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SHORT COMMUNICATION

Range extension of Catedral spiny-chest frog, *Alsodes gargola* Gallardo, 1970: a record in an unknown road of the Chilean Patagonia

Extensión de distribución de la rana de pecho espinoso del Catedral, *Alsodes gargola* Gallardo, 1970: un registro en una ruta desconocida de la Patagonia Chilena

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ABSTRACT

This article reports the presence of *Alsodes gargola* Gallardo, 1970 in Paso Las Golondrinas, south of Lago Verde village, Aysén Region, Chile. To validate our finding, we made a phylogenetic analysis with the mitochondrial gene cytochrome b. This record extends the distribution range of this species, which inhabits Chile and Argentina, 130 km to the south. The finding emphasizes the importance of conducting samplings in little-known zones of Chilean Patagonia, especially in those that totally lack antecedents on their herpetofauna.

Keywords: endangered species, Lago Carlota National Reserve, Lago Verde, Patagonian frogs, unknown roads.

RESUMEN

En este artículo se reporta la presencia de *Alsodes gargola* Gallardo, 1970 en Paso Las Golondrinas, al sur del poblado de Lago Verde, Región de Aysén, Chile. Para validar nuestro hallazgo, hicimos un análisis filogenético con el gen mitocondrial citocromo b. Este registro amplía el rango de distribución de esta especie, que habita en Chile y Argentina, 130 km al sur. El hallazgo enfatiza la importancia de realizar muestreos en zonas poco conocidas de la Patagonia Chilena, en especial en aquellas que carecen totalmente de antecedentes sobre su herpetofauna.

Palabras clave: especies en peligro, Lago Verde, ranas patagónicas, Reserva Nacional Lago Carlota, rutas desconocidas.

The genus *Alsodes* Bell, 1843 currently comprises 19 species (Frost 2022), most of which are distributed on the western slopes of the southern Andes, in the temperate forests of central-south Chile (18 species; Correa 2019); the genus is also marginally found in Argentina with five species (Frost 2022).

Alsodes gargola Gallardo, 1970 is a Patagonian frog, whose type locality is Tonchek Lake, Cerro Catedral, Province of Río Negro, Argentina (Gallardo 1970). In this country, *Alsodes gargola* is widely distributed, being present in the provinces of Neuquén, Río Negro (in several localities within Nahuel

Huapi National Park), and Chubut (Los Alerces and Lago Puelo National Parks) (Úbeda *et al*. 2012). Initially, Cei (1976) divided this species in two subspecies: *A. gargola gargola* and *A. gargola neuquensis*, but the second subspecies, present in Neuquén Province (volcanic tablelands of Lonco Luan), was recognized as a distinct species (*Alsodes neuquensis*) following a phylogenetic analysis (Blotto *et al*. 2013), being therefore the unique endemic *Alsodes* species of Argentina. *Alsodes gargola* has an elevational range of 200-2,000 m a.s.l. and it is the anuran that can live at the highest altitude in Patagonia (Úbeda 2021). It lays a reduced number of eggs (between 30 to 40, Gallardo 1970) and has a very long larval development, up to four years (Logares & Úbeda 2004, Baffico & Úbeda 2006, Úbeda & Casanovas 2012, Úbeda 2021).

In Chile, the presence of *A. gargola* was recently validated by Blotto *et al*. (2013), with four specimens from Futaleufú (Los Lagos Region). There is a previous record within the same area, a little further south, in Río Correntoso (Futaleufú National Reserve), initially cataloged as *Alsodes verrucosus* (Elgueta *et al*. 2006), but according to the phylogenetic analysis of Correa *et al*. (2018, 2020) that population also corresponds to *A. gargola*.

Given these antecedents, the aim of this article is reporting the third record of *Alsodes gargola* in Chile, made during a herpetofaunal survey carried out near of Lago Verde village, Aysén Region. Furthermore, this is the southernmost record of this species, including both Argentina and Chile.

