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FILOGEOGRAFÍA DE *Batrachyla taeniata* (GIRARD, 1855) E INFLUENCIA DEL PAISAJE SOBRE LA ESTRUCTURA GENÉTICA DE SUS POBLACIONES

Tesis para optar al grado de Doctor en Sistemática y Biodiversidad

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AUTORIZACIÓN

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RESUMEN GENERAL

Batrachyla taeniata es un anuro endémico de los bosques templados de *Nothofagus* del sur de Chile y Argentina. En Chile, tiene una amplia distribución que va desde la provincia de Aconcagua en la región de Valparaíso hasta la provincia Capitán Prat en la región de Aysén. Además, está presente en tres islas cercanas al continente, Quiriquina, Mocha y Chiloé. Esta especie es de particular interés desde un punto de vista biogeográfico, ya que, se encuentra en dos regiones ecológicas diferentes: Región Mediterránea y Oceánica, abarcando aproximadamente más de 1.500 km de norte a sur. En su distribución norte está asociada a bosques relictos costeros hidrófilos y mesófilos (ej: Zapallar y Quintero) rodeados de vegetación semiárida y en su distribución sur asociada a bosques húmedos y sombríos en ambientes saturados de humedad. La ecología térmica de *B. taeniata* revela que esta especie es termoconformista como la mayoría de los anfibios, debido a un compromiso entre las condiciones térmicas y balance de agua que reducen la capacidad de estos animales para regular la temperatura. La evidencia ecológica sugiere que existen diferencias en los caracteres reproductivos entre las poblaciones de distribución norte y distribución sur. Estas diferencias estarían relacionadas con la variabilidad climática, particularmente disponibilidad de agua, producto de la presencia o ausencia de lluvias,

confiriéndole a las poblaciones de su distribución norte una notable capacidad adaptativa.

La distribución actual de *B. taeniata* abarca áreas de alta heterogeneidad topográfica, que incluyen áreas de mayor estabilidad y de menor estabilidad histórica relativa, debido a que han sido expuestas reiteradas veces a las glaciaciones. A pesar de que esta especie ha sido asociada a bosque de *Nothofagus*, su presencia en el norte ha sido explicada como un relicto, producto de los avances del bosque junto con la especie hacia latitudes menores durante las glaciaciones del Pleistoceno. Sin embargo, esta hipótesis no ha sido evaluada y por otra parte, las relaciones florísticas que aún perduran entre el bosque Valdiviano y Chile central-norte se remontan a una época anterior a la diversificación de las actuales asociaciones del bosque sureño. La evidencia genética sugiere que existe una correlación significativa entre la distancia genética y distancia geográfica. La población del norte de su distribución estaría altamente estructurada con relación a las poblaciones del sur.

En esta tesis, por medio de inferencias filogeográficas, demográficas y modelos de nicho, estudiamos si los mecanismos históricos como los ciclos glaciales han condicionado la distribución de sus linajes y patrones de contracción – expansión, tanto genéticos como geográficos (Capítulo I). Para ello, se realizó

un análisis filogeográfico de la especie, en función de la variabilidad genética de sus poblaciones. Mediante este análisis se buscó inferir el origen de las poblaciones actuales con sus respectivas rutas de recolonización y se evaluó el efecto contracción-expansión demográfica y geográfica. En segundo lugar, en el contexto actual de las poblaciones, se evaluó si la diversidad genética y estructura poblacional depende del grado de conectividad entre poblaciones, su ubicación dentro del rango geográfico que ocupa la especie y la heterogeneidad del paisaje (Capítulo II). Para ello, se realizó un análisis demográfico y de genética del paisaje, mediante el uso de marcadores moleculares microsatélites, evaluando si en las poblaciones de *B. taeniata* se cumple el Patrón poblacional Central-Periférico de Mayr (CPH).

Los resultados generales de esta investigación identifican dos grandes grupos genealógicos dentro de la distribución latitudinal de *B. taeniata* separados por el Río Biobío y asociados en el caso del grupo norte a la ecorregión Mediterránea y en el caso del grupo sur, a la ecorregión Oceánica. Los clados del grupo sur han experimentado expansiones demográficas desde el Pleistoceno (~ 0.04 mya), comenzando desde una época anterior a la LGM, por lo que este último evento glacial no tuvo un efecto directo en la reducción de sus tamaños efectivos (Ne). Por otro lado, el inicio de signos sólidos de crecimiento demográfico se evidenció para los clados asociados al rango central de

distribución a partir de ~ 0.01 mya, en contraste con el clado norte, ya que no muestran signos evidentes de expansión demográfica, ajustándose a un modelo de tamaño poblacional constante. El modelo de paleodistribución muestra signos de un proceso de contracción asociado con el LGM seguido de una expansión geográfica posterior. La tendencia potencial del patrón de cambio en el tamaño de la distribución de la especie es LIG> LGM <actual. Por lo que, en esta primera parte, se revela que las poblaciones del grupo sur de *B. taeniata* se encuentran en un posible proceso de expansión demográfica desde antes de la LGM y de expansión geográfica desde el LGM (Capítulo I). Con relación a la información revelada por las poblaciones mediante el uso de microsatélites, se encontró soporte estadístico para mostrar que en general, existe una diferenciación y estructura genética en la especie, de acuerdo con sus patrones de distribución norte-sur. Este patrón de diferenciación se asocia principalmente en poblaciones que se dan en la periferia del rango de distribución, dado el menor número de alelos, baja heterocigosidad, mayor grado de estructuración, alto valor de consanguinidad y baja tasa de migración con respecto a las poblaciones que se distribuyen en el centro. Además, existe una alta correlación entre la distancia genética y geográfica, por lo que las poblaciones se ajustan a un modelo de aislamiento por distancia, apoyado por la distancia geográfica que tienen las poblaciones hacia el centro del rango de distribución, cumpliéndose la CPH para *B. taeniata*.

Palabras claves: Batrachylidae**,** ADN Mitocondrial, diversidad genética, demografía, modelos de nicho ecológico, microsatélites, genética del paisaje, Hipótesis Central-Periférica (CPH).

INTRODUCCIÓN GENERAL

Historia Natural de América del Sur y su efecto en la biodiversidad

La historia natural de América del Sur ha sido fluctuante y compleja, debido a los cambios climáticos y geomorfológicos ocurridos durante fines del Terciario y comienzos del Cuaternario (Mc. Culloch et al. 2000; Thorson 1999; Villagrán et al. 2004). Es por esto que durante los últimos años las consecuencias bióticas derivadas de los eventos volcánicos y orogenéticos así como, las cíclicas glaciaciones ocurridas durante el Cenozoico han recibido considerable atención (Rabassa et al. 2005) dado que han ayudado a modelar el paisaje y provocado efectos sobre la distribución y diversificación de la fauna (Smith-Ramírez 2004; Ruzzante et al. 2008).

Las glaciaciones durante el Pleistoceno cubrieron vastas áreas a ambos lados de los Andes (Heusser et al. 1999; Moreno et al. 1999; Lamy et al. 2007). La evidencia sugiere que en el sur de América del Sur, las glaciaciones ocurrieron desde el final del Mioceno, aproximadamente hace 6 Ma, con un efecto acentuado en el relieve andino durante el Pleistoceno a través de al menos cinco grandes glaciaciones de eventos fríos durante el último millón de años, con la Gran Glaciación Patagónica (GGP) (Rabassa et al. 2011; Victoriano 2020). Durante este período, los Andes Patagónicos estuvieron cubiertos por una capa continua de hielo montañoso, desde los 37 ° S hasta el Cabo de Hornos (56 ° S) (Rabassa et al. 2011). En Chile, el

Último Máximo Glacial (LGM), el evento frío más reciente, extendió esta capa de hielo marginalmente hacia el norte entre 38° S y 34° S (Heusser 2003).

El creciente interés por entender los procesos históricos que han intervenido en la biología evolutiva de los organismos que habitan el territorio patagónico ha sido relativamente documentado, ejemplo de ello son los trabajos realizados en marsupiales (Himes et al. 2008), roedores (Palma et al. 2012; Rodríguez-Serrano et al. 2006) lagartijas (Vidal et al. 2012; Victoriano et al. 2008; Morando et al. 2007) y anfibios (Nuñez et al. 2011). La mayoría de estos estudios han intentado evaluar el efecto de las glaciaciones sobre los procesos de contracción-expansión poblacional y geográfica. Es así como los estudios filogeográficos han revelado patrones congruentes dentro y entre plantas vasculares y vertebrados terrestres, como la presencia de refugios en bajas latitudes y la expansión postglacial a latitudes mayores (Sérsic et al. 2011). De acuerdo con lo anterior, un grupo de especies tolerantes a las bajas temperaturas se refugiaron en los límites o bordes de la capa de hielo (Cosacov et al. 2010). Por otra parte, un grupo de especies o poblaciones de estas se encontraban fuera del efecto directo de los glaciales, pudiendo posteriormente utilizar el territorio disponible que dejó la retracción del hielo durante los periodos interglaciales, para expandir sus rangos geográficos (Sérsic et al. 2011; Nuñez et al. 2011).

Patrones espaciales de variabilidad genética intrapoblacional de anfibios en Chile

De los diversos estudios filogeográficos con vertebrados, la mayoría de los anfibios presenta diferenciación filogeográfica profunda, básicamente por su poca vagilidad (Vázquez-Domínguez 2007). Sin embargo, en el norte de Chile, Victoriano et al. (2015) en la especie *Telmatobius marmoratus*, confirmaron un escenario reciente de expansión poblacional y eventos de alta conectividad a una edad más temprana que el final del Último Máximo Glacial, probablemente asociados con períodos más lluviosos en las tierras altas. En el centro de Chile, Vásquez et al. (2013) en *Rhinella arunco*, mostraron que, a pesar de presentar un bajo nivel de estructura filogeográfica, se destaca un efecto de las glaciaciones del Pleistoceno sobre la diversidad genética intrapoblacional del extremo sur de su área de distribución. Por otra parte, las glaciaciones afectaron directamente a la especie *Eupsophus calcaratus*, un anfibio de los bosques templados de Chile. En su estudio filogeográfico, se recuperaron seis linajes fuertemente sustentados, que fueron afectados por los cambios climáticos del Pleistoceno (Núñez et al. 2011).

Las generalizaciones sobre patrones filogeográficos compartidos entre especies han sido derivados de las inferencias de la historia demográfica a partir de datos genéticos e información geográfica (Victoriano et al. 2008; Vidal et al. 2012). Esto ha permitido estimar, cualitativa o cuantitativamente, los cambios del tamaño de las poblaciones (de expansión demográfica) y de

sus rangos geográficos (expansión espacial) (Camargo et al. 2013). En *E. calcaratus* (Nuñez et al. 2011) se observaron aumentos demográficos, pero sin evidencia de expansión en su rango. La historia demográfica de las especies es reflejada en los patrones de variación genética dentro y entre poblaciones (Gutenkunst et al. 2009), de este modo la estimación de parámetros poblacionales refleja cómo estos linajes han sido afectados por el cambio climático, la presencia de barreras geográficas, disponibilidad de recursos y/o aislamiento por distancia.

Modelos de nichos ecológicos: Proyecciones en tiempo y espacio

La historia de vida cuaternaria de las especies ha determinado su rango actual y grado de la distribución de variación genética presente (Pastorino 2002). La información del registro fósil puede ayudar a reconstruir las distribuciones históricas de algunos taxa (Carstens & Richards 2007). Sin embargo, la mayoría de los taxa carecen de fósiles suficientes, por esta razón, el modelado paleodistribucional puede proporcionar valiosos datos geográficos espaciales referentes a las distribuciones ancestrales (Carstens & Richards 2007) y apreciar los cambios a través del tiempo de los taxa (Martínez-Meyer & Peterson 2006; Camargo et al. 2013). De esta manera, proyectando modelos de distribución actuales sobre coberturas climáticas pasadas y los datos de ocurrencia de especies, mediante procedimientos estadísticos o de aprendizaje automático, se puede predecir si las especies han conservado, reducido, fragmentado o bien cambiado su distribución

geográfica (Anderson et al. 2003; Martínez-Meyer et al. 2004; Peterson et al. 2004; Roberts & Hamann 2012). La estimación de distribuciones pasadas mediante el uso de modelos climáticos se ha utilizado para investigar preguntas sobre extinciones, ubicaciones de refugios, rutas de migración o para proponer hipótesis sobre la estructura de la población pasada de especies en estudios filogeográficos (Nogués-Bravo 2009). Por ejemplo, pueden usarse para explorar cómo las condiciones climáticas del LGM afectaron las distribuciones pasadas y actuales (Kukwa & Kolanowska 2016; Fuentes-Hurtado et al. 2016; Banaccorso et al. 2006).

Estos modelos tienen limitaciones importantes que necesitan ser consideradas en la interpretación de sus resultados (Roberts & Hamann 2012), debido a que la solidez de ellos depende de los enfoques utilizados para desarrollar el modelo, la estabilidad de los nichos ecológicos, así como restricciones en las distribuciones geográficas a través del tiempo y en diferentes contextos de la comunidad (Peterson et al. 2004). Por esta razón es necesario caracterizar adecuadamente el nicho de la especie (Neerincks et al. 2010). Esta situación se da cuando el modelo predice bien la distribución conocida de la especie, es decir, las variables utilizadas son adecuadas y la especie está en equilibrio con el ambiente (Martínez-Meyer & Peterson 2006). Por ello, si en una especie, coincide su distribución potencial con su distribución real, entonces es probable que esté limitada principalmente por los parámetros abióticos de su distribución (Munguía et al. 2008). Esta limitación geográfica puede estar relacionada con el

conservadurismo de nicho (NC) (Wiens & Graham 2005), que es la tendencia de las especies a mantener estables en el tiempo caracteres de sus nichos ecológicos cuando se enfrentan a condiciones ambientales nuevas (Martínez-Meyer & Peterson 2006; Wiens & Donoghue 2004). En el contexto del modelamiento de nicho ecológico, estos caracteres se refieren a los requerimientos de las tolerancias de las especies a factores del ambiente que limitan sus rangos geográficos (Wiens et al. 2010),

El algoritmo basado en máxima entropía (Maxent) es un método de aprendizaje automático de propósito general con una formulación matemática simple y precisa para el modelado de distribución de especies (Phillips et al. 2006). Tiene como objetivo predecir la idoneidad ambiental de la especie en función de las variables ambientales dadas, su predicción es continua y su evaluación se realiza mediante el valor del área bajo la curva ROC (AUC) (Phillips et al. 2006; Phillips et al. 2004). Estos modelos pueden ser utilizados en la evaluación de la biodiversidad, el diseño de reservas, el manejo del hábitat y la restauración, los planes de conservación de especies y hábitats y la predicción de los efectos del cambio ambiental en las especies y los ecosistemas (Franklin 2009) como por ejemplo los eventos de contracción y expansión geográfica a través del tiempo (Camargo et al. 2013).

Patrón poblacional Central-Periférico (CPH) de Mayr (1963)

Las características del paisaje pueden afectar en gran medida los patrones de dispersión y flujo genético entre las poblaciones y, por tanto, moldear la dinámica de la población y sus trayectorias evolutivas (Funk et al. 2005). En los últimos años, se han desarrollado herramientas estadísticas para revelar la acción de flujo de genes durante la divergencia. La migración entre poblaciones hace que ellas tiendan a homogeneizarse entre sí y que parezcan más como una sola población (Pinho & Hey 2010), ya que el flujo de genes las uniformiza y contrarresta la diferenciación genética que pudiera ocurrir por el aislamiento geográfico (Slatkin 1987). Muchos estudios han encontrado que la diferenciación genética entre poblaciones aumenta con la distancia geográfica entre ellas (Brieva & Formas 2001; Gallardo et al. 2011). Por esto, existe un creciente interés en la cuantificación de la estructura genética de las poblaciones, a través de sus áreas de distribución geográfica (Eckert et al. 2008).

Desde un punto de vista intrapoblacional, la hipótesis del Patrón poblacional Central-Periférico (CPH) propuesto por Mayr (1963), predice que las poblaciones periféricas reducen la variabilidad genética, y poseen una mayor diferenciación genética que las poblaciones geográficamente centrales (Dai & Fu 2011), por lo que a menudo, se asume que merecen una mayor prioridad de conservación respecto a las poblaciones centrales. Lo anterior dado como resultado de un tamaño efectivo poblacional (Ne) inferior, una

mayor deriva genética, efecto fundador, fragmentación y mayor aislamiento (Eckert et al. 2008; Pandey & Rajora 2012). Comprender la partición de la varianza genética en poblaciones periféricas y centrales puede arrojar más luz sobre los efectos de la deriva genética y el flujo de genes en la estructura de la población y, por lo tanto, mejorar los intentos de conservar dicha diversidad (Cires et al. 2013).

Al evaluar la interacción entre la tasa de migración, la deriva genética y el tiempo de establecimiento de las poblaciones, se observa que, la baja tasa de migración y distancia de migración máxima son los parámetros que sustentan la hipótesis del CPH. Junto a esto, se ha inferido que las barreras geográficas entre las poblaciones insulares y continentales bloquean el flujo de genes y aumentan las distancias genéticas entre ellas (Formas & Brieva 2000; Brieva & Formas 2001; Vidal & Ortiz 2009). La teoría genética predice que los niveles de variación genética se incrementan con el tamaño de la población efectiva (Frankham 1996), por tanto, las poblaciones insulares y periféricas tienen un mayor riesgo de extinción que las poblaciones continentales (centrales) debido a que muestran una menor variación genética y menor tamaño efectivo poblacional que las del continente de distribución central (Frankham 1997). Los estudios sugieren que los factores ecológicos pueden tener un fuerte impacto tanto en el tamaño poblacional *per se* y en variación genética intrapoblacional, incluso a pequeña escala (Liu et al. 2013). Estos niveles de variación intrapoblacional puden ser cuantificados usando Genética del Paisaje, un área de investigación que

integra la genética de poblaciones, la ecología del paisaje y estadística espacial (Gallardo et al. 2011; Garrido-Garduño & Vázquez-Domínguez 2013). Esta área examina los procesos contemporáneos que afectan a la variación genética (Knowles 2009). De esta manera permite describir patrones genéticos espaciales y los procesos que pueden originar esos patrones diferenciándose de otros enfoques genéticos, como la filogeografía, ya que tiende a centrarse en los procesos a escalas espaciales y temporales más finas (Manel et al. 2003). La persistente influencia de la historia del uso del suelo y la perturbación natural en los ecosistemas contemporáneos se ha hecho evidente, es por ello que análisis realizados a múltiples escalas han demostrado la importancia del patrón de paisaje para muchos taxa (Turner 2005). Dada la importancia de la diversidad genética para la viabilidad de la población, la combinación de datos ecológicos y genéticos puede ayudar a identificar los factores que limitan el tamaño de la población y determinar la potencial conservación de las poblaciones (Liu et al. 2013).

El modelo de estudio

Los anfibios por la gran dependencia a su hábitat (Duellman & Trueb 1994) y capacidades relativamente bajas de dispersión, son a menudo filopátricos (Jehle & Artzen 2002). Ellos son un excelente sustrato para el estudio de los efectos ambientales sobre los procesos evolutivos que dieron conformación a la distribución de la fauna actual, porque sus poblaciones tienden a mostrar una mayor estructura genética con respecto a otros grupos (Johns &

Avise 1998), dando lugar a distintas poblaciones, las que pueden representar entidades genéticas únicas, a pesar de la proximidad geográfica entre ellas (Jehle & Artzen 2002).

Los bosques templados de *Nothofagus* del sur de Chile y Argentina se caracterizan por una fauna de anuros endémica (Formas 1979) entre las que destaca el género *Batrachyla* Bell, 1843. Este género está constituido por cinco especies: *B. antartándica, B. fitzroya, B. leptopus, B. nibaldoi, B. taeniata*. De éstas, *B. taeniata* es una especie que tiene una amplia distribución en Chile y marginalmente en Argentina. En Chile, se extiende desde la provincia de Aconcagua en la región de Valparaíso hasta la provincia Capitán Prat en la región de Aysén (Sallaberry et al. 1981; Brieva & Formas 2001) (Fig. 3). Se ha reportado frecuentemente desde Concepción hacia el sur y la mayoría de las localidades son de baja altura, entre 0 y 300 m; en la Cordillera de Nahuelbuta y en Lonquimay sobrepasan los 1000 m (Cei 1962). Además, presenta distribuciones insulares en a lo menos tres islas cercanas al continente, Quiriquina, Mocha y Chiloé (Sallaberry et al. 1981). Desde un punto de vista filogeográfico esta especie es de particular interés, ya que se encuentra en dos regiones ecológicas diferentes: Región Mediterránea y Oceánica (di Castri 1968), abarcando una distribución latitudinal más de 1.500 km (Puente-Torres & Simonetti 2016). En su distribución norte se encuentra asociada a bosques relictos costeros hidrófilos y mesófilos (Zapallar y Quintero) rodeados de vegetación semiárida (Cei 1962; Formas 1979). La presencia de estos bosques ha sido

considerada producto de migraciones de procedencia austral, ocurridas durante las glaciaciones pleistocénicas (Villagrán & Armesto 1980; Cei & Capurro 1952). Bajo este contexto, el efecto de las glaciaciones del Pleistoceno en las poblaciones de *B. taeniata* generan un escenario de múltiples alternativas a evaluar. Las poblaciones pudieron: 1) emigrar hacia el norte junto con el bosque; 2) refugiarse en los límites o bordes de la capa de hielo en la Cordillera de la Costa, al norte del paralelo 41; 3) refugiarse sobre los glaciares, al sur del paralelo 41; 4) emigrar desde el este a oeste para utilizar el territorio disponible dejado por la retracción del hielo durante cada período interglacial (Formas 1979).

Como se ha mencionado, a pesar de que *B. taeniata* se encuentra en dos regiones ecológicas diferentes (Mediterránea y Oceánica) la ecología térmica revela que esta especie es termoconformista como la mayoría de los anfibios, debido a un compromiso entre las condiciones térmicas y balance de agua que reducen la capacidad de estos animales para a regular la temperatura (Iturra-Cid & Ortiz 2010). La evidencia ecológica sugiere que existen diferencias en los caracteres reproductivos entre las poblaciones del Chile central y sur. Estas diferencias estarían relacionadas con variabilidad climática, particularmente disponibilidad de agua, producto de la presencia o ausencia de lluvias, confiriéndole a las poblaciones de Chile central una notable capacidad adaptativa (Díaz et al. 1987) para sobrevivir en una zona con menor precipitación y mayor temperatura ambiental. Por otra parte, la evidencia genética sugiere que existe una correlación significativa entre la

distancia genética y distancia geográfica. Al parecer las poblaciones al norte de su distribución estarían altamente estructuradas con relación a las poblaciones del sur (Brieva & Formas 2001).

B. taeniata muestra una distribución continua desde Concepción al sur, en contraste con sus poblaciones de Chile central en donde es menos frecuente y sus poblaciones están más aisladas geográficamente (Correa et al. 2014).

El problema a estudiar

Si se considera la historia geoclimática y heterogeneidad ambiental del rango de distribución que abarca *B. taeniata*, así como características ecológicas encontradas en la especie, cabe plantearse las siguientes preguntas: ¿el último máximo glacial promovió divergencia genética entre las poblaciones de su rango de distribución norte y poblaciones del sur?, si las poblaciones de su rango norte de distribución no han tenido la influencia directa de las glaciaciones, ¿se habrían mantenido éstas estables en el tiempo a diferencia de las poblaciones de distribución sur que exhibirían una expansión poblacional y geográfica?, ¿se cumplirá el Patrón poblacional Central-Periférico de Mayr (CPH)? y finalmente, ¿el paisaje influye en el grado de estructuración poblacional?

Batrachyla taeniata que se caracteriza por ser uno de los anfibios chilenos con mayor rango de distribución. Por ello, con estos antecedentes, el presente trabajo busca evaluar los patrones filogeográficos y demográficos de las poblaciones de *B. taeniata* presentes en Chile. En primer lugar, por medio de inferencias filogeográficas, demográficas y modelos de nicho, estudiamos si los mecanismos históricos como los ciclos glaciales han condicionado la distribución de sus linajes y patrones de contracción – expansión, tanto genéticos como geográficos (Capítulo I). Para ello, se realizó un análisis filogeográfico de la especie, en función de la variabilidad genética de sus poblaciones. Mediante este análisis se buscó inferir el origen de las poblaciones actuales con sus respectivas rutas de recolonización y se evaluó el efecto contracción-expansión demográfica y geográfica. En segundo lugar, en el contexto actual de las poblaciones, se evaluó si la diversidad genética y estructura poblacional depende del grado de conectividad entre poblaciones, su ubicación dentro del rango geográfico que ocupa la especie y la heterogeneidad del paisaje (Capítulo II). Para ello, se realizó un análisis demográfico y de genética del paisaje, mediante el uso de marcadores moleculares microsatélites, evaluando si en las poblaciones de *B. taeniata* se cumple el Patrón poblacional Central-Periférico de Mayr (CPH).

Bibliografía

Anderson, R., Lew, D. & A.T. Peterson. 2003. Evaluating predictive models of species' distributions: criteria for selecting optimal models. Ecological Modelling 162: 211-232.

Banaccorso, E., Koch, I. & A.T. Peterson. 2006. Pleistocene fragmentation of Amazon species'ranges. Diversity and Distributions 12: 157-164.

Brieva, L.M. & J.R. Formas. 2001. Allozyme variation and geographic differentiation in the Chilean leptodactylid frog *Batrachyla taeniata* (Girard, 1854). Amphibia-Reptilia 22:413-420.

Camargo, A., Werneck, F.P., Morando, M., J W. Sites, J.R. & L. J. Avila. 2013. Quaternary range and demographic expansion of *Liolaemus darwinii* (Squamata: Liolaemidae) in the Monte Desert of Central Argentina using Bayesian phylogeography and ecological niche modelling. Molecular Ecology 22: 4038–4054.

Carstens, B. & C. Richards. 2007. Integrating coalescent and ecological niche modeling in comparative phylogeography. Evolution 61-6:1439-1454.

Cei, J.M. 1962. Batracios de Chile. Ediciones de la Universidad de Chile, Santiago. 128 pp.

Cei, J.M. & L.F. Capurro.1952. Biología y Desarrollo de *Eupsophus taeniatus* Girard Investigaciones Zoológicas Chilenas. 4:159-182.

Cires, E., Pérez, R., Bueno, A. & J.A. Fernández. 2013. Genetic diversity in peripheral and central populations of the Cantabrian endemism *Genista legionensis* (Pau) M. Laínz (Fabaceae). Anales del Jardín Botánico de Madrid 70(1): 91-96.

Correa, C., Cisternas, J., Velásquez, N., Lobos, G. & J. San Martín-Órdenes 2014. Distribution extension, new records and historical ocurrence of *Batrachyla taeniata* (Girard, 1854) (Anura: Batrachylidae) in Chile. Herpetology notes 7: 703-706.

Cosacov, A., Sérsic, A., Sosa, V., Jhonson, L. & A. Cocucci .2010. Multiple periglacial refugia in the Patagonian steppe and post-glacial colonization of the Andes: the phylogeography of Calceolaris polyrhiza. Journal of Biogeography 37:1463-1477.

Dai, Q. & J. Fu. 2011. When central populations exhibit more genetic diversity than peripheral populations: A simulation study. Chinese Sci Bull. 56(24): 2531-2540.

Díaz, N., Sallaberry, N. & J. Valencia.1987. Microhabitat and Reproductive traits in Populations of the frog, *Batrachyla taeniata*. Journal of Herpetology 2(4):317-323.

Di Castri, F. 1968. Esquisse écologique du Chile. In: Biologie de l'Amérique Australe, p. 7-82. Delamare Deboutteville, C., Rapoport, E., Eds, Paris, C.N.R.S.

Duellman, W.E. & L. Trueb. 1994. Biology of Amphibians. McGraw-Hill, New York.

Eckert, C., Samis, K.E. & S.C. Lougheed. 2008. Genetic variation across species' geographical ranges: the central–marginal hypothesis and beyond. Molecular Ecology 17:1170–1188.

Formas, J.R. 1979. La herpetofauna de los bosques temperados de Sudamérica: 341-379. En: The South American herpetofauna (Ed. W.E. Duellman), pp. 341-379. Monograph 7, Museum of Natural History, University of Kansas, Lawrence, Kansas.

Formas, J.R. & L.M. Brieva. 2000. Population genetics of the Chilean frog *Batrachyla leptopus* (Leptodactylidae). Genetics and Molecular Biology 23(1): 43-48.

Frankham, R. 1997. Do island populations have less genetic variation than mainland populations? Heredity 78: 311—327.

Frankham, R. 1996. Relationship of Genetic Variation to Population Size in Wildlife. Conservation Biology 10(6): 1500-1508.

Franklin, J. 2009. Mapping species distributions: Spatial inference and prediction. Cambridge, UK: Cambridge University Press. In press.

Fuentes-Hurtado, M., Hof, A.R. & R. Jansson. 2016. Paleodistribution modeling suggests glacial refugia in Scandinavia and out-of-Tibet rango expansión of the Arctic fox. Ecology and Evolution 6(1): 170-180.

Funk, W.C., Blouin, M.S., Corn P.S, Maxell B.A, Pilliod D.S., Amish S. & F.W. Allendorf. 2005. Population structure of Columbia spotted frogs (*Rana*

luteiventris) is strongly affected by the landscape. Molecular Ecology 14: 483-496.

Gallardo, C., Hernández, H., Diniz-Filho, J., Palma, E. & M. Méndez. 2011. Relationship between the genetic structure of the Andean toad *Rhinella spinulosa* (Anura: Bufonidae) and the northern Chile landscape (21°- 24° S). Revista Chilena de Historia Natural 84 (3):391-406.

Garrido-Garduño, T. & E. Vázquez-Domínguez. 2013. Genetic, spatial and connectivity methods of analysis in landscape genetics. Rev. Mex. Biodiv. 84 (3): 1031-1054.

Guisan, A. & K.E. Zimmermann. 2000. Predective habitat distribution models in ecology. Ecological Modelling 135: 147-186.

Gutenkunst, R.N., Hernandez, R.D., Williamson, S.H. & C.D. Bustamante. 2009. Inferring the joint demographic history of multiple populations from multidimensional SNP frequency data. PLoS Genetics 5(10): 1-11.

Heusser, C.J., Heusser, L.E. & T.V. Lowell. 1999. Paleoecology of the southern Chilean Lake District-Isla Grande de Chiloé during middlelate Llanquihue glaciation and deglaciation. Geografiska Annaler Series 81A: 231-284.

Heusser, C.J. 2003. Ice age Southern Andes. A Chronicle of paleoecological events. Developments in Quaternary science, 3. Jim Rose (Serie editor). Elsevier BV.Amsterdam. The Netherlands. 243 pp.

Himes, C., Gallardo, M. & G. Kenagy. 2008. Historical biogeography and post-glacial recolonization of South American temperate rain forest by the relectual marsupial Dromiciops gliroides. Journal of Biogeography 35: 1415-1424

Iturra-Cid, M. & J.C. Ortiz. 2010. *Batrachyla taeniata* (NCN). Body temperature. Herpetological Review 41(3): 333.

Jehle, R. & W. Artzen. 2002. Review: microsatellite markers in amphibian conservation genetics. Herpetological journal 12: 1-9.

Johns, G.C. & J.C., Avise. 1998. A comparative summary of genetic distances in the vertebrates from the mitochondrial cytochrome b gene. Mol. Biol. Evol. 15: 1481–1490.

Knowles, L.L. 2009. Statistical phylogeography. Annual Review of Ecology, Evolution, and Systematics 40: 593–612.
Kukwa, M. & M. Kalanowska. 2016. Glacial refugia and the prediction of future habitat coverage of the South American lichen species *Ochrolechia austroamericana*. Sci. Rep. 6: 38779; doi:10.1038/srep38779.

Lamy, F., Kaiser, J., Arz, H.W., Hebbeln, D., Ninnemann, U., Timm, O.A Timmermann & J.R. Toggweiler. 2007. Modulation of the bipolar seesaw in the Southeast Pacific during Termination 1. Earth and Planetary Science Letters 259 (3-4): 400-413.

