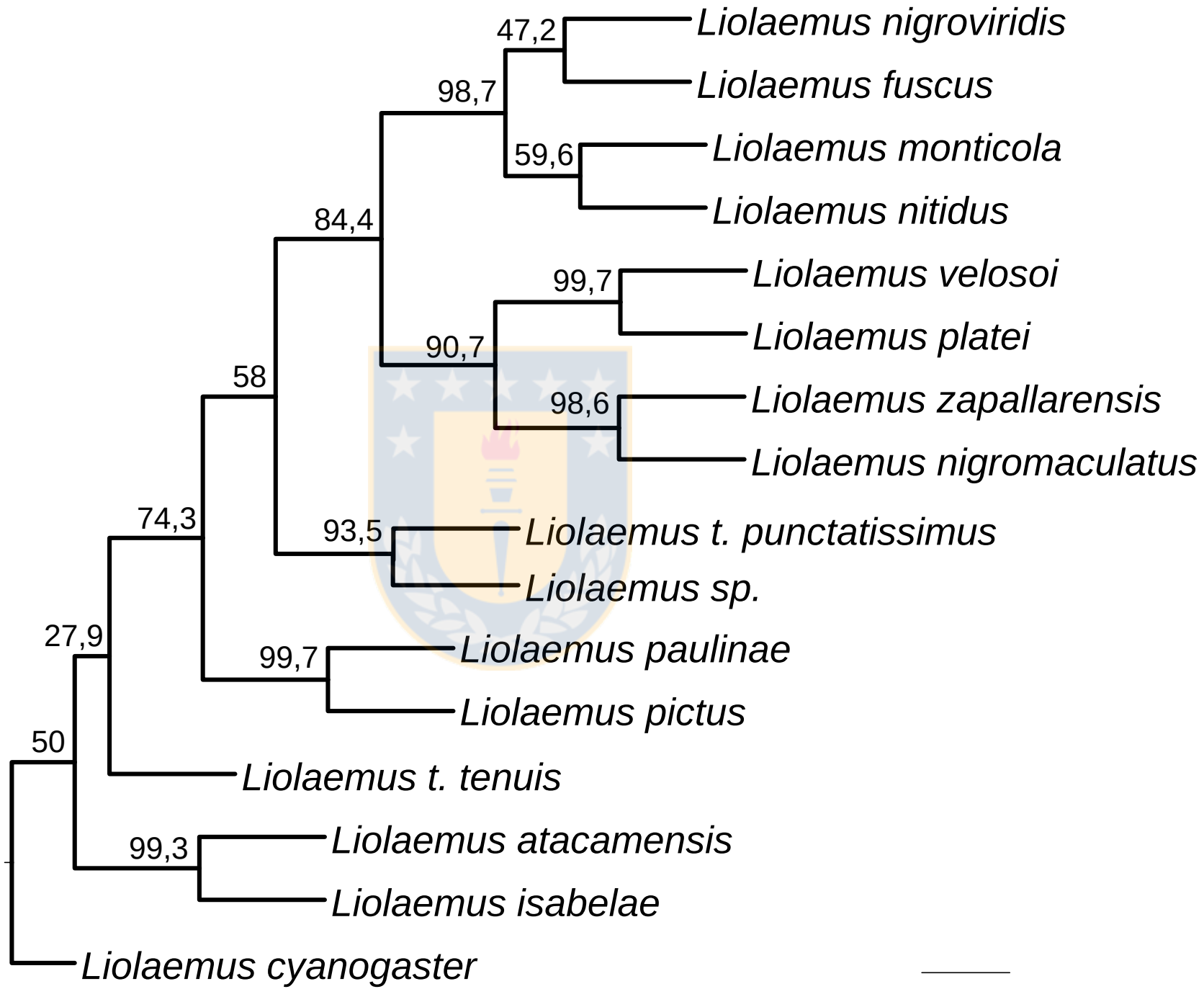
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Liolaemus nigroviridis
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Liolaemus platei
Liolaemus zapallarensis
Liolaemus nigromaculatus
Liolaemus t. punctatissimus
Liolaemus sp.
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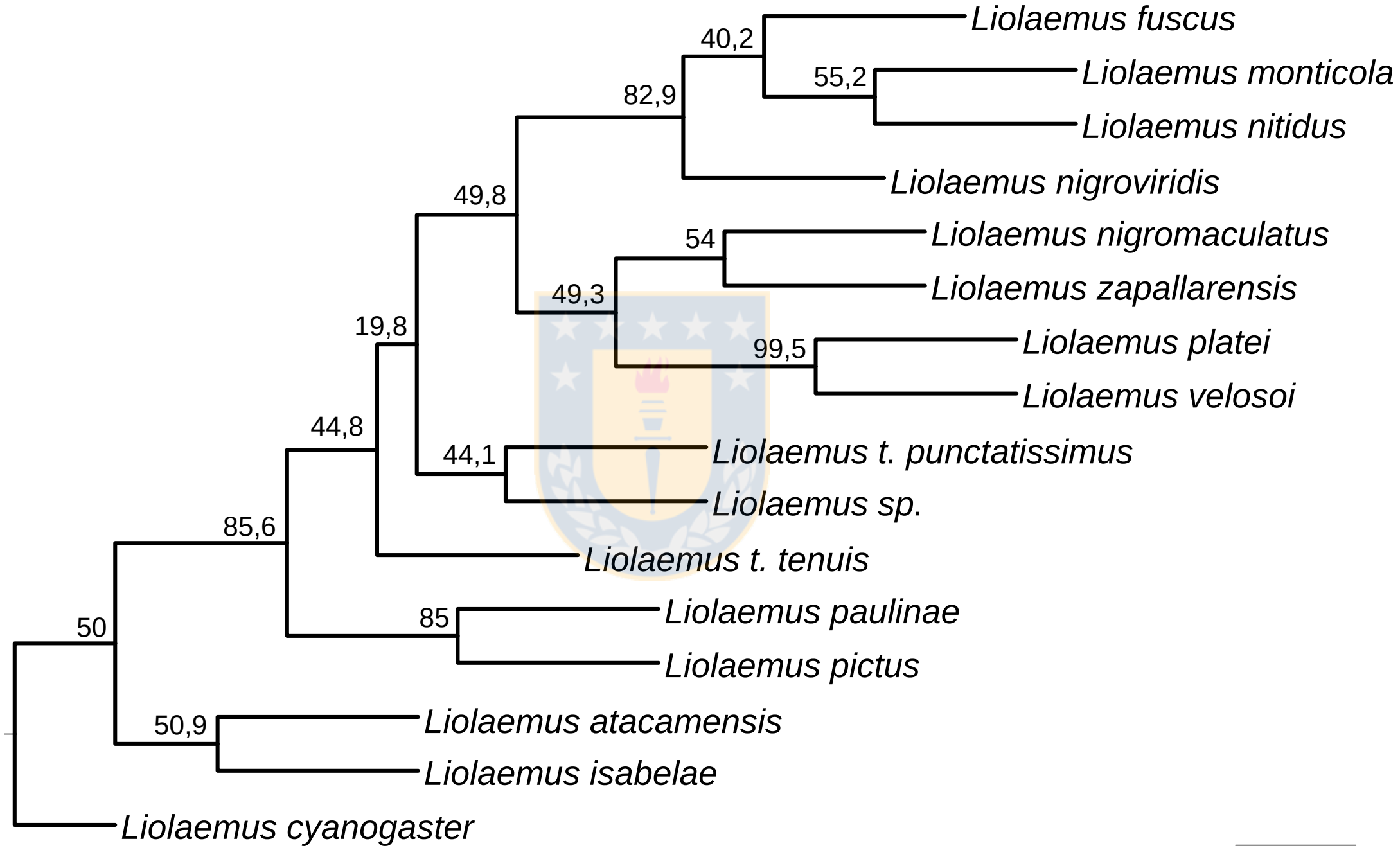
Liolaemus fuscus