Herpetofaunal diurnal surveys were conducted during the second week of February 2021, in a secondary road known only for residents (opened in summer season and passable only in four-wheel drive vehicles), between Lago Verde and Villa La Tapera. The landscape is dominated by native Andean temperate grassland of *Nassauvia dentata-Senecio portalesianus* (Luebert & Pliscoff 2017). In each survey point, we made transects (200 meters long, with sampling time between 30 minutes and 1.5 hours), and observations and records of frogs were documented (by three authors). The active search included the manipulation of stones, lumbers, trunks and shrubs, to record hidden frogs. The frogs registered were measured, photographed, released in the same place of the record and one individual of *Alsodes* sp. was collected. Measurements were taken using a Vernier caliper to the nearest millimeter. Surveys were conducted under permit RE N°298/2021, issued by Servicio Agrícola y Ganadero, SAG. Frogs were identified reviewing published scientific papers and field guides (*e.g*. Gallardo 1970, Charrier 2019).

The individual of *Alsodes* sp. collected (code MNHN/HERP 5863, Museo Nacional de Historia Natural, Santiago, Chile) was a subadult male with the following measurements: (i) snouth-vent length: 33 mm; (ii) head length: 10 mm; (iii) head width: 13 mm; (iv) femur length: 13 mm (right hindlimb); (v) tibia length (right hindlimb): 15 mm. The record was made 21 km south of Lago Verde: 44°25'50" S, 71°51'00" W (1,216 m a.s.l.), in a small lagoon 2.15 km north of Paso Las Golondrinas (3 km from the border with Argentina) and 9 km north-west of Lago Carlota National Reserve (Fig. 1). The lagoon (unnamed), apparently shallow, measures approximately 290 m long and 45 m wide, and has no associated streams (we walked the entire periphery of the lagoon). The landscape is mountainous with several small to medium-sized lagoons (Fig. 2A). In the vicinity there are large wooded patches of *Nothofagus* sp. The specimen was found under rocks, which cover the shore of the lagoon edge (Fig. 2B). Under these rocks (of medium size), a large number of amphipods were found (which could be part of the diet of post-metamorphic specimens). In addition to *Alsodes* sp., an adult specimen of Chile four-eyed frog (*Pleurodema thaul*) was found in the lagoon, also hidden under rocks.

The specimen was identified as *Alsodes gargola* based on the following morphological characters given by Gallardo (1970): robust appearance; head wider than long, prominent eyes, with circular pupils; snout short, canthus rostralis evident but short; nostrils closer to snout than eye; eardrum not visible; rounded toe tips; internal metatarsal tubercle elongated, the external one rounded; back of body (including femurs and tibiae) with small granulations (Fig. 3A); ventrally smooth, with pectoral patch markings (Fig. 3B); dorsal coloration light brown, with some darker spots and dark cross bars on the legs; clear, yellowish ventral coloration.

The specimen was defined as subadult due to its size (33 mm), significantly smaller than the adults, between 48 mm (for the typical adult male, Gallardo 1970) and 70 mm (Úbeda 1998). Morphologically similar species to *Alsodes gargola* from Aysén Region are *A. australis* Formas, Úbeda, Cuevas & Núñez, 1997, and *A. coppingeri* (Günther 1881). Therefore, we made a phylogenetic analysis to validate our record. Briefly, we extracted total DNA from the tongue of the collected specimen with a commercial kit and sequenced a fragment of the mitochondrial gene cytochrome b with primers MVZ15-L (Moritz *et al*. 1992) and CytbAR-H (Goebel *et al*. 1999). The PCR protocol for amplifying this fragment is described in Correa *et al*. (2013). Then, we carried out a Bayesian phylogenetic analysis, including nine specimens of *A. gargola*: four from Futaleufú (Blotto *et al*. 2013), one from Futaleufú National Reserve (Correa *et al*. 2018, specimen DBGUCH 3372; in Correa *et al*. 2020 the locality appears only as Futaleufú), two from Laguna Tonchek (type locality, Argentina, Blotto *et al*. 2013) and two from other two Argentinian localities, Arroyo Zanjón Hondo (Blotto *et al*. 2013) and vicinity of Refugio Neumeyer, Nahuel Huapi

National Park (Zhang *et al*. 2013). Moreover, representatives of other 13 species of the genus were included (Blotto *et al*. 2013, Charrier *et al*. 2015, Correa *et al*. 2018). There is a cytochrome b sequence from an additional species, *A. kaweshkari*, which has been included in some phylogenetic analyses (*e.g*. Formas *et al*. 2008, Barrasso *et al*. 2022), but was not included here because there are doubts about its correct identification (see discussion in Appendix S3 of Blotto *et al*. 2013). The phylogenetic analysis was performed in MrBayes v3.2.7a (Ronquist *et al*. 2012), applying independently to each codon position a reversible-jump method to explore the space of all General Time Reversible sub-models, plus gamma and proportion of invariable sites parameters. The analysis consisted of four independent chains run for 20 million generations, sampled every 1000 generations. The first 25% of generations was conservatively discarded as burn-in.