Liu, Y., Webber. S., Bowgen. K., Schmaltz, L., Bradley, K., Halvarsson, P., Abdelgadir, M. & M. Griesser. 2013. Environmental factors influence both abundance and genetic diversity in a widespread bird species 3(14): 4683-4695.

Manel, S., Schwartz, M., Luikart, G. & P. Taberlet. 2003. Landscape genetics: Combining landscape ecology and population genetics. Trends in Ecology and Evolution 18: 189-197.

Martínez-Meyer, E. & A.T. Peterson. 2006. Conservatism of ecological niche characteristics in North American plant species over the Pleistocene-to-Recent transition. Journal of Biogeography 33:1779-1789.

Martínez-Meyer, E., Peterson, A.T. & W.W. Hargrove. 2004. Ecological niches as stable distributional constraints on mammal species, with implications for Pleistocene extinctions and climate change projections for biodiversity. Global Ecology and Biogeography 13: 305-314.

Mayr, E. 1963. Populations, Species and Evolution. An Abridgment of Animal species and evolution. The Belknap Press of Harvard University Press Cambridge, Massachusetts and London, England. 797pp.

MC. Culloch, R.D., Bentley, M.J. & R.S. Purves. 2000. Climatic inferences from glacial and palaeoecological evidence at the last glacial termination, southern South America. Journal of Quaternary Science 15: 409–417.

Morando, M., Avila, L.J., Turner C. R. & J.W. Sites, Jr. 2007. Molecular evidence for species complex in the Patagonian lizard *Liolaemus bibronii* and phylogeography of the closely related *Liolaemus gracilis* (Squamata: Liolaemini). Molecular phylogenetics and evolution 43(3): 952-973.

Moreno, P.I., Jacobson, G.L., Andersen, B., LowelL, T.V. & G.H. Denton. 1999. Abrupt vegetation and climate changes during the last glacial

maximum and the last Termination in the Chilean Lake District: A case study from Canal de la Puntilla (41ºS). Geografiska Annaler 81A: 285–311.
Munguía, M., Peterson, A.T. & V. Sánchez-Cordero. 2008. Dispersal limitation and geographical distributions of mammal species. Journal of Biogeography 35: 1879-1887.

Nogués-Bravo, D. 2009. Predicting the past distribution of species climatic niches. Global Ecology and Biogeography 18: 521-531.

Núñez, J.J., Wood, N.K., Rabanal, F.E., Fontanella, F.M. & J.W. Sites, Jr. 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 (2011) 343–352.

Ortiz. J.C. 1989. Una vision caleidoscopica de los recursos naturales. Facultad de Ciencias Biológicas y de Recursos Naturales. Dirección de Extensión. Universidad de Concepción. Sociedad de Biología de Chile, Regional Concepción. N°2 56 pp.

Palma, E., Boric-Bargetto, D., Torres-Pérez, F., Hernández, C. & T. Yates. 2012. Glaciation Effects on the Phylogeographic Structure of *Oligoryzomys longicaudatus* (Rodentia: Sigmodontinae) in the Southern Andes. PLoS ONE 7(3): e32206. doi:10.1371/journal.pone.0032206.

Pandey, M. & O.P. Rajora. 2012. Genetic diversity and differentiation of core vs. peripheral populations of eastern white cedar, Thuja occidentalis (Cupressaceae). American Journal of Botany 99(4): 690–699. 2012.

Pastorino, M.J. & L.A Gallo. 2002. Quaternary evolutionary history of *Austrocedrus chilensis*, a cypress native to the Andean-Patagonian forest. Journal of Biogeography 29: 1167-1178.

Pefaur, J. & J. Yáñez. 1980. Ecología descriptiva de la isla Mocha (Chile) en relación al poblamiento de vertebrados. Museo Nacional de Historia Natural, Boletín 37: 103-112.

Peterson, A.T., Martinez-Meyer, E., Gonzalez-Salazar, C. & P. W. Hall. 2004. Modeled climate change effects on distributions of Canadian butterfly species. Canadian Journal of Zoology 82:851-858.

Phillips, S.J., Anderson R.P. & R.E. Schapire. 2006. Maximum entropy modeling of species geographic distributions. Ecological Modelling 190: 231-259.

Phillips, S.J., Dudík, M. & R.E. Schapire. 2004. A maximum entropy approach to species distribution modeling. Princeton University. Princeton, NJ.

Pinho, C. & J. Hey. 2010. Divergence with Gene Flow: Models and Data. Annual Review of Ecology, Evolution, and Systematics 41: 215-230.

Puente-Torres, S. & J.A. Simonetti. 2016. *Batrachyla taeniata* (Girard, 1855): filling the distribution gap and describing a new type of habitat for this species in central Chile. Check List 12(4): 1-3.

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.

Rabassa, J., Coronato A. & Salemme . 2005. Chronology of the late Cenozoic Patagonian glaciation and their correlation with biostratigraphic units of the Pampean Region (Argentina). Journal of South American Earth Science 81-103.

Rodríguez-Serrano, E., Cancino, R.A. & R.E. Palma. 2006. Molecular phylogeography of *Abrothrix olivaceus* (Rodentia: Sigmodontinae) in Chile. Journal of Mammalogy 87(5): 971 -980.

Roberts, D.R. & A. Hamann. 2012. Predicting potencial climate change impacts with bioclimate envelope models: a palaeoecological perspective. Global Ecology and Biogeography 21: 121-133.

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.

Sallaberry, M., Valencia, J. & N. Díaz. 1981. Distribución y ambiente de *Batrachyla taeniata* (Girard) en Chile (Anura - Leptodactylidae). Boletín del Museo Nacional de Historia Natural (Chile) 38: 61-67.

Sérsic, A.N., Cosacov, A., Cocucci, A.A., et al. 2011. Emerging phylogeographical patterns in plants and terrestrial vertebrates from Patagonia. Biological Journal of the Linnean Society 103: 475–494.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. Science 236: 787-792.

Smith-Ramírez, C. 2004. The Chilean coastal range: a vanishing center of biodiversity and endemism in South American temperate rain forests. Biodivers. Conserv. 13: 373–393.

Tavera, J. & C. Veyl. 1958. Reconocimiento geológico de la isla Mocha. Publicación Depto. Geología, Univ. de Chile, Santiago 12: 157-188.
Thorson, R.M. 1999. The glacial limit in South America and its rol in biogeography: Darwin´s observations. Ciencia al día 4:1-15.

Vásquez, D., Correa C., Pastenes L., Palma, E. & M. Méndez. 2013. Low phylogeographic structure of *Rhinella arunco* (Anura: Bufonidae), an endemic amphibian from the Chilean Mediterranean hotspot. Zoological Studies 52:35.
Vázquez-Domínguez, E. 2007. Filogeografia y vertebrados Cap. 14. Ecología Molecular. Eguiarte L, Souza V, Aguirre X (Compiladores). 571pp.

Victoriano, P. 2020. Phylogeography of Chilean Lizards: Histories of Genetic Diversification on the Western Slope of Southern Andes, in Lizards of Patagonia: Diversity, Systematics, Biogeography and Biology of the Reptiles at the End of the World, M. Morando and L.J. Avila, Editors. 2020, Springer International Publishing: Cham. p. 255-291.

Victoriano, P., Muñoz-Mendoza, C., Sáez P.A., Salinas H.F., Muñoz-Ramírez C., et al. 2015. Evolution and Conservation on Top of the World: Phylogeography of the Marbled Water Frog (*Telmatobius marmoratus* Species Complex; Anura, Telmatobiidae) in Protected Areas of Chile. Journal of Heredity: 546-559

Victoriano, P., Ortiz, J.C., Benavides, E., Adams, B.J. & J.W, Sites, Jr. 2008. Comparative phylogeography of codistributed species of Chilean Liolaemus (Squamata: Tropiduridae) from the central-southern Andean range. Mol. Ecol. 17: 2397–416.

Vidal, M., Moreno, P.I. & E. Poulin. 2012. Genetic diversity and insular colonization of *Liolaemus pictus* (Squamata, Liolaeminae) in north-western Patagonia. Austral Ecology 37: 67–77.

Villagrán, C., Armesto, J.J., Hinojosa, F., Cuvertino, J., Pérez, C. & C. Medina 2004. El enigmático origen del bosque relicto de Fray Jorge. Ediciones Universidad de La Serena, La Serena, Chile 1: 3- 43.

Villagrán, C. & J.J. Armesto. 1980. Relaciones florísticas entre las comunidades relictuales del Norte Chico y la zona central con el bosque del

sur de Chile. Boletín del Museo Nacional de Historia Natural, Chile 37: 87-101.

Wiens, J.J., Ackerly, D.D., Allen, A.P., Anacker, B.L., Buckley, L.B., et al. 2010. Niche conservatism as an emerging principle in ecology and conservation biology. Ecology Letters 13: 1310-1324.

Wiens, J.J. & C.H. Graham. 2005. Niche Conservatism: Integrating Evolution, Ecology, and Conservation Biology. Annu.Rev. Ecol.Evol. Syst. 36: 519-539.

Wiens, J.J. & M.J. Donoghue. 2004. Historical biogeography, ecology and species richness. TRENDS in Ecology and Evolution 19(12): 639-644.

HIPÓTESIS DE INVESTIGACIÓN

CAPÍTULO 1: Inferencias Filogeográficas

Dado que *B. taeniata* posee una amplia distribución a lo largo de Chile, la cual incluye zonas que han sido glaciadas y no glaciadas. Las poblaciones actuales se debieron originar a partir de refugios en la costa debido a los eventos glaciales del Cuaternario. La recolonización se produjo desde el nor-oeste, repoblando las áreas sur y este, esto generó en las poblaciones de distribución sur un efecto de contracción-expansión, tanto demográfica como geográfica, en contraste con las poblaciones del norte de su distribución que se han mantenido relativamente estables en el tiempo.

HIPÓTESIS 1: La dinámica del Cuaternario dada por los ciclos glaciales han condicionado la distribución de linajes y generaron patrones de contracción-expansión tanto poblacional como geográfica en la especie *B. taeniata*

PREDICCIONES:

1. Alta estructura genética asociada a su distribución geográfica latitudinal
2. Signos de expansión demográfica y bajos niveles de diversidad y estructura genética en las poblaciones distribuidas en el rango sur.
3. Signos estabilidad demográfica en poblaciones distribuidas en el rango norte.
4. Presencia de refugios en el área cercana a la cordillera de la Costa
5. Evidencia de recolonización desde el noroeste y repoblación hacia las áreas sur y este.

CAPITULO 2: Inferencias demográficas

II. Considerando la información geográfica, ecológica y genética de la especie, se espera que la distancia geográfica y características del paisaje han influido en la estructuración de sus poblaciones, donde las poblaciones de la periferia de su distribución norte y sur muestran menor diversidad genética y mayor estructura genética, producto de la baja o nula tasa de migración entre ellas, cumpliéndose a esta escala el Patrón poblacional Central-Periférico de Mayr (CPH).

HIPÓTESIS 2: En el contexto actual de las poblaciones, la diversidad genética poblacional depende del grado de conectividad entre poblaciones, de la historia de los tamaños poblacionales y de la heterogeneidad del paisaje que ocupan, viéndose más afectadas las poblaciones que se ubican en la periferia latitudinal del rango de distribución de la especie.

PREDICCIONES:

1. Un alto número de poblaciones estimadas relacionadas al gradiente latitudinal norte-sur.
2. Alta distancia y estructura genética en las poblaciones de *B. taeniata*,
3. Bajos valores del número de alelos, heterocigosidad y tasas de migración, en las poblaciones ubicadas en la periferia del rango de distribución norte y sur de la especie.
4. Altos valores de estructuración, distancia genética y consanguinidad en las poblaciones ubicadas en la periferia del rango de distribución norte y sur de la especie.

5. Relación positiva entre la distancia geográfica (Km) que tienen las poblaciones hacia el centro del rango de distribución de la especie y la estructura genética.

OBJETIVOS

Objetivo general

Evaluar el efecto de los cambios climáticos del Cuaternario sobre los patrones de diversidad genética y geográfica y el efecto del paisaje actual sobre la estructura genética de sus poblaciones.

Objetivos específicos

1. Generar una base de datos genética (ADN mitocondrial y nuclear, microsatélites) y geográfica para *B. taeniata* (Capítulo I y II).
2. Evaluar relaciones filogeográficas de las poblaciones de *B. taeniata* (Capítulo I).
3. Cuantificar la variación genética intra e inter poblacional (Capítulo I y II).
4. Evaluar el proceso de expansión demográfica mediante el cambio del tamaño efectivo de sus poblaciones a través del tiempo (Capítulo I).
5. Evaluar el proceso de expansión del rango mediante un análisis filogeográfico de difusión continúa (Capítulo I).
6. Evaluar el evento de expansión geográfica mediante un modelo de paleo-distribución desde el pasado interglacial (120, 21 Ma y actual) (Capítulo I).
7. Estimar parámetros demográficos mediante el uso de marcadores moleculares microsatélites (Capítulo II).
8. Evaluar el efecto del flujo génico en un escenario actual (Capítulo II).
9. Evaluar si en la especie se cumple la CPH de Mayr (Capítulo II).

CAPÍTULO I

ESTUDIO FILOGEOGRÁFICO DE LA RANITA DE ANTIFAZ *Batrachyla taeniata* (ANURA: BATRACHYLIDAE)

PHYLOGEOGRAPHIC STUDY OF THE MASKED FROG *Batrachyla taeniata* (ANURA: BATRACHYLIDAE)

Phylogeographic study of the masked frog *Batrachyla taeniata* (Anura: Batrachylidae)

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Abstract

Evidence suggests that southern South America has been subject to periodic climatic fluctuations. Glacial events have occurred since the end of the Miocene (6 Ma), with an accentuated effect on the Andean relief during the Pleistocene. The organisms that inhabit this territory have been strongly influenced by glaciers in the last million years, modifying the geographic distributions of the species of flora and fauna, especially in the areas that were covered by ice. Phylogeographic studies have revealed congruent patterns between species of vascular plants and terrestrial vertebrates, such as the presence of refuges at low latitudes and postglacial expansion at higher latitudes. The objective of our study was to evaluate the effect of the

Quaternary climatic changes on the patterns of genetic and geographic diversity in *Batrachyla taeniata*, an anuran with wide distribution in Chile associated with different ecological regions. Using two mitochondrial regions (control region and cytochrome b), we implemented a Bayesian approach to determine the phylogenetic relationships of the gene trees and estimate the age of the Most Recent Common Ancestor (MRCA). A haplotype network was reconstructed for both genes. A discriminant analysis of principal components (DAPC) was done to obtain the best separation of individuals within groups. We used an analysis of molecular variance (AMOVA) to determine the level of population structure among the previously defined groups. Population size fluctuations over time were estimated for each clade and range expansion was determined by continuous diffusion phylogeographic analysis and by species distribution modelling. The results identified two large genealogical groups in the latitudinal distribution of *B. taeniata*, separated by the Biobío River—the northern group associated with the Mediterranean ecoregion and the southern group with the Oceanic ecoregion of evergreen forests. The northernmost clade fits a model of constant population size, in contrast to the clades that make up the southern group, which have experienced demographic expansions since the Pleistocene (~ 0.04 Mya). The clades associated with the central range of distribution also show solid signs of population growth from ~ 0.01 Mya. The potential trend of the pattern of change in the distribution area of the species over time is Last Interglacial (LIG) > Last Glacial Maximum (LGM) < Current.

Keywords: Amphibians, Batrachylidae, Mitochondrial DNA, genetic diversity, Species distribution modelling.

Introduction

The growing interest in understanding the historical processes that have affected the evolutionary biology of the organisms that inhabit the Patagonian territory has been relatively well documented. Examples of this are the studies carried out on marsupials [1], rodents [2, 3] lizards [4-8] and amphibians [8-10]. These phylogeographic studies can provide valuable information on the historical processes underlying diversification in this region [11]. Most of these studies have tried to evaluate the effect of glaciations on population and geographic contraction-expansion processes.

The evidence suggests that in southern South America glaciations occurred since the end of the Miocene, approximately 6 Ma, with an accentuated effect on the Andean relief during the Pleistocene through at least five major glaciations over the last million years, with the Great Patagonian Glaciation (GPG) [15, 16]. During this period, the Andes in Patagonia were covered by a continuous mountain ice sheet from 37°S to Cape Horn (56°S) [15]. The most recent cold event In Chile, the Last Glacial Maximum (LGM), extended this ice sheet marginally northward between 38° S and 34° S [17].

77 Congruent patterns have been revealed between species of vascular plants
78 and terrestrial vertebrates, such as refuges at low latitudes and postglacial
79 expansion at higher latitudes [12]. This suggests that a group of species
80 tolerant to low temperatures survived in small refuges within the ice sheet
81 limits or edges [13], promoting genetic structure. Another (non-exclusive)
82 possibility is that groups of species or refugia source populations of those
83 same species were outside the direct effect of glaciers; they used the
84 available territory left by the retraction of the ice during each interglacial
85 period to expand their geographic ranges. This climate dynamics promoted
86 later genetic divergence. The Last Glacial Maximum produced patterns of
87 population genetic variation associated with historical divergence explained
88 by the isolation of their populations followed by a later differentiation
89 explained by geographic range expansion towards the areas that were
90 previously covered by ice sheets [9, 14]. These long periods of isolation
91 among some populations of the South American taxa may be reflected in the
92 deep phylogeographic divisions observed within some groups like mammals,
93 birds, fish, invertebrates; plant groups were structured mainly during the
94 Pleistocene, while other groups like most of the herpetofauna underwent an
95 intraspecific lineage split much earlier, during the Pliocene or Miocene [11].

96

97 Most amphibians present deep phylogeographic differentiation, basically due
98 to their low vagility [18]. However, in northern Chile, Victoriano et al. (2015)
99 [19] confirmed a recent population expansion scenario and high connectivity
100 events in *Telmatobius marmoratus* at an age younger than the end of the

Last Glacial Maximum, probably associated with more rainy periods in the highlands. In central Chile, Vásquez et al. (2013) [20] showed that *Rhinella arunco*, despite presenting a low level of phylogeographic structure, showed an effect of the Pleistocene glaciations on the intrapopulation genetic diversity in the extreme south of its distribution area. The glaciations directly affected the species *Eupsophus calcaratus*, an amphibian from the temperate forests of Chile, Six strongly supported lineages were recovered in the phylogeographic study of Nuñez et al (2009), which were affected by the climate changes of the Pleistocene [9].

The widely distributed Chilean species constitute a good model to evaluate the joint effects of geographic complexity, glacial cycles and fluctuating river volumes on the intraspecific differentiation of populations and phylogeographic structure [7]. The temperate *Nothofagus* forests of southern Chile and Argentina have a wide distribution and an endemic anuran fauna [21], including the genus *Batrachyla* Bell, 1843, formed of five species: *B. antartándica, B. fitzroya, B. leptopus, B. nibaldoi, B. taeniata*. *B. taeniata* has a wide distribution in Chile and marginally in Argentina. In Chile it extends from the Aconcagua province in the Valparaíso Region to the Capitán Prat province in the Aysén Region [22-24]. This distribution includes two different ecological regions, the Mediterranean and Oceanic Regions [25]. It has been frequently reported from Concepción to the south (mainly the Oceanic Region), in contrast to its populations in Mediterranean central Chile, where it is less frequent and its populations are more geographically isolated [24, 26].

Most of the localities are at low elevation, between 0 and 300 m; but in the Cordillera de Nahuelbuta and Lonquimay they exceed 1000 m [27]. It also has an insular distribution on at least three islands near the mainland, Quiriquina, Mocha and Chiloé [23]. Its presence in central Chile is associated with hydrophilic and mesophilic coastal relict forests (Zapallar and Quintero) surrounded by semi-arid vegetation [21, 27]. The presence of these forests in the area has been explained as a product of migrations of southern origin during the Pleistocene [28, 29]. According to this, Formas (1979) [21] hypothesized that *B. taeniata* migrated north along with the forest, which would explain its presence in the area. However, the floristic relationships between the Valdivian forest and north-central Chile date back to a time before the diversification of the current southern forest associations [28, 30]. A parallel situation is observed comparing the flora of Isla Mocha with the forests of southern Chile. Its flora would represent a remnant of that existing on the Concepción coast before diversification of the current Valdivian forest associations [28].

The species' evolutionary background indicates a possible most recent common ancestor with *B. leptopus* from 13.11 Mya [31]. The only genetic study of *B. taeniata* (allozyme markers) showed genetic differentiation related to the geographic distance between the northernmost population of its range distribution (Quintero) and the centre and southern group populations [22]. This makes it a good model to understand the consequences of the Quaternary dynamics on intraspecific processes such as the genetic

149 differentiation of their populations. According to the above, a scenario is
150 presented that according to Victoriano (2020) [16] constitutes a good natural
151 experiment.

152

153 Considering the geographic and historical setting of *B. taeniata*, the objective
154 of this study is to evaluate the phylogeographic relationships of continental
155 populations and determine the effect of Quaternary climate change and
156 geography on patterns of the genetic diversity of the species. The following
157 predictions will be tested: (1) High genetic structure associated with its
158 latitudinal geographic distribution (2) Signs of demographic expansion and
159 low levels of diversity and genetic structure in the populations distributed in
160 the southern range (3) Signs demographic stability in populations distributed
161 in the northern range (4) Presence of refuges in the area near the Cordillera
162 de la Costa (5) Evidence of recolonization from the northwest towards the
163 southern and eastern areas.

164

165 **Materials and Methods**
166

167 **Sample collection**
168

169 This study is based on 138 specimens of *B. taeniata*, from the Valparaíso
170 region to the Aysén region. These samples were obtained from the Museum
171 of Zoology of the University of Concepción (MZUC-UCCC) and field
172 campaigns (Fig 1, S1 Table). The new specimens sampled were captured

with capture permits of the Agricultural and Livestock Service (authorization SAG-3350/2015 and 4557/2018). All captures were made in accordance with the protocols approved by the Bioethics Committee of the University of Concepción (Chile). The new individuals collected were deposited in the herpetological collection of the Museum of Zoology of the University of Concepción (MZUC-UCCC).

DNA extraction, PCR amplification, and sequencing

For DNA extraction, oral mucosa tissue was used from field campaigns and muscle tissue from samples from the herpetological collection of the Museum of Zoology of the University of Concepción. (MZUC). The extracted tissue was treated with the commercial Wizard ® SV Genomic (Promega) kit following the manufacturer's instructions. The mitochondrial DNA control region (CR) was amplified by PCR using the ControlJ-L (CTAACGTTTCACGAAGATGGAA) and ControlP-H (GTCCATAGATTCASTTCCGTC AG) primers [32]. The cytochrome b (cyt b) gene region was amplified with primers MVZ15-L [33] and CytbAR-H [32]. PCR amplifications were performed in a total volume of 25.5 µL containing 5X Buffer Taq (Promega), 25mM $MgCl_2$ (Promega), 2.5 mM dNTPs (Promega), 10 µM of each primer (Macrogen), 0.5 U μL^{-1} Taq DNA Polymerase (Promega) and ca. 100 ng μL^{-1} DNA. The thermocycler conditions for all loci were an initial denaturation of 5 min at 94 °C, followed by 40 cycles of 45 s 92 °C, 1 min at the optimum annealing temperature of 53

°C, an extension of 45s at 72 °C and a final extension of 10 min at 72 °C. PCR products were detected on 1.0% agarose gels were sent to the sequencing service of MACROGEN, Seoul, South Korea to purify and sequence. The sequences were edited and aligned in the Codoncode Aligner v 4.2.2 program [34]. We also verified the sequences with the DnaSP v6 [35] program to translate them into amino acids. Finally, sequences representing all haplotypes were submitted to GenBank with accession numbers (genebank access numbers).

Genetic diversity, genealogical analysis and time estimates

Previous to the phylogeographic analysis, it was verified whether the aligned sequences of the data set present saturation of the substitutions using the Xia test [36] in the DAMBE v5.3.25 program [37]. To determine if the sequences approach neutral evolution, the Tajima D neutrality test was performed [38] in the DnaSP v6 [35] program. This test assumes that populations are in mutation-drift and migration-drift equilibrium. Standard indices (haplotype diversity Hd, nucleotide diversity π) were estimated for each clade of the genealogical analysis. The mean values of genetic divergence for all pairs between locations were estimated based on p-distances using the Mega 6 program [39].

A Bayesian approach was implemented to determine the phylogenetic relationships of the gene trees. Non-redundant sequences of CR (n=101)

221 and cyt b (n= 44) were used in this analysis. Two specimens of *B. leptopus*
222 (GenBank Accession number: MH301135.1 and MH301136.1 for CR;
223 MH301156.1 and MH301157.1 for cyt b) were chosen as outgroup taxa for
224 phylogenetic reconstruction of *B. taeniata*.

225

226 The appropriate nucleotide substitution models (HKY+I+G for CR and
227 GTR+I+G for cyt b) were estimated in both cases with the JModelTest 2
228 software [40, 41] based on the Akaike Information Criterion. Phylogenetic
229 trees were reconstructed with the Bayesian Markov Chain Monte Carlo
230 approach implemented in BEAST 1.10.1 program [42], which provides a
231 flexible framework for hypothesis testing with time-structured molecular
232 sequence data [43]. The Bayes factor analysis indicated that genealogical
233 trees reconstructed under a strict clock model received decisive nodal
234 support compared to uncorrelated models. The analysis was run for 100
235 million generations and 1000 generations were retained, resulting in a file of
236 100,000 trees. The first generations (20%) were removed as burn-in. All trees
237 below the observed stationary level were discarded. These trees were
238 summarized in a maximum *a posteriori* tree with TreeAnotator and visualized
239 in FigTree v1.4.4.

240

241 The age of the most recent common ancestor (MRCA) of the main clades of
242 *B. taeniata* was estimated with Beast 1.8.2 [42] using the CR and Cyt-b
243 matrix. We used prior Neo-batrachian mutation rates of 0.37917 per million

years [44] with a standard deviation of 0.05 [9, 45]. The model used for this analysis was the GTR+I+G with a strict clock log normal clock, selected using Bayes factor [46] and the Yule speciation process. Analyses were run for 100 million generations and sampled every 1000 iterations, discarding the first 20%.

In addition, a haplotype network was reconstructed for both CR and Cyt-b matrices, as it allows visualizing the relationships between sequences, using statistical parsimony algorithms implemented in Network v4.6.1.1. [47]

Genetic structure and historical demographic changes

A discriminant analysis of principal components (DAPC) was done with the R package adegenet v.2.1.3 [49], which defines discriminant axes that obtain the best separation of individuals within predefined groups by locality and makes a probabilistic assignment of individuals to each group [49]. This model-free multivariate analysis approach is a valuable tool to investigate and visualize the presence of genetic clusters, since it maximizes between-group variation and minimizes within-group variation [49].

Previous to this analysis, the most probable number of populations was estimated with the Geneland v4.0.3 program [50], however the results did not

allow the detection of differentiated genetic groups within the spatial distribution of *B. taeniata* (S3 Fig).

To determine the level of population structure among the previously defined groups we implemented an analysis of molecular variance (AMOVA), using the software ARLEQUIN v3.5 [51]. Intra-population genetic variation (F_{SC}), inter-group variation (F_{CT}), inter-population and intra-group variation (F_{ST}) were estimated. The populations and the individuals assigned to them were made based on the results obtained in the genealogy and the genetic clusters obtained from the DAPC. The groups were divided into those populations contained the two large clades obtained in the gene genealogy (I and II), with a total of six clades.

The demographic history of each clade was inferred by pairwise mismatch distribution analysis [52]. This analysis was implemented in DnaSP v6 together with the Raggedness statistic [53].

Population size fluctuations over time were estimated for each clade identified by genealogy using the Bayesian Skyline Plot (BSP) method implemented in Beast [42]. We used a substitution rate of 0.37917 per million years [44], which has been used previously for intraspecific analysis in *B. leptopus* [10]. We ran 100 $x10^6$ generations and discarded the first 10% as

burn-in. Estimated distributions were used to generate confidence intervals representing phylogenetic uncertainty and coalescence [42]; Tracer v.1.7[46] was used to monitor parameter estimates and ESS values.

Range expansion was determined by continuous diffusion phylogeographic analysis in BEAST 1.7.4 [42] with the CR data set using both a time-homogeneous Standard Random Walk (SRW) and a time-heterogeneous Relaxed Random Walk (RRW) model with log-normally distributed rate variation [54]. Input xml files were prepared according to the examples in the tutorials on the BEAST website. Sub-sampling was done to select a representative of each haplotype found in a specific locality [55]. For each model we performed two independent runs of 200 million generations, sampled every 1000 generations. Parameter traces were inspected with Tracer to check stationary, high ESS and convergence between runs. We compared the SRW and RRW models using BF estimated with the path and stepping-stone sampling values; the marginal likelihood of each was estimated 500000 steps, with 5000 burn-in. Tree files were annotated with TreeAnnotator 1.7.4 [42] to estimate a maximum clade credibility (MCC) tree after removing the burn-in. The ancestral locations over time were visualized using Google Earth Pro v.7.3. and SPREAD v.1.0.7[56].

Paleo-distribution modelling

A presence database was constructed for *B. taeniata* with 151 points (S4 Fig), based on records found in the literature, the Zoology Museum at the University of Concepción (MZUC) and own data. The data matrix was edited in Arcmap 10.2 (Esri, Redlands, California, USA), to eliminate duplicated data and those that were too aggregated, to obtain a better representation of niche and distribution. A total of 119 known locations for *B. taeniata* were used as occurrence data in ArcGIS 10.2 (Esri). Final occurrence data were split randomly into two equal portions: 50% with which to calibrate the model and 50% to evaluate model predictions.

The information on average climate conditions for Chile and the bordering areas of Argentina was obtained from the WorldClim database, version 1.4 (www.worldclim.org) [57] with a spatial resolution of 30 arc seconds (~1 km) in the whole area of study for current times (between 1960 and 1990) and the Last Interglacial (LIG), and with a resolution of 2.5 minutes to Last Glacial Maximum (LGM). For the LGM we used two general circulation models, since they use different data and algorithms [58]. For these projections we used the models CCSM4 and MIROC-ESM. The bioclimate layers were limited to the area termed M. This area was estimated as the polygon between 25°5'18.71'' to 56°37'11.81'' S and 76°27'15.3'' to 64°41'45.19''W, covering the known occurrences and to ensure all sites in the probable area are within the range

334 of species dispersal in the past projections. We delineated M using Arcmap
335 10.2 (Esri, Redlands, California, USA).

336

337 We selected three sets of variables for the construction of models. The first
338 set included all 19 variables (Table S4). The second set was defined by the
339 exploration of the previous set with pairwise correlation r <0.75, which was
340 evaluated using Pearson's correlation coefficient, false discovery rate and
341 multi-collinearity test in R using the fuzzySim package [59]. When two
342 variables were highly correlated, we selected the one assumed to be most
343 important biologically [60, 61]. The ten selected variables were bio2, bio4,
344 bio7, bio8, bio9, bio12, bio13, bio14, bio17, bio19. The third set of variables
345 included the four variables (bio4, bio8, bio9 and bio19) that contributed most
346 to the prediction of the models (Table S4). This was achieved by calculating
347 the percentages of contribution relative to the current model and the results
348 of a jack-knife test. Thus, the three data sets consisted of 19, 10, and four
349 variables.

350

351 We used MaxEnt 3.4.3 [62] to evaluate the effect of climate change on
352 habitat suitability of *B. taeniata*. The niche models were generated via the
353 Kuenm package in R [63]. We explored a combination of 20 regularization
354 parameter values (0.1-1.0 at intervals of 0.1; 1-6 at intervals of 1; 8; and 10),
355 29 sets of model response types (i.e. all possible combinations of linear,
356 quadratic, product, threshold, and hinge responses), and the three sets of

environmental variables described above. In all, 1581 candidate models were created; each was evaluated based on statistical significance: pROC [64, 65], omission rate (OR) and the Akaike information criterion corrected (AICc) [66].

The candidate models were first reduced based on partial ROC tests [64], removing non-significant models from further consideration. Using an acceptable calibration omission rate of E=5%, all models with omission rates above 0.05 were removed [63]. Finally, models were filtered by lowest values of the AICc [66].