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Liolaemus velosoi
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Liolaemus sp.
Liolaemus t. tenuis
Liolaemus paulinae
Liolaemus pictus
Liolaemus atacamensis
Liolaemus isabelae
Liolaemus cyanogaster
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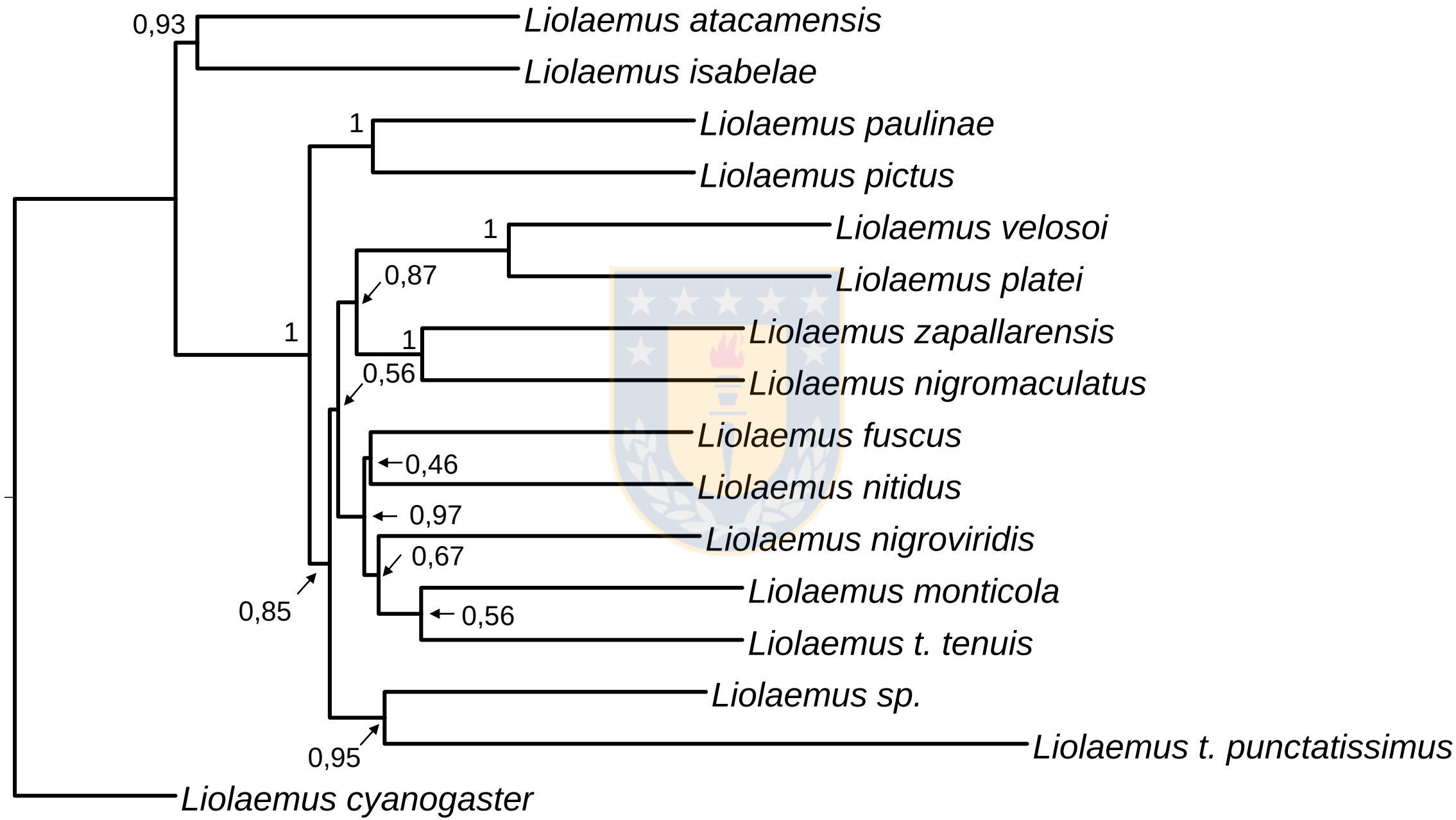
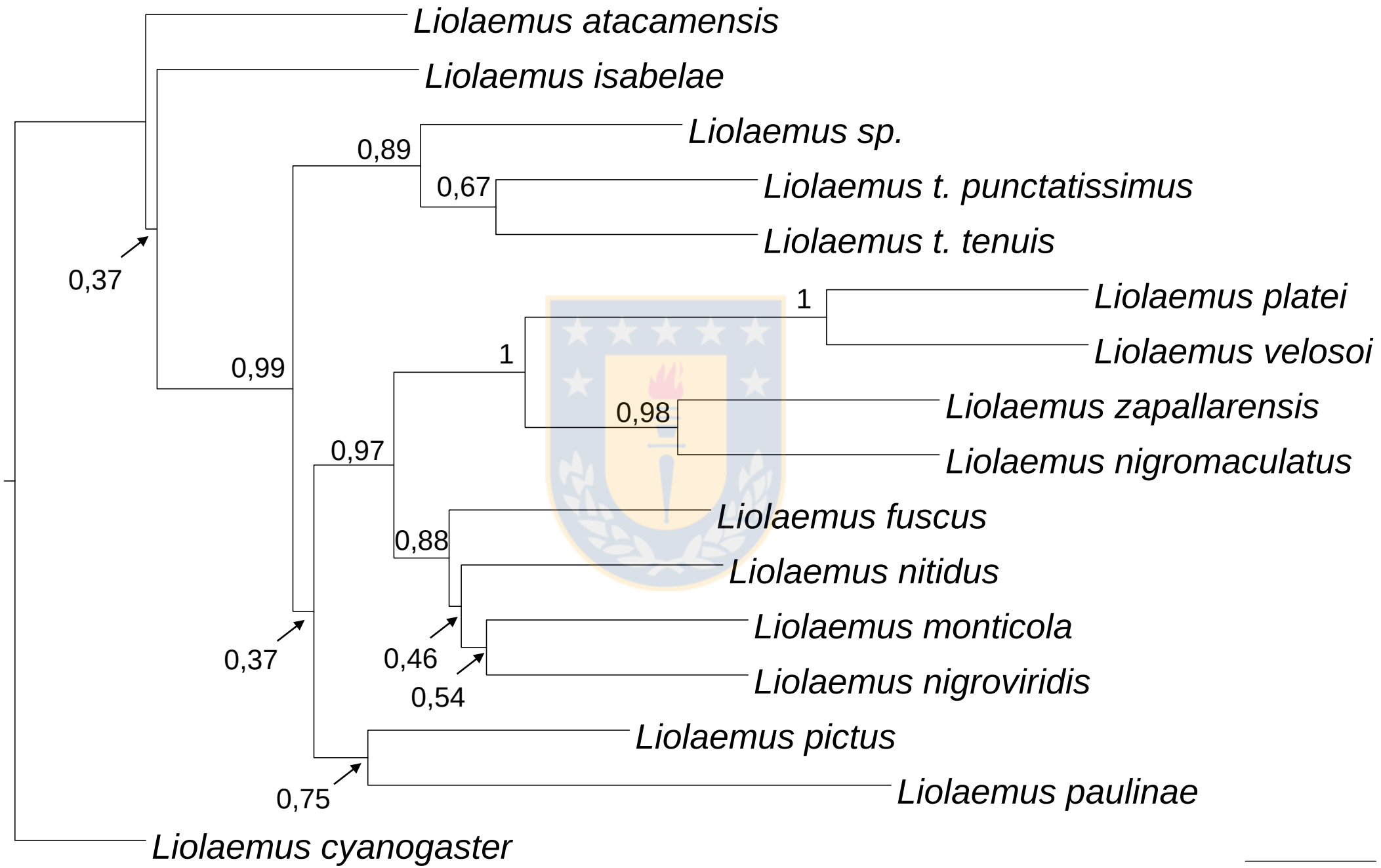