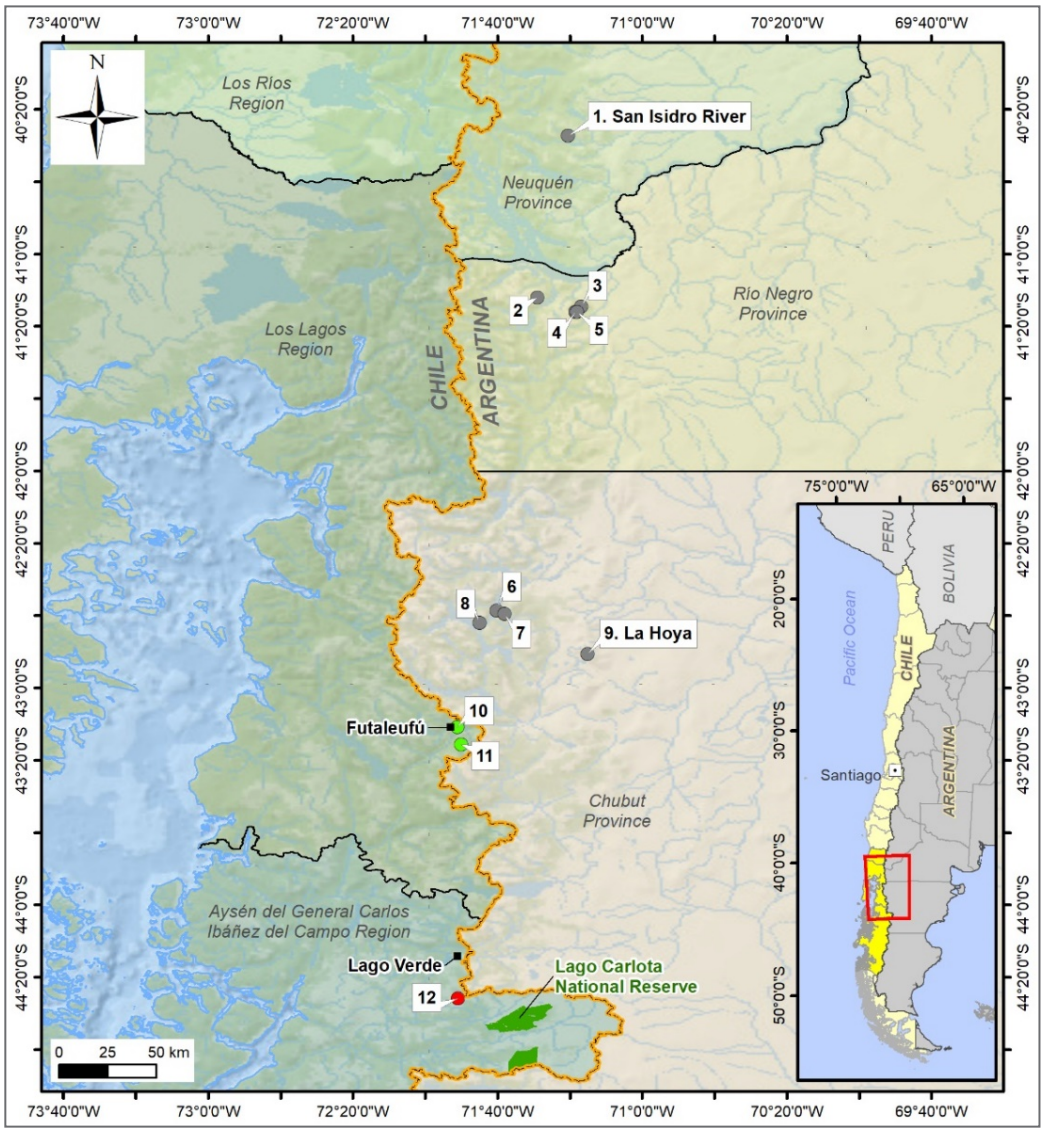

Figure 1. Distribution map of *Alsodes gargola* in Argentina and Chile. Gray dots: published records of *A. gargola* in Argentina (1 to 9). Green dots: published records of *A. gargola* in Chile; Futaleufú (10) and Futaleufú National Reserve (11). Red dot: new record at Paso Las Golondrinas, south of Lago Verde, Aysén Region, Chile (12). The numbers for each locality follow Table 1. / Mapa de distribución de *Alsodes gargola* en Argentina y Chile. Puntos grises: registros publicados de *A. gargola* en Argentina (1 