Final models were created using parameter settings selected in kuenm, with 10 bootstrap replicate analyses of available occurrence data. We transferred final models to Chile and the bordering areas of Argentina under the present-day conditions and past scenarios described above, permitting extrapolation, and clamping in Maxent projections. We used the median values across replicates as an estimate of the present-day spatial distribution of suitable and unsuitable conditions across the 10 replicate analyses for each final parameter set [67]. From models transferred to past conditions, we obtained three results: one per LIG and two per LGM. Results for each LGM were maintained separate to represent two scenarios of potential changes of suitable areas.

Additionally, in order to test the climatic similarity between calibration area (M) and projections for the past periods, we used the mobility-oriented parity (MOP) metric [68] based on 10% sampling of the reference region (M). This method is used for representing levels of similarity between the calibration area and the specific scenario of projection [63].

Results

Genetic diversity, genealogical analysis and time estimates

We obtained 485 base pairs (bp) of control region (CR) mtDNA sequences for 138 individuals (34 localities); 64 sequences of 971 bp of cytochrome (cyt b) and 58 sequences for the concatenated matrix with 1356 bp. Sequence lengths were not biased geographically.

Two well-supported deep clades were obtained in the topology of the CR gene tree. Clade I includes haplotypes distributed in the northern range (between 32° and 37°S), while clade II includes haplotypes distributed in the southern range (between 37° and 48°S)., Two well-supported subclades were obtained in clade I (A and B). Subclade A includes localities of the northern limit of the distribution; subclade B contains localities of the central range of distribution of this species north of the Biobío River (lower course of the river: 36° 49' S; 73° 11' W). Four subclades were obtained in clade II (C, D, E and F). Subclade C includes localities mainly from the Coast Range

403 in the Biobío Region. Subclade D is restricted to an area in the Araucanía
404 and Los Ríos Regions. Finally, Subclades E and F occur in the southern
405 distribution limit, in the Los Lagos and Aysén Regions (Fig 2).

406

407 The genealogy obtained from cyt b also recovers two well-supported deep
408 clades (I and II). However, it did not recover the C and D subclades.
409 Subclades A, B, E and F were corroborated with this marker and with the
410 concatenated matrix (S1 and S2 Figs).

411

412 The divergence of the main clades of *B. taeniata* (Clade I + Clade
413 II) occurred during the Pleistocene, approximately ~0.229 Mya [95%
414 confidence interval (CI) = 0.145-0.323]. Divergence of the A and B subclades
415 would have happened within the northern group, approximately ~0.086 Mya
416 [95% CI = 0.052-0.121]. The first divergence within the southern group
417 between C+D and E+F subclades was ~0.115 Mya [95% CI = 0.070-0.162].
418 The separation between C and D clades was approximately ~0.105 Mya [95%
419 CI = 0.058-0.139], and between E and F was approximately ~0.068 Mya
420 [95% CI = 0.041-0.097].

421

422 The haplotype network was highly congruent with the Bayesian genealogy.
423 We recovered two main haplogroups corresponding to mtDNA clades I and
424 II, separated by 42 mutational steps in the CR network and separated by 57

steps in the cyt b network. The two haplogroups (clades I and II) for the CR do not share haplotypes and are dendritic in shape. The haplogroups obtained from cyt b share only two haplotypes and have a dendritic shape (Fig 3).

The number of CR haplotypes per subclade ranged from 6 (A) to 20 (D). A total of 104 nucleotide sites were polymorphic. The highest estimate of haplotype diversity was found in the total subclades. The highest nucleotide diversity was detected in subclade A and the lowest in subclade B (Table 1). Negative values were found for Tajima´s D in all subclades except B but only were significant in subclades C, D, and F. Fu´ Fs index was negative and significant only for subclades C, D and E. Rozas´ R2 test was positive but non-significant for all subclades (Table 1). P-distance values were correlated with the geographic distance between localities (Table S2).

Genetic structure and historical demographic changes

The CR-based DAPC analysis identified six clusters (K=6; Fig 4, S3 Table). The groups are congruent with the genealogical tree and are distributed in a north-south latitudinal gradient. Cluster A occurs in the northern limit of distribution (Quintero) and areas that historically have been associated with a gap in the distribution of *B. taeniata.* Cluster B includes the area north of the Biobío River and is a transition zone of the ecological regions described by di Castri (1968) Concepción (Fig 1). Cluster C includes localities of coastal

449 zones northwest of the Cordillera de Nahuelbuta. Cluster D extends through
450 intermediate and eastern areas in its northern limit and the coastal regions in
451 its southern boundary (Valdivia). Cluster E includes localities of the Los
452 Lagos Region and borders the sea (Puerto Montt, Carelmapu). Finally,
453 Cluster F covers the extreme south of the distribution of this species.

454

455 All values were significant for the CR- and cyt b-based Amova test ($p<0.001$).
456 High genetic variation (62.39%) was observed between groups for the CR
457 marker (I and II major clades). The variation among clusters within groups
458 was 14.46%, and variation within clusters was 23.15%. The same pattern of
459 variation was observed for cyt b (Table 2).

460

461 The mismatch distribution analysis (Fig 5) shows relatively unimodal patterns
462 for clusters B, C, D, E and F, but only with low raggedness values for clusters
463 C and D, however not significant. Cluster A showed a multi-modal distribution
464 and a high but not significant raggedness index (Table 1).

465

466 Reconstruction of the demographic history by Bayesian Skyline Plot BSP (Fig
467 6) does not suggest a reduction in effective size in the B. *taeniata*
468 populations but showed a signal of effective size growth for clusters B, C, D,
469 E, and F. Cluster B showed a low signal of changes in effective population
470 sizes from 5000 Ybp (years before present). Cluster C showed a constant

growth in population size until about 8000 Ybp, followed by a strong signal of expansion until the present. Cluster D showed a signal of population expansion starting around 12500 Ybp. Cluster E showed constant population growth which began about 25000 Ybp, but with a stronger signal after 8000 Ybp. Cluster F began to increase 15000 Ybp, with a weak signal of growth 6000 Ybp. Cluster A did not show changes in effective population size (Ne) over time from 30000 Ybp.

Historical geographic distribution changes

Comparing the SRW and RRW models based on marginal likelihood estimates indicates that the variable diffusion rate model through genealogy branches fits the data better than the constant rate model. The marginal log likelihood values for the RRW were -2332.4 and -2333.6 and for the SRW runs were -3413.8 and -3414.0, for path and stepping-stone sampling, respectively. The resulting $\log_e$ Bayes Factor ~ 1081 represents robust evidence in favour of the RRW model. This model was used to reconstruct phylogeographic diffusion patterns based on the MCC tree.

The reconstruction shows the origin of the expansion located south of the Biobio River, on the western side of the Coast Range, ~110000 years ago. However, the uncertainty associated with this ancestral location of *B. taeniata* is the 80% -HPD area range between 36.668° and 37.681° S latitude and from 73.531° to 72.867° W longitude. This diffusion model

suggests a first dispersal event towards the southeast in the intermediate depression, in the area of Araucanía, starting ~103000 Ybp. This was followed by two long-distance colonisations 63000 Ybp: i) to the south in the Los Lagos region and ii) to the north of its distribution range (Quintero). In the next phase, expansions would have occurred to the southwest along the coast ~ 40000 Ybp (Los Ríos and Biobío regions) (Fig 7). Subsequently, multiple dispersal events occurred towards the coast in Biobio and towards the coast and Andes Range in Araucanía ~10000 Ybp. The southward expansion would have begun ~10000 years ago from the western area of the Seno de Reloncaví (Fig 7).

Paleo-distribution modelling

The initial jack-knife analysis, correlation coefficients, false discovery rate and multi-collinearity indicated that the variable that contributed the most to the model was Precipitation of Coldest Quarter (bio19), followed by Mean Temperature of Driest Quarter (bio9), Temperature Seasonality (bio4), Mean Temperature of Wettest Quarter (bio8) and Precipitation of Driest Quarter (bio17) (Table S4). The cumulative contribution of these five factors was 89.7%. The response curves show that between ~ 220 and 1000 mm^3 precipitation in the coldest season increases the species' probability of presence. The probability increased with mean temperature of the driest quarter greater than 9 °C, with a maximum of 23 °C. Suitability increased with temperature seasonality less than 18 °C. The mean temperature of the

519 wettest quarter increased the probability of presence with temperatures
520 above 5 °C and below 10 °C.

521

522 We assessed 1581 candidate models in model calibration, all of which were
523 statistically significant compared to a null model of random prediction. Two of
524 these significant models met the omission criterion of 5% (S5 Fig). Finally,
525 among the significant, low-omission models, the model with the minimum
526 AICc value was one with a regularization parameter value of 2, quadratic,
527 product, and threshold features and the second set of environmental
528 variables.

529

530 The median of the selected climatic model suggested by the modelling
531 identified areas with different suitability levels from the Valparaíso Region in
532 areas near the coast to the Aysén Region, including the large island of
533 Chiloé, fitting the current presence record of *B. taeniata*. Highly suitable
534 areas were concentrated in the coastal area of the Biobío Region and coastal
535 and intermediate zones through Araucanía, Los Rios, Los Lagos and Chiloé
536 Island. Suitability declined toward the northern range of distribution of the
537 species and mountainous areas. The suitability area in the Valparaíso
538 Region is smaller and is concentrated along the coast (Quintero) (Fig 8D).
539 The selected model showed low uncertainty over the entire predicted
540 distribution area (S6 Fig).

541

Paleo-distribution modelling in LIG suggests a greater range extent for *B. taeniata* from the Valparaíso Region to Los Lagos, mainly in coastal and intermediate areas with high habitat suitability values (Fig 8A). Predictions at the LGM based on the MIROC climatic model suggest a smaller range of habitat suitability. A similar prediction was obtained with the CCSM climatic model at the LGM. Both models restrict the optimal area to the south-central zone and outside the continental limit, because the models consider lower sea levels during that glacial phase. The range of distribution retracted to the west towards lower and coastal areas. The MIROC model suggests higher suitability in intermediate zones and medium probabilities towards the south (8B and 8C Figs).

The selected model presented a greater suitability area than in the LGM (for both models), suggesting postglacial range expansion towards the east and south from the central distribution and to a lesser degree towards the Andes Range in the south.

Discussion

Our results support six lineages strongly supported by posterior probabilities and the haplotype network, contained in two major genealogical groups in the latitudinal distribution of *B. taeniata* separated by the Biobío River. The northern group is associated with the Mediterranean ecoregion and the southern group with the Oceanic evergreen forest ecoregion (Fig 1). The

566 separation limit of these groups (Biobío River) has also been described as a
567 geographic barrier for the populations of other species [3, 7, 16]. Fluvial
568 systems have previously been hypothesized as probable barriers for other
569 species, promoting intraspecific differentiation [16]. River barriers could
570 support the six lineages identified latitudinally, since historically the volume of
571 water in most of the rivers in southern Chile was influenced by the climate
572 changes of the Pleistocene [69] and the successive advances of habitat for
573 *B. taeniata* towards the north during the glaciations and subsequent
574 interglacial retraction may have generated disintegration of habitats in the
575 north, producing populations with restricted distribution such as cluster A.
576 This cluster is isolated in the south by the Mataquito and Maule Rivers.
577 Cluster B is isolated in the south by the Biobío River; cluster C to the north by
578 the Biobío River and to the east by the Nahuelbuta Range, an area that has
579 been identified as a glacial refuge for several species [12]. This is supported
580 by the high haplotype diversity found in cluster C. Cluster D in the south is
581 isolated by the Valdivia and Bueno rivers; cluster E in the south by the
582 continental geographic limit and the Pacific Ocean. This cluster is isolated by
583 the Seno de Reloncaví in the north and incorporates the southern geographic
584 limit of the species. Finally, cluster F that has a more recent origin is
585 separated by the geography of the extreme south.

586

587 The AMOVA test confirmed this pattern of variation among *B. taeniata*
588 populations, with a high percentage of variation between groups (Cluster I
589 and II) and less differentiation between populations (K=6) within groups

590 (Table 2). These results may indicate that *B. taeniata* has had medium-range
591 dispersal since the beginning of the Pleistocene, giving rise to the wide
592 distribution of the species in Chile. Divergence time estimates support this
593 idea, given that divergence of the major clades of *B. taeniata* (Clade I +
594 Clade II) occurred ~0.229 Mya, followed by more recent intraspecific
595 diversification events. Our spatial projection of the diffusion through time
596 indicates that the first dispersal event of the species would have started
597 ~103000 Ybp since coastal area in Arauco towards the southeast in the
598 intermediate depression in Araucanía, and a later long-distance colonization
599 to the south and north of the distribution range (Fig 7). These results also
600 indicate greater historical connectivity in the southern populations compared
601 to the demographic behaviour of the northern populations. This is consistent
602 with estimates of demographic history (based on Bayesian Skyline Plots and
603 mismatch distribution) that show signals of growth of effective size for
604 clusters C, D, E, and F (southern populations), low signal of growth in
605 effective population size in cluster B and stability of effective size for cluster
606 A, fitting the constant population size model.

607 As has been estimated in other species [7, 9], the paleodistribution model
608 shows signs of a contraction process associated with the LGM followed by a
609 subsequent geographic expansion to the south and east. The potential trend
610 of the pattern of change in the size of the distribution of the species is LIG >
611 LGM < Current.

612

613 Phylogeographic studies on anurans are still scarce, especially for those
614 species that inhabit temperate forests and Patagonia. However, the
615 phylogeographic descriptions of the biota of southern South America, for
616 example marsupials, rodents, lizards and amphibians [1, 2, 6, 7, 9, 10],
617 reveal that the responses to Quaternary dynamics were varied and mostly
618 species-specific [70]. The general pattern for these species suggests that
619 populations affected by changes in their habitat associated with glacial
620 events experience rapid population expansion as a previously glacial area is
621 colonized or recolonizes [9, 55, 71]. If divergence is associated with these
622 events, newly founded populations should present low levels of genetic
623 diversity compared to source populations reduced to refuges or that
624 remained stable over time [71]. Cluster F of *B. taeniata*, which is located at
625 the southern end of its distribution range (Aysén), is consistent with the
626 indicators of genetic variability that show low values of nucleotide diversity
627 (π) and high values of haplotype diversity (Hd) compared to the other clades
628 of the southern group in areas that were not directly affected by Pleistocene
629 glaciations. There are signs of population growth since the late Pleistocene
630 and early Holocene for all the clusters of the southern group, showing recent
631 demographic growth patterns similar to those of other anurans that inhabit
632 Patagonia (*B. leptopus* and *E. calcaratus*) [9, 10]. However, although in *E.*
633 *calcaratus* has identifying signs of demographic expansion consistent with
634 LGM glacial retreat, there was no evidence of significant range expansion [9].
635 Our result of demographic growth is reinforced in *B. taeniata* with ecological
636 niche model analyses. The paleo-distribution modelling shows signs of a

637 contraction process associated with the LGM followed by a subsequent
638 geographic expansion, and is consistent with the influence of climate change
639 on species dependent on temperature. Also, the result of the models in the
640 past projections is very similar to the estimates for other species, where the
641 distribution pattern during the LGM was concentrated outside the limits of the
642 ice sheet and near the Pacific coast in south-central Chile [7, 12, 72].
643 According to the model, the most suitable areas would not only have been
644 distributed near the current Chilean coast, but *B. taeniata* would also have
645 extended its distribution towards the west in areas currently under the sea.
646 The latter considers that during the LGM the sea level dropped approximately
647 130 m [16, 73]. The projections of both models (MIROC and CCSM) suggest
648 the presence of the classic refuge in the Coast Range described by Sérsic
649 (2011) [12]. This indicates that the phylogeographic processes to which *B.*
650 *taeniata* has been exposed would have covered mainly two glacial events of
651 the Pleistocene (Middle Plestiocene <260>150 ka and LGM ca. 25 ka) and
652 Holocene climate changes (Last Glacial 16-11.6 ka) [15] in an extremely
653 heterogeneous geographical scenario. The initial divergence within *B.*
654 *taeniata* occurred between the northern group and southern group
655 approximately 0.220 Mya, a period associated with glacial del Middle
656 Plestiocene (<260>150 ka) [15] suggesting that diversification of this
657 species major lineages may have occurred earlier than reported in other
658 frogs such as *B. leptopus* and *E. calcaratus*, however, later divergence
659 events in the southern group are associated with the divergence range of the
660 deep clades inferred in *B. leptopus* (0.107 Mya and *E. calcaratus* 0.188 Mya)

661 [9,10]. The most recent divergence event between cluster E and cluster F
662 (~0.068 Mya) is concordant with a time within Llanquihue Glaciation (~0.070
663 Mya) [76]. This event was reported previously as a probable event that
664 generated divergence within *E. calcaratus* [10].

665

666 Although this study did not consider insular samples (for example the Island
667 of Chiloé), it would be expected that this population would show a geographic
668 origin from different gene pools as has been found in *L. pictus* and *B.*
669 *leptopus* [6, 8], where the genetic results indicated at least three source
670 populations from Antillanca (Osorno), Chaitén (Futaleufú) and northwest
671 Chiloé Island.

672

673 This study indicates that the populations of the southern group of *B. taeniata*
674 are possibly in a process of demographic expansion since before the LGM
675 (ca. 25 Kya) and geographic expansion since the late Pleistocene and early
676 Holocene. Despite being a species with wide availability of habitat towards
677 the south, the populations of its northern range (clade A) that conform to
678 constant population size models are restricted.

679

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681

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References

1. Himes CMT, Gallardo MH, Kenagy GJ. Historical biogeography and post-glacial recolonization of South American temperate rain forest by the relictual marsupial *Dromiciops gliroides.* Journal of Biogeography. 2008; 35(8): 1415-1424.

2. Rodríguez-Serrano E, Cancino RA, Palma RE. Molecular phylogeography of *Abrothrix olivaceus* (Rodentia: sigmodontinae) in Chile. Journal of Mammalogy. 2006; 87(5): 971-980.

3. Palma RE, Boric-Bargetto D, Torres-Pérez F, Hernández CE, Yates TL. Glaciation Effects on the Phylogeographic Structure of Oligoryzomys longicaudatus (Rodentia: Sigmodontinae) in the Southern Andes. PLoS ONE. 2012; 7(3):e32206. doi: 10.1371/journal.pone.0032206.

4. Morando M, Avila LJ, Turner CR, Sites Jr JW. 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. 2007; 43(3): 952-973. doi: 10.1016/j.ympev.2006.09.012

5. Victoriano PF, Ortiz JC, Benavides E, Adams BJ, Sites Jr JW. Comparative phylogeography of codistributed species of Chilean *Liolaemus* (Squamata: Tropiduridae) from the central-southern Andean range. Molecular Ecology. 2008; 17(10): 2397-416. doi: 10.1111/j.1365-294X.2008.03741.x

6. Vidal MA, Moreno PI, Poulin E. Genetic diversity and insular colonization of Liolaemus pictus (Squamata, Liolaeminae) in north-western Patagonia. Austral Ecology. 2012; 37(1): 67-77.

7. Muñoz-Mendoza C, D´Elía G, Panzera A, Villalobos-Leiva A, Sites Jr JW, Victoriano PF. Geography and past climate changes have shaped the evolution of a widespread lizard from the Chilean hotspot. Molecular Phylogenetics and Evolution. 2017; 116: 157-171.

8. Vidal M, Ibañez S, Moreno PI, Poulin E. Phylogeography of a Patagonian lizard and frog: Congruent signature of southern glacial refuges. Austral Ecology. 2016; 41: 399-408. doi: 10.1111/aec.12325

9. Nuñez JJ, Wood NK, Rabanal F, Fontanella FM, Sites Jr JW. Amphibian phylogeography in the Antipodes: Refugia and postglacial colonization explain mitochondrial haplotype distribution in the Patagonian frog *Eupsophus calcaratus* (Cycloramphidae). Molecular Phylogenetics and Evolution. 2011; 58(2): 343-352. doi: 10.1016/j.ympev.2010.11.026

10. Nuñez JJ, Suárez-Villota EY, Querencia CA, Olivares AP, Sites Jr JW. Phylogeographic analysis and species distribution modelling of the wood frog *Batrachyla leptopus* (Batrachylidae) reveal interglacial diversification in south western Patagonia. PeerJ. 2020; 8: e9980. doi: 10.7717/peerj.9980

11. Turchetto-Zolet AC, Pinheiro F, Salgueiro F, Palma-Silva C. Phylogeographical patterns shed light on evolutionary process in South America. Molecular Ecology. 2013; 22(5): 1193-213. doi: 10.1111/mec.12164

12. Sérsic AN, Cosacov A, Cocucci AA, Johnson LA, Pozner R, Avila LJ, et al. Emerging phylogeographical patterns of plants and terrestrial vertebrates from Patagonia. Biological Journal of the Linnean Society. 2011; 103(2): 475-494. doi: 10.1111/j.1095-8312.2011.01656.x

13. Cosacov A, Sérsic AN, Sosa V, Johnson LA, Cocucci AA. Multiple periglacial refugia in the Patagonian steppe and post-glacial colonization of the Andes: The phylogeography of *Calceolaria polyrhiza*. Journal of Biogeography, 2010; 37: 1463-1477. doi: 10.1111/j.1365-2699.2010.02307.x

14. Knowles LL. Did the pleistocene glaciations promote divergence? Tests of explicit refugial models in montane grasshopprers. Molecular Ecology. 2001; 10(3): 691-701. doi: 10.1046/j.1365-294x.2001.01206.x

15. Rabassa J, Coronato A, Martínez O. Late Cenozoic glaciations in Patagonia and Tierra del Fuego: an updated review. Biological Journal of the Linnean Society. 2011; 103: 316-335. doi: 10.1111/j.1095-8312.2011.01681.x

16. Victoriano PF. Phylogeography of Chilean Lizards: Histories of Genetic Diversification on the Western Slope of Southern Andes. In: Morando M, Avila LJ, editors. Lizards of Patagonia: Diversity,

Systematics, Biogeography and Biology of the Reptiles at the End of the World: Springer International Publishing; 2020. pp. 255-291.

17. Heusser CJ. Ice age southern Andes: A chronicle of palaeoecological events 3st ed. Elsevier Press; 2003

18. Vásquez-Domínguez E, Filogeografía y vertebrados. In: Eguiarte LE, Souza V, Aguirre X, editors. Ecología Molecular: Instituto Nacional de Ecología, Universidad Autónoma de México; 2007. pp. 441-461

19. Victoriano PF, Muñoz-Mendoza C, Sáez PA, Salinas HF, Muñoz-Ramírez C, Sallaberry M, et al. Evolution and Conservation on Top of the World: Phylogeography of the Marbled Water Frog (*Telmatobius marmoratus* Species Complex; Anura, Telmatobiidae) in Protected Areas of Chile. The Journal of Heredity. 2015; 106(1): 546-559.

20. Vásquez D, Correa C, Pastenes L, Palma RE, Méndez MA. Low phylogeographic structure of *Rhinella arunco* (Anura: Bufonidae), an endemic amphibian from the Chilean Mediterranean hotspot. Zoological Studies. 2013; 52(1): 1-11

21. Formas J. La herpetofauna de los bosques temperados de Sudamérica, In: Duellman W, editor. The South American herpetofauna, Museum of Natural History, University of Kansas, Lawrence, Kansas. Monograph 1979. pp. 341-379.

22. Brieva L, Formas J. Allozyme variation and geographic differentiation in the Chilean leptodactylid frog *Batrachyla taeniata* (Girard, 1854). Amphibia-Reptilia. 2001; 22: 413-420.

23. Sallaberry M, Valencia J, Díaz N. Distribución y ambiente de *Batrachyla taeniata* (Girard) en Chile (Anura - Leptodactylidae). Boletín del Museo Nacional de Historia Natural (Chile). 1981; 38: 61-67.

24. Correa C, Cisternas J, Velásquez N, Lobos G, San Martín-Órdenes J. Distribution extension, new records and historical ocurrence of *Batrachyla taeniata* (Girard, 1854) (Anura: Batrachylidae) in Chile. Herpetology notes. 2014; 7: 703-706.

25. Di Castri F. Esquisse écologique du Chile. Biologie de l'Amérique Australe, ed. C. Delamare-Deboutteville and E. Rapoport. 1968, Paris, C.N.R.S.

26. Cisternas-Medina I, Ortiz JC, Ferrada S. Development and characterization of microsatellite molecular markers for the eye mask frog *Batrachyla taeniata* (Girard, 1855). Genetics and Molecular Research. 2019; 18 (2): gmr16039960.

27. Cei, J., Batracios de Chile, ed. E.d.l.U.d. Chile. Santiago 1962

28. Villagrán C, Armesto JJ, Relaciones florísticas entre las comunidades relictuales del Norte Chico y la zona central con el bosque del sur de Chile. Boletín del Museo Nacional de Historia Natural (Chile). 1980; 37: 87-101.

29. Cei J, Capurro L. Biología y Desarrollo de *Eupsophus taeniatus* Girard Investigaciones Zoológicas Chilenas. 1958; 4: 159-182.

30. Villagrán C, Abarzúa AM, Armesto JJ. Nuevas evidencias paleobotánicas y filogeográficas de la historia Cuaternaria de los bosques subtropical-templados de la Cordillera de la Costa de Chile. In: Smith-Ramírez C, Saqueo SA, edits. Biodiversidad y Ecología de los Bosques Costeros de Chile. Editorial Universidad de Los Lagos, Chile. 2019. pp. 3-21.

31. Feng YJ, Blackburn DC, Liang D, Hillis DM, Wake DB, Cannatella DC, et al. Phylogenomics reveals rapid, simultaneous diversification of three major clades of Gondwanan frogs at the Cretaceous–Paleogene boundary. Proceedings of the National Academy of Sciences. 2017; 114(29): E5864-E5870. doi: 10.1073/pnas.1704632114

32. Goebel AM, Donnelly JM, Atz ME. PCR primers and amplification methods for 12S ribosomal DNA, the control region, cytochrome oxidase I, and cytochrome b in bufonids and other frogs, and an overview of PCR primers which have amplified DNA in amphibians successfully. Molecular Phylogenetics Evolution. 1999; 11(1): 163-99. doi: 10.1006/mpev.1998.0538

33. Moritz C, Schneider CJ, Wake DB. Evolutionary relationships within the *Ensatina eschscholtzii* complex confirm the ring species interpretation. Systematic Biology. 1992; 41: 273-291. doi: doi.org/10.1093/sysbio/41.3.273

34. CodonCode Corporation. 2018. Codon Code Aligner v. 9.01. Avaliable: https://www.codoncode.com/aligner/index.htm. Accessed 26 May 2020

35. Rozas J, Sánchez-DelBarrio JC, Messeguer X, Rozas R. DnaSP, DNA polymorphism analyses by the coalescent and other methods, Bioinformatics. 2003; 19(18): 2496-2497. doi: 10.1093/bioinformatics/btg359

36. Xia X, Li C, Yang Q. Routine analysis of molecular data with software DAMBE. Fundamental Concepts and Methodology in Molecular Palaeontology. Science Publishers, China. 2003; 149-167

37. Xia X, Xie Z. DAMBE: Data Analysis in Molecular Biology and Evolution. Journal of Heredity. 2001; 92: 371-373.

38. Tajima F. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics. 1989; 123(3): 585-595.

39. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution. 2013; 30(12): 2725-2729. doi: 10.1093/molbev/mst197

40. Darriba D, Taboada GL, Doallo R, Posada D. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods. 2012; 9(8): 772.

41. Guindon S, Gascuel O. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Systematic Biology. 2003. 52(5): 696-704. doi: 10.1080/10635150390235520

42. Drummond AJ, Suchard MA, Xie D, Rambaut A. Bayesian Phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution. 2012; 29(8):1969-1973. doi: 10.1093/molbev/mss075

43. Rambaut A, Ho SY, Drummond AJ, Shapiro B. Accommodating the Effect of Ancient DNA Damage on Inferences of Demographic Histories. Molecular Biology and Evolution. 2008; 26(2): 245-248. doi: 10.1093/molbev/msn256

44. Irisarri I, San Mauro D, Abascal F, Ohler A, Vences M, Zardoya R. The origin of modern frogs (Neobatrachia) was accompanied by acceleration in mitochondrial and nuclear substitution rates. BMC Genomics. 2012; 13(1): 1-19

45. Menezes L, Canedo C, Batalha-Filho H, Garda AA, Gehara M, Napoli MF. Multilocus Phylogeography of the Treefrog *Scinax eurydice* (Anura, Hylidae) Reveals a Plio-Pleistocene Diversification in the Atlantic Forest. PLoS ONE. 2016. 11(6): e0154626. doi: 10.1371/journal.pone.0154626

46. Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. Systematic Biology. 2018. 67(5): 901-904. doi: 10.1093/sysbio/syy032

47. Bandelt HJ, Forster P, Röhl A. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution, 1999; 16(1): 37-48. doi: 10.1093/oxfordjournals.molbev.a026036

48. Jombart T. Adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics. 2008; 24(11): 1403-1405. doi: 10.1093/bioinformatics/btn129

49. Jombart T, Ahmed I. Adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. Bioinformatics. 2011; 27(21): 3070-3071. doi: 10.1093/bioinformatics/btr521

50. Guillot G, Mortier F, Estoup A. GENELAND: a computer package for landscape genetics. Molecular Ecology Notes. 2005; 5(3): 712-715. doi: 10.1111/j.1471-8286.2005.01031.x

51. Excoffier L, Lischer HE. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources. 2010; 10(3): 564-567. doi: 10.1111/j.1755-0998.2010.02847.x

52. Rogers AR, Harpending H. Population growth makes waves in the distribution of pairwise genetic differences. Molecular Biology and Evolution. 1992; 9(3): 552-569. doi: 10.1093/oxfordjournals.molbev.a040727

53. Harpending HC. Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. Human Biology, 1994; 66(4): 591-600.

54. Lemey P, Rambaut A, Welch JJ, Suchard MA. Phylogeography Takes a Relaxed Random Walk in Continuous Space and Time. Molecular Biology and Evolution. 2010; 27(8): 1877-1885. doi: 10.1093/molbev/msq067

55. Camargo A, Werneck FP, Morando M, Sites Jr JW, Avila LJ. Quaternary range and demographic expansion of *Liolaemus darwinii* (Squamata: Liolaemidae) in the Monte Desert of Central Argentina using Bayesian phylogeography and ecological niche modelling. 2013; 22(15): 4038-4054.

56. Bielejec F, Rambaut A, Suchard MA, Lemey P. SPREAD*:* Spatial Phylogenetic Reconstruction of Evolutionary Dynamics. Bioinformatics, 2011; 27(20): 2910-2912. doi: 10.1093/bioinformatics/btr481

57. Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology: A Journal of the Royal Meteorological Society. 2005; 25(15): 1965-1978. doi: 10.1002/joc.1276

58. Guevara L, Morrone JJ, León-Paniagua L. Spatial variability in species' potential distributions during the Last Glacial Maximum under different Global Circulation Models: Relevance in evolutionary biology. Journal of Zoological Systematics and Evolutionary Research. 2019; 57(1): 113-126. doi: 10.1111/jzs.12238

59. Barbosa AM. FuzzySim: applying fuzzy logic to binary similarity indices in ecology. Methods in Ecology and Evolution. 2015; 6(7): 853-858. doi: 10.1111/2041-210X.12372

60. Qin A, Liu B, Guo Q, Bussmann RW, Ma F, Jian Z, et al. Maxent modeling for predicting impacts of climate change on the potential distribution of *Thuja sutchuenensis* Franch., an extremely endangered conifer from southwestern China. Global Ecology and Conservation. 2017;10: 139-146. doi: 10.1016/j.gecco.2017.02.004

61. Bourke J, Busse K, Bohme W. Potential effects of climate change on the distribution of the endangered Darwin's frog. North-Western Journal of Zoology. 2018; 14(2): 165-170.