0,93
Liolaemus atacamensis
Liolaemus isabelae
1
Liolaemus paulinae
Liolaemus pictus
1
Liolaemus velosoi
0,87
Liolaemus platei
1
1
Liolaemus zapallarensis
0,56
Liolaemus nigromaculatus
Liolaemus fuscus
0,46
Liolaemus nitidus
0,97
Liolaemus nigroviridis
0,67
0,85
Liolaemus monticola
0,56
Liolaemus t. tenuis
Liolaemus sp.
0,95
Liolaemus t. punctatissimus
Liolaemus cyanogaster
0.3


Liolaemus atacamensis
Liolaemus isabelae
Liolaemus sp.
0,89
0,67
Liolaemus t. punctatissimus
Liolaemus t. tenuis
0,37
1
Liolaemus platei
Liolaemus velosoi
1
0,99
0,98
Liolaemus zapallarensis
Liolaemus nigromaculatus
0,97
Liolaemus fuscus
0,88
Liolaemus nitidus
Liolaemus monticola
0,37
0,46
0,54
Liolaemus nigroviridis
Liolaemus pictus
0,75
Liolaemus paulinae
Liolaemus cyanogaster
0.5

DISCUSIÓN GENERAL

Filogeografía

Los resultados de esta tesis proveen una explicación posible para el origen y diversificación dentro de *Liolaemus tenuis sensu lato*, dejando en evidencia la gran diversidad entre y dentro de sus poblaciones. Se puede concluir que la filogeografía de *L. tenuis* se asocia con los ciclos glaciales del Pleistoceno; durante los avances glaciales las poblaciones del rango sur fueron extirpadas, desplazadas o restringidas a refugios, mientras que durante los interglaciares, los individuos recolonizaron las áreas nuevamente disponibles a partir de refugios y de zonas estables (e.g. la zona costera). La ocurrencia de un clado somero en la filogenia, que representa poblaciones del sur del rango distribucional de la especie, así como la presencia de una mayor diversidad genética en áreas no glaciadas (centro y norte), son algunos de los indicadores del impacto que las glaciaciones ejercieron en *L. tenuis*. Este tipo de patrones poblacionales también pueden observarse en otros taxa presentes en la región (e.g. *Liolaemus pictus*, Vera-Escalona et al. 2012; *Eupsophus*, Núñez et al. 2011; peces del género *Diplomystes*, Muñóz-Ramírez et al. 2014; *Galaxias*, Ruzzante et al. 2008; plantas: Muellner et al. 2005), lo cual deja en evidencia los procesos comunes a los cuales estos taxa han sido sujetos.

La estructura filogeográfica de *L. tenuis* (Capítulo 1) muestra clados geográficamente estructurados, con linajes alopátridos en las zonas centro y norte, sugerentes de una historia de procesos vicariantes, y congruentes a su vez con el mayor grado de fragmentación del territorio en esta región. Por otra parte, en el rango sur, dos linajes co-distribuidos de divergencias profundas dan cuenta de procesos de expansión reciente y contacto secundario. Los indicadores demográficos evidencian una mayor estabilidad poblacional a lo largo del tiempo, mayor diversidad haplotípica y estructura genética en las poblaciones de la región centro-norte respecto a aquellas del rango sur, donde se observa una menor diversidad, mayor cantidad de haplotipos redundantes, y signos de expansión poblacional, indicando que estas poblaciones son probablemente colonizadores recientes. Sin embargo, se infieren reducciones en el tamaño efectivo tanto en las poblaciones de los rangos norte como sur de *Liolaemus tenuis*, en períodos correspondientes a la Glaciación Llanquihue (50.000-100.000 años) y el Último Máximo Glacial (UMG, ~20.000 años). Con respecto a las reducciones poblacionales en el rango sur, éstas fueron causadas probablemente por efecto directo del avance glacial, patrón que ya había sido reportado para *Liolaemus*

pictus, especie simpátrica con *L. tenuis* en esta región (Vera-Escalona et al. 2012). Sin embargo, hacia el norte de los 35°S la lengua de hielo se fue haciendo cada vez más angosta, quedando restringida principalmente a las cumbres montañosas a la altura de la latitud 33°S (Heusser 2003). Por lo tanto, las poblaciones del rango norte de *L. tenuis* sufrieron en menor intensidad y duración los efectos del avance de los hielos (efectos periglaciales). En estas latitudes, sin embargo, los efectos del relieve se vuelven más importantes debido a las mayores alturas en la Cordillera de los Andes y de la Costa, donde la línea de hielo se hizo presente a partir de los 800 metros durante las glaciaciones (Fox 1993), reduciendo posiblemente el rango altitudinal de distribución de *L tenuis* (que en condiciones actuales alcanza los 1800 metros). Adicionalmente, el glaciar del Valle del Maipo, que durante las últimas glaciaciones alcanzó el valle Central (Brüggen 1950; Vuilleumier 1971), probablemente actuó en conjunto con lo anterior reduciendo tamaños poblacionales en esta región. Finalmente, dichos eventos producto de la orogenia, sumado a una circulación atmosférica glacial más vigorosa, habría reforzado el efecto de sombra de lluvias en Chile Central, afectando la distribución de los bosques (Villagrán 1994) y por consecuencia también las poblaciones de *L. tenuis*.

Fuera de los límites del UMG, las áreas costeras en Chile central habrían actuado como refugios para *L. tenuis*, lo cual también ha sido reportado para plantas y otros animales en base a registros palinológicos, variación de isoenzimas y diversidad genética (Villagrán et al. 1996; Premoli et al. 2003; Victoriano et al. 2008; Sérsic et al. 2011). Sin embargo, dentro de los límites del UMG, los resultados sugieren que algunas poblaciones del rango sur persistieron en refugios, a partir de los cuales expandieron sus rangos durante los interglaciares. En este trabajo, estas áreas de estabilidad histórica se identificaron mediante la integración de datos moleculares (ramas largas, haplotipos exclusivos, alta diversidad) con modelajes de nicho, donde se observa una alta probabilidad de hábitat tanto actual como durante el UMG, en contraposición a áreas de inestabilidad histórica, donde ocurre un desplazamiento en la probabilidad de ocurrencia durante el UMG respecto a la actual, acompañada de haplotipos redundantes, ramas cortas, y baja diversidad. De esta forma, en este trabajo se encuentra evidencia que apoya al área de Lonquimay (~38º33'S) como posible refugio pleistocénico, lo cual fue originalmente propuesto por Heusser (2003). Vera-Escalona y colaboradores (2012) encuentran evidencia de un refugio intraglacial para

Liolaemus pictus en el área de Malalcahuello, adyacente al valle de Lonquimay. Esto sería congruente con lo reportado por Sérsic y colaboradores (2011), quienes señalan que regiones andinas entre los 39º y 43ºS habrían albergado varios refugios intraglaciares.