al 9). Puntos verdes: registros publicados de *A. gargola* en Chile; Futaleufú (10) y Reserva Nacional Futaleufú (11). Punto rojo: nuevo registro en Paso Las Golondrinas, al sur de Lago Verde, Región de Aysén, Chile (12). Los números de cada localidad siguen la Tabla 1.

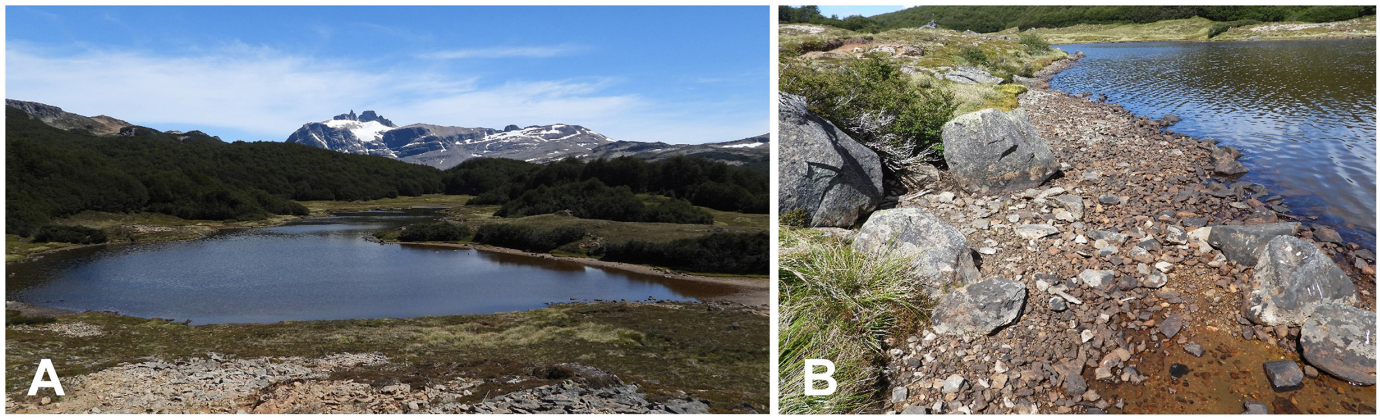

Figure 2. Landscape (A) and microhabitat (B) in which *Alsodes gargola* was found in Paso Las Golondrinas, Aysén Region, Chile. / Paisaje (A) y microhábitat (B) en que *Alsodes gargola* fue encontrado en Paso Las Golondrinas, Región de Aysén, Chile.