62. Phillips SJ, Anderson RP, Schapire RE. Maximum entropy modeling of species geographic distributions. Ecological Modelling. 2006; 190(3): 231-259. doi: 10.1016/j.ecolmodel.2005.03.026

63. Cobos ME, Peterson AT, Barve N, Osorio-Olvera L. Kuenm: an R package for detailed development of ecological niche models using Maxent. PeerJ. 2019; 7: e6281. doi: 10.7717/peerj.6281

64. Peterson AT, Papeş M, Soberón J. Rethinking receiver operating characteristic analysis applications in ecological niche modeling. Ecological Modelling. 2008; 213(1): 63-72.

65. Lobo J, Jiménez-Valverde A, Real R. AUC: a misleading measure of the performance of predictive distribution models. Journal of Global Ecology and Biogeography. 2008; 17(2): 145-151. doi: 10.1111/j.1466-8238.2007.00358.x

66. Warren DL, Seifert SN. Ecological niche modeling in Maxent: the importance of model complexity and the performance of model selection criteria. Ecological Applications. 2011; 21(2): 335-342. doi: 10.1890/10-1171.1

67. Behroozian M, Ejtehadi H, Peterson AT, Memariani F, Mesdaghi M. Climate change influences on the potential distribution of *Dianthus polylepis* Bien. ex Boiss. (Caryophyllaceae), an endemic species in the Irano-Turanian region. PLoS ONE, 2020; 15(8): e0237527. doi: 10.1371/journal.pone.0237527

68. Owens HL, Campbell LP, Dornak LL, Saupe EE, Barve N, Soberón J, et al. Constraints on interpretation of ecological niche models by limited environmental ranges on calibration areas. Ecological Modelling. 2013; 263: 10-18. doi: 10.1016/j.ecolmodel.2013.04.011

69. Sallaberry-Pincheira N, Garin C, González-Acuña D, Sallaberry MA,, Vianna JA. Genetic divergence of Chilean long-tailed snake (*Philodryas chamissonis*) across latitudes: Conservation threats for different lineages. Diversity and Distributions. 2011; 17: 152-162

70. Valdez L, D'Elía G. Genetic Diversity and Demographic History of the Shaggy Soft-Haired Mouse *Abrothrix hirta* (Cricetidae; Abrotrichini). Frontiers in Genetics. 2021; 12184). doi: 10.3389/fgene.2021.642504

71. Hewitt GM. Post-glacial re-colonization of European biota. Biological Journal of the Linnean Society. 2008; 68(1-2): 87-112. doi: 10.1111/j.1095-8312.1999.tb01160.x

72. Vera-Escalona I, D'Elía G, Gouin N, Fontanella FM, Muñoz-Mendoza C, Sites Jr JW, et al.Lizards on Ice: Evidence for Multiple Refugia in Liolaemus pictus (Liolaemidae) during the Last Glacial Maximum in the Southern Andean Beech Forests. PLoS ONE, 2012; 7(11): e48358. doi: 10.1371/journal.pone.0048358

73. Davies BJ, Darvill CM, Lovell H, Bendle JM, Dowdeswell JA, Fabel D, et al.The evolution of the Patagonian Ice Sheet from 35 ka to the present day (PATICE). Earth-Science Reviews. 2020; 204: 103152. doi: 10.1016/j.earscirev.2020.103152

74. Capurro L. Distribución de *Eupsophus taeniatus* Girard (Anura, Cystignathidae) en Chile. Investigaciones Zoológicas Chilenas. 1953. 1(10): 14-15.

75. Donoso-Barros R. Catálogo herpetológico chileno. Boletín del Museo Nacional de Historia Natural. 1970; 31: 49-124.

76. Rabassa J, Clapperton CH. Quaternary glaciations of the Southern Andes. Quaternary Sci. Rev. 1990. 9: 153-174.

Tables

Table 1. Measures of genetic diversity and demographic expansion for mtDNA (RC) clades. Numbers in bold indicate statistically significant values.

	Genetic variability					Demographic expansion			
Phylogroup	**N**	**S**	**H**	**Hd**	**π**	**Fu**	**D**	**R2**	**r**
A	8	34	6	0.929	0.02634	1.39	-1.11	0.147	0.15
B	20	12	10	0.879	0.00735	-2.23	0.04	0.130	0.07
C	28	36	17	0.947	0.01323	**-4.58**	**-1.54**	0.098	0.02
D	23	33	20	0.988	0.01057	**-14.52**	**-1.69**	0.070	0.01
E	13	27	11	0.974	0.01541	**-3.37**	-1.12	0.118	0.08
F	9	16	7	0.917	0.00966	-1.41	**-1.65**	0.195	0.07
Total	101	104	58	0.981	0.05337	**-10.56**	0.13	0.108	0.075

*P < 0.05. N, number of individuals; S, number of polymorphic sites; H, number of haplotypes; Hd, Haplotype diversity; π, nucleotide diversity; Fu, Fu's test; D, Tajima test; R2, Ramos-Onsis Roza's test; r, raggedness index.

Table 2. Amova results for control region (RC) and cytochrome b (cyt b)

Source of Variation	df	Sum of Squares	Variance components	Percentage of variation
Among groups				
CR	1	799.976	17.77743 Va	62.39
cyt b	1	689.651	23.54254 Va	66.95
Among populations within groups	4	286.129	4.12172 Vb	14.46
CR	3	208.647	7.88373 Vb	22.42
cyt b				
Within populations				
CR	95	626.727	6.59712 Vc	23.15
cyt b	52	194.422	3.73888 Vc	10.63
Total CR	100	1712.832	28.49627	
Total cyt b	56	1092.719	35.16506	

P < 0.0001 Fixation indices for CR: F_{SC}=0.38453; F_{ST}=0.76849*; F_{CT}=0.62385* and for cyt b: F_{SC}=0.67831; F_{ST}=0.89368; F_{CT}=0.66948

Figures

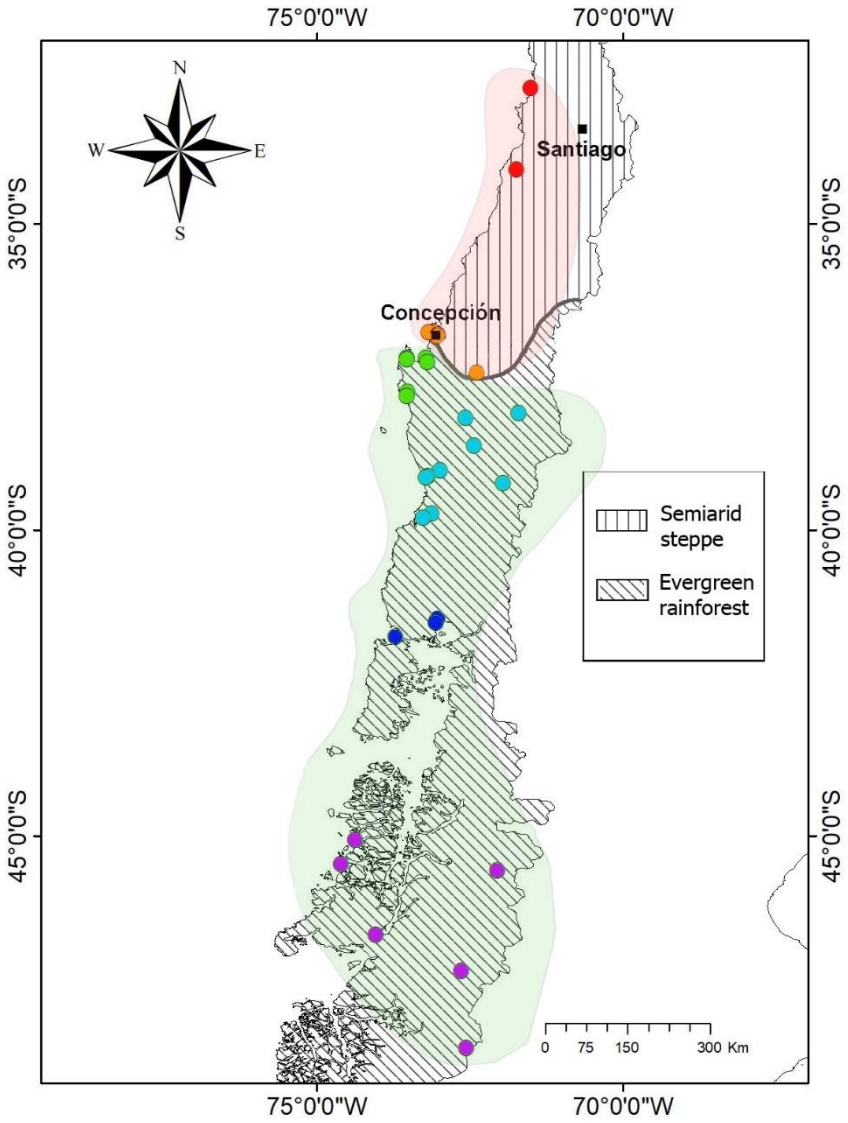


Fig 1. Geographic distribution of *B. taeniata* included in this study. The circles correspond to the localities studied (S1 Table) and the colours to the clusters obtained in the genealogical analysis (Fig 2). Red = Cluster A; Orange = Cluster B; Green = Cluster C; Light blue = Cluster D; Blue = Cluster E and Violet = Cluster F. The light red and light green polygons cover the localities of the two deep clades obtained in the genealogical analysis. The crossed-out areas correspond to the ecological regions described by di Castri (1968) and were extracted and modified from Brieva & Formas (2001).

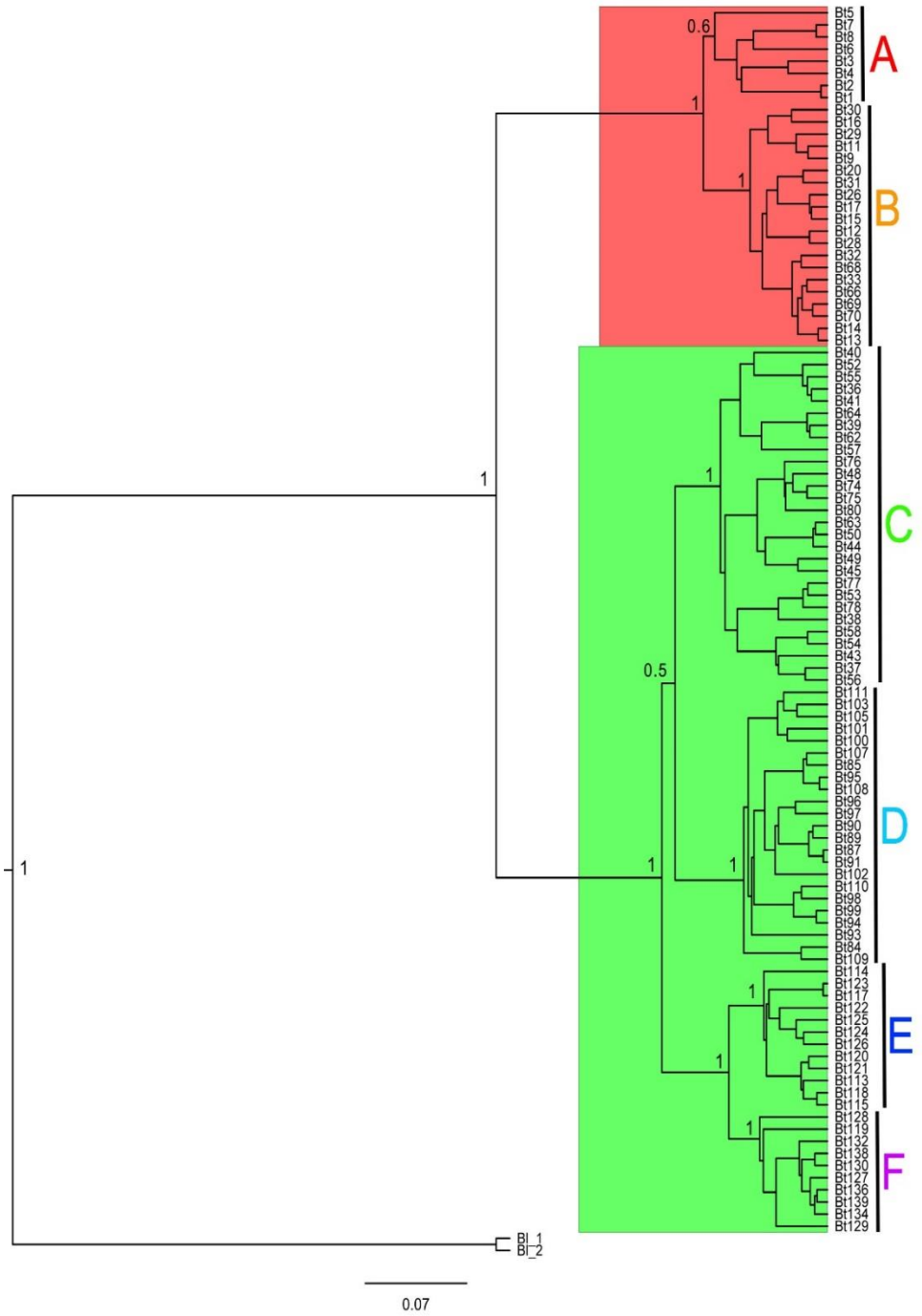


Fig 2. Genealogical relationships of control region *B. taeniata* haplotypes as inferred by Bayesian analysis. The support values (Bayesian Posterior Probabilities) shown by significant nodes.

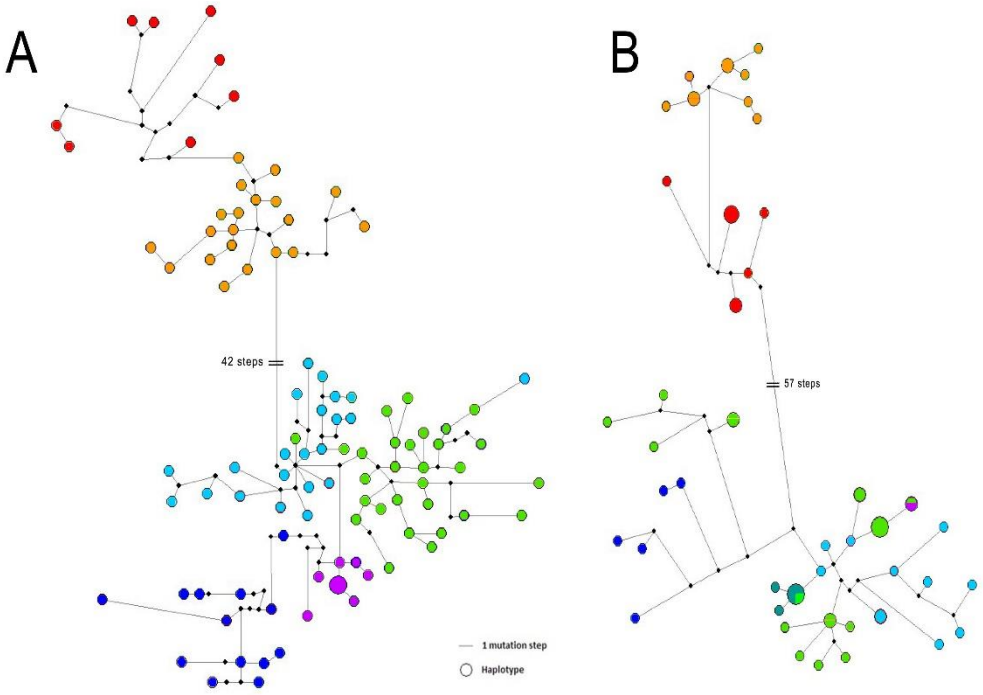


Fig 3. Median-joining network of mitochondrial control region (A) and cytochrome b (B) haplotypes of *B. taeniata.* Circle size in the network is proportional to haplotype sampling frequency. Color indicates the clusters obtained in the genealogical analysis.

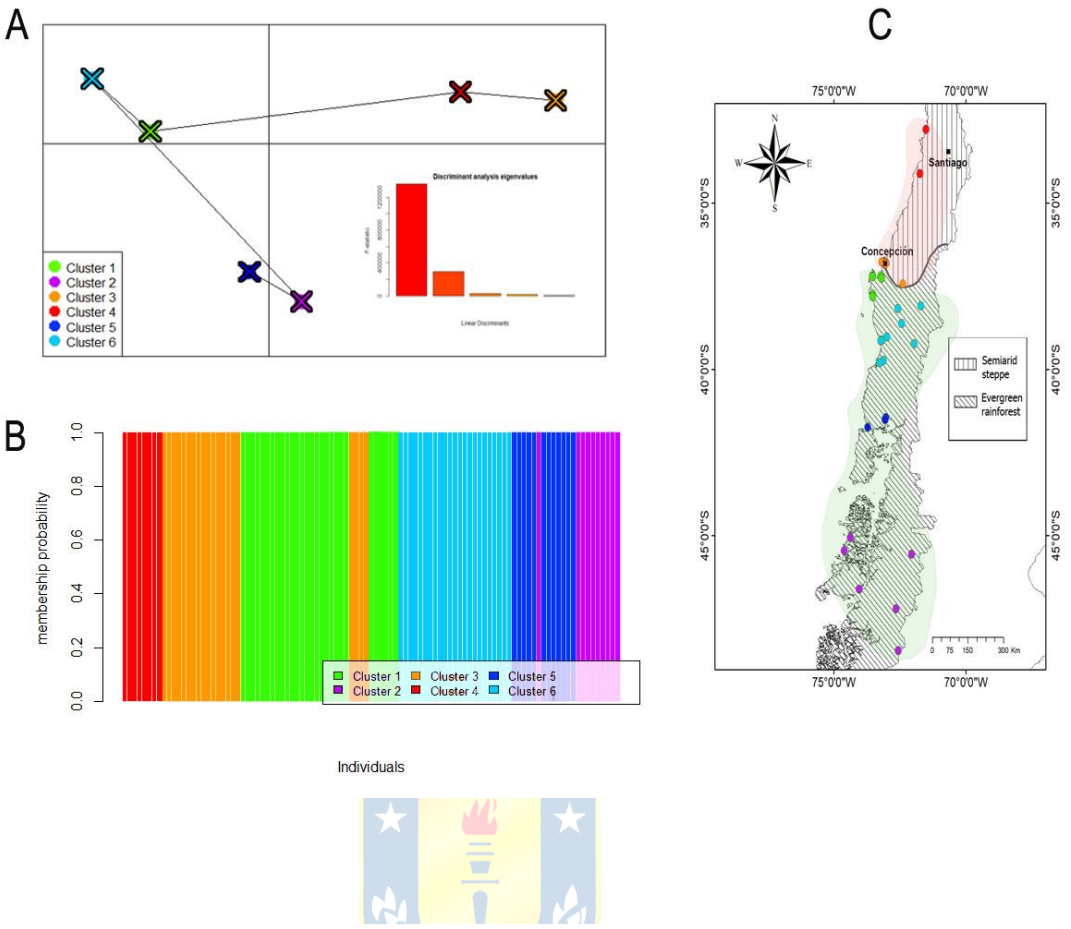


Fig 4. A) Discriminant analysis of principal components. Fifty main components and three discriminant functions were retained. "X" indicate the clusters identified by the study. The correspondence of the clusters with the clades obtained in the genealogy is the following: Cluster 1=C; Cluster 2=F; Cluster 3=B; Cluster 4=A; Cluster 5=E; Cluster 6=D. B) Graph of probability membership of the individuals for each cluster. The colour represents the cluster obtained in Fig 4A. (S3 Table). C) Fig 1 Geographic distribution of *B. taeniata* included in this study; the circles correspond to the localities studied (S1 Table).

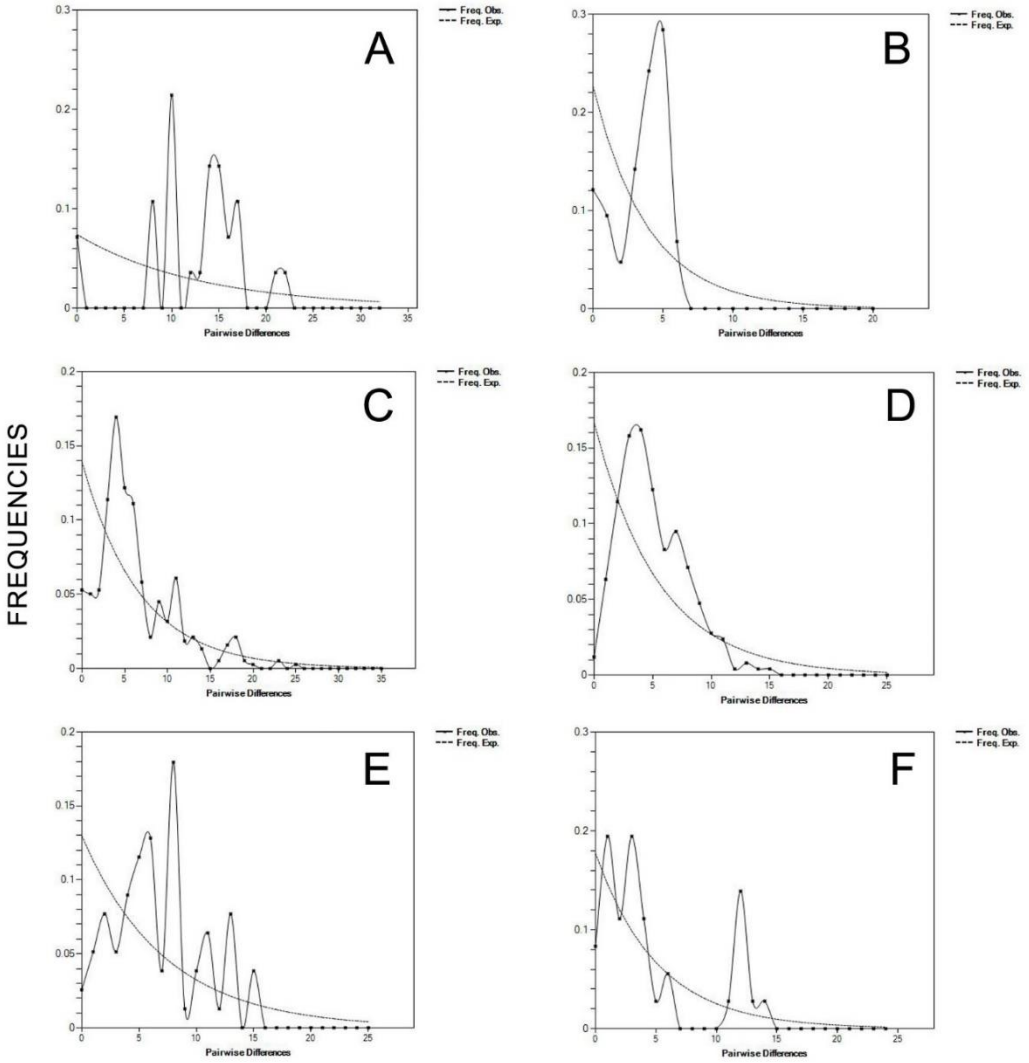


Fig 5. Mismatch distribution of observed frequencies of pairwise differences among *B. taeniata* clades for mitochondrial sequences (Control region).

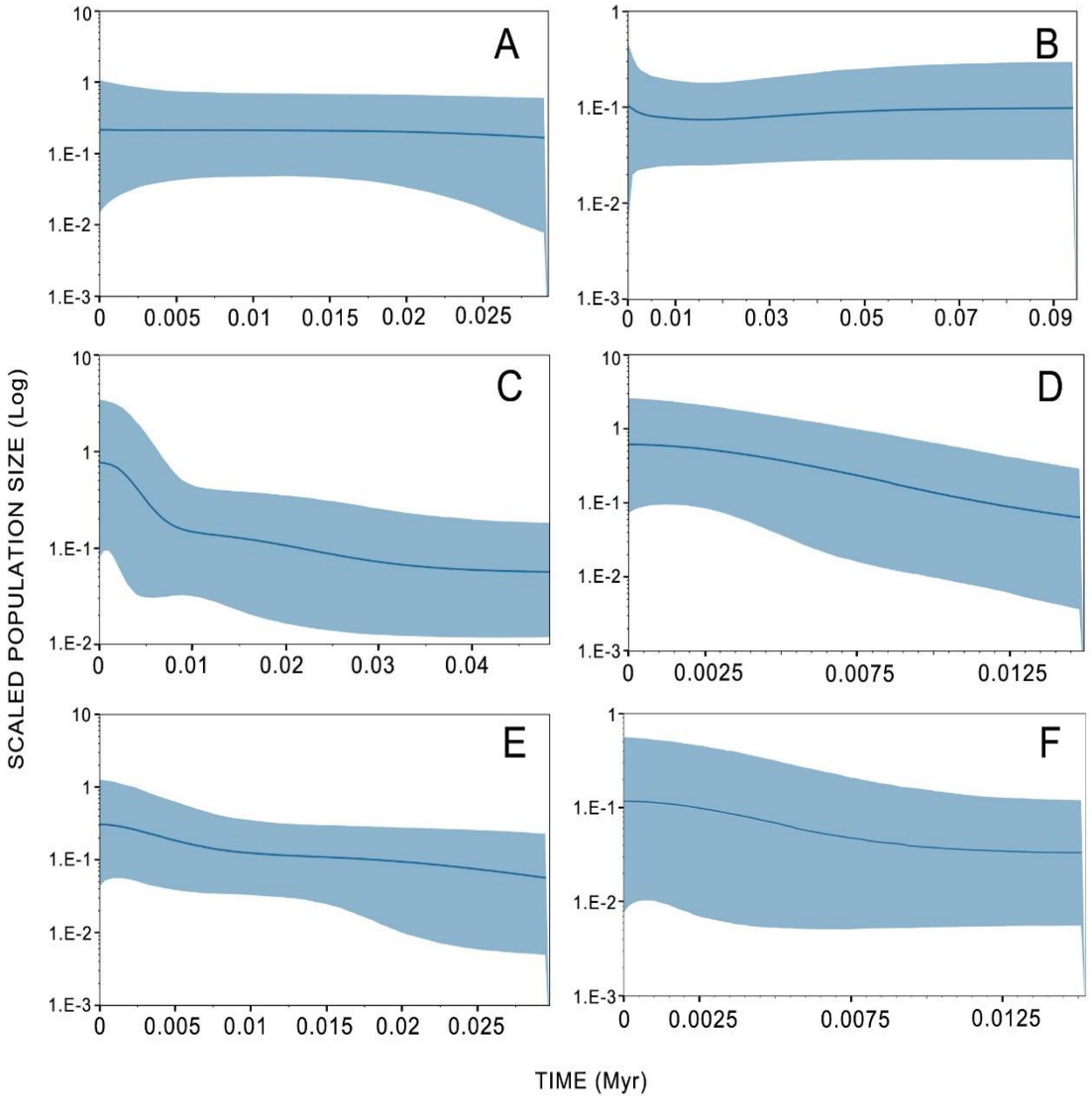


Fig 6. Bayesian skyline plot (BSP) for each clade of *B. taeniata* based on mitochondrial sequences (Control region). Light-blue area represents 95% confidence intervals for Ne.

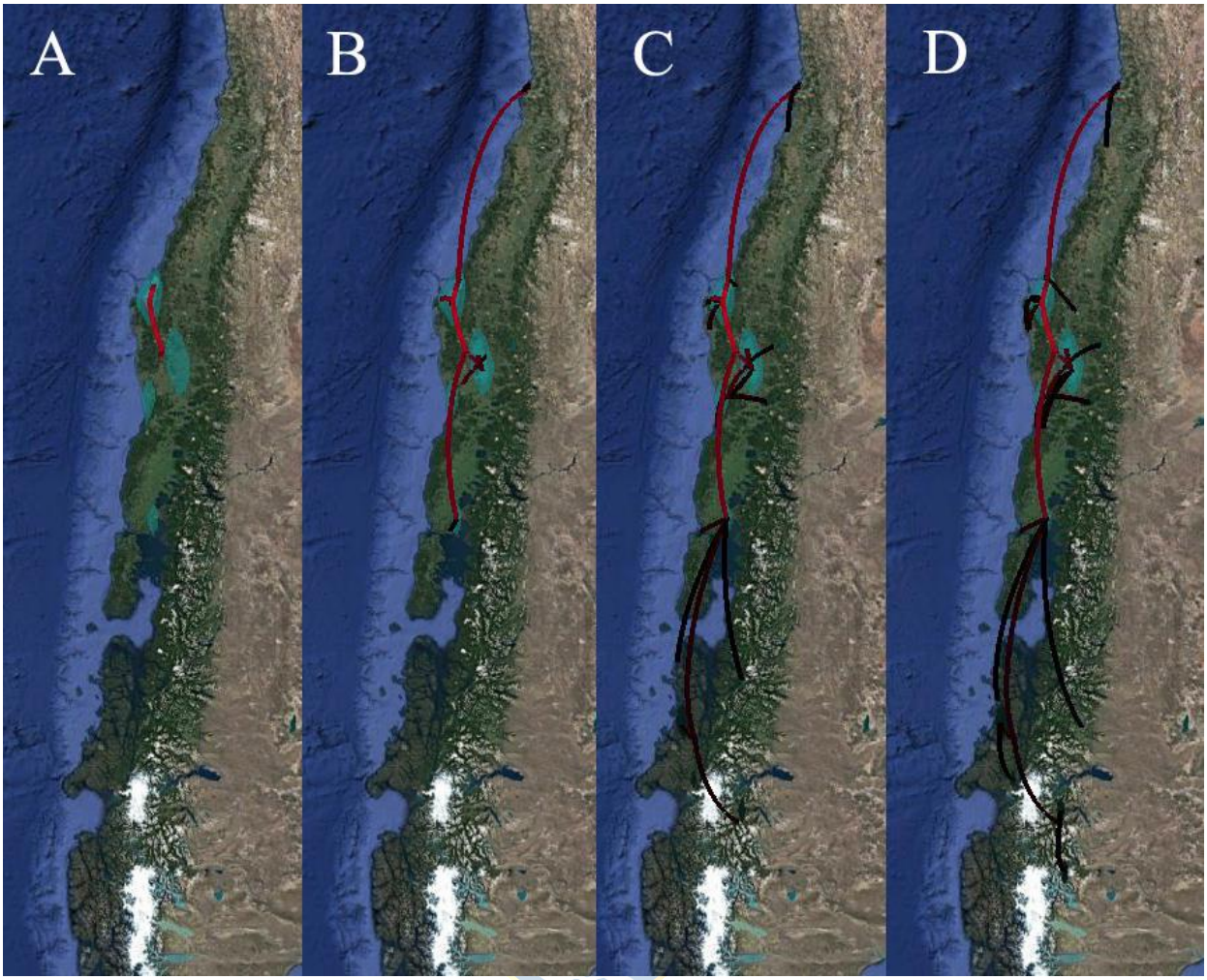


Fig 7. Spatial projection of the diffusion pattern through time, based on the maximum clade credibility (MCC) tree estimated with a Bayesian phylogeographic analysis in Beast (RRW model) at four time slices: A) ~103kya, B) ~40 kya, C) ~10 kya, D) current. The red lines represent the branches of the MCC tree with a gradient from dark to light colour indicating older *vs*. younger branches. The blue regions represent the 80% HPD uncertainty in the location of ancestral branches with a gradient between clear and dark representing older *vs*. younger diffusion events.