Adicionalmente, se identifican dos quiebres filogeográficos consistentes con dos ríos importantes que cruzan Chile de este a oeste, y que marcan los límites de regiones biogeográficas; el Maipo (~33ºS) y el Biobío (~36ºS). Nuestros resultados indican que los niveles flujo génico histórico a ambos lados de estos dos ríos son muy bajos. El rol de los ríos como determinantes de quiebres filogeográficos ya había sido reportado en *Liolaemus* tanto en Argentina (e.g. Morando et al. 2003, 2007) como en Chile (e.g. Tórres-Pérez et al. 2007). Particularmente, los ríos Maipo y Biobío ya habían sido aludidos en la literatura como barreras al flujo genético en varias especies de vertebrados (e.g. Chesser 1999; Tórres-Pérez et al. 2007; Sallaberry-Pincheira et al. 2011), lo cual se corrobora en el presente trabajo para *Liolaemus tenuis*. Si bien no se descarta que otros ríos en Chile estén afectando la distribución de la diversidad genética dentro de *L. tenuis*, el Maipo y Biobío serían los que ejercen un mayor impacto, probablemente debido a que los mismos marcan la transición entre distintas regiones bioclimáticas; el Río Maipo se ubica aproximadamente en el límite sur de la zona mediterránea semiárida, y el Río Biobío el límite de las zonas mediterránea húmeda y perhúmeda (Di Castri 1968). Al norte del Río Maipo altos niveles de aridez son acompañados de una vegetación del tipo de estepas arbustivas, bosques esclerófilos y matorrales. A su vez, hacia el sur va disminuyendo progresivamente la aridez y la temperatura, mientras que aumentan los vientos y las precipitaciones, pasando de vegetación del tipo sabana arbolada a bosque valdiviano dominado por *Notophagus* (Di Castri 1968; Etienne 1985). Por lo tanto, las diferencias en clima y composición florística a ambos lados de los ríos Maipo y Biobío, en conjunción con el efecto de barrera que los mismos ejercen podrían ser los determinantes la distribución geográfica de las poblaciones de *L. tenuis*.

Para concluir esta sección, nuestros resultados muestran que *Liolaemus tenuis* es una especie cuya demografía ha sido afectada por los cambios climáticos asociados a los ciclos glaciares, en conjunción a barreras topográficas y fluviales. La forma en la cual esta especie ha respondido a dichos cambios ha sido diferencial a lo largo de su distribución, siendo más afectadas las poblaciones de su rango sur (~35º-40ºS) así como las regiones

andinas. Las poblaciones del rango norte fueron afectadas en cierta medida, probablemente por efectos periglaciares, mientras que las poblaciones centrales se mantuvieron más estables. Finalmente, en base a las distancias genéticas, valores de *Fst* y a la ocurrencia en simpatría de dos clados divergentes en el rango sur, se predice que *L. tenuis* probablemente constituya un complejo de especies, lo cual fue evaluado en el capítulo 2.

Delimitación de especies

Nuestros análisis de delimitación de especies (Capítulo 2), los cuales integran datos genéticos multilocus, morfológicos y ambientales, sugieren con gran apoyo estadístico, la presencia de dos especies válidas dentro de *Liolaemus tenuis sensu lato*. Adicionalmente, los resultados de este trabajo sugieren que ambos taxa podrían ser diferenciados en base a la forma de su cabeza, atributo considerado bajo fuerte selección en lagartijas ya que estaría asociado con actividades de índole ecológica (e.g. alimentación, uso de microhábitat) y reproductiva (e.g. cópula, interacciones agonísticas entre machos) (Kaliontzopoulou et al. 2012).

La primera especie validada (especie candidata *Liolaemus tenuis tenuis*) incluye únicamente las poblaciones del rango norte de la especie, tal y como fue originalmente descrita, con Salamanca como límite norte, y el río Maipo como límite sur. Como se mencionó anteriormente, en esta región la Cordillera de los Andes alcanza mayores alturas, acompañadas por cordilleras montañosas transversales (e.g. Cordón Cantilla, ca. 2100 metros) y ríos que cruzan el territorio de este a oeste. Lo anterior hace que esta área presente un alto grado de fragmentación, lo cual promueve la estructuración de poblaciones y por lo tanto altos niveles de diversidad beta (Whittaker 1956). La segunda especie delimitada (la cual incluiría las especies candidatas *Liolaemus tenuis punctatissimus* y *Liolaemus sp*.) incluye poblaciones del sur del Río Maipo hasta Valdivia aproximadamente, región donde los Andes alcanzan menores alturas y el territorio presenta una mayor conectividad, favoreciendo procesos de dispersión y flujo génico entre las poblaciones. Esta especie presentaría entonces un rango distribucional mucho más amplio que *Liolaemus tenuis tenuis*, y ocurriría en un territorio mucho más interconectado.

Diversificación de Liolaemus tenuis sensu lato

Según nuestros resultados, el origen de *Liolaemus tenuis sensu lato* remonta al Pleistoceno medio (ca. 1.82 mya; mya=millones de años). Dado que la topología actual de Chile Central (~33º-36°S) comenzó a forjarse en el Mioceno temprano (Ghosh et al. 2006; Farías et al. 2008), se descarta el levantamiento de los Andes como proceso subyacente a la diversificación dentro de este linaje. Sin embargo, en el Plioceno tardío-Pleistoceno temprano se registran ya glaciaciones, las cuales, mediante los cambios que produjeron a nivel del clima, paisaje y nivel del mar, en conjunción con los eventos tectónicos y geomorfológicos que experimentó la región, probablemente tuvieron un rol central en los procesos de diversificación que atravesó *Liolaemus tenuis*. Si bien la glaciación más importante en esta región ocurrió hace solo un millón de años (Gran Glaciación Patagónica = GPG) (Rabassa et al. 2011), hay evidencia de varias glaciaciones que tuvieron lugar entre la Primera Glaciación Patagónica (PGP; ~3.5 mya) y la GPG. La extensión y duración de dichos eventos, así como el tiempo de los avances y retrocesos glaciares no se conocen con precisión para las glaciaciones previas a la GPG (Pre-GPG). Sin embargo, sí hay evidencia de una glaciación datada entre los 2.05-1.86 mya (Mercer, 1976), lo cual sería coincidente con los tiempos de diversificación estimados para los dos taxa delimitados (Capítulo 2; Fig. 5). Se considera que las glaciaciones que tuvieron lugar a partir de 1.8 mya cubrieron completamente la Patagonia, desde los 36° a los 56°S (McCulloch et al. 2000; Hulton et al. 2002). Adicionalmente, tal como se mencionaba en la sección anterior, hay evidencia que sugiere que el Río Maipo habría jugado un rol en la diversificación dentro de *L. tenuis sensu lato*. La cuenca de este río se encuentra en los Andes, donde se almacenó mucha agua en forma de hielo durante los períodos glaciares. Durante las deglaciaciones, con el aumento de la temperatura y el derretimiento de los hielos, el caudal del Río Maipo (así como de otros ríos que nacen en los Andes), habrían aumentado considerablemente, reforzando el efecto de barrera. Por lo tanto, es posible que las glaciaciones Pre-GPG hayan jugado un rol en la diversificación de *Liolaemus tenuis sensu lato*, las cuales en conjunción con barreras topográficas como cadenas montañosas transversales y ríos dieron lugar a procesos de diversificación en alopatría.