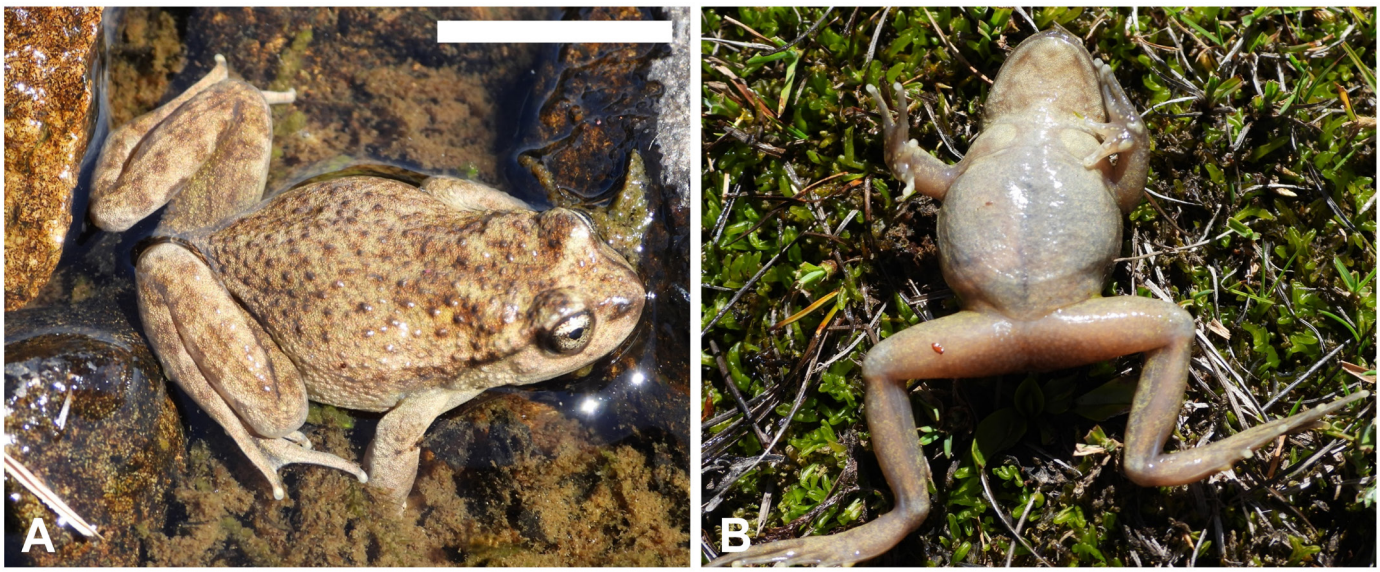

Figure 3. Subadult male of *Alsodes gargola* found in Paso Las Golondrinas, Aysén Region, Chile. (A) Dorsal view. White bar represents 16.5 mm. (B) Ventral view. / Macho subadulto de *Alsodes gargola* encontrado en Paso Las Golondrinas, Región de Aysén, Chile. (A) Vista dorsal. La barra blanca representa 16,5 mm. (B) Vista ventral.

An alignment of 1003 nucleotide sites was obtained, although with numerous gaps at the ends because some sequences were shorter in length (a few sequences are 385 bases long). Despite this, the topology of the Bayesian consensus tree (Fig. 4) is fully consistent with the results of previous analyses of the genus performed with the same gene (Correa *et al*. 2018, 2020). The same groups of species were recovered with high support, as well as most of the recognized species of the genus. All the *A. gargola* samples were grouped with maximum support (posterior probability = 1), including the Paso Las Golondrinas sample (ON584164; Fig. 4), which confirms its assignment to this species according to the external morphology examination (see above).

Regarding the geographic location, this new record is 130 km south of Río Correntoso (Futaleufú National Reserve), Los Lagos Region, Chile (Fig. 1). In Argentina, the species extends from Río San Isidro (40°27'18" S) to La Hoya, Cordón de Esquel (42°50'42" S) and covers three provinces: Neuquén, Río Negro and Chubut; while in Chile it is found in the regions of Los Lagos and Aysén (considering this new record, Table 1).

Table 1. Geographic localities with published records of *Alsodes gargola* in Argentina and Chile. The number of each locality corresponds to those indicated in Figure 1. / Localidades geográficas con registros publicados de *Alsodes gargola* en Argentina y Chile. El número de cada localidad corresponde a las indicadas en la Figura 1.