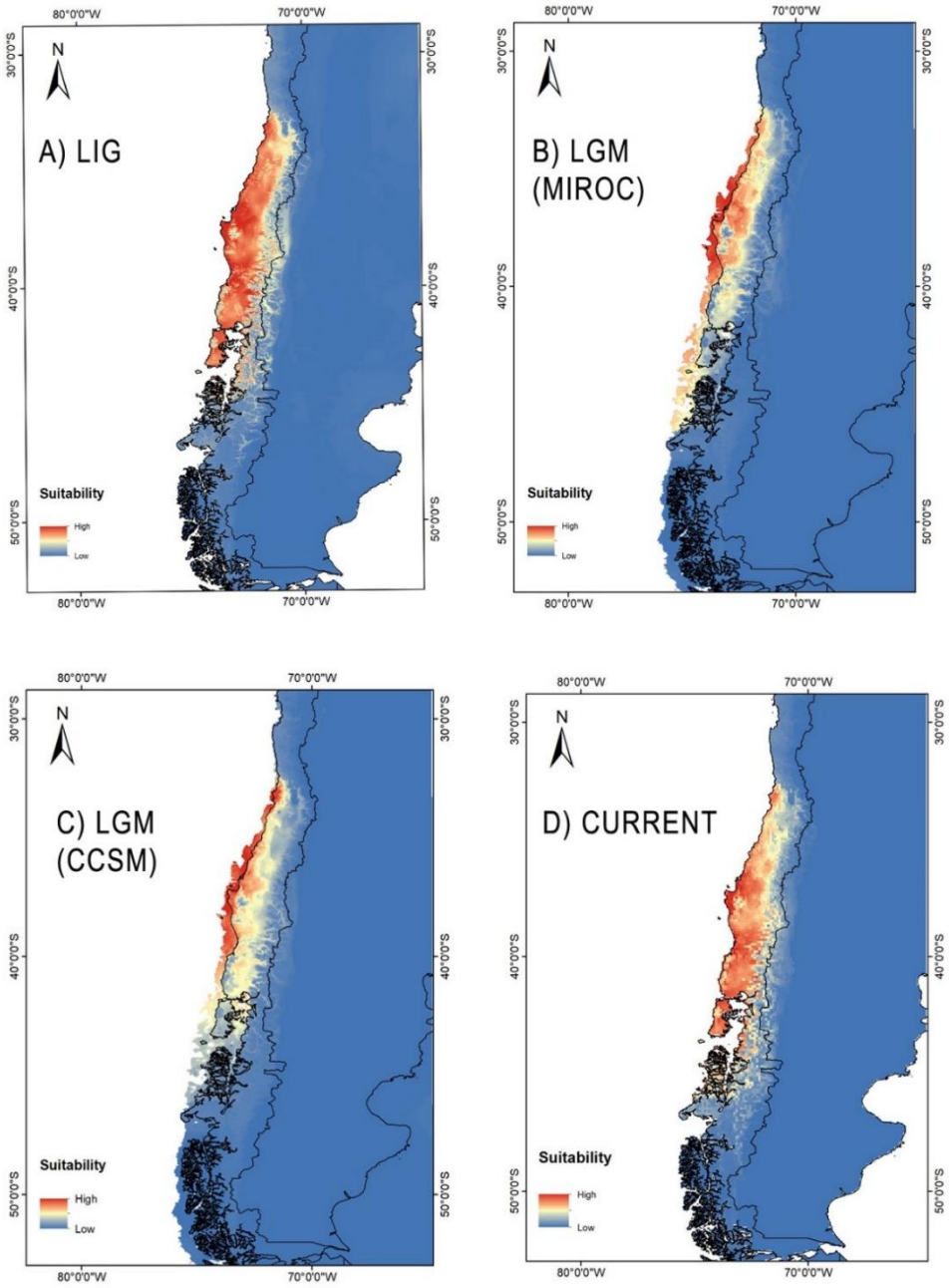


Fig 8. Maps of predicted climatic suitability of *B. taeniata* across Quaternary climatic fluctuations (A-C) and current (D). Warmer colours indicate higher climatic suitability.

Supplementary material: Tables

S1 Table. Collection localities for *Batrachyla taeniata*. geographic coordinates and haplotype codes for control region (CR) and cytochrome b (cyt b)

				Samples	
	Locality	**Latitude**	**Longitude**	**CR**	**cyt b**
1	Quintero	-32.79493	-71.51062	Bt1. Bt2. Bt3. Bt4. Bt5. Bt6	Bt1. Bt2. Bt3. Bt4. Bt5. Bt6
2	Litueche	-34.1166667	-71.7333333	Bt7. Bt8	Bt7. Bt8
3	Hualpén	-36.7833333	-73.0833333	Bt9. Bt10. Bt11. Bt12. Bt13. Bt13. Bt14. Bt15. Bt16	Bt9. Bt10. Bt11. Bt12. Bt16
4	Parque Botánico Hualpén	-36.7833333	-73.1666667	Bt17. Bt18. Bt19. Bt20. Bt21. Bt22. Bt23	Bt17. Bt23
5	UdeC	-36.8166667	-73.0166667	Bt24	
6	Cerro Caracol	-36.8333333	-73.0333333	Bt25. Bt26. Bt27. Bt28. Bt29. Bt30. Bt31. Bt31. Bt32. Bt33	Bt25
7	San Pedro de la Paz	-36.8166667	-73.1	Bt34	
8	Horcones. Laraquete	-37.20017	-73.21361	Bt35. Bt36. Bt37. Bt38. Bt39. Bt40. Bt41. Bt42	Bt35. Bt36. Bt38. Bt39. Bt40. Bt41
9	Codigua. Laraquete	-37.26082	-73.19267	Bt43. Bt44. Bt45	Bt43. Bt44. Bt45
10	La Cal. Arauco	-37.2	-73.5333333	Bt46. Bt47. Bt48. Bt49. Bt50. Bt51. Bt52. Bt53. Bt54	Bt48. Bt49
11	Río Tubul. Arauco	-37.2166667	-73.5333333	Bt55. Bt56. Bt57. Bt58. Bt59. Bt60. Bt61. Bt62. Bt63. Bt64. Bt65	Bt56. Bt58. Bt59
12	Los Ángeles	-37.439127	-72.380131	Bt66. Bt67. Bt68. Bt69. Bt70. Bt71	Bt66. Bt67. Bt68. Bt70
13	Curanilahue. Predio Descabezado	-37.75	-73.5166667	Bt72. Bt73. Bt74. Bt75	Bt73. Bt74
14	Curanilahue. Predio San Enrique	-37.8166667	-73.5333333	Bt76. Bt77. Bt78. Bt79. Bt80. Bt81. Bt82. Bt83	Bt76. Bt82. Bt83
15	Pemehue	-38.1	-71.7	Bt84	Bt84
16	El Pajal. Traiguén	-38.18333	-72.566666	Bt85. Bt86. Bt87. Bt88. Bt89. Bt90. Bt91. Bt92. Bt93	Bt85. Bt86. Bt88. Bt90

		33	7		
17	Muco. Lautaro	-38.6333333	-72.4333333	Bt94. Bt95. Bt96. Bt97. Bt98	Bt96.
18	Santa Amelia	-39.033793	-72.987559	Bt99	Bt99
19	Entre Laguna Toltén-Hualpín	-39.127242	-73.186916	Bt100	Bt100
20	Fundo Montemar	-39.153045	-73.21539	Bt101. Bt102	
21	Quelhue	-39.235983	-71.958632	Bt103. Bt104. Bt105. Bt106	Bt103. Bt104. Bt106
22	Pichihuape. Valdivia	-39.740101	-73.125314	Bt107. Bt108	
23	Valdivia	-39.802409	-73.26096	Bt109. Bt110. Bt111	Bt109. Bt110. Bt111
24	Bosque camino a Mehuín			Bt112	
25	Fundo Lagunita. El Roble	-41.456866	-73.031866	Bt113. Bt114	
26	Chinquihue. Pto Montt	-41.516667	-73.05	Bt115. Bt116. Bt117. Bt188. Bt119. Bt120. Bt121. Bt122. Bt123	Bt119. Bt122. Bt123
27	Carelmapu	-41.7467	-73.713	Bt124. Bt125. Bt126	
28	Isla Kent. Aysén	-45.066297	-74.376082	Bt128	Bt128
39	Los Torrones. Aysén	-45.566618	-72.043503	Bt129	Bt129
30	Parque Nacional Laguna San Rafael	-46.616666	-74.033333	Bt130. Bt131	
31	Cuenca Río Baker 1	-47.2069444	-72.634444	Bt132. Bt133	
32	Villa O'Higgins	-48.468333	-72.555833	Bt134	
33	Aysén	-45.466667	-74.6	Bt136. Bt137. Bt138. Bt139	

S2 Table. Genetic divergence (p-distance) between subclades estimated for the CR. Values on the diagonal are intra-subclade p-distances.

Subclade	A	B	C	D	E	F
A	0.0160					
B	0.0280	0.0080				
C	0.0900	0.0910	0.0080			
D	0.0950	0.0950	0.0200	0.0090		
E	0.1100	0.1130	0.0440	0.0420	0.0150	
F	0.1060	0.1060	0.0300	0.0230	0.0280	0.0040

S3 Table. Membership assigned by DPCA analysis for the CR marker

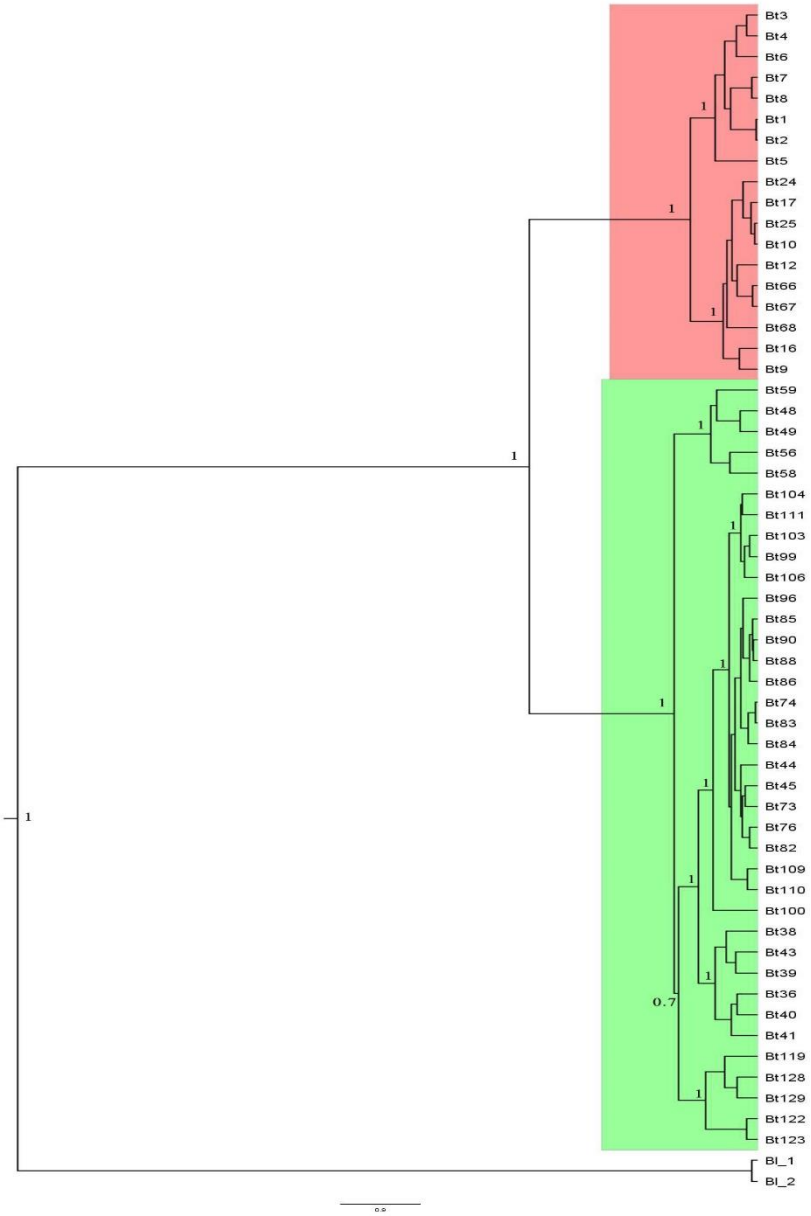
Individual	Cluster	Individual	Cluster	Individual	Cluster	Individual	Cluster
Bt1	4	Bt38	1	Bt76	1	Bt111	6
Bt2	4	Bt39	1	Bt77	1	Bt113	5
Bt3	4	Bt40	1	Bt78	1	Bt114	5
Bt4	4	Bt41	1	Bt80	1	Bt115	5
Bt5	4	Bt43	1	Bt84	6	Bt117	5
Bt6	4	Bt44	1	Bt85	6	Bt118	5
Bt7	4	Bt45	1	Bt87	6	Bt119	2
Bt8	4	Bt48	1	Bt89	6	Bt120	5
Bt9	3	Bt49	1	Bt90	6	Bt121	5
Bt11	3	Bt50	1	Bt91	6	Bt122	5
Bt12	3	Bt52	1	Bt93	6	Bt123	5
Bt13	3	Bt53	1	Bt94	6	Bt124	5
Bt14	3	Bt54	1	Bt95	6	Bt125	5
Bt15	3	Bt55	1	Bt96	6	Bt126	5
Bt16	3	Bt56	1	Bt97	6	Bt127	2
Bt17	3	Bt57	1	Bt98	6	Bt128	2
Bt20	3	Bt58	1	Bt99	6	Bt129	2
Bt26	3	Bt62	1	Bt100	6	Bt130	2
Bt28	3	Bt63	1	Bt101	6	Bt132	2
Bt29	3	Bt64	1	Bt102	6	Bt134	2
Bt30	3	Bt66	3	Bt103	6	Bt136	2
Bt31	3	Bt68	3	Bt105	6	Bt138	2
Bt32	3	Bt69	3	Bt107	6	Bt139	2
Bt33	3	Bt70	3	Bt108	6		
Bt36	1	Bt74	1	Bt109	6		
Bt37	1	Bt75	1	Bt110	6		

S4 Table. List of environmental variables used in niche modelling and their percentage contributions.

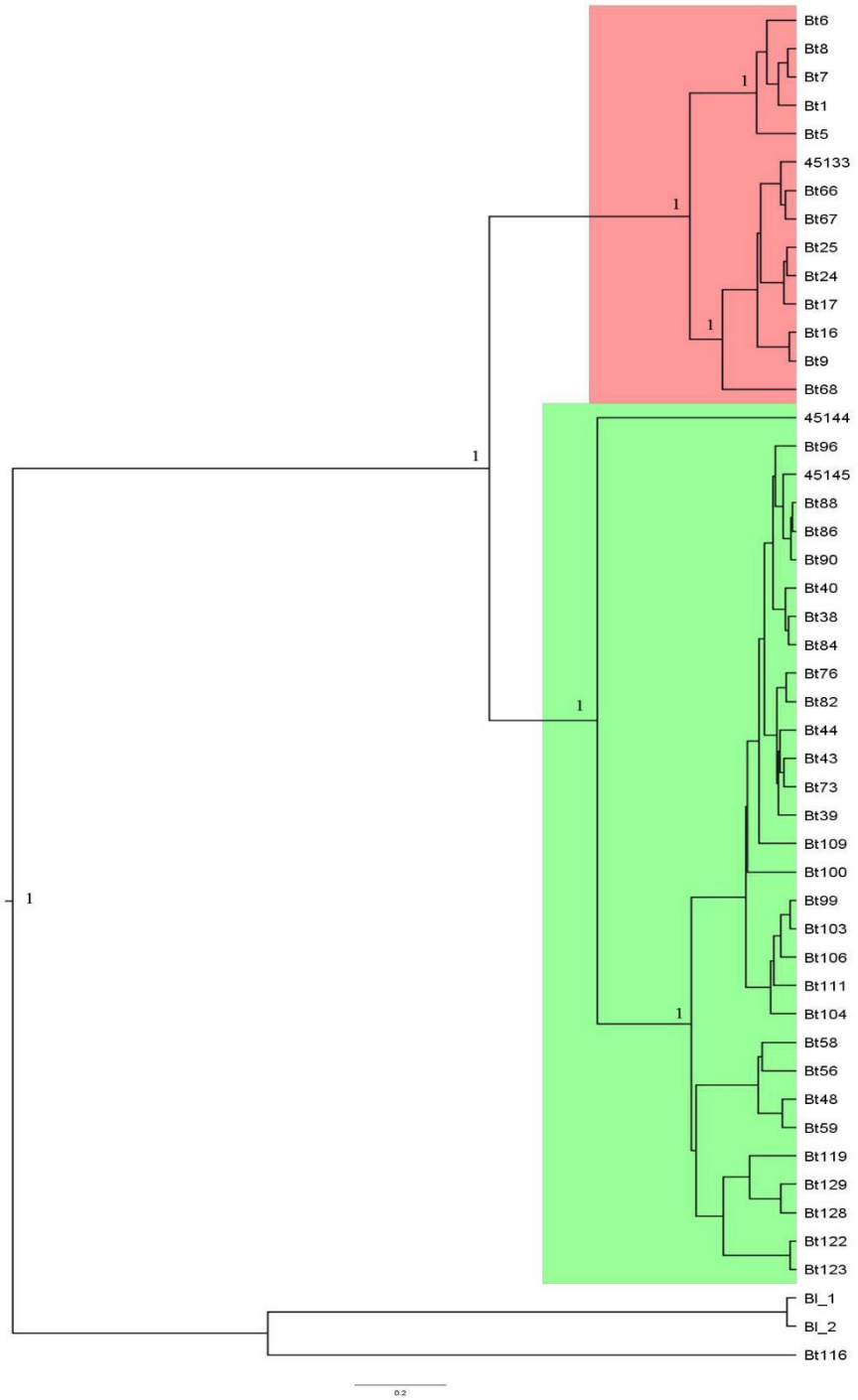
Code	Environment variables	Unit	% contribution
Bio1	Annual Mean Temperature	◦C	
Bio2	**Mean Diurnal Range (Mean of monthly (max temp – min temp))**	**◦C**	**1.8**
Bio3	Isothermality (Bio2/Bio7) (* 100)	-	
Bio4	**Temperature Seasonality (standard deviation *100)**	**C of V**	**4.9**
Bio5	Max Temperature of Warmest Month	◦C	
Bio6	Min Temperature of Coldest Month	◦C	
Bio7	**Temperature Annual Range (Bio5-Bio6)**	**◦C**	**1.6**
Bio8	**Mean Temperature of Wettest Quarter**	**◦C**	**3.3**
Bio9	**Mean Temperature of Driest Quarter**	**◦C**	**17.2**
Bio10	**Mean Temperature of Warmest Quarter**	**◦C**	**0.5**
Bio11	Mean Temperature of Coldest Quarter	◦C	
Bio12	**Annual Precipitation**	**◦C**	**1.4**
Bio13	**Precipitation of Wettest Month**	**mm**	**1.7**
Bio14	**Precipitation of Driest Month**	**mm**	**1.0**
Bio15	Precipitation Seasonality (Coefficient of Variation)	mm	
Bio16	**Precipitation of Wettest Quarter**	**mm**	**0.9**
Bio17	**Precipitation of Driest Quarter**	**mm**	**2.0**
Bio18	Precipitation of Warmest Quarter	mm	
Bio19	**Precipitation of Coldest Quarter**	**mm**	**62.3**

The highlighted variables. selected through False Discovery Rate and multicollinearity testing. were used in modeling.

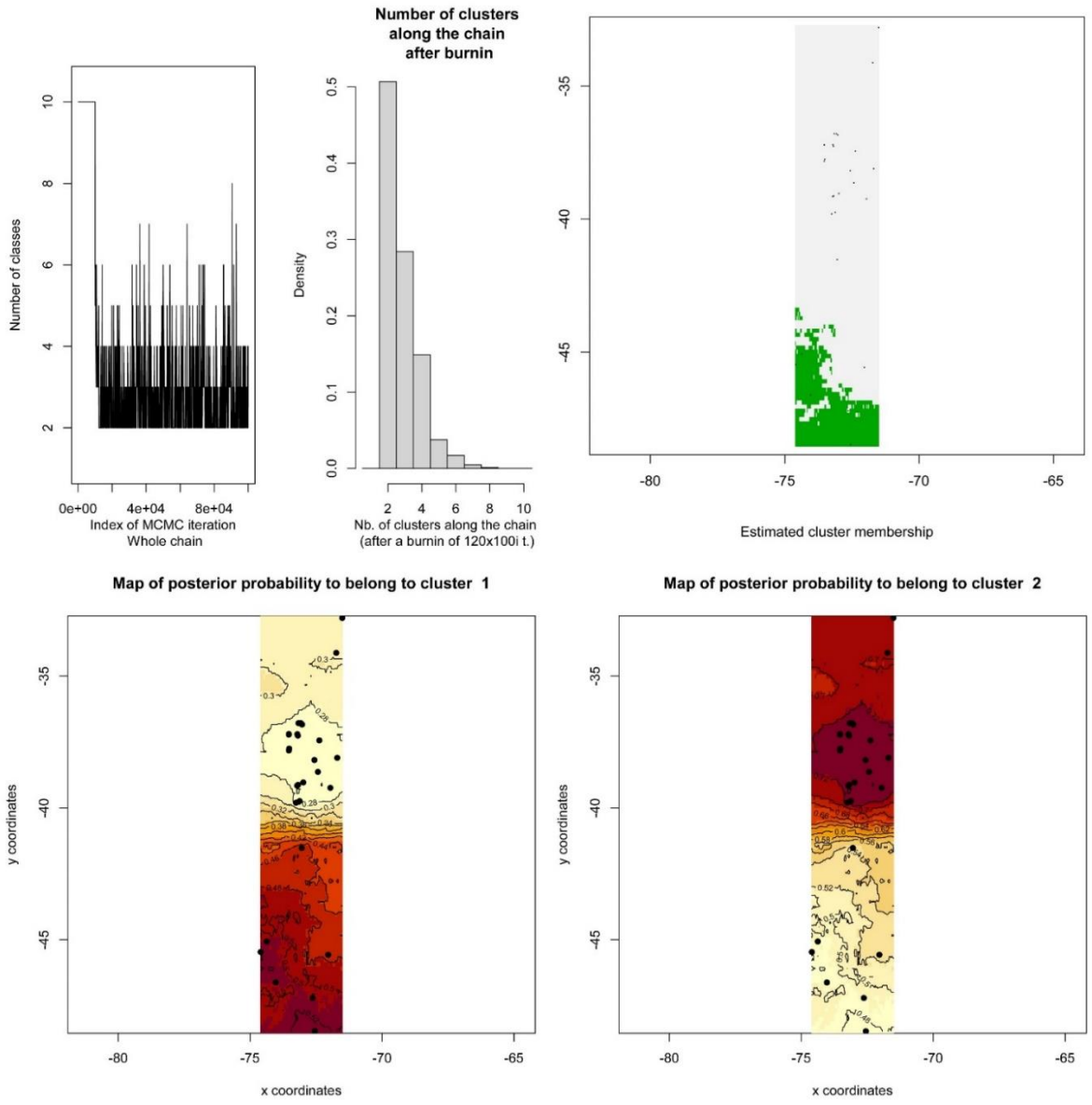
Supplementary material: Figures



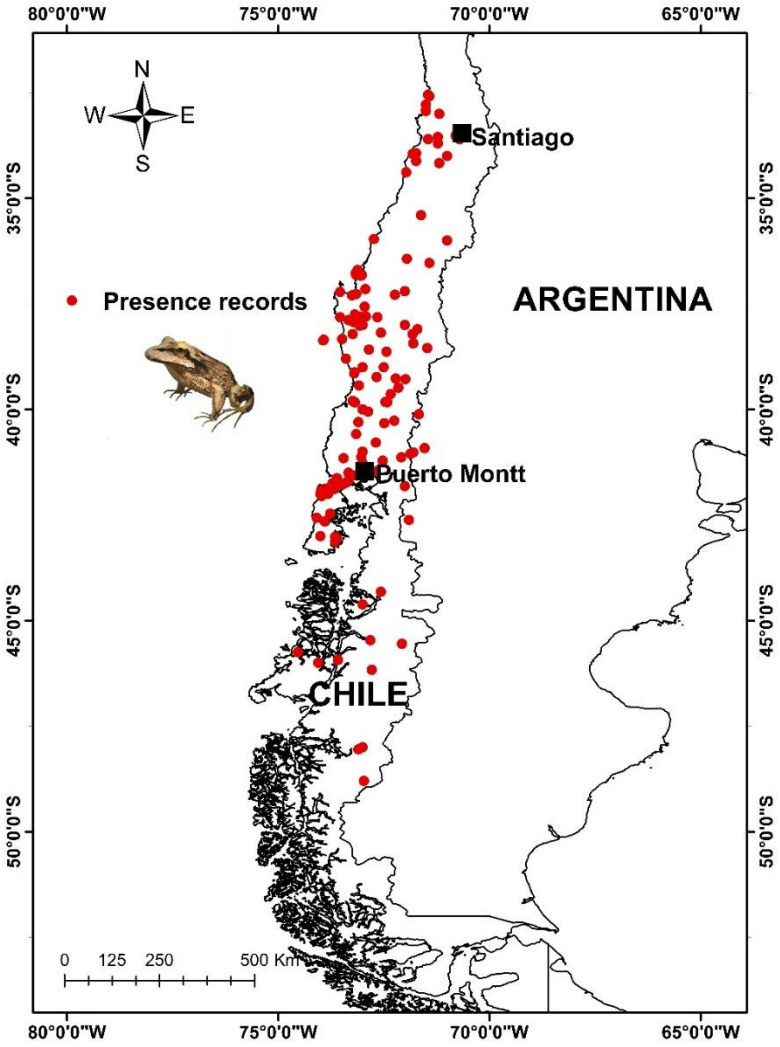
S1 Fig. Genealogical relationships of concatenated (control region + cytochrome b) *B. taeniata* haplotypes as inferred by Bayesian analysis. The support values (Bayesian Posterior Probabilities) shown by major nodes. The red and green circles on the map indicates the locations sampled (mtDNA) for geographic groups identified by the tree topology.



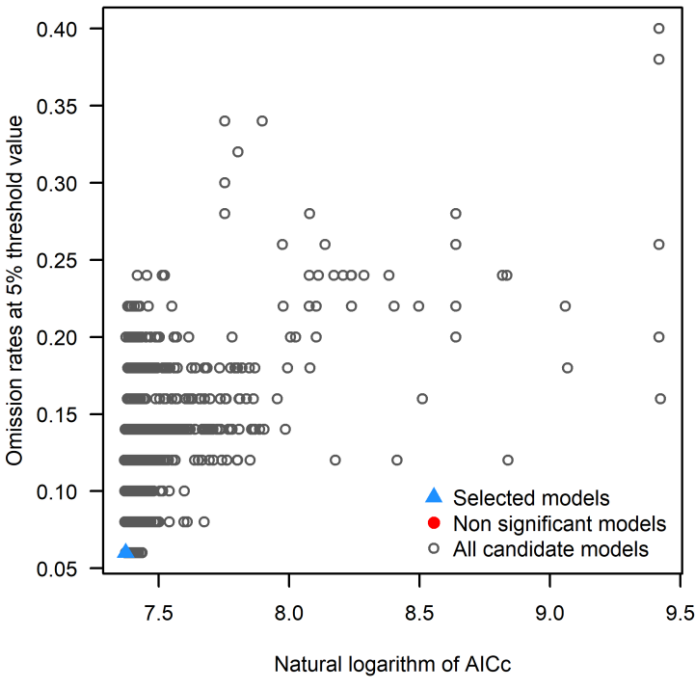
S2 Fig. Genealogical relationships of cyt b *B. taeniata* haplotypes as inferred by Bayesian analysis. The support values (Bayesian Posterior Probabilities) are shown by major nodes.



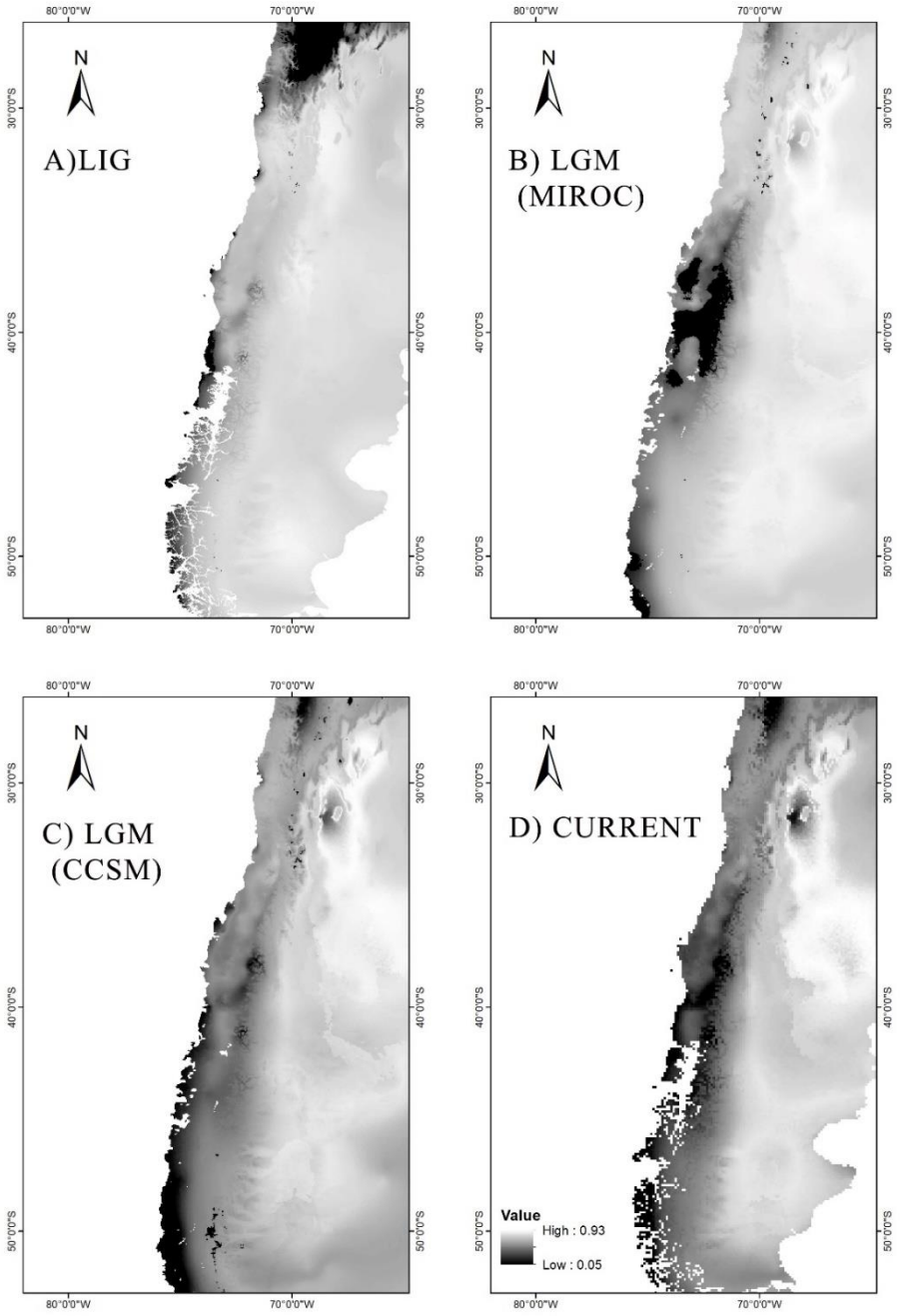
S3 Fig. Number of clusters. maps of cluster membership. and posterior probability for each cluster based on mitochondrial sequences (Control region) in Geneland analyses. The estimated cluster membership represents the modal cluster assignment of each pixel (B) and coloured contours show regions of high (light yellow) to low (red) posterior probability of membership in cluster 1 (C) and Cluster 2 (D).



S4 Fig. Present distribution of *B. taeniata* based on the records from literature. museums and personal observations between the years 1952 and 2018 used for niche modelling.



S5 Fig. Models selected in the calibration of the analysis.



S6 Fig. Extrapolation risk analyses using the MOP metric for paleo-distribution (A-C) and current models. Values in raster files range from zero to one. where zero represents strict extrapolative areas and other values represent levels of similarity between the calibration area and the specific scenario of projection.