Alcances taxonómicos

En este trabajo elevamos las dos subespecies descritas dentro de *Liolaemus tenuis sensu lato*, *Liolaemus tenuis tenuis* y *Liolaemus tenuis punctatissimus* a nivel de especie. Adicionalmente, se redefinen sus rangos de distribución ya que no son concordantes con las descripciones originales, las cuales postulaban que ambas subespecies se solapaban entre los 36º y 39º grados de latitud sur (de la Región del Biobío a Los Ríos). El nuevo re-arreglo propuesto en esta tesis, considera dos especies para las cuales no hay evidencia de simpatría; *Liolaemus tenuis* ocurriría en las regiones de Coquimbo, Valparaíso y Metropolitana, y *Liolaemus punctatissimus* en las regiones de O'Higgins, Maule, Biobío, Araucanía y Los Ríos. Los cambios taxonómicos aquí propuestos tendrán importantes implicancias en el ámbito de la conservación de estas especies, dado que la distribución de *Liolaemus tenuis sensu stricto* se reduce de forma considerable. Adicionalmente, dado que las poblaciones argentinas que anteriormente eran clasificadas dentro de *Liolaemus tenuis sensu lato* ahora se incluyen dentro de *Liolaemus punctatissimus*, *Liolaemus tenuis* se vuelve una especie endémica de Chile.

Comentarios sobre la aparente coloración críptica entre L. tenuis y L. punctatissimus

Las subspecies dentro de *Liolaemus tenuis sensu lato* fueron originalmente descritas en base a patrones de coloración, un carácter que puede variar mucho dentro y entre especies y por lo tanto no puede ser considerado un indicador confiable sobre las relaciones evolutivas (Burbrink et al. 2000). Normalmente es la selección sexual la fuerza evolutiva que se encuentra detrás de la coloración; en especies en las cuales los machos son coloridos, este color suele ser indicativo de la calidad del macho (Hamilton & Zuk 1982; Folstad & Karter 1992) y hay evidencia de que las hembras basan su elección de pareja en base a este carácter (Lancaster et al. 2007, 2009). Estudios previos no han logrado encontrar diferencias en los patrones de coloración entre las dos subspecies descritas de *Liolaemus tenuis*, proponiendo en base a esto que se trata de una única especie de amplia distribución (Vidal et al. 2007). Considerando los resultados aquí encontrados, y el hecho de que *L. tenuis* and *L. punctatissimus* serían especies hermanas, es posible que haya una fuerte selección asociada a los patrones de coloración de los machos. Probablemente, dado que ambos taxa se encuentran en alopatría, esta coloración críptica se ha podido mantener a

lo largo del tiempo (en oposición a si la especiación se hubiera dado en simpatría, caso en el cual se esperaría un desplazamiento de caracteres; Brown & Wilson 1956), y las especies hayan desarrollado otros mecanismos de aislamiento reproductivo, como por ejemplo post-cigóticos. Se postula que procesos como retención de polimorfismos ancestrales, selección estabilizadora o plasticidad fenotípica podrían estar detrás de este tipo de patrones (Zamudio et al. 2016). Sin embargo, algunos estudios sugieren que las poblaciones del sur de *L. tenuis sensu lato* (lo que sería ahora *L. punctatissimus*), que están asociadas a bosques más densos, serían más coloridos que los machos de las poblaciones del norte (*L. tenuis*) (Müller & Hellmich 1933; Donoso-Barros 1966; Hellmich 1950; Vidal et al., 2007), con lo cual sigue abierta la posibilidad de que estos dos taxa efectivamente puedan ser diferenciados en base a su coloración. Un estudio detallado, teniendo en cuenta la distribución propuesta para *L. tenuis* y *L. punctatissimus* en este trabajo, sería necesario para establecer si ambas especies pueden ser efectivamente diferenciadas en base a su coloración o si representan una coloración críptica.

Sobre la incongruencia entre la filogenia mitocondrial y multilocus

Cabe señalar que la filogenia mitocondrial (Capítulo 1; Fig. 1) no recupera las mismas relaciones que el árbol de especies multilocus (Capítulo 2; Fig. S1). En la primera, se infiere que el linaje que en el Capítulo 2 llamamos *L. t. tenuis*, es hermano de *L. t. punctatissimus* con una probabilidad *a posteriori* moderada (Subclado I.2, Fig. 1, pp=0.77). Este subclado es a su vez hermano del linaje que nombramos *Liolaemus sp.* (Clado II) con una probabilidad *a posteriori* alta (pp=1). Por otra parte, el árbol multilocus no apoya estas relaciones, ya que recupera a *L. t. punctatissimus* y *L. sp.* como linajes hermanos (pp=1). Este tipo de discordancias es esperable debido al tiempo mayor de coalescencia que requieren los marcadores nucleares (incluidos en las inferencias del árbol de especies) respecto a los mitocondriales (Moore 1995; Palumbi et al. 2001). Sin embargo, hay otras explicaciones posibles, además de la estocasticidad coalescente (Kingman 2000). El ADN mitocondrial es un genoma haploide, que refleja la historia matrilineal de una especie al ser heredado por línea materna. En casos de diferencias en la dispersión entre los sexos, como cuando ocurre filopatría de las hembras, puede no reflejar la historia evolutiva de la especie, mostrando por ejemplo clados estructurados inferidos con ADN mitocondrial y

una estructura genética baja con marcadores nucleares (e.g. Seppä et al. 2006). Por otra parte, si los eventos de divergencia poblacional fueron múltiples y poco espaciados temporalmente, el simple hecho de realizar inferencias basadas en un único marcador, sea de naturaleza nuclear o mitocondrial, probablemente lleve a la inferencia de un árbol "incorrecto" debido a la repartición incompleta de linajes. Otras explicaciones plausibles pero raramente consideradas en estos contextos es el llamado "*allele surfing*" (Klopfstein et al. 2006; Excoffier & Ray 2008), en el cual luego de una expansión poblacional a partir de una pequeña población (e.g. a partir de un refugio), alelos de baja frecuencia podrían alcanzar altas frecuencias rápidamente llevando a una alta estructuración de estas poblaciones. Esto tendría como consecuencia una incongruencia entre filogenias estimadas con estos marcadores y filogenias estimadas con marcadores en los cuales no se dio este fenómeno.

A modo de conclusión, en este trabajo de identifican dos linajes dentro de *Liolaemus tenuis sensu lato*, *Liolaemus tenuis* y *Liolaemus punctatissimus*, los cuales pueden ser diferenciados en base a datos genéticos, morfológicos y ambientales. Estos linajes habrían divergido en el Pleistoceno medio, posiblemente por pulsos vicariantes, generados por la acción conjunta de los ríos y las oscilaciones entre glaciales e interglaciales (glaciaciones Pre-GPG). El árbol de especies estimado difiere de las relaciones entre linajes que sugiere la topología de la filogenia mitocondrial, lo cual es posiblemente explicado por estocasticidad coalescente. Los árboles de genes estimados a partir de los marcadores nucleares están menos resueltos que el árbol mitocondrial pero aun así puede apreciarse la tendencia general que agrupa a los haplotipos pertenecientes a *L. tenuis*. Por otra parte, estudios que aborden más exhaustivamente las diferencias morfológicas entre *L. tenuis* y *L. punctatissimus* serían necesarios, ya que aquí solo fueron consideradas diferencias en cuanto a la forma de la cabeza. En este mismo sentido, sería necesario re-evaluar (ya que la distribución de ambas especies ha sido re-definida) si existe una diferencia en patrones de coloración entre ambas especies.

Relaciones filogenéticas en el subgénero *Liolaemus*

Los arreglos filogenéticos de las especies del subgénero *Liolaemus* inferidos en este trabajo (Capítulo 3) no son congruentes con aquellos propuestos en base a caracteres morfológicos. Los resultados confirman que las

secciones *nigromaculatus* y *chiliensis* dentro de *Liolaemus sensu stricto* (subgénero *Liolaemus*) no son monofiléticas, como tampoco lo son ninguno de los grupos de especies aquí considerados (*alticolor-bibronii*, *pictus*, *nigromaculatus*, *nigroviridis*). En términos generales, los tres conjuntos de datos utilizados (elementos ultraconservados, genes codificantes y ambos) apoyaron la misma filogenia, independiente del método utilizado para su inferencia. El hecho de haber obtenido resultados consistentes, independiente de la naturaleza de los datos y de la aproximación utilizada sugiere que este tipo de datos filogenómicos puede ser usado de forma efectiva para generar inferencias robustas en *Liolaemus*.

Algunos de los resultados obtenidos confirman relaciones filogenéticas propuestas en base a un solo locus, cómo la cercana relación de parentesco (aunque no resuelta) entre las especies simpátridas *Liolaemus fuscus*, *L. montícola*, *L. nitidus* y *L. nigroviridis*, recuperada también por Schulte et al. (2000), Schulte & Moreno-Roark (2010), Guerrero et al. (2013), Schulte (2013) y Troncoso-Palacios et al. (2015). Sin embargo, estas especies son clasificadas en secciones y grupos distintos (grupos *montícola* y *nigroviridis* pertenecen a la sección *nigromaculatus* mientras que los grupos *alticolor-bibronii* y *robertmertensi* pertenecen a la sección *chiliensis*). La validez de los grupos *montícola* y *robertmertensi* no pudo ponerse a prueba dado que se incluyó solo un representante de cada grupo, y un mínimo de dos es necesario para evaluar monofilia. Las dos especies incluidas del grupo *nigroviridis* (*L. isabelae* y *L. nigroviridis*) y *alticolor-bibronii* (*L. fuscus* y *L. paulinae*) señalan que estos grupos no son naturales ya que ambos pares de especies no forman clados exclusivos. Cabe señalar que *L. isabelae* y *L. nigroviridis*, así como *L. fuscus* y *L. paulinae* no ocurren en simpatría; *Liolaemus isabelae* habita únicamente en la región de Atacama, mientras que *L. nigroviridis* se encuentra restringida a los alrededores de la Región de Valparaíso y Metropolitana. Por su parte, *L. fuscus* presenta una amplia distribución, desde La Serena a Concepción aproximadamente, pero la misma no se solapa con la distribución de *L. paulinae*, restringida a los alrededores de Calama. Filogenias recientes basadas en ADN ya cuestionaban la inclusión de estas especies en los mismos grupos, dado que en ninguna filogenia *L. fuscus* es inferida como hermana de *L. paulinae* ni *L. isabelae* hermana de *L. nigroviridis*. Guerrero et al. (2013) y Schulte (2013) recuperan a *L. isabelae* como hermana de *L. paulinae*, lo cual es incongruente con nuestros resultados, dado que *L. isabelae* está más cercanamente emparentada con *L. atacamensis* y *L. nigroviridis* aparece en el mismo clado que *L. fuscus*.

Las filogenias estimadas recuperan también un clado compuesto por cuatro de las cinco especies muestreadas del grupo *nigromaculatus*, recuperando a su vez y de forma consistente, a las especies del grupo "*platei*" (dentro del grupo *nigromaculatus; L. velosoi* y *L. platei*) como especies hermanas, congruente con otras filogenias mitocondriales (e.g. Troncoso-Palacios et al. 2015). Los resultados implican que el grupo *nigromaculatus* es polifilético, a menos que se excluya de este grupo a *L. atacamensis*, que aparece más cercanamente emparentada con *L. isabelae*. En dicho caso, el grupo *nigromaculatus* formaría un grupo natural, con alto soporte estadístico. Otro resultado esperado, especialmente considerando las conclusiones del capítulo 2, fue que los dos linajes pertenecientes a *Liolaemus punctatissimus* (denotados como *Liolaemus t. punctatissimus* y *L. sp*.) siempre se recuperan como linajes hermanos. Lamentablemente, debido a un bajo número de datos recuperados para *Liolaemus tenuis sensu stricto*, no pudo inferirse su posición en la filogenia. Por otra parte, se recuperaron algunas relaciones inesperadas, es decir, relaciones que no han sido reportadas en ninguno de los trabajos publicados a la fecha. Un ejemplo es la relación *L. pictus-L. paulinae*, inferida de forma consistente a través de los distintos análisis y con distintas combinaciones de datos, y con alto soporte estadístico. Sin embargo, ambas especies presentan distribuciones muy disyuntas, y al ser el muestreo realizado una submuestra de la diversidad total de especies del género *Liolaemus*, consideramos que esta relación es probablemente artificial.

El mensaje más trascendente que se desprende de esta sección es reafirmar la incongruencia entre los grupos propuestos en base a caracteres morfológicos y los clados recuperados en base a secuencias de ADN, lo cual ya había sido sugerido por otros trabajos que se basaron en filogenias estimadas con secuenciación Sanger para marcadores mitocondriales (e.g. Troncoso-Palacios et al. 2015). El incremento en la cantidad de loci para inferencias filogenéticas es ventajoso ya que aumenta el poder estadístico y permite la resolución de discordancias que surgen de analizar y comparar filogenias en base a locus individuales. Por lo tanto, el presente trabajo, al ser realizado con un número ampliamente superior de marcadores y de distintas naturalezas, en combinación con diversas aproximaciones metodológicas (incluyendo el primer árbol de especies estimado para el subgénero *Liolaemus*), confirma las aseveraciones anteriores, dejando en evidencia lo inapropiado de la taxonomía actual en cuanto a la clasificación en grupos de las lagartijas del subgénero *Liolaemus.* Si bien solo incluimos una pequeña

submuestra de la gran diversidad de este grupo, y por lo tanto los alcances de los resultados presentan restricciones, en este trabajo se presenta la primera perspectiva filogenómica para el subgénero *Liolaemus*. Se espera que las relaciones aquí inferidas sirvan como "generadoras de hipótesis", dado que puede ser considerado un punto de partida para futuros estudios, que sirvan para indicar por ejemplo las regiones de la filogenia que necesitan ser más muestreadas para lograr una taxonomía más clara, como re-definir grupos en base a afinidades evolutivas, entre otros. Adicionalmente, esta filogenia podría dar lugar a estudios más finos, que intenten comprender los procesos de especiación detrás de este género tan rico y diverso de lagartijas.