CAPÍTULO II

INFLUENCIA DEL PAISAJE SOBRE LA ESTRUCTURA GENÉTICA DE LAS POBLACIONES DE *Batrachyla taeniata* (BATRACHYLIDAE): UN ENFOQUE CON MARCADORES MICROSATÉLITES

INFLUENCE OF THE LANDSCAPE ON THE GENETIC STRUCTURE OF THE POPULATIONS OF *Batrachyla taeniata* (BATRACHYLIDAE): AN APPROACH WITH MICROSATELLITE MARKERS

Influence of the landscape on the genetic structure of the populations of *Batrachyla taeniata* (Batrachylidae): An approach with microsatellite markers

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Abstract

The Central-Peripheral Hypothesis (CPH) predicts a high value of the genetic diversity and genetic flow in the populations that located at the center of the species' geographic distribution. So, it is often assumed that the peripherical populations deserve a higher conservation priority over central populations. Here, we examine the CPH for *Batrachyla taeniata* using 11 loci microsatellites to characterize and estimate the structure and genetic differentiation of their populations. Since this amphibian has a wide distribution in Chile, but discontinuous in the northern range, we evaluated whether genetic differentiation fits a distance isolation model, and whether

population structure is related to environmental factors using a generalized linear model. In this study, we found statistical support to show that in general there is a differentiation and structure genetic in the species, according to their north-south distributional patterns. The major differentiation is associated mainly in populations that occur on the periphery of the distribution range (north and south), supporting CPH hypothesis for the species *B. taeniata*. In addition, exist a high correlation between genetic and geographical distance, so the populations conform to a distance isolation model, supported by the sizes of geographical distance that the populations have towards the center of the distribution range.

Keywords: Amphibian, genetic diversity, environmental factors, CPH

Introduction

Landscape characteristics can greatly affect patterns of dispersal and gene flow between populations and thus shape population dynamics and their evolutionary trajectories (Funk et al., 2005). In recent years, statistical tools have been developed to reveal the action of gene flow during divergence. Migration between populations makes them tend to homogenize with each other and appear more like a single population (Pinho & Hey, 2010), since the flow of genes standardizes them and counteracts the genetic differentiation that could occur by geographical isolation (Slatkin, 1987). Many studies have found that genetic differentiation between populations

increases with the geographic distance between them (Brieva & Formas, 2001; Gallardo et al., 2011). For this reason, there is a growing interest in quantifying the genetic structure of populations, across their geographical range, in order to understand why species can exhibit stable limits in their distribution, along with assessing the conservation value of peripheral populations (Eckert et al., 2008).

From an intrapopulation point of view, the Central-Peripheral population Pattern (CPH) hypothesis proposed by Mayr (1963) , predicts that peripheral populations reduce genetic variability, and have greater genetic differentiation than geographically central populations (Dai & Fu, 2011), so it is often assumed that they deserve a higher conservation priority over central populations. This as a result of a lower effective population size (Ne), greater genetic drift, founder effect, fragmentation, and greater isolation (Eckert et al., 2008; Pandey & Rajora, 2012). Understanding the partitioning of genetic variance into peripheral and central populations may shed more light on the effects of genetic drift and gene flow on population structure and thus improve attempts to preserve such diversity (Cires et al., 2013). However, most research on CPH focuses on geographical distance rather than ecological distance (Duncan et al., 2015).

When evaluating the interaction between the migration rate, genetic drift, and the time of establishment of populations, it is observed that the low

migration rate and maximum migration distance are the parameters that support the CPH hypothesis. Along with this, it has been inferred those geographical barriers between island and continental populations block the flow of genes and increase the genetic distances between them (Brieva & Formas, 2001; Formas & Brieva, 2000; Vidal & Ortiz, 2009). Genetic theory predicts that levels of genetic variation increase with effective population size (Frankham, 1997), therefore, island and peripheral populations have a higher risk of extinction than continental (central) populations because they show less genetic variation and smaller effective population size than those of the centrally distributed continent (Frankham, 1997). Studies suggest that ecological factors can have a strong impact on both population size *per se* and intrapopulation genetic variation, even on a small scale (Liu et al., 2013). These levels of intrapopulation variation can be quantified using Landscape Genetics, a research area that integrates population genetics, landscape ecology and spatial statistics (Gallardo et al., 2011; Garrido-Garduño & Vázquez-Domínguez, 2013). This area examines contemporary processes affecting genetic variation (Knowles, 2009). In this way it allows to describe spatial genetic patterns and the processes that can originate those patterns differing from other genetic approaches, such as phylogeography, since it tends to focus on the processes at finer spatial and temporal scales (Manel et al., 2003). The persistent influence of the history of land use and natural disturbance on contemporary ecosystems has become evident, which is why analyses at multiple scales have demonstrated the importance of landscape patterns for many taxa (Turner, 2005). Given the importance of genetic

diversity for population viability, combining ecological and genetic data can help identify factors that limit population size and determine the potential conservation of populations (Liu et al., 2013).

Since ecological factors can have a strong impact on the variation and genetic structure of populations, the widely distributed Chilean species are submitted to a variety of environmental conditions determined by physiography, vegetation, height above sea level, where these conditions may perform divergent selective pressures (Di Castri, 1968). *Batrachyla taeniata* is a species that has a wide distribution in Chile and marginally in Argentina. In Chile, it extends from the Aconcagua province in the Valparaíso Region to the Capitán Prat province in the Aysén region, covering different environments and including different climatic regions (Mediterranean and Oceanic) (Di Castri, 1968). In the northern sector (32° 54'S and 37° 00'S), most localities are in the coastal plains, and only some are in the central valley. Further south (37°00'S and 42°25'S), it reaches the foot of the Andes and Argentine territory. Most of the localities are low in height on sea level between 0 and 300 m. More than 71% of the known localities are between 37°S and 43°S and are associated with Evergreen rainforest unlike the northern towns, associated with Semiarid steppe with marked contrasts in the distribution of precipitation and annual temperature (Sallaberry et al., 1981).

Given the landscape attributes exposed for the species *B. taeniata*, the objective of this study is to evaluate: 1) the hypothesis of the Centre-

126 Peripheral population Pattern of Mayr (CPH); 2) the influence of the
127 landscape on population structuring.

128

129 **Materials and Methods**
130

131 **Sample collection**
132

133 This study is based on 83 specimens of *B. taeniata*. These samples were
134 obtained from the Museum of Zoology of the University of Concepción
135 (MZUC-UCCC) and field campaigns (Table S1. Fig.1). The new specimens
136 sampled were captured with the capture permits of the Agricultural and
137 Livestock Service (authorization SAG-3350/2015 and 4557/2018). All
138 captures were made in accordance with the protocols approved by the
139 Bioethics Committee of the University of Concepción (Chile). The new
140 individuals collected were deposited in the herpetological collection of the
141 Museum of Zoology of the University of Concepción (MZUC-UCCC).

142

143 **DNA extraction, PCR amplification, and fragment analysis**
144

145 Through optimizations of Kit Promega Wizard Purification Systems, we
146 extracted DNA of 83 individuals of *B. taeniata* from the sampled localities for
147 use in the isolation of microsatellite loci. Obtaining microsatellites was carried
148 out following the protocol of Cisternas-Medina et al. (2019). The PCR
149 products were run on an Applied BioSystems 3730 DNA Analyzer sequencer

in Macrogen, Korea, Seoul. Alleles were scored using the PeakScanner version 1.0 (Applied Biosystems).

The number of alleles (A), Size the range of observed alleles in base pairs (bp), number of individual genotyped (N), observed heterozygosity (Ho), expected heterozygosity (He) and inbreeding coefficient (FIS) were tested for by using GenALEx 6.5 (Peakall & Smouse, 2006).

Genetic structure

The Geneland v4.0.3 program (Guillot et al., 2012) was used to determine the most probable number of populations under the Hardy-Weinberg assumption and the equilibrium linkage between loci (Guillot et al., 2004). For this, five independent chains were run with populations ranging between 1 and 10. The best model was chosen that represented the allele frequency and spatial model of coordinates through a model test. Each run consisted of one million iterations, a "thinning interval" of 1000 and a "burn-in" phase of 200 iterations. The posterior probability parameter plot was plotted to evaluate the convergence between the runs, and the posterior estimates of the number of populations were compared. Population membership probability maps were created in a spatial domain defined by 100 pixels in both X and Y axes. This allowed the detection of differentiated genetic groups within the spatial distribution of *B. taeniata*.

A discriminant analysis of principal components (DAPC) was done with the R package Adegenet v.2.1.3 (Jombart, 2008), which defines discriminant axes along for obtaining the best separation of individuals within predefined groups and allows making a probabilistic assignment of individuals to each group (Jombart & Ahmed, 2011). This model-free multivariate analysis approach is a valuable tool to investigate and visualize genetic clusters' presence, since it maximizes between-group variation and minimizes within-group variation (Jombart & Ahmed, 2011). We run the analysis following the tutorial and recommendations of authors (http://adegenet.r-forge.r-project.org/files/tutorial-dapc.pdf). The results of PCA eigenvalues and DAPC were plotted with RStudio v.

To determine the level of population structure among the defined groups we implemented an analysis of molecular variance (AMOVA), using the software ARLEQUIN v3.5 (Excoffier & Lischer, 2010). Intra-population genetic variation (Fsc), inter-group variation (Fct) and inter-population variation, intra-group (Fst) were estimated. The populations and the individuals assigned to them were made based on the results obtained in the genealogy and the genetic clusters obtained from the Geneland and DAPC analysis. The criteria were taken to separate the groups by their location in their range of distribution (Northern vs Central vs South distribution range).

BayesAss 3.0.4 software (Wilson & Rannala, 2003) calculated gene flow per generation between pairs of populations. This analysis indicates recent migrants among pairs of populations in a bidirectional way, expressed in the frequency of individuals moving between each population per generation. The acceptance rates for the proposed changes to the parameters migration rates, allele frequencies and inbreeding coefficients will be adjusted on changing the values of the respective mixing parameters (Rannala, 2007).

Landscape and genetic structure

A Mantel test was performed to assess the statistical relationship between genetic distance and geographic distance. This analysis was performed on Arlequin (Excoffier & Lischer, 2010).

To assess the relative importance of the landscape on the population structure, we examined the interactions between landscape characteristics, represented using different types of environments, and population genetic structure patterns. To this end, the degree to which the observed structure is explained by environmental heterogeneity was evaluated (Foll & Gaggiotti, 2006). These analyses were performed in the GESTE program (Foll & Gaggiotti, 2006) which implements a Bayesian method to evaluate the effect that biotic and abiotic environmental factors (geographic distance sizes, temperature, altitude, local population, etc.) have on the genetic structure of

218 populations. To do this, it estimates the Fst values for each population and
219 relates them to environmental factors using a generalized linear model.

220

221 The six-factor model of environmental effects, including latitude, longitude,
222 elevation above sea level, precipitation of coldest quarter, mean temperature
223 of driest quarter, Euclidean distance on the genetic characteristics of *B.*
224 *taeniata* within the population was used. The values for the precipitation of
225 coldest quarter (bio19) and mean temperature of driest quarter (bio9) were
226 obtained from the climate data site https://www.worldclim.org and they were
227 chosen because they are the variables that most contribute to habitat
228 suitability in previous studies (niche modelling). To evaluate the isolation of
229 populations from the distribution center was estimated as a factor Euclidean
230 distance using the ArcGIS 9.2 software (ESRI 2006). The model of the
231 highest posterior probability was identified with GESTE 2.0 software, which
232 best explained the genetic structures of the analysed.

233

234 **Results**
235

236 **Genetics structure and diversity**
237

238 The microsatellite loci based Geneland analysis identified nine clusters (K=9;
239 Fig. 2.1; 2.2). They include (a): Cluster-1 that occurs in the north limit of
240 distribution (Quintero), (b): Cluster-2 corresponding to Litueche, (c): Cluster-3
241 corresponding to Hualpén, (d): Cluster-4 occur in the Concepción, (e):

242 Cluster-5 corresponding to La Cal, (f): Cluster-6 corresponding to El Pajal,
243 (g): Cluster-7 occur in Valdivia, (h): Cluster-8 corresponding to Puerto Montt,
244 and (i): Cluster-9 that including localities of Aysén, in the south limit of
245 distribution (Fig.1).

246

247 Discriminant analysis of principal components (DAPC) also identifies nine
248 clusters (K=9; Fig. S1) but the assignment of individuals varies in cluster 3
249 identified by Geneland analysis (Table S2).

250

251 The average characteristics of 11 microsatellite loci for the nine populations
252 of *B. taeniata* are presented in Table S3. Overall, the number of alleles per
253 locus was in the range of 10 to 34. In all the populations, the observed
254 heterozygosity (Ho) was less than expected heterozygosity (He) and the
255 values of the fixation index (F) for all populations were positive values,
256 indicating a deficit of heterozygotes, Table 1.

257

258 The analysis of genetic distance between populations (Clusters) of *B.*
259 *taeniata* indicate that population 8 (Cluster-8) varies over 29 % with
260 populations 1 to 7, and ~ 28% with the population 9 (Table 2). Structuring
261 indices (FST) between pairs of populations are between 0.085 and 0.438
262 indicating low gene flow and greater genetic distance between more
263 geographically isolated populations (Table 3).

For the microsatellite loci based Amova test, all values were significant ($p<0.001$). High genetic variation (46.66%) was observed within individuals. The variation within the population was 28.34%, and among populations within groups was 19.61%. Low genetic variation (5.38%) was observed within groups (Table 4).

The gene flow estimated in BayesAss (Table S4) indicates low values in estimated migration rates among the populations of *B. taeniata.* Among the populations of the northern distribution range (Pop 1 and Pop 2) the maximum migration rate is 0.0854 individuals per generation in Pop 2 from Pop 1. On the other hand, individuals in Pop 1 of Pop 2 reach a rate of 0.0224. With respect to the populations of the central distribution range, the highest migration rate reaches a value of 0.1431 individuals per generation in Pop 7, coming from Pop 6, followed by a rate of 0.0398 individuals per generation in Pop 4 of Pop 3. For populations in the southern range, the maximum migration rates are 0.1282 individuals per generation of Pop 9 from Pop 5.

The values of the inbreeding coefficient estimated in BayesAss show high values for the populations: Pop1, Pop2, Pop5, Pop6, Pop7, Pop8 and Pop9 and lower values for the populations: Pop 3 and Pop4 (Table S5). These values indicate that there is a decrease in the number of heterozygotes in most populations, consistent with the low values of migration rates.

Landscape and genetic structure

The Mantel test revealed a significant pattern of isolation by distance (r = 0.646, p < 0,0049) for the nine *B. taeniata* populations of this study.

The analysis in Geste evaluated 64 possible models to explain the genetic differentiation of *B. taeniata* populations. The highest probability model (*P* = 0.153) includes a constant and Euclidean distance (model 33) (Fig. S2B). The estimated σ^2 is 0.860 (HPDI = [0.321; 2.74]), which indicates that Euclidean distance of the populations towards the center of the distribution range of the species explains well genetic differentiation. In the total posterior probability, the Euclidean distance factor comprised 43 %, and the longitude 32%, followed by altitude factors 21% and Mean temperature of the driest quarter 20% (Table S6.)

Discussion

Genetics structure and diversity of populations

The genetic study of *B. taeniata* using microsatellites provides relevant information for the knowledge of the species and the genetic structure of its populations. This type of information is essential for Chilean amphibians, since to date, studies related to population genetics cover very few species (Correa et al., 2016). Our results show differentiation and genetic structure in the species, according to its north-south distribution patterns. The pattern of

312 differentiation of their populations is mainly associated with those that are
313 located on the periphery (north and south) of their range of distribution.

314

315 Our results are consistent with previous genetic studies conducted on the
316 species (Brieva & Formas, 2001; Cisternas-Medina et al., 2019). Brieva and
317 Formas (2001) using allozyme variation identified three clusters of
318 populations from individuals from nine localities of populations according to
319 their north-south distributional patterns. In this study, the analysis made in
320 Geneland recovered nine clusters of populations from thirteen localities (Fig.
321 1, Fig. 2.1; 2.2). These clusters also follow a north-south pattern according to
322 their latitudinal distributions. The nuclear data reported here also estimate
323 nine clusters in the DPCA analysis (Fig. S1, Table S2), supporting the
324 presence of a variation for the species at the latitudinal level. This pattern of
325 variation is to be expected because Anura are species that have a restricted
326 dispersal capacity that tends to promote differentiation (Duellman, 1999), so
327 they tend to present a greater genetic structure in their populations with
328 respect to other vertebrates (Semlitsch & Skelly, 2007). This north-south
329 latitudinal variation pattern in *B. taeniata* may be influenced by the different
330 environmental conditions to which the species is subjected since it is found in
331 the Mediterranean and Oceanic bioclimatic regions (Di Castri, 1968). In its
332 northern distribution, it is associated with hydrophilic and mesophilic coastal
333 relict forests (eg. Zapallar and Quintero) surrounded by semi-arid vegetation
334 and in its southern distribution associated with humid and shady forests in
335 humidity saturated environments. This climatic variability that encompasses

the species, given particularly by the availability of water, temperature and habitat of swamps and temporary pools, give the populations of its northern distribution a remarkable adaptive capacity linked to the reproductive characteristics of *B. taeniata* (Sallaberry et al., 1981).

The Center-Periphery Hypothesis (CPH) predicts that populations located on the periphery of a species' range should have lower levels of genetic variation than those in the center of the range (Cires et al., 2013; Dai & Fu, 2011; Mayr, 1963). In this study, populations located in the center of the range have a higher number of effective alleles (Na) and alleles (Ne), compared to populations located in the north and south of their distribution (Table 1). Moreover, although the levels of observed heterozygosity are lower for all estimated populations, the expected heterozygosity levels are higher for centrally distributed populations. The low values in estimated migration rates among the populations of *B. taeniata,* are associated with the high values of inbreeding calculated for most of their populations (Table S4). The lowest inbreeding coefficients (Table S5) were obtained for the Pop3 (Hualpén) and Pop4 (Concepción) populations, where the species has historically been most frequently reported. However, Pop5 (Arauco) showed high inbreeding coefficient values, despite being a population associated with the center of the species' range of distribution.

About the genetic distances between populations, the most significant distance was estimated between Pop8 and the rest of the populations; and Pop9 (Aysén) with Pop1 (Quintero) and Pop2 (Litueche) (Table 2). The Fst index is a measure of population differentiation due to genetic structure, and a value above 0.15 can be considered as significant in differentiating populations (Frankham et al., 2002). Therefore, there was significant differentiation between populations pairs given that Fst indices were greater than 0.15 except for the Fst value between Pop 3 and Pop4, which would be directly associated with the geographical distances between populations. Under this context, a greater degree of structuring between Pop1 and Pop9 would be expected; however, the structuring index between Pop 2 and Pop 9 was the highest, with a value of 0.692. But due to the low number of individuals of pop2, care must be taken when interpreting the information (Table 3).

The standardized variance of allele frequencies indicated a high level of genetic differentiation within individuals. (Fit = 0.53336, p<0,00001). On average, 46.66% of the total variance of allele frequencies was attributed to genetic differentiation within individuals, while 28.33% was found within populations. The proportion of the variance in the subpopulation contained in an individual Fis was of 0.37784, p<0,00001. This value is associated with the excess of homozygotes found within the populations, supporting the high levels of inbreeding in these populations of *B. taeniata* (Table S5), but lower

than that recorded in the sister species *B. leptopus* with alloenzymes, since it presented a Fis value of 0.640 ($P < 0.005$) (Formas & Brieva 2000).

Landscape and genetic structure

According to the Mantel test results, the genetic variation at the level of the entire distribution (range) of the species conforms to a distance isolation model, so geographical distance has importance in limiting the dispersion of the species. This relationship is coincident and supports what was reported by Brieva & Formas (2001). This positive relationship has been observed in other species of Chilean amphibians of wide distribution such as *Rhinella spinulosa* (Gallardo et al., 2011; Méndez et al., 2004) and in other vertebrates such as *Philodryas chamissonis* (Sallaberry-Pincheira et al., 2011), *Abrothrix longipilis* (Valdez et al., 2020), *Abrothrix olivacea brachiotis* (Zepeda et al., 2019), contrary to the negative and significant correlation found in *B. leptopus* (Formas & Brieva, 2000).

Understanding the processes and patterns of gene flow and local adaptation requires detailed knowledge of how landscape characteristics structure populations (Manel et al., 2003). In the context of the biology of *B. taeniata,* it is assumed that temperature and precipitation have a direct effect on the structure and differentiation of their populations since their reproduction depends directly on these variables (Jara et al., 2019). However, our results indicate that the highest probability model that relates the Fst values of each

405 population with environmental factors using a generalized linear model,
406 includes a constant and Euclidean distance (Table S6, Fig.S2B), therefore,
407 for the populations incorporated in this study, it is observed that those that
408 occur at greater distances from the center of the distribution range of the
409 species have higher values of genetic structure (Fig.S2). In addition, the
410 longitude factor, the second that has the highest subsequent probability
411 (Table S4). In amphibians, the longitude and altitude also tend to promote
412 structuring, mainly the isolation that occurs between populations (Funk et al.,
413 2005; Gallardo et al., 2011) evidencing contrasting demographic processes
414 in other vertebrates (Victoriano, 2020).

415

416 **Conclusion**
417

418 According to the information revealed by the populations incorporated in this
419 study, we found statistical support to show that in general there is a
420 differentiation and structure genetic in the species, according to their north-
421 south distributional patterns. This pattern of differentiation is associated
422 mainly in populations that occur on the periphery of the distribution range
423 (north and south), given that these populations show: a lower number of
424 alleles, low heterozygosity, a greater degree of structuring, high inbreeding
425 value and low migration rate with respect to the populations that are
426 distributed in the center. In addition, we found that there is a high correlation
427 between genetic and geographical distance, so the populations conform to a
428 distance isolation model, supported by the sizes of geographical distance

that the populations have towards the center of the distribution range. In general, this study supports CPH for the species B. *taeniata.*

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References

Brieva, L., & Formas, J. (2001). Allozyme variation and geographic differentiation in the Chilean leptodactylid frog *Batrachyla taeniata* (Girard, 1854). *Amphibia-Reptilia, 22*, 413-420.

Cires, E., Pérez, R., Bueno, A., & Fernández Prieto, J. A. (2013). Genetic diversity in peripheral and central populations of the Cantabrian endemism Genista legionensis (Pau) M. Laínz (Fabaceae). *Anales del Jardín Botánico de Madrid, 70*, 91-96. doi:10.3989/ajbm.2313

Cisternas-Medina, I., Ortíz, J., & Ferrada-Fuentes, S. (2019). Development and characterization of microsatellite molecular markers for the eye mask frog *Batrachyla taeniata* (Girard, 1855). *Genet. Mol. Res.,18* (2):gmr16039960

Correa, C., Donoso, J.P., & Ortiz, J.C. (2016). Estado de conocimiento y conservación de los anfibios de Chile: una síntesis de los últimos 10 años de investigación. *Gayana*, *80*, 103-124.

Dai, Q., & Fu, J. (2011). When central populations exhibit more genetic diversity than peripheral populations: A simulation study. *Chinese Science Bulletin, 56*(24), 2531-2540. doi:10.1007/s11434-011-4605-x

Di Castri, F. (1968). *Esquisse écologique du Chile.* . Paris, C.N.R.S.

Duellman, W. (1999). *Patterns of Distribution of Amphibians: A Global Perspective*. Baltimore and London.

Duncan, S. I., Crespi, E. J., Mattheus, N. M., & Rissler, L. J. (2015). History matters more when explaining genetic diversity within the context of the core–periphery hypothesis. *24*(16), 4323-4336. doi:https://doi.org/10.1111/mec.13315

Eckert, C. G., Samis, K. E., & Lougheed, S. C. (2008). Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. *Mol Ecol, 17*(5), 1170-1188. doi:10.1111/j.1365-294X.2007.03659.x

Excoffier, L., & Lischer, H. E. (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Resour, 10*(3), 564-567. doi:10.1111/j.1755-0998.2010.02847.x

Foll, M., & Gaggiotti, O. (2006). Identifying the Environmental Factors That Determine the Genetic Structure of Populations. *Genetics, 174*, 875-891. doi:10.1534/genetics.106.059451

Formas, R., & Brieva, L. M. (2000). Population genetics of the Chilean frog Batrachyla Leptopus (Leptodactylidae). *Genetics and Molecular Biology, 23*. doi:10.1590/S1415-47572000000100007

Frankham. R-, Ballou. J.D., Briscoe, D.A. Introduction to Conservation Genetics. Cambridge: Cambridge University Press; 2002.

Frankham, R. (1997). Do island populations have less genetic variation than mainland populations? *Heredity, 78*(3), 311-327. doi:10.1038/hdy.1997.46

Funk, W. C., Blouin, M. S., Corn, P. S., Maxell, B. A., Pilliod, D. S., Amish, S., & Allendorf, F. W. (2005). Population structure of Columbia spotted frogs (Rana luteiventris) is strongly affected by the landscape. *Molecular ecology, 14*(2), 483-496. doi:10.1111/j.1365-294x.2005.02426.x

Gallardo, C. E., Hernández, H. J., Diniz-Filho, J. A. F., Palma, R. E., & Méndez T, M. A. (2011). Relationship between the genetic structure of the Andean toad *Rhinella spinulosa* (Anura: Bufonidae) and the northern Chile landscape (21°- 24° S). *Revista Chilena de Historia Natural, 84*, 391-406.

Garreaud, R., Alvarez-Garreton, C., Barichivich, J., Boisier, J. P., Christie, D., Galleguillos, M., Zambrano-Bigiarini, M. (2017). The 2010-2015 megadrought in central Chile: Impacts on regional hydroclimate and vegetation. *Hydrology and Earth System Sciences, 21*, 6307-6327. doi:10.5194/hess-21-6307-2017

Garrido-Garduño, T., & Vázquez-Domínguez, E. (2013). Métodos de análisis genéticos, espaciales y de conectividad en genética del paisaje. *Revista mexicana de biodiversidad, 84*, 1031-1054.

Guillot, G., Mortier, F., & Estoup, A. (2004). Geneland: A program for landscape genetics. *Mol. Ecol. Notes,* 5, *712–715.*

Guillot, G., Renaud, S., Ledevin, R., Michaux, J., & Claude, J. (2012). A Unifying Model for the Analysis of Phenotypic, Genetic, and Geographic Data. *Systematic Biology, 61*(6), 897-911. doi:10.1093/sysbio/sys038

Jara, F., Cuello. M., & Úbeda, C. (2019). Afrontando el invierno: La rana de ceja corta se reproduce y desarrolla en condiciones climáticas adversas; Administración de Parques Nacionales. Parque Nacional Nahuel Huapi. *Macroscopia, 8;1*(2), 16-21.

Jombart, T. (2008). Adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics, 24*(11), 1403-1405. doi:10.1093/bioinformatics/btn129

Jombart, T., & Ahmed, I. (2011). Adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. *Bioinformatics, 27*(21), 3070-3071. doi:10.1093/bioinformatics/btr521

Knowles, L.L. (2009). Statistical Phylogeography. *Annual Review of Ecology, Evolution, and Systematics 40*(1), 593-612. doi:10.1146/annurev.ecolsys.38.091206.095702

Liu, Y., Webber, S., Bowgen, K., Schmaltz, L., Bradley, K., Halvarsson, P., Abdelgadir, M., Griesser, M. (2013). Environmental factors influence both abundance and genetic diversity in a widespread bird species. *Ecology and Evolution 3*(14), 4683-4695. doi:https://doi.org/10.1002/ece3.856

Manel, S., Schwartz, M., Luikart, G., & Taberlet, P. (2003). Landscape genetics: combining landscape ecology and population genetics. Trends Ecol Evol 18: 189-197. *Trends in Ecology & Evolution, 18*, 189-197. doi:10.1016/S0169-5347(03)00008-9

Mayr, E. (1963). Animal species and evolution. Havard University Press.

559 Méndez, M., Soto M, E., Correa, C., Veloso, A., Vergara, E., Sallaberry, M.,
560 & Iturra, P. (2004). Morphological and genetic differentiation among
561 Chilean populations of *Bufo spinulosus* (Anura: Bufonidae). *Revista*
562 *Chilena De Historia Natural, 77*. doi:10.4067/S0716-
563 078X2004000300014
564
565 Pandey, M., & Rajora, O. (2012). Genetic diversity and differentiation of core
566 vs. peripheral populations of eastern white cedar, *Thuja occidentalis*
567 (Cupressaceae). *American journal of botany, 99*, 690-699.
568 doi:10.3732/ajb.1100116
569
570 Peakall, R., & Smouse, P.E. (2006). Genalex 6: genetic analysis in Excel.
571 Population genetic software for teaching and research. *6*(1), 288-295.
572 doi:https://doi.org/10.1111/j.1471-8286.2005.01155.x
573
574 Pinho, C., & Hey, J. (2010). Divergence with Gene Flow: Models and Data.
575 *41*(1), 215-230. doi:10.1146/annurev-ecolsys-102209-144644
576
577 Rannala, B. (2007). BayesAss Edition 3.0 User's Manual. *University of*
578 *California Davis*.
579
580 Semlitsch, R.D., & Skelly, DK., (2007). Ecology and conservation of pool-
581 breeding amphibians (Calhoun AJK & D. PG Eds.). London: CRC
582 Press.
583
584 Sallaberry, M., Valencia, J., & Díaz, N. (1981). Distribución y ambiente de
585 *Batrachyla taeniata* (Girard) en Chile (Anura - Leptodactylidae).
586 *Boletín del Museo Nacional de Historia Natural* (Chile) 38: 61-67.
587 Sallaberry-Pincheira, N., Garin, C., González-Acuña, D., Sallaberry, Vianna,
588 M.A., & (2011). Genetic divergence of Chilean long-tailed snake
589 (*Philodryas chamissonis*) across latitudes: Conservation threats for
590 different lineages. *Diversity and Distributions, 17*, 152-162.
591
592 Slatkin, M. (1987). Gene flow and the geographic structure of natural
593 populations. *Science, 236*(4803), 787-792.
594 doi:10.1126/science.3576198
595
596 Turner, M. (2005). Landscape Ecology: What Is the State of the Science? *SM*
597 *Annu. Rev. Ecol. Evol. Syst, 1502*, 319-344.
598 doi:10.1146/annurev.ecolsys.36.102003.152614
599
600 Valdez, L., Quiroga-Carmona, M., & D'Elía, G. (2020). Genetic variation of
601 the Chilean endemic long-haired mouse *Abrothrix longipilis* (Rodentia,
602 Supramyomorpha, Cricetidae) in a geographical and environmental
603 context. *PeerJ, 8*, e9517. doi:10.7717/peerj.9517
604

Victoriano, P. F. (2020). Phylogeography of Chilean Lizards: Histories of Genetic Diversification on the Western Slope of Southern Andes. In M. Morando & L. J. Avila (Eds.), *Lizards of Patagonia: Diversity, Systematics, Biogeography and Biology of the Reptiles at the End of the World* (pp. 255-291). Cham: Springer International Publishing.

Vicuna, s., Garreaud, R., & McPhee, J. (2011). Climate change impacts on the hydrology of snowmelt driven basin in semiarid Chile. *Climatic Change, 105*, 469-488. doi:10.1007/s10584-010-9888-4

Vidal, M.A., & Ortiz, J.C. (2009). Amphibia, Anura, Ceratophryidae, *Batrachyla leptopus* Bell, 1843: insular distribution extension, Chile. *Check List, 5*(4), 869-871. doi:10.15560/5.4.869

Wilson, G.A., & Rannala, B. (2003). Bayesian inference of recent migration rates using multilocus genotypes. *Genetics, 163*(3), 1177-1191.

Zepeda, P. S., Rodríguez-Serrano, E., Torres-Pérez, F., Celis-Diez, J. L., & Palma, R.E. (2019). Genetic variability and structure of the Olive Field Mouse: a sigmodontine rodent in a biodiversity hotspot of southern Chile. *PeerJ, 7*, e6955. doi:10.7717/peerj.6955

Tables

Table 1. Mean and Standar Error (SE) over Loci for each population (Pop) of *B. taeniata*.