CONCLUSIONES

Los resultados aquí obtenidos dejan en evidencia que:

-Los eventos tectónicos y climáticos que se desarrollaron durante el Plioceno-tardío (Neógeno) al Pleistoceno temprano (Cuaternario) parecen dar cuenta de los procesos de diversificación dentro de *L. tenuis*.

-Las glaciaciones pleistocénicas impactaron la demografía de *L. tenuis sensu lato*, sobre todos la de aquellas poblaciones que ocurren al sur de los 36°S, las cuales exhiben signos de reducciones poblacionales seguidas por expansiones y contacto secundario.

-La diversidad dentro de *L. tenuis sensu lato* estaba siendo subestimada, ya que la especie contiene diversidad correspondiente a dos especies válidas.

-Como consecuencia, se elevaron las dos subespecies existentes a especies, con una re-definición de sus rangos distribucionales. La misma, hizo que *L. tenuis sensu stricto* pasara a ser una especie con un rango muy reducido, y es ahora especie endémica de Chile.

-El uso integrado de información genética, morfológica y ecológica hace no solo más robustas las inferencias en cuanto a los límites de las especies sino que hace más completa la descripción de nuevas especies (e.g. su identificación, probables proceso subyacentes en la historia de su diversificación y definición de nuevos rangos distribucionales).

-Las relaciones evolutivas inferidas en este trabajo a partir de datos filogenómicos no es congruente con las filogenias inferidas en base a caracteres morfológicos. Las secciones *nigromaculatus* y *chiliensis* contenidas dentro de *Liolaemus sensu stricto* son polifileticas. Los grupos estudiados (e.g. *nigromaculatus*, *alticolor-bibronii*, etc) son también polifiléticos. Una urgente re-definición de estos grupos y secciones es necesaria, sobre todo debido al alto ritmo de descripción de nuevas especies de *Liolaemus*.

BIBLIOGRAFÍA

Brown, W.L. & Wilson, E.O. 1956. Character displacement. Systematic Zoology 5, 49–64.

Brüggen, J., 1950. Fundamentos de la Geologia de Chile. Santiago: Instituto Geográfico Militar.

Burbrink, F.T., Lawson, R. & Slowinski, J.B. 2000. Mitochondrial DNA phylogeography of the polytypic North American rat snake (*Elaphe obsoleta*): a critique of the subspecies concept. Evolution 54, 2107–2118.

Chesser R.T. 1999.Molecular systematics of the rhinocryptid genus *Pteroptochos*. Condor 439-446.

Donoso-Barros, R. 1966. Reptiles de Chile. Universidad de Chile.

Etienne, M. 1985. La forêt mediterranéenne du Chili. Forêt méditerranéenne, t. VII, n.1, 1985.

Excoffier, L. & Ray, N. 2008. Surfing during population expansions promotes genetic revolutions and structuration. Trends in Ecology & Evolution, 23(7), 347-351.

Farías M., Charrier R., Carretier S., Martinod J., Fock A., Campbell D., Cáceres J. & D. Comte. 2008. Late Miocene high and rapid surface uplift and its erosional response in the Andes of central Chile (33–35 S). Tectonics, 27(1).

Folstad, I. & Karter A.K. 1992. Parasites, bright males and the immune-competence handicap. American Naturalist 139, 603–22.

Fox, A. N. 1993. Snowline altitude and climate in the central Andes (5-280S) at present and during the late Pleistocene glacial maximum. Cornell University, August.

Ghosh P., Garzione C.N. & J.M. Eiler. 2006. Rapid uplift of the Altiplano revealed through 13C–18O bonds in paleosol carbonates. Science, 311: 511–515.

Guerrero, P. C., Rosas, M., Arroyo, M. T. & Wiens, J. J. 2013. Evolutionary lag times and recent origin of the biota of an ancient desert (Atacama–Sechura). Proceedings of the National Academy of Sciences, 110, 11469-11474.

Hamilton, W.D. & Zuk, M. 1982. Heritable true fitness and bright birds: a role for parasites? Science 218, 384–7.

Hellmich, W. 1950. Die Eidechsen der Ausbeute Schröder (Gattung *Liolaemus*, Iguan.) (Beiträge zur Kenntnis der Herpetofauna Chiles XIII). Veröffentlichungen der Zoologischen Staatssammlung München 1.

Heusser, C.J. 2003. Ice age southern Andes, A cronichle of paleoecological events.Developments in quaternary science, 3. Elsevier, 238 pp

Hulton, N.R.J., Purves, R.S., McCulloch, R.D., Sugden, D.E. & Bentley M.J. 2002. The last glacial maximum and deglaciation in southern. South America. Quaternary Science Reviews, 21, 233–241.

Kaliontzopoulou, A., Adams, D. C., van der Meijden, A., Perera, A., & Carretero, M. A. 2012. Relationships between head morphology, bite performance and ecology in two species of *Podarcis* wall lizards. Evolutionary Ecology, 26(4), 825-845.

Kingman, J.F.C. 2000. Origins of the coalescent: 1974–82. Genetics, 156, 1461–1463.

Klopfstein, S., Currat, M. & Excoffier, L.2006. The fate of mutations surfing on the wave of a range expansion. Molecular Biology and Evolution 23, 482–490.

Lancaster, L.T., McAdam, A.G., Wingfield, J.C. & Sinervo, B.R. 2007. Adaptive social and maternal; induction of antipredator dorsal patterns in a lizard with alternative social strategies. Ecology Letters 10, 798–808. DOI: 10.1111/j.1461-0248.2007.01069.x

Lancaster, L.T., Hipsley, C.A. & Sinervo, B. 2009. Female choice for optimal combinations of multiple male display traits increases offspring survival. Behavioral Ecology 20(5), 993–9. DOI: https://doi.org/10.1093/beheco/arp088

Lobo, F. 2005. Las relaciones filogenéticas dentro del grupo *chiliensis* (Iguania: Liolaemidae: *Liolaemus*): sumando nuevos caracteres y taxones. Acta zoológica llloana, 49, 65-87.

Lobo, F., Espinoza, R. E. & Quinteros, S. 2010. A critical review and systematic discussion of recent classification proposals for liolaemid lizards. Zootaxa, 2549, 1-30.

McCulloch, R.D., Bentley, M.J., Purves, R.S., Hulton, N. R. J., Sugdeni, D.E. & C. M. Clapperton. 2000 Climatic inferences from glacial and palaeoecological evidence at the last glacial termination, southern South America. Journal of Quaternary Science, 15, 409–417.

Mercer, J. H. 1976. Glacial history of southernmost South America. Quaternary Research 6(2): 125-166.

Moore, W.S. 1995. Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. Evolution, 49, 718–726.

Morando, M., Avila, L.J. & Sites J.J.W. 2003. Sampling strategies for delimiting species: genes, individuals, and populations in the *Liolaemus elongatus-kriegi* complex (Squamata: Liolaemidae) in Andean-Patagonian South America. Systematic Biology 52: 159–185.