Pop		N	Na	Ne	Ho	He	F
Pop 1	**Mean**	6	4	2.94	0.52	0.61	0.16
	SE	0	0	0.34	0.11	0.05	0.15
Pop 2	**Mean**	2	1	1.16	0.14	0.15	0.00
	SE	0	0	0.16	0.07	0.06	0.20
Pop 3	**Mean**	30	14	8.05	0.69	0.81	0.16
	SE	1	2	1.38	0.08	0.05	0.06
Pop 4	**Mean**	8	7	5.48	0.70	0.77	0.07
	SE	0	1	0.67	0.08	0.05	0.10
Pop 5	**Mean**	9	5	3.63	0.31	0.59	0.59
	SE	0	1	0.71	0.11	0.08	0.12
Pop 6	**Mean**	8	6	3.88	0.43	0.69	0.38
	SE	1	0	0.55	0.08	0.04	0.10
Pop 7	**Mean**	4	4	3.05	0.47	0.54	0.17
	SE	0	1	0.60	0.11	0.09	0.11
Pop 8	**Mean**	6	4	2.80	0.33	0.54	0.42
	SE	0	0	0.44	0.08	0.08	0.11
Pop 9	**Mean**	3	2	1.41	0.19	0.26	0.21
	SE	0	0	0.22	0.09	0.07	0.20

N=Sample Size, Na=No. Alleles, Ne=No. Effective Alleles, Ho=Observed Heterozygosity, He=Expected, uHe=Unbiased Expected Heterozygosity, and F=Fixation Index

Table 2. Genetic distance between populations (Clusters) of *B. taeniata.*

Pop 1	Pop 2	Pop 3	Pop 4	Pop 5	Pop 6	Pop 7	Pop 8	Pop 9	
18.60									**Pop 1**
24.25	7.00								**Pop 2**
27.53	29.22	19.44							**Pop 3**
27.44	27.31	25.29	20.93						**Pop 4**
30.60	32.50	28.82	26.70	19.84					**Pop 5**
27.40	27.35	23.53	23.39	26.08	18.71				**Pop 6**
25.90	28.20	23.18	24.08	25.60	22.60	15.80			**Pop 7**
31.81	33.83	30.59	29.04	33.27	29.88	29.23	19.60		**Pop 8**
28.83	31.50	26.06	24.00	25.88	26.13	24.95	27.92	13.00	**Pop 9**

Table 3. FST structuring indexes between pairs of populations of *B. taeniata.*

Pop 1	Pop 2	Pop 3	Pop 4	Pop 5	Pop 6	Pop 7	Pop 8	Pop 9	
0.000									**Pop 1**
0.332	0.000								**Pop 2**
0.164	0.352	0.000							**Pop 3**
0.160	0.323	0.085	0.000						**Pop 4**
0.236	0.468	0.162	0.142	0.000					**Pop 5**
0.201	0.377	0.100	0.111	0.168	0.000				**Pop 6**
0.279	0.510	0.188	0.217	0.272	0.206	0.000			**Pop 7**
0.248	0.465	0.182	0.175	0.256	0.184	0.254	0.000		**Pop 8**
0.413	0.692	0.312	0.296	0.369	0.329	0.403	0.417	0.000	**Pop 9**

Table 4. Amova results for microsatellites.

Source of variation	Percentage variation
Among groups	5.38556
Among population within groups	19.61029
Within populations	28.33986
Within individuals	46.66430

Indice	Value fixation índice over all loci
FIS	0.37784 *
FSC	0.20727 *
FCT	0.05386 *
FIT	0.53336 *

P < 0.00001

Figures

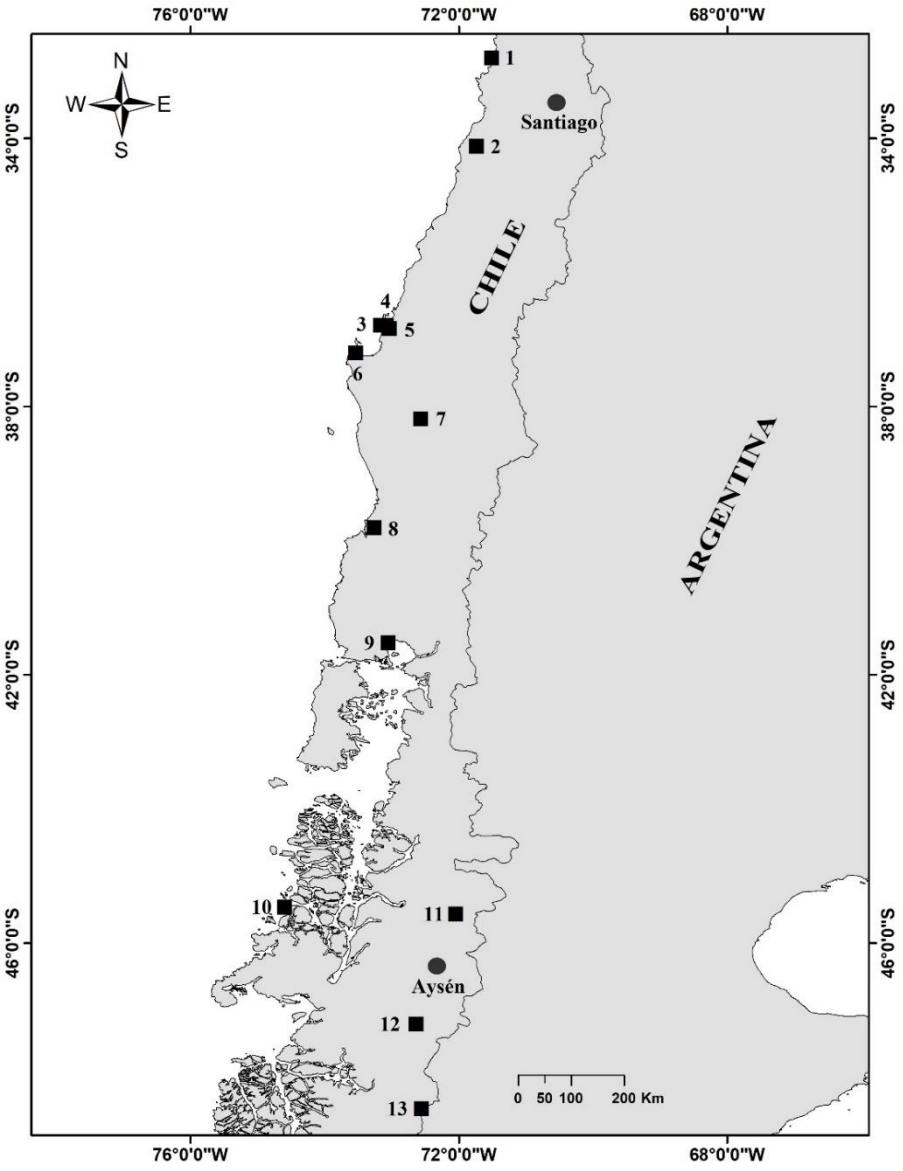

Figure 2. Geographic distribution of *B. taeniata* samples included in this study. Open squares correspond to localities studied (Supplement Table 1).

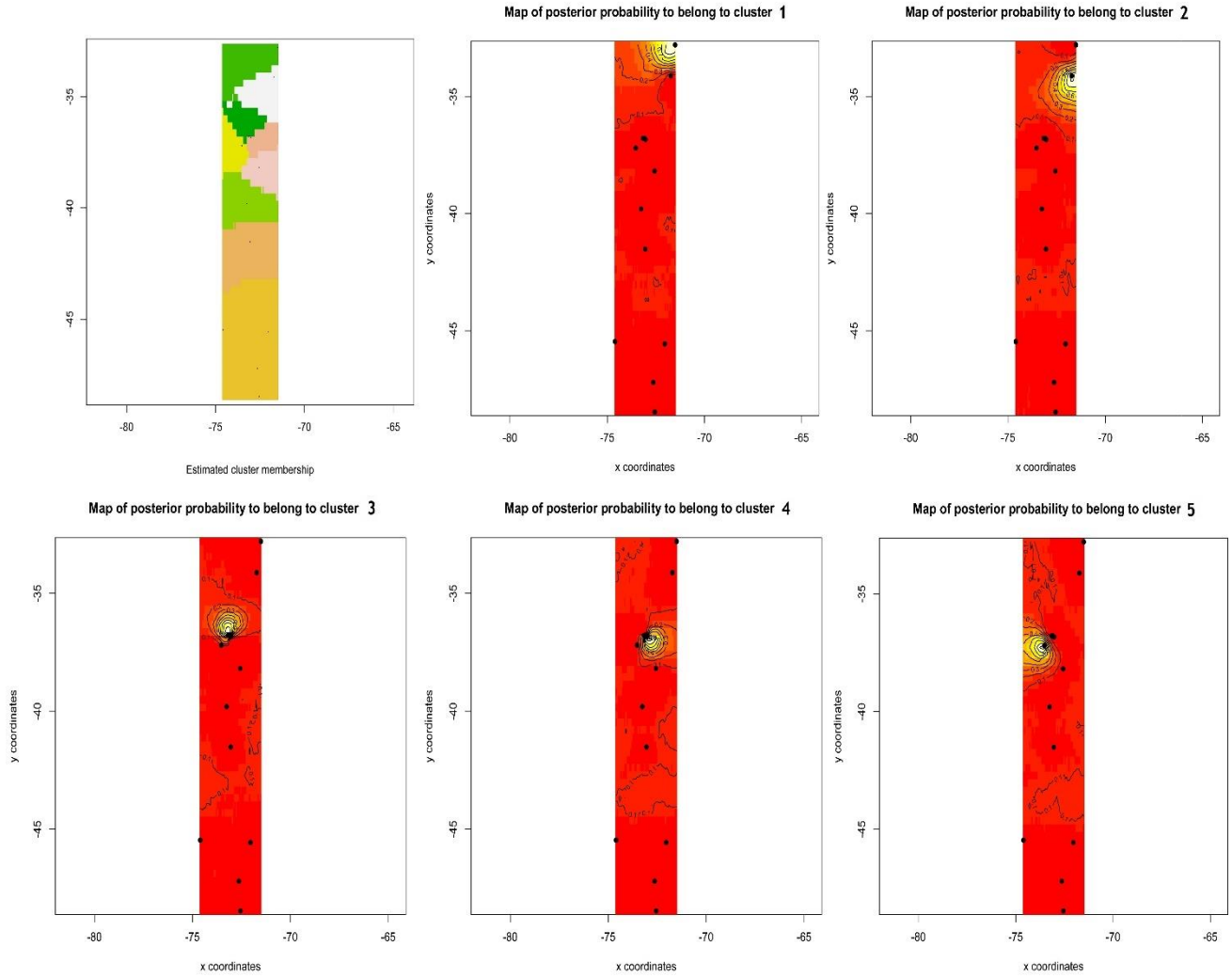

Figure 3.1 Number of clusters based on microsatellites markers. Maps of cluster membership and posterior probability for each cluster obtained in Geneland analyses were shown in the figure. The estimated cluster membership represents the modal cluster assignment of each pixel (A), and coloured contours show regions of high (light yellow) to low (red) posterior probability of membership for clusters 1 to 5.

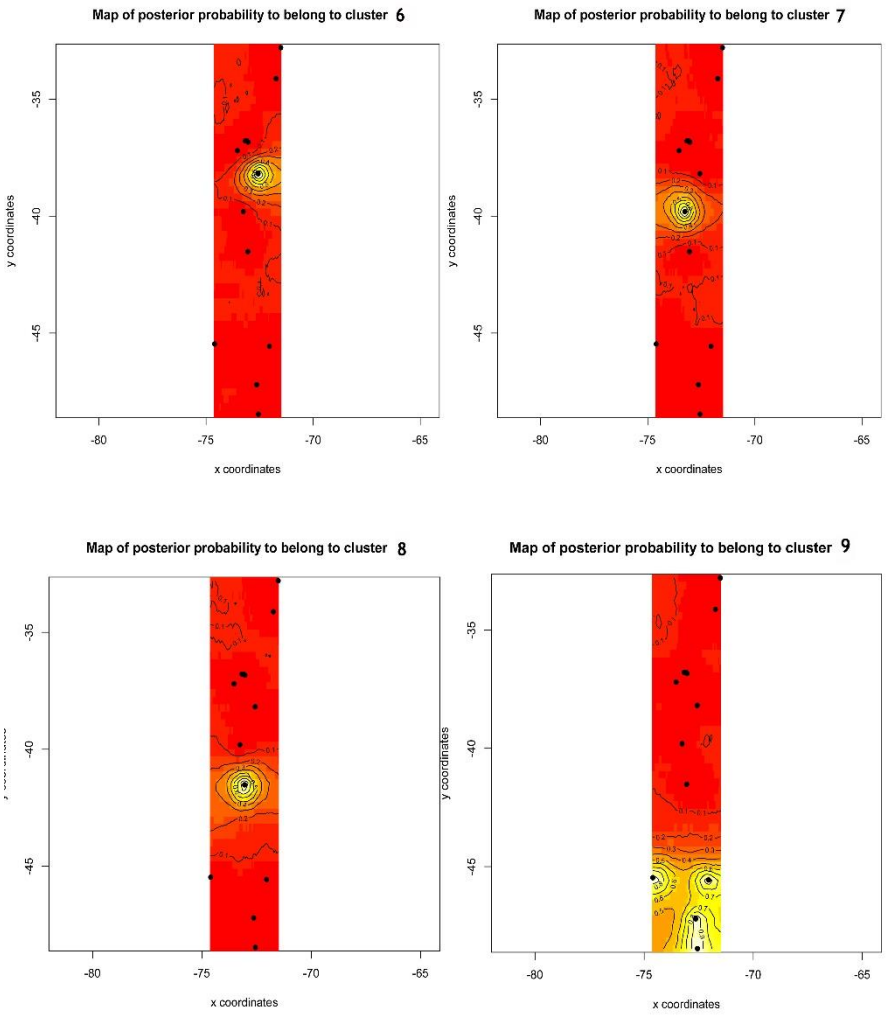

Figure 4.2 Clusters 6 to 9 based on microsatellites markers. Coloured contours show regions of high (light yellow) to low (red) posterior probability of membership.

Supplementary material: Tables

Table S. 1. Collection localities for *Batrachyla taeniata,* geographic coordinates, and cluster codes for microsatellites marker.

	Locality	Latitude	Longitude	N	Cluster by Geneland
1	Quintero	-32.794930	-71.510620	6	1
2	Litueche	-34.116667	-71.733333	2	2
3	Parque Botánico Hualpén	-36.783333	-73.166666	23	3
4	Hualpén	-36.786220	-73.077144	9	3
5	Cerro Caracol	-36.846090	-73.042487	8	4
6	La Cal	-37.215853	-73.539021	10	5
7	El Pajal	-38.183333	-72.566667	10	6
8	Valdivia	-39.802409	-73.260960	5	7
9	Chinquihue	-41.521878	-73.058785	6	8
10	Aysén	-45.566618	-72.043503	1	9
11	Aysén	-45.510975	-74.405288	1	9
12	Aysén	-48.468333	-72.555833	1	9
13	Aysén	-47.206944	-72.634444	1	9

Table S. 2. Assignment of individuals by Geneland and DAPC analysis.

Sample	ID Pop by Geneland	ID Pop by Adegenet	ID Pop in order latitudinal
1	2	4	1
2	2	4	1
3	2	4	1
4	2	4	1
5	2	4	1
6	2	4	1
7	9	4	2
8	9	4	2
9	1	7	3
10	1	8	3
11	1	6	3
12	1	6	3
13	1	6	3
14	1	6	3
15	1	6	3
16	1	6	3
17	1	7	3

18	1	8	3
19	1	7	3
20	1	8	3
21	1	8	3
22	1	6	3
23	1	6	3
24	1	8	3
25	1	7	3
26	1	8	3
27	1	8	3
28	1	7	3
29	1	6	3
30	1	8	3
31	1	7	3
32	1	6	3
33	1	6	3
34	1	7	3
35	1	8	3
36	1	8	3
37	1	8	3
38	1	7	3
39	1	7	3
40	1	6	3
41	7	6	4
42	7	6	4
43	7	6	4
44	7	6	4
45	7	6	4
46	7	6	4
47	7	6	4
48	7	6	4
49	4	2	5
50	4	2	5
51	4	2	5
52	4	2	5
53	4	2	5
54	4	2	5
55	4	2	5
56	4	2	5
57	4	9	5
58	4	6	5
59	8	5	6
60	8	5	6
61	8	5	6

62	8	9	6
63	8	9	6
64	8	9	6
65	8	9	6
66	8	9	6
67	8	9	6
68	8	9	6
69	3	5	7
70	3	5	7
71	3	5	7
72	3	5	7
73	3	5	7
74	6	3	8
75	6	3	8
76	6	3	8
77	6	3	8
78	6	3	8
79	6	3	8
80	5	1	9
81	5	1	9
82	5	1	9
83	5	1	9

Table S. 3. Average characteristics of microsatellites loci of *B. taeniata* populations.

Pop	Locus	N	Na	Ne	I	Ho	He	uHe	F
Pop 1	**LocBat1**	6	3	2.182	0.888	0.167	0.542	0.591	0.692
	LocBat4	6	5	3.130	1.358	0.833	0.681	0.742	-0.224
	LocBat12	6	3	2.667	1.028	1.000	0.625	0.682	-0.600
	LocBat17	6	3	2.667	1.028	0.167	0.625	0.682	0.733
	LocBat9	6	2	1.180	0.287	0.167	0.153	0.167	-0.091
	LocBat25	6	4	2.667	1.127	0.167	0.625	0.682	0.733
	LocBat27	6	4	2.880	1.199	0.667	0.653	0.712	-0.021
	LocBat34	6	5	4.235	1.517	0.833	0.764	0.833	-0.091
	LocBat31	6	4	2.880	1.199	0.167	0.653	0.712	0.745
	LocBat54	6	3	2.323	0.918	0.500	0.569	0.621	0.122
	LocBat43	6	8	5.538	1.907	1.000	0.819	0.894	-0.220
Pop 2	**LocBat1**	2	1	1.000	0.000	0.000	0.000	0.000	N/D
	LocBat4	2	1	1.000	0.000	0.000	0.000	0.000	N/D
	LocBat12	2	1	1.000	0.000	0.000	0.000	0.000	N/D
	LocBat17	2	1	1.000	0.000	0.000	0.000	0.000	N/D
	LocBat9	2	1	1.000	0.000	0.000	0.000	0.000	N/D

	LocBat25	2	2	1.600	0.562	0.500	0.375	0.500	-0.333
	LocBat27	2	2	2.000	0.693	0.000	0.500	0.667	1.000
	LocBat34	2	2	1.600	0.562	0.500	0.375	0.500	-0.333
	LocBat31	2	1	1.000	0.000	0.000	0.000	0.000	N/D
	LocBat54	2	2	1.600	0.562	0.500	0.375	0.500	-0.333
	LocBat43	0	0	0.000	0.000	0.000	0.000	0.000	N/D
Pop 3	**LocBat1**	31	11	4.598	1.923	0.355	0.783	0.795	0.547
	LocBat4	29	8	2.386	1.277	0.379	0.581	0.591	0.347
	LocBat12	32	20	11.441	2.700	0.875	0.913	0.927	0.041
	LocBat17	32	10	6.919	2.077	0.688	0.855	0.869	0.196
	LocBat9	31	5	1.765	0.839	0.355	0.433	0.441	0.181
	LocBat25	30	12	4.663	1.899	0.700	0.786	0.799	0.109
	LocBat27	31	19	12.645	2.692	0.839	0.921	0.936	0.089
	LocBat34	32	20	12.800	2.738	0.969	0.922	0.937	-0.051
	LocBat31	25	13	6.983	2.184	0.520	0.857	0.874	0.393
	LocBat54	31	14	8.736	2.351	0.935	0.886	0.900	-0.056
	LocBat43	29	26	15.574	3.025	0.966	0.936	0.952	-0.032
Pop 4	**LocBat1**	8	6	4.571	1.630	0.250	0.781	0.833	0.680
	LocBat4	8	9	7.111	2.079	0.500	0.859	0.917	0.418
	LocBat12	8	9	7.111	2.079	1.000	0.859	0.917	-0.164
	LocBat17	8	7	5.333	1.787	0.625	0.813	0.867	0.231
	LocBat9	8	2	1.600	0.562	0.500	0.375	0.400	-0.333
	LocBat25	8	6	4.414	1.635	0.875	0.773	0.825	-0.131
	LocBat27	8	9	6.737	2.047	1.000	0.852	0.908	-0.174
	LocBat34	8	11	9.143	2.307	1.000	0.891	0.950	-0.123
	LocBat31	8	5	2.560	1.190	0.375	0.609	0.650	0.385
	LocBat54	8	6	4.571	1.630	0.625	0.781	0.833	0.200
	LocBat43	8	9	7.111	2.079	1.000	0.859	0.917	-0.164
Pop 5	**LocBat1**	10	2	1.220	0.325	0.000	0.180	0.189	1.000
	LocBat4	10	2	1.342	0.423	0.100	0.255	0.268	0.608
	LocBat12	10	3	1.852	0.802	0.000	0.460	0.484	1.000
	LocBat17	10	3	1.515	0.639	0.000	0.340	0.358	1.000
	LocBat9	10	4	2.381	1.089	0.000	0.580	0.611	1.000
	LocBat25	9	5	2.104	1.080	0.111	0.525	0.556	0.788
	LocBat27	6	7	5.538	1.820	0.667	0.819	0.894	0.186
	LocBat34	8	8	6.737	1.981	0.625	0.852	0.908	0.266
	LocBat31	10	9	7.143	2.068	0.400	0.860	0.905	0.535
	LocBat54	10	6	3.636	1.522	0.500	0.725	0.763	0.310
	LocBat43	10	10	6.452	2.085	1.000	0.845	0.889	-0.183
Pop 6	**LocBat1**	7	6	4.455	1.611	0.286	0.776	0.835	0.632
	LocBat4	10	5	1.724	0.895	0.400	0.420	0.442	0.048
	LocBat12	10	7	3.704	1.557	0.400	0.730	0.768	0.452
	LocBat17	10	6	2.778	1.330	0.200	0.640	0.674	0.688
	LocBat9	10	3	1.869	0.819	0.200	0.465	0.489	0.570

	LocBat25	9	5	3.447	1.382	0.111	0.710	0.752	0.843
	LocBat27	7	6	2.722	1.352	0.571	0.633	0.681	0.097
	LocBat34	5	7	5.556	1.834	0.600	0.820	0.911	0.268
	LocBat31	10	8	5.128	1.846	0.300	0.805	0.847	0.627
	LocBat54	9	7	3.306	1.532	0.667	0.698	0.739	0.044
	LocBat43	6	9	8.000	2.138	1.000	0.875	0.955	-0.143
Pop 7	**LocBat1**	3	5	4.500	1.561	1.000	0.778	0.933	-0.286
	LocBat4	5	4	1.923	0.940	0.600	0.480	0.533	-0.250
	LocBat12	3	4	3.600	1.330	0.333	0.722	0.867	0.538
	LocBat17	0	0	0.000	0.000	0.000	0.000	0.000	N/D
	LocBat9	5	3	1.852	0.802	0.200	0.460	0.511	0.565
	LocBat25	5	5	2.500	1.228	0.400	0.600	0.667	0.333
	LocBat27	5	5	4.545	1.557	0.800	0.780	0.867	-0.026
	LocBat34	5	7	6.250	1.887	1.000	0.840	0.933	-0.190
	LocBat31	5	3	1.852	0.802	0.200	0.460	0.511	0.565
	LocBat54	5	7	5.556	1.834	0.600	0.820	0.911	0.268
	LocBat43	5	1	1.000	0.000	0.000	0.000	0.000	N/D
Pop 8	**LocBat1**	6	4	3.429	1.309	0.833	0.708	0.773	-0.176
	LocBat4	6	2	1.385	0.451	0.000	0.278	0.303	1.000
	LocBat12	6	4	2.667	1.127	0.333	0.625	0.682	0.467
	LocBat17	6	4	1.714	0.837	0.333	0.417	0.455	0.200
	LocBat9	6	3	2.571	1.011	0.000	0.611	0.667	1.000
	LocBat25	6	1	1.000	0.000	0.000	0.000	0.000	N/D
	LocBat27	6	5	3.789	1.468	0.667	0.736	0.803	0.094
	LocBat34	6	5	3.600	1.424	0.333	0.722	0.788	0.538
	LocBat31	6	3	1.412	0.566	0.167	0.292	0.318	0.429
	LocBat54	6	5	3.273	1.352	0.500	0.694	0.758	0.280
	LocBat43	6	7	6.000	1.864	0.500	0.833	0.909	0.400
Pop 9	**LocBat1**	1	1	1.000	0.000	0.000	0.000	0.000	N/D
	LocBat4	0	0	0.000	0.000	0.000	0.000	0.000	N/D
	LocBat12	4	2	1.280	0.377	0.250	0.219	0.250	-0.143
	LocBat17	4	3	2.667	1.040	0.000	0.625	0.714	1.000
	LocBat9	4	2	1.280	0.377	0.250	0.219	0.250	-0.143
	LocBat25	4	2	1.280	0.377	0.250	0.219	0.250	-0.143
	LocBat27	4	1	1.000	0.000	0.000	0.000	0.000	N/D
	LocBat34	4	3	2.462	0.974	1.000	0.594	0.679	-0.684
	LocBat31	3	2	1.385	0.451	0.333	0.278	0.333	-0.200
	LocBat54	4	2	1.600	0.562	0.000	0.375	0.429	1.000
	LocBat43	4	2	1.600	0.562	0.000	0.375	0.429	1.000

* N=Sample Size, Na=No. Alleles, Ne=No. Effective Alleles, I=Information Index, Ho=Observed Heterozygosity, He=Expected, uHe=Unbiased Expected Heterozygosity, and F=Fixation Index. N/D= No Data

Table S. 4. Migration rates between pairs of *B. taeniata* populations. The indices represent the fraction of individuals in the receiving population who are migrants from the population of origin per generation.

		SOURCE POP								
		Pop 1	**Pop 2**	**Pop 3**	**Pop 4**	**Pop 5**	**Pop 6**	**Pop 7**	**Pop 8**	**Pop 9**
RECEPTOR POP	**Pop 1**	**0.8216** (0.0412)	0.0224 (0.0209)	0.0218 (0.0203)	0.0228 (0.0212)	0.0225 (0.0208)	0.0220 (0.0202)	0.0223 (0.0210)	0.0222 (0.0208)	0.0224 (0.0210)
	Pop 2	0.0854 (0.0454)	**0.6966** (0.0275)	0.0301 (0.0271)	0.0305 (0.0276)	0.0355 (0.0339)	0.0306 (0.0280)	0.0306 (0.0281)	0.0303 (0.0273)	0.0304 (0.0276)
	Pop 3	0.0080 (0.0077)	0.0081 (0.0080)	**0.9347** (0.0204)	0.0082 (0.0081)	0.0084 (0.0079)	0.0081 (0.0079)	0.0082 (0.0080)	0.0082 (0.0078)	0.0081 (0.0079)
	Pop 4	0.0199 (0.0187)	0.0197 (0.0184)	0.0398 (0.0302)	**0.8191** (0.0426)	0.0224 (0.0211)	0.0197 (0.0187)	0.0198 (0.0187)	0.0203 (0.0191)	0.0193 (0.0180)
	Pop 5	0.0170 (0.0163)	0.0173 (0.0164)	0.0176 (0.0167)	0.0180 (0.0171)	**0.8594** (0.0368)	0.0178 (0.0169)	0.0182 (0.0170)	0.0173 (0.0164)	0.0175 (0.0168)
	Pop 6	0.0175 (0.0166)	0.0174 (0.0166)	0.0173 (0.0162)	0.0194 (0.0182)	0.0192 (0.0184)	**0.8564** (0.0379)	0.0175 (0.0167)	0.0177 (0.0169)	0.0176 (0.0169)
	Pop 7	0.0235 (0.0221)	0.0236 (0.0221)	0.0243 (0.0226)	0.0236 (0.0223)	0.0237 (0.0220)	0.1431 (0.0428)	**0.6907** (0.0225)	0.0238 (0.0221)	0.0237 (0.0223)
	Pop 8	0.0221 (0.0208)	0.0217 (0.0201)	0.0222 (0.0208)	0.0221 (0.0204)	0.0221 (0.0207)	0.0227 (0.0211)	0.0224 (0.0209)	**0.8226** (0.0410)	0.0221 (0.0208)
	Pop 9	0.0258 (0.0240)	0.0253 (0.0235)	0.0259 (0.0240)	0.0259 (0.0243)	0.1282 (0.0430)	0.0255 (0.0238)	0.0258 (0.0241)	0.0252 (0.0235)	**0.6924** (0.0239)

Table S. 5. Inbreeding Coefficients for the populations of *B. taeniata*.

Population	Inbreeding Coefficients
Pop 1	0.4896 (0.0718)
Pop 2	0.5009 (0.2888)
Pop 3	0.2172 (0.0293)
Pop 4	0.2073 (0.0685)
Pop 5	0.6999 (0.0486)
Pop 6	0.4379 (0.0526)
Pop 7	0.4988 (0.2891)
Pop 8	0.5772 (0.0740)
Pop 9	0.4972 (0.2900)

Table S. 6. Relationship between genetic differentiation and environmental factors.

Factor	Total posterior probability
Latitude, °	0.148
Longitude, °	0.324
Elevation above sea level, m	0.225
Precipitation of coldest quarter, mm^3	0.133
Mean temperature of driest quarter, °C	0.209
Euclidean distance, Km	0.430

Supplementary material: Figures

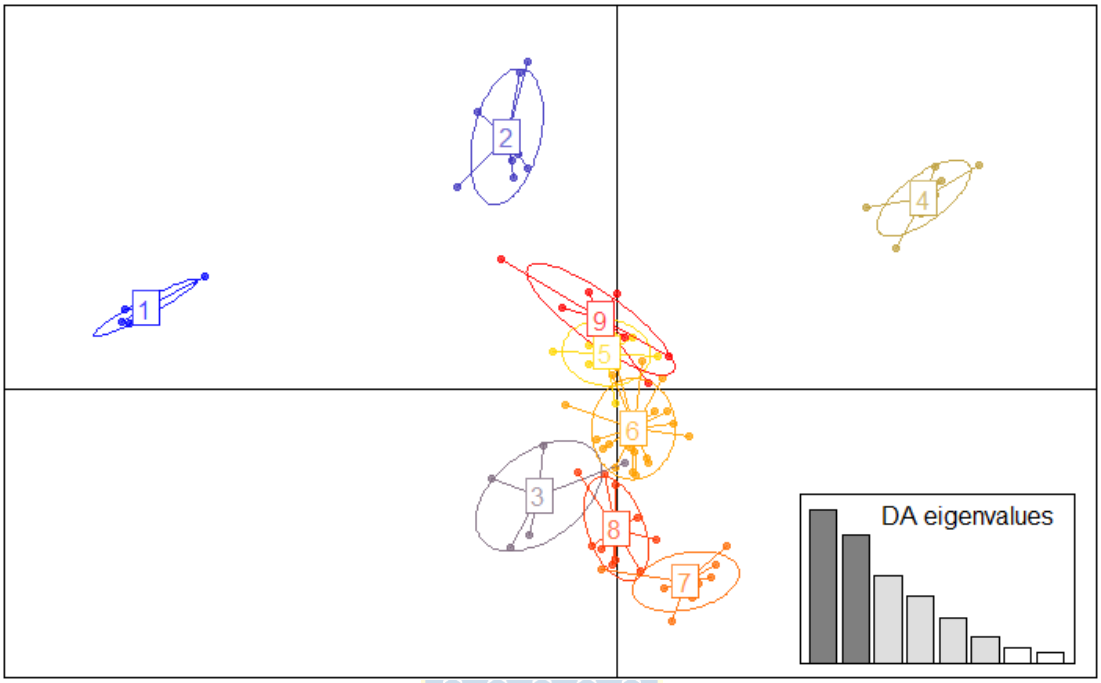

Figure S. 1. Discriminant analysis of principal components for *B. taeniata* populations.

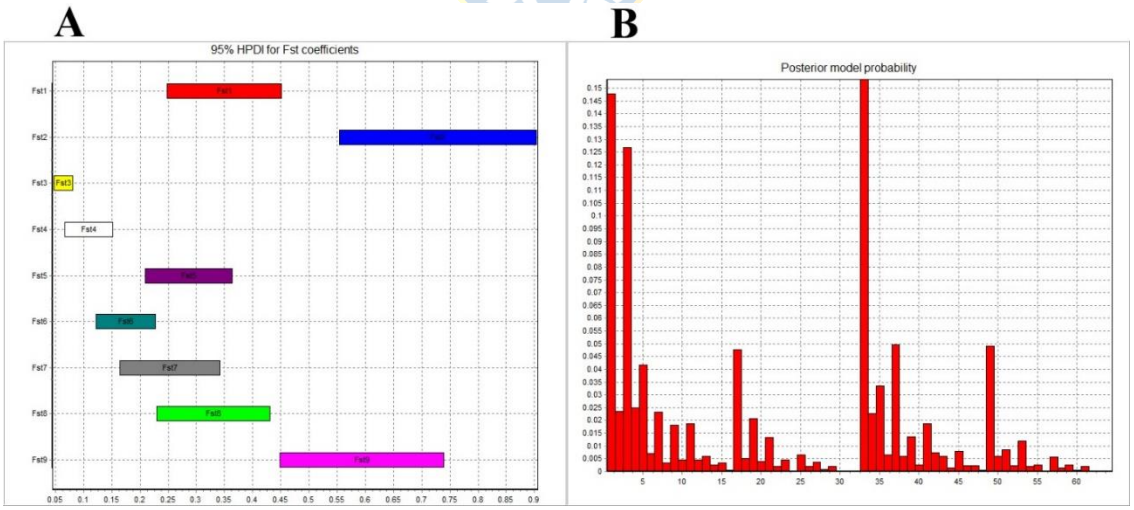

Figure S. 2. A) Degree of uncertainty of the estimation of the Fst coefficient (95% HPDI) in the populations of *B. taeniata*. B) Posterior models probability estimated in GESTE program. The graph shows the best model (33).

CONCLUSIONES GENERALES

- Se identifican dos grandes grupos genealógicos dentro de la distribución latitudinal de *B. taeniata* separados por el Río Biobío y asociados en el caso del grupo norte a la ecorregión Mediterránea y en el caso del grupo sur, a la ecorregión Oceánica, con una evidente diferenciación y estructura genética de acuerdo con sus patrones de distribución norte-sur.

- El clado extremo del grupo norte no muestra signos evidentes de expansión demográfica, ajustándose a un modelo de tamaño poblacional constante. En contraste a los clados que conforman el grupo sur, los cuales han experimentado expansiones demográficas desde el Pleistoceno (~ 0.04 mya), comenzando desde una época anterior a la LGM, por lo que este último evento glacial no tuvo un efecto directo de reducción de sus tamaños efectivos (Ne). Asimismo, los clados asociados al rango central de distribución muestran signos sólidos de crecimiento demográfico para los a partir de ~0.01 mya.

- El modelo de paleodistribución muestra signos de un proceso de contracción asociado con el LGM seguido de una expansión geográfica posterior. La tendencia potencial del patrón de cambio en el tamaño de la distribución de la especie es LIG> LGM <actual.

- Los resultados con microsatélites no recuperan la divergencia observada en los análisis históricos (ADN mitocondrial).

- Las poblaciones de *B. taeniata* presentan un patrón general de diferenciación genética asociado con mayor diversidad en el centro del rango de distribución y menor diversidad en los extremos norte y sur.

- Las estimaciones del número de alelos, heterocigosidad, grado de estructuración, consanguinidad y tasa de migración, respaldan una mayor diferenciación de las poblaciones ubicadas en la periferia del rango de distribución norte y sur de la especie.

- Las poblaciones se ajustan a un modelo de aislamiento por distancia. Y a su vez, aumenta la estructuración genética de las poblaciones a medida que existen mayores magnitudes de distancia geográfica (Km) dadas por la ubicación de cada población, hacia el centro del rango de distribución de la especie.

- En la especie *B. taeniata* se cumple el Patrón Poblacional Central-Periférico de Mayr (CPH).

ANEXO

GMR

Development and characterization of microsatellite molecular markers for the eye mask frog *Batrachyla taeniata* (Girard, 1855)

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ABSTRACT

Batrachyla taeniata (Batrachylidae) is a small frog from Chile and Argentina with a distributional range that spans more than 1500 km. However, their populations are threatened due to the change in land use, deforestation, and human settlements. Through massive sequencing runs in ION Torrent PGM, was built DNA fragment library, from which we finally isolated and characterized 25 microsatellite loci from 40 individuals. The number of alleles per locus ranged from 2 to 23, allele sizes varied between 110 and 268 bp, observed heterozygosity ranged from 0.205 to 1.000 and the expected heterozygosity between 0.21 and
0.93. We found evidence of deviations of HWE for 10 microsatelliteloci, linked to high inbreeding coefficients, indicating a loss of heterozygosity in those particular loci. There was no linkage disequilibrium found within the microsatellite loci tested through the sequential Bonferroni correction. These microsatellite markers can be

used to evaluate population problems such as low diversity and high genetic structure, with the purpose of optimize future conservation strategies for the populations of this species.

Keywords: *Batrachyla taeniata*; Microsatellites; ION Torrent; fragment analysis.

INTRODUCTION

Batrachyla taeniata (Anura: Batrachylidae) is a small frog species endemic to the temperate forests of Nothofagus that has a wide distribution in Chile and marginally in Argentina. In Chile, it extends from the Aconcagua province in the Valparaíso region to the Capitán Prat province in the Aysén region (Sallaberry et al., 1981; Brieva and Formas 2001; Correa et al., 2014). In addition, it presents island distributions in at least three islands near the continent, Quiriquina, Mocha and Chiloé (Sallaberry et al., 1981). This species is of particular interest from a biogeographical point of view, since it has been found in two different ecological regions: the Mediterranean and Oceanic regions (di Castri, 1968) covering approximately more than 1,500 km from north to south. In its northern distribution is associated with hydrophilic coastal relict forests and mesophiles (ej: Zapallar y Quintero) surrounded by semi-arid vegetation and in the southern distribution with humid and shady forests in environments saturated with humidity (Cei, 1962; Formas, 1979). This species has been frequently reported from Concepción to the south, in contrast to the populations in central Chile where is less frequent and more geographically isolated (Correa et al., 2014). The populations of central Chile (Quintero) present high values of distance and genetic differentiation with respect to the populations of the southern distribution group (Brieva and Formas, 2001). The species has been categorized as “Least Concern” in the International Union for Conservation of Nature (IUCN) red list (2015), despite the fact that Chilean law considers it "Almost Threatened" by the Classification Regulation of Species (RCE) (2014) because northern populations are more affected by the change in land use, deforestation and human settlement. These modifications in the landscape can have a strong effect on the patterns of dispersion and genetic flow of the species, this due to that amphibians have a restricted dispersion capacity that tends to promote differentiation (Duellman, 1999; Funk et al., 2005). For this reason, they show a greater genetic structure in their populations compared to other groups of species (Semlitsch et al., 2008).

The microsatellites are a type of molecular marker widely used in population genetics, conservation biology and evolutionary biology (Abdul-Muneer, 2014). These correspond to fragments of DNA sequence that consist of tandem repetitions of basic units that vary between 1 to 10 nucleotides in length and are repeated throughout the genome (Hamada et al., 1982; Vieira et al., 2016). The genetic variation of many microsatellite loci is characterized by high heterozygosity and polymorphisms (Litt and Luty, 1989; Ellegren, 2004), being a versatile tool for the identification of genetically deteriorated populations (Arif et al., 2011). Consequently, the use of this type of DNA markers can help to establish better management and protection plans for the conservation of biological populations. In the present study, we developed 25 microsatellite markers for *B. taeniata* with the purpose of using them in future population studies to investigate the genetic structure and diversity of their populations for conservation purposes.

MATERIALS AND METHODS

Samples

For the study DNA samples was obtained of specimens coming from Hualpén Botanic Park (36°47'S; 73°09'W),surroundings Hualpén (36°47'S; 73°05'W) Concepción, river headboard Tubul (37°12'S; 73°32'W) La Cal (37°12'S; 73°31'W) Arauco, Chile.

DNA preparation, sequencing and obtaining microsatellite markers

Through optimizations of Kit Promega Wizard Purification Systems, we extracted DNA of 27 individuals of *B. taeniata* from the sampled localities for use in the isolation of microsatellite loci. By quantifying DNA, we select 8 samples from different locations to perform the massive sequencing. The initial amount of DNA used was 100 ng, quantified spectrophotometrically (Nanodrop) and fluorimetrically (Qubit). DNA fragmentation at 400 bp was performed with the Covaris S220 sonicator. The DNA fragment library was constructed using the Ion Xpress Plus gDNA Fragment Library Kit (Thermo Scientific, manual MAN0009847, review C.0). For the preparation of library, emulsion and chip sequencing, the manufacturer's protocols were followed without modifications (Thermo Scientific, P.N. MAN0009847 revision D.0, MAN0010902 revision A.0 and MAN0009816 revision D.0). The readings in Fastq format were filtered using the Prinseq program (Schmieder and Edwards, 2011). The readings that passed the filters were transformed to Fasta format and were clustered at 90% identity by CD-HIT (Li et al., 2001). Subsequently, the MISA program (MISA webpage) was used for the identification and localization of dinucleotide microsatellites with at least 6 repetitions and tri /tetra-nucleotides with at least 5 repetitions. The identification of theprimers was done with Primer3 (Rozen and Skaletsky, 2000) with a range in amplicon size between 100-500 bp and an optimum of 350 bp, length of the primer of 19-25 bp with an optimum of 21 bp, Melting Temperature (TM) between 53-57°C with an optimum of 55°C and a GC percentage between 30-80%.

Microsatellite Genotyping

Of the 221 repetitive motifs obtained, 60 pairs of primers were selected, and these were tested for amplification using DNA from 10 individuals. PCR amplifications were performed in a total volume of 8 uL containing 10X Buffer Taq (Thermo Scientific), 25 mM $MgCl_2$ (Thermo Scientific), 2.5 mM dNTPs (Promega), 10 uM of each primer (Macrogen), 0.5 U uL-1 Taq DNA Polymerase (Thermo Scientific) and ca. 100 ng uL-1 DNA. Thethermocycler conditions for all the loci was with an initial denaturation of 5 min at 95°C, followed by 20 cycles of 30 s 95°C, 30 s at the optimum annealing temperature of 65-55°C and a final extension of 30 s at 72°C. In each cycle, the temperature was decreased in 0.5°C and the following conditions were continued: 30 s of denaturation at 95°C, 30 s of annealing at 55°C, 30 s of extension at 72°C and a final extension of 5 min at 72°C.

The PCR products were separated by 2.5% electrophoresis in agarose gels (BM-0120 Winkler), which ran between 60 min and 90 min. A first approximation of the microsatellite loci was estimated with the molecular weight marker Bench Top 100 pb DNA ladder (Promega).

For fragment analysis, 30 pairs of primers were selected, which were marked with four fluorophores (6FAM, PET, VIC, and NED). The selected microsatellites were amplified again using DNA from 40

individuals from the locality of Hualpén with the same initial conditions. The PCR products were run on an Applied BioSystems 3730 DNA Analyzer sequencer in Advanced Analysis Center, University of Guelph, Canada.

Statistical Analyses

Alleles were scored using the PeakScanner version 1.0 (Applied Biosystems). The number of alleles (A), Size the range of observed alleles in base pairs (bp), number of individual genotyped (N), observed heterozygosity (Ho), expected heterozygosity (He) and inbreeding coefficient (FIS) were tested for by using GenALEx 6.5 (Peakall and Smouse, 2006). Tests for deviations from the Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium were made in GENEPOP v 4.5 (Rousset, 2008). We also compare the structure of two populations separated by urbanization in the locality of Concepción in the Structure v 2.3.4 software (Pritchard et al., 2010) with the 11 most polymorphic microsatellites developed in this work.

RESULTS

Twenty-five of the pairs of primers tested amplified high-quality PCR products that exhibited polymorphisms. The characteristics of the loci are reported in Table 1. Most of the microsatellite primers contained dinucleotide and tetranucleotide repeat units. The number of alleles (A) varied between 2 and 23 (mean, 9.52). The sizes of the alleles varied between 110 to 268 bp, the heterozygosity observed (Ho) varied between 0.21 and 1.00 (mean, 0.71) and the expected heterozygosity (He) between 0.21 and 0.93 (mean, 0.68).

Table 1: Characterization of microsatellites for *Batrachyla taeniata.*

Locus	Repeat motif	Primer sequences (5'-3')	A	Size (bp)	N	H_o	H_e	F IS		GenBank Accession No
LocBat1	(AT)7	F: TTTCTTCCTATAGACCAGTG R: GGTATTGAGGCTACATCTTA	13	162-180	39	0.410	0.801	0.488	**	MK910322
LocBat4	(CA)9	F: GATACCAGGTAATTGTTGC R: ACCTGCTACTTCCTCTATTT	10	110-130	29	0.414	0.587	0.296	**	MK910323
LocBat12	(GA)14	F: GAATAAAGCCAGTGTGAAC R: GTAAAGGACCCTTAGAAAAG	21	123-164	40	0.900	0.919	0.021		MK910324
LocBat17	(AT)11	F: GGGGACACTTTATAATCAG R: AATGACAGACGTCAGTAGAC	10	142-161	34	0.676	0.854	0.208	**	MK910325
LocBat9	(TA)8	F: CTAATAACGTAAGTGCAACC R: GTTGTAACGAGTAAGTCCAT	8	171-220	34	0.441	0.510	0.136	**	MK910326
LocBat14	(AT)9	F: ATGTGCTCACAGAAGAAAG R: GATATACACCCTTGGTCCT	5	135-144	39	0.923	0.625	-0.477		MK910327
LocBat27	(TGC)8	F: GGACTGAATGTTCTTGTAGT R: ATTAAACTGTTCTAGGAGGC	22	132-169	39	0.846	0.922	0.083		MK910328
LocBat18	(TAA)8	F: GATTACTTCAGTGCTAGTCAG R: TAGAGCTATTTGATCAGAGG	15	200-235	38	0.737	0.869	0.152		MK910329
LocBat19	(TAC)7	F: TACAGTTACCAAGAGTCCC R: GATAGCAAGGTTAGTGGTAA	8	150-166	39	0.641	0.757	0.153	**	MK910330
LocBat20	(CTG)7	F: GTTGAGACCACAAATCAAG R: TTAGTTGGAGTCTCTAATGC	2	188-191	40	1.000	0.500	-1.000		MK910331
LocBat7	(AC)9	F: CAATACATCAGACTTTCTCC R: TATTTGGTCCTAGTGAAGAG	9	154-167	34	0.765	0.726	-0.054		MK910332

LocBat22	(TCC)7	F: CTGTGTACATTTTCTTGGG R: GAGCAGATGCTTGTATCTAT	2	129-135	40	1.000	0.500	-1.000		MK910333
LocBat25	(CAT)9	F: CTAACATAGTGGTCATGGAT R: CATATTCACTAAAGCAGAGC	13	137-165	36	0.667	0.815	0.182	**	MK910334
LocBat36	(TAGA)9	F: AGAGATAAGGAAAGTTGGG R: CCATCTATCTACCTAACTCCTA	6	116-140	40	0.625	0.494	-0.266		MK910335
LocBat43	(AT)8	F: TGTACCTCACCTAAGAAAGA R: GGAAGGGAATAATACTTCTG	17	164-180	38	0.395	0.897	0.560	**	MK910336
LocBat31	(AGTT)10	F: TAGATCTCAACAGGAACAAG R: AGGTTAGACTTTTAAGCCAC	13	129-188	32	0.563	0.855	0.342	**	MK910337
LocBat37	(GTCT)6	F: AGATAGGAGCTTTAGGATTC R: GTACTCTGAAGTTGTGGTAAC	3	132-137	40	1.000	0.620	-0.613		MK910338
LocBat34	(ATAG)6	F: ATACAGACTCAAAGTGTCGT R: CCACCAATGTTAGTTTACC	23	177-268	40	0.975	0.933	-0.045		MK910339
LocBat52	(CTG)5	F: TAAATACCCATGTAGTCCAG R: AATTTCTTACCCGCTACTC	2	137-144	39	0.974	0.500	-0.950		MK910340
LocBat51	(GA)7	F: TCCAGTTAGATAAGCGTTAC R: ACATATAGCACTTCTACCCTC	6	172-179	39	0.205	0.214	0.040		MK910341
LocBat53	(CTG)6	F: CCTTTCTGATGCTACACTAC R: TAGTTTGGAGTCTCTAATGC	3	248-251	36	1.000	0.514	-0.947		MK910342
LocBat54	(ATA)6	F: GGCTCTATTGATGACAATC R: CTCTGTAAGTCTGCTTTTGT	14	126-159	38	0.921	0.881	-0.046		MK910343
LocBat55	(ATT)6	F: TACCTACATGGGATGATTAC R: GTCAATCCAAGTTAGTTGC	4	122-135	29	0.207	0.599	0.655	**	MK910344
LocBat 56	(AGG)6	F: CTACTGTGTCAAGCTGATAAC R: TAGTGTAGCTGCTGTGTGTAT	6	142-158	30	0.600	0.734	0.182	**	MK910345
LocBat59	(GTCT)6	F: AGATAGGAGCTTTAGGATTC R: CTCTGAGTTGTGGTAACCT	3	129-133	39	1.000	0.500	-1.000		MK910346

A = Number of alleles, Size the range of observed alleles in base pairs (bp), N = Number of individual genotyped, H_o = Observedheterozygosity, He = Expected heterozygosity, FIS = Inbreeding coefficient

** Significant evidence ($p < 0.01$) against Hardy-Weinberg equilibrium model

We found evidence of deviations of HWE for 10 microsatellite loci, linked to high inbreeding coefficients (FIS), indicating a loss of heterozygosity in those particular loci. The inbreeding coefficient values were significantly different from zero ($p < 0.05$) with negative FIS value detected for the loci LocBat 14, LocBat 20, LocBat7, LocBat22, LocBat36, LocBat37, LocBat34, LocBat52, LocBat53, LocBat54, and LocBat59, indicating that the population possess a greater number of heterozygotes in those particular loci, supported by the observed heterozygosity values (Ho).

There was no linkage disequilibrium found within the microsatellite loci tested through the sequential Bonferroni correction. Nevertheless, significant deviations from the HWE expectations in the population reflected the occurrence of heterozygote deficiency.

Genetic summary statistics of eleven microsatellite loci from for two population of *B. taeniata* (Table 2) show thatin both populations the Ho was less than He (average Ho = 0.699: He = 0.832 and Ho = 0.627: He = 0.807). The values of the inbreeding coefficient were low for both populations, however, the population of the city of Hualpén shows a higher number of loci with high FIS (average FIS = 0.239).

Table 2: Genetic summary statistics of eleven microsatellite loci from two locations of *Batrachyla taeniata* in Concepción

Locus	**Hualpén botanic park** (N=30)				**Hualpén city** (N=10)			
	A	H_o	H_e	FIS	A	H_o	H_e	FIS
LocBat1	11	0.414	0.761	0.456	7	0.400	0.780	0.487
LocBat4	8	0.435	0.542	0.197	6	0.333	0.694	0.520

LocBat12	18	0.867	0.901	0.038	13	1.000	0.905	-0.105
LocBat17	8	0.640	0.811	0.211	7	0.778	0.827	0.060
LocBat27	18	0.931	0.903	-0.031	8	0.600	0.840	0.286
LocBat18	15	0.793	0.866	0.084	8	0.556	0.809	0.313
LocBat25	12	0.643	0.798	0.195	8	0.750	0.797	0.059
LocBat43	16	0.464	0.893	0.480	8	0.200	0.825	0.758
LocBat31	13	0.640	0.864	0.259	5	0.286	0.673	0.576
LocBat34	23	0.967	0.935	-0.034	11	1.000	0.890	-0.124
LocBat54	13	0.900	0.878	-0.025	8	1.000	0.836	-0.196
Average	*14*	*0.699*	*0.832*	*0.167*	*8*	*0.627*	*0.807*	*0.239*

A = Number of alleles, H_o = Observed heterozygosity, H_e = Expected heterozygosity, FIS = Inbreeding coefficient N = Sample size

The genetic distance value of Nei among the population of Hualpén botanic park and Hualpén city was from 0.416. However, the estimates in STRUCTURE indicate that the best K value for both previously separated groups is 1 (Table 3), indicating the presence of a single population (likelihood values k = 1: -2194.5, k = 2: -2276.2, k = 3: - 3081.4).

Table 3: Estimate of the best value of K in STRUCTURE software

K	Ln prob. of data	Mean value of Ln likelihood	Variance of Ln likelihood
1	-2194.5	-2161.5	66.0
2	-2276.2	-2090.6	371.2
3	-3081.4	-2039.2	2084.5

K= Number of populations, Ln=Likelihood.

DISCUSSION

The advances in the massive sequencing have allowed great advantages for the development of molecular markers of microsatellite type (Sung-Yin et al., 2018; Zhang et al., 2018). These markers are one of the most widely explored genetic markers in several research areas due to their robustness and high polymorphism (Senan et al., 2014; Zhuang et al., 2017) and are an excellent tool to know the structure of natural populations and help the conservation of the species (Mu-Yeong et al., 2017; Nakahama et al., 2018).

A good model for investigating the genetics structure of wild animal populations areamphibians (Beebee, 2005), because are highly dependent on their habitat (Duellman and Trueb, 1994) with relatively low dispersal capacities (Jehley and Arntzen, 2002). In addition, their populations show a greater genetic structure with respect to other groups (Johns and Avise, 1998), giving rise to populations that can represent unique genetic entities, between relatively short geographic distances (Jehle and Artzen, 2002; Beebee, 2005).

The genetic variability of a natural population is important for its continuity over time (Lacy, 1997), like itsgenetic structure, therefore, the combination of ecological and genetic data can help identify the factors that limitthe size of the population and determine the potential conservation of them (Liu et al., 2013). However, these studies are not yet represented in the conservation efforts for Chilean amphibians' populations. Despite the advantages of microsatellite markers for population

analysis, only a few species count on their development and characterization, including Pleurodema thaul (D'elía et al., 2014), Rhinoderma darwinii (Fuentes et al., 2014), Telmatobufo bullocki (Moreno-Puig et al., 2014), Telmatobius chusmisensis (Fabres et al., 2018).

In this study, we developed for the first time a set of twenty-five polymorphic microsatellites for B. taeniata. As seen in the results, the number of alleles (A) varied between 2 and 23 (mean, 9.52), finding highly polymorphic alleles such as those reported in P. thaul where the number of alleles per locus varied between 7 and 22(D'elía et al., 2014). As mentioned, the heterozygosity observed (Ho) varied between 0.21 and 1.00 (mean, 0.71), with higher values per locus than reported in P. thaul (D'elía et al., 2014), R. darwinii (Fuentes et al., 2014), T. bullocki (Moreno-Puig et al., 2014) and T. chusmisensis (Fabres et al., 2018).

In this work was no linkage disequilibrium found within the microsatellite loci tested through the sequential Bonferroni correction. Nevertheless, significant deviations from the HWE expectations in the population reflected the occurrence of heterozygote deficiency, which can be given because of high inbreeding within the population, a scenario that has been demonstrated in other Chilean amphibians (Fuentes et al., 2014).

As mentioned, the genetic structure of populations is not always reflected in the geographical proximity of individuals (Evanno et al., 2005). However, in the analysis carried out for individuals of *B. taeniata* from two locations separated by high human urbanization, they show that they still correspond to the same population (thebest k = 1). This indicates that individuals still share ancestry of the same ancestral population, perhaps due to a recent temporary separation of the analyzed localities.

CONCLUSION

It is important to advance in the development of obtaining genetic information from this species to improve strategies and prioritize the conservation of their populations, especially the northern populations that are more affected by the change in land use, deforestation and human settlement. In summary, we report twenty-five microsatellite markers in *B. taeniata* identified by massive sequencing. These microsatellite markers represent a valuable resource for future population analysis for conservation purposes.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abdul-Muneer PM (2014) Application of microsatellite markers in conservation genetics and fisheries management: recent advances in population structure analysis and conservation strategies. Genet Res Int 2014: 1-11. https://doi.org/10.1155/2014/691759

Arif IA, Khan HA, Bahkali AH, Al Homaidan AA, et al. (2011) DNA marker technology for wildlifeconservation. Saudi J Biol Sci 18: 219-225. https://doi.org/10.1016/j.sjbs.2011.03.002

Beebee TJC (2005) Conservation genetics of amphibians. Heredity 95: 423-427.https://doi.org/10.1038/sj.hdy.6800736

Brieva LM and Formas JR (2001) Allozyme variation and geographic differentiation in the Chilean leptodactylid frog *Batrachyla taeniata* (Girard, 1854). Amphib-Reptil 22: 413-420. https://doi.org/10.1163/15685380152770372

Cei JM (1962) Batracios de Chile. Ediciones Universidad de Chile, Santiago. CVIII + 128 pp.

Correa C, Cisternas J, Velásquez N, Lobos G, et al. (2014) Distribution extension, new records and historical occurrence of *Batrachyla taeniata* (Girard, 1854) (Anura: Batrachylidae) in Chile. Herpetol Notes 7: 703- 706.

D'elía G, Beasley RR, Lance SL, Jones KL, et al. (2014) Development of polymorphic microsatellite markers for the Pleuroderma thaul. Conserv Genet Resour 6: 747-749. https://doi.org/10.1007/s12686-014-0206-8

Di Castri F and Hajek E (1976) Bioclimatología de Chile. Ediciones Universidad Católica de Chile. Lira 140 Santiago, Chile. 128 pp.

Duellman WE (1999) Patterns of Distribution of Amphibians: A Global Perspective. The Johns HopkinsUniversity Press, Baltimore and London. 633 pp.

Duellman WE. and Trueb L (1994) Biology of Amphibians. The Quarterly Review of Biology 69: 532-532. https://doi.org/10.1086/418798

Ellegren H (2004) Microsatellites: simple sequences with complex evolution. Nat Rev Genet 5: 435-445.https://doi.org/10.1038/nrg1348

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software structure: A simulation study. Mol Ecol 14: 2611-2620. https://doi.org/10.1111/j.1365-294x.2005.02553.x

Fabres A, Fibla P, Araya C, Sallaberry M, et al. (2018) Development and characterization of 22 polymorphic microsatellites of the Andean frog Telmatobius chusmisensis (Anura, Telmatobius) and

cross amplification in seven Chilean species of the genus. Mol Biol Rep 45: 1533-1538. https://doi.org/10.1007/s11033- 018-4228-2

Formas JR (1979) The South American Herpetofauna: Its origen, evolution, and dispersal (ed: Duellman,
W. E.), pp. 314-379. Museum of Natural History, University of Kansas, Monograph 7, Lawrence, Kansas. https://doi.org/10.5962/bhl.title.3207Fuentes-Navarrete M, Gómez-Uchida D, Gallardo-Escarate C, Canales-Aguirre C, et al. (2014) Development of microsatellites for Southern Darwin's frog Rhinoderma darwinii (Duméril & Bibron, 1841). Conserv Genet Resour 6: 1035-19037. https://doi.org/10.1007/s12686-014-0279-4

Funk WC, Blouin MS, Corn PS, Maxell BA, et al. (2005) Population structure of Columbia spotted frogs (Rana luteiventris) is strongly affected by the landscape. Mol Ecol 14: 483-496. https://doi.org/10.1111/j.1365- 294x.2005.02426.x

Jehle R and Artzen W (2002) Review: microsatellite markers in amphibian conservation genetics. HerpetolJ 12: 1-9.

Johns GC and Avise JC (1998) A comparative summary of genetic distances in the vertebrates from themitochondrial cytochrome b gene. Mol Biol Evol 15: 1481-1490. https://doi.org/10.1093/oxfordjournals.molbev.a025875

Hamada H, Petrino MG, Kakunaga T (1982) A novel repeated element with Z-DNA-forming potential is widely found in evolutionarily diverse eukaryotic genomes. PNAS 79: 6465-6469. https://doi.org/10.1073/pnas.79.21.6465

IUCN SSC Amphibian Specialist Group (2015) *Batrachyla taeniata*. The IUCN Red List of Threatened Species. https://doi.org/10.2305/iucn.uk.2015-4.rlts.t56334a79812277.en

Lacy RC (1997) Importance of Genetic Variation to the Viability of Mammalian Populations. J Mamal 78:320-335. https://doi.org/10.2307/1382885

Li W, Jaroszewski J, Godzik A (2001) Clustering of highly homologous sequences to reduce the size oflarge protein database. Bioinformatics 17: 282-283. https://doi.org/10.1093/bioinformatics/17.3.282

Litt M and Luty JA (1989) A hypervariable microsatellite revealed by in vitro amplification of dinucleotiderepeat within the cardiac muscle actin gene. Am J Hum Genet 44: 397-401.

Liu Y, Webber S, Bowgen K, Schmaltz L, et al., (2013) Environmental factors influence both abundance and genetic diversity in a widespread bird species. Ecol Evol 3: 4683-4695. https://doi.org/10.1002/ece3.856

MISA – MIcroSAtellite identification tool. https://doi.org/10.1093/benz/9780199773787.article.b00123495

Moreno-Puig V, Yildirim Y, Brunton DH (2014) Development of microsatellite markers for the critically endangered frog Telmatobufo bullocki and cross-species amplification in two related species. Conserv

GenetResour 6: 883-884. https://doi.org/10.1007/s12686-014-0231-7

Mu-Yeong L, Hea CM, Hey SJ, Eui-Geun S, et al. (2017) Characterization of 33 microsatellite markers and development of multiplex PCR for yellow-throated marten (Martes flavigula). Genet Mol Res 17: gmr16039854.

Nakahama N, Ito O, Kaneko S, Matsuki Y, et al. (2018) Development of microsatellite markers for the endangered orchid Calanthe izu-insularis (Orchidaceae). Genes Genet. Syst. 93: 31-35. https://doi.org/10.1266/ggs.17-00021

Peakall R and Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software forteaching and research. Mol Ecol Notes 6: 288-295. https://doi.org/10.1111/j.1471-8286.2005.01155.x

Pritchard JK, Wen X, Falush D (2010) Documentation for Structure software: Version 2.3. Department ofHuman Genetics. University of Chicago, USA.

Rousset F (2008) GENEPOP'007: a complete re-implementation of the GENEPOP software for Windowsand Linux. Mol. Ecol. Resour. 8: 103-106. https://doi.org/10.1111/j.1471-8286.2007.01931.x

Rozen S and Skaletsky H (2000). Primer3 on the WWW for General Users and for Biologist Programmers. Bioinformatics Methods and Protocols. Humana Press, New Jersey, 365-386. https://doi.org/10.1385/1-59259-192- 2:365

Sallaberry M, Valencia J, Diaz N (1981). Distribución y ambiente de *Batrachyla taeniata* (Girard) en Chile(Anura - Leptodactylidae). Bol Mus Nac Hist Nat 38: 61-67.

Schmieder R and Edwards R (2011) Quality control and preprocessing of metagenomic datasets.
Bioinformatics 27: 863-864. https://doi.org/10.1093/bioinformatics/btr026

Semlitsh R and Skelly D (2008) Ecology and Conservation of Pool-Breeding Amphibians. In: Calhoun AJK, deMaynadier PG (eds) Science and conservation of vernal pools in northeastern North America. CRC Press, Boca Raton, pp 127-148.

Senan S, Kizhakayil D, Sasikumar B, Sheeja TE (2014) Methods for Development of Microsatellite Markers: An Overview. Not Sci Biol 6: 1-13. https://doi.org/10.15835/nsb.6.1.9199

Sung-Yin Y, Wai-Ling F, Wenhua SCh, Chia-Min H, et al. (2018) Development of Novel Polymorphic Microsatellite Markers in Catch Bowl Coral, Isopora palifera (Scleractinia; Acroporidae) Using Next-generation Sequencing. Zool Stud 57: 32

Vieira MLC, Santini L, Diniz AL, Munhoz CF (2016). Microsatellite markers: what they mean and why they are so useful. Genet Mol Biol 39: 312-328. https://doi.org/10.1590/1678-4685-gmb-2016-0027

Zhang F, Wang Y, Xu M, Wei L, et al. (2018). Development and characterization of twenty-eight polymorphic microsatellite for rice field eel (Monopterus albus) using RAD tag sequencing. Genet Mol Res 17: gmr16039880. https://doi.org/10.4238/gmr16039880

Zhuang YF, Wang ZF and Wu LF (2017) New set of microsatellites for Chinese tallow tree, Triadicasebifera. Genet. Mol Res 16: gmr16029624.