Morando, M., Avila, L.J., Turner, C.R. & Sites J.W. Jr. 2007. Molecular evidence for a species complex in the Patagonian lizard *Liolaemus bibronii* and phylogeography of the closely related *Liolaemus gracilis* (Squamata: Liolaemini). Molecular Phylogenetics and Evolution 43: 952–973.

Muellner A.N., Tremetsberger K., Stuessy T. & Baeza C.M. 2005. Pleistocene refugia and recolonization routes in the southern Andes: insights from *Hypochaeris palustris* (Astraceae, Lactuceae). Molecular Ecology, 14, 203–212.

Müller, L. & Hellmich, W. 1933. Beiträge zur Kenntnis der Herpetofauna Chiles. VIII. Bemerkungen über *Liolaemus tenuis* (Duméril et Bibron). Zool. Anz. 104 (11/12), 305-310.

Muñóz-Mendoza, C., D´Elia, G., Panzera, A., Mendez, M., Villalobos-Leiva, A., Sites, J.W.Jr., Victoriano, P. F. en prensa. Geography and past climate changes have shaped the evolution of a widespread lizard from the Chilean hotspot. Aceptado con modificaciones en: Molecular Phylogenetics and Evolution.

Muñoz-Ramírez, C. P., Unmack, P. J., Habit, E., Johnson, J. B., Cussac, V. E. & Victoriano, P. 2014. Phylogeography of the ancient catfish family Diplomystidae: biogeographic, systematic, and conservation implications. Molecular Phylogenetics and Evolution, 73, 146-160.

Nuñez, J. J., Wood, N. K., Rabanal, F. E., Fontanella, F. M. & Sites, J. W. 2011. Amphibian phylogeography in the Antipodes: Refugia and postglacial colonization explain mitochondrial haplotype distribution in the Patagonian frog *Eupsophus calcaratus* (Cycloramphidae). Molecular Phylogenetics and Evolution, 58(2), 343-352.

Palumbi, S.R., Cipriano, F. & Hare, M.P. 2001. Predicting nuclear gene coalescence from mitochondrial data: the three-times rule. Evolution, 55, 859–868.

Pamilo K. & Nei, M. 1988. Relationships between gene trees and species trees. Molecular Biology and Evolution 5(5): 568–583.

Pincheira-Donoso, D. & Núñez, H. 2005. Las especies chilenas del género *Liolaemus*Wiegmann, 1834 (Iguania Tropiduridae: Liolaeminae). Taxonomía, sistemática y evolución. Publicación ocasional del Museo Nacional de Historia Natural, 59, 7-486.

Premoli, A.C., Vergara, R., Souto, C.P., Lara, A. & Newton, A.C. 2003. Lowland valleys shelter the ancient conifer *Fitzroya cupressoides* in the Central Depression of Southern Chile. Journal of the Royal Society of New Zealand, 33, 623–631.

Rabassa J., Coronato A. & O. Martínez. 2011. Late Cenozoic glaciations in Patagonia and Tierra del Fuego: an updated review. Biological Journal of the Linnean Society 103: 316–335.

Ruzzante, D. E., Walde, S. J., Gosse, J. C., Cussac, V. E., Habit, E., Zemlak, T. S.& Adams, E. D. 2008. Climate control on ancestral population dynamics: insight from Patagonian fish phylogeography. Molecular Ecology, 17(9), 2234-2244.

Sallaberry-Pincheira N., Garin C. F., González-Acuña D., Sallaberry M. A. & J.A. Vianna.2011. Genetic divergence of Chilean long-tailed snake (*Philodryas chamissonis*) across latitudes: conservation threats for different lineages. Diversity and Distributions 17(1): 152-162.

Schulte, J. A. 2013. Undersampling taxa will underestimate molecular divergence dates: an example from the South American lizard clade Liolaemini. International journal of evolutionary biology, 2013, 1-12.

Schulte Ii J. A., Macey R. J., Espinoza R. E. &A. Larson. 2000. Phylogenetic relationships in the iguanid lizard genus *Liolaemus*: multiple origins of viviparous reproduction and evidence for recurring Andean vicariance and dispersal. Biological Journal of the Linnean Society 69(1): 75-102.

Schulte, J. A. & Moreno-Roark, F. 2010. Live birth among Iguanian lizards predates Pliocene–Pleistocene glaciations. Biology letters, 6, 216-218.

Seppä, P., Fernandez-Escudero, I., Gyllenstrand, N. & Pamilo, P. 2006. Obligatory female philopatry affects genetic population structure in the ant *Proformica longiseta*. Insectes sociaux, 53(3), 362-368.

Sérsic, A., A. Cosacov, A.A. Cocucci, L. Johnson, R. Pozner, L.J. Avila, J.W. Sites & Morando, M. 2011. Emerging phylogeographical patterns of plants and terrestrial vertebrates from Patagonia. Biological Journal of the Linnean Society 103, 475 – 494.

Torres-Pérez F., Lamborot M., Boric-Bargetto D., Hernandez C. E., Ortiz J. C. & R.E. Palma. 2007. Phylogeography of a mountain lizard species: An ancient fragmentation process mediated by riverine barriers in the *Liolaemus monticola* complex (Sauria: Liolaemidae). Journal of Zoological Systematics and Evolutionary Research 45: 72–81.

Troncoso-Palacios, J., Elorza, A. A., Puas, G. I. & Alfaro-Pardo E. 2016. A new species of *Liolaemus* related to *L. nigroviridis* from the Andean highlands of Central Chile (Iguania, Liolaemidae). ZooKeys, 555, 91.

Vera-Escalona, I., D'Elía, G., Gouin, N., Fontanella, F. M., Muñoz-Mendoza, C., Sites Jr, J. W. & Victoriano, P. F. 2012. Lizards on ice: evidence for multiple refugia in *Liolaemus pictus* (Liolaemidae) during the last glacial maximum in the southern Andean beech forests. PloS one, 7(11), e48358.

Victoriano, P. F., Ortiz, J. C., Benavides, E., & Adams, B. J. 2008. Comparative phylogeography of codistributed species of Chilean *Liolaemus* (Squamata: Tropiduridae) from the central-southern Andean range. Molecular Ecology, 17(10), 2397-2416.

Vidal, M., Ortiz, J.C. & Labra A., 2007. Sexual and geographic variation of color patterns in *Liolaemus tenuis* (Squamata, Liolaeminae). Gayana 71(1), 27-33.

Villagrán, C. M. 1994. Quaternary history of the Mediterranean vegetation of Chile. En Ecology and biogeography of Mediterranean ecosystems in Chile, California, and Australia (pp. 3-20). Springer New York.

Villagrán, C., Moreno, P. & Villa, R. 1996: Antecedentes palinológicos acerca de la historia cuaternaria de los bosques chilenos. En: Armesto, J. J.; Villagrán, C.; Arroyo, M. K. ed. Ecología de los bosques nativos de Chile. Santiago, Chile, Editorial Universitaria. Pp. 51-70.

Vuilleumier, B.S. 1971. Pleistocene changes in the fauna and flora of South America. Science 173, 771-780.

Whittaker, R. H. 1956. Vegetation of the Great Smoky Mountains. Ecological Monographs. 26: 1–80