N°	Country	Region/Province	Locality	Latitude (S)	Longitude (W)	Altitude (m a.s.l.)	Reference
1	Argentina	Neuquén	Río San Isidro	40°27'18"	71°20'28"	1,070+	Úbeda (1998)
2	Argentina	Río Negro	Laguna Schmoll, Laguna Tonchek, Cerro Catedral	41°12'	71°29'	1,750-1,940	Gallardo (1970), Baffico & Úbeda (2006), Logares & Úbeda (2006), Úbeda (2021)
3	Argentina	Río Negro	Valle de los Perdidos	41°14'32"	71°16'54"	1,375	Baffico & Úbeda (2006), Casanovas & Úbeda (2006)
4	Argentina	Río Negro	Cerro Challhuaco	41°15'59"	71°18'25"	1,750	Baffico & Úbeda (2006)
5	Argentina	Río Negro	Bosque Challhuaco	41°16'	71°18'	1,300	Baffico & Úbeda (2006)
6	Argentina	Chubut	Población Neira, East shore of Lago Rivadavia	42°38'36"	71°40'18"	550	Úbeda (2002)
7	Argentina	Chubut	0.5 km Laguna Neira	42°39'34"	71°38'07"	1,120	Úbeda (2002)
8	Argentina	Chubut	Arroyo Zanjón Hondo	42°42'	71°45'	890	Formas *et al.* (1997), Blotto *et al.* (2013)
9	Argentina	Chubut	La Hoya, Cordón de Esquel	42°50'42"	71°15'05"	1,420	Úbeda (2000)
10	Chile	Los Lagos	Futaleufú*	43°10'51"	71°51'09"	350-496	Blotto *et al.* (2013), C. Correa (pers. obser.)
11	Chile	Los Lagos	Reserva Nacional Futaleufú (Río Correntoso)**	43°15'35"	71°50'05"	920	Elgueta *et al.* (2006)
12	Chile	Aysén	Paso Las Golondrinas	44°25'50"	71°51'00"	1,216	This publication

Notes: +Estimated altitude from Google Earth. *Correa *et al.* (2006) included in their phylogenetic analyses a specimen labeled *Alsodes* sp. (DBGUCH 3372), which according to the analyses by Correa *et al.* (2018, 2020) corresponds to *A. gargola*; actually, that specimen comes from Futaleufú National Reserve, record published in Elgueta *et al.* (2006); Blotto *et al.* (2013) included specimens from Futaleufú but did not specify the coordinates; the coordinates included in this table correspond to personal observations of C. Correa. **Elgueta *et al.* (2006) published this record as *A. verrucosus*, without providing coordinates; the coordinates and altitude in the table correspond to the approximate location according to the map of the Reserve that appears in Elgueta *et al.* (2006). / +Altitud estimada de Google Earth. *Correa *et al.* (2006) incluyeron en sus análisis filogenéticos un ejemplar rotulado como *Alsodes* sp. (DBGUCH 3372), que de acuerdo a los análisis de Correa *et al.* (2018, 2020) corresponde a *A. gargola*; en realidad, ese ejemplar proviene de la Reserva Nacional Futaleufú, registro publicado en Elgueta *et al.* (2006); Blotto *et al.* (2013) incluyeron ejemplares de Futaleufú, pero no especificaron las coordenadas; las coordenadas incluidas en esta tabla corresponden a observaciones personales de C. Correa. **Elgueta *et al.* (2006) publicaron este registro como *A. verrucosus*, sin proporcionar coordenadas; las coordenadas y altitud de la tabla corresponden a la ubicación aproximada de acuerdo al mapa de la Reserva que aparece en Elgueta *et al.* (2006).

The distribution of *A. gargola* in Chile apparently is limited to border areas with Argentina, as Futaleufú (Blotto *et al*. 2013) and our record in Paso Las Golondrinas. This would differentiate it from the other *Alsodes* species of the Aysén Region, which occur in central areas or closer to Patagonian channels and fjords (Charrier 2019). The new record is located in a landscape of mountain lakes, which resembles it to the records of *A. gargola* in Argentina (*e.g*. Gallardo 1970, Úbeda 2021). These records have been reported in environments such as pools, streams and small alpine oligotrophic lakes which remain superficially frozen, from four to eight months per year, with minimal temperatures in winter ranging between 0 to 3 °C, so the most optimal survival strategy is one that implies a very slow larval development of up to four years ("overwintering"; Logares & Úbeda 2004, 2006, Úbeda & Casanovas 2012, Úbeda 2021).

The new record near Paso Las Golondrinas is the third record of *A. gargola* in Chile, and represents a new species of amphibian for the Aysén Region. Our record extends the geographic distribution of *A. gargola* 130 km south of its known occurrence in Futaleufú National Reserve (Chile) and 183 km south of its known southern record in Argentina. In

this last country, *A. gargola* has a wide geographic distribution, encompassing the provinces of Neuquén, Río Negro and Chubut (the nine records indicated in Table 1, in addition to several other records non published formally, such as Lago Nuevo, Cerro Cuyín Manzano, Cerro López, Paso Vuriloche, Estancia El Cóndor, Cerro Lago, Lago Puelo and Río Tigre; Úbeda 1998). Historically, the localities of La Atravesada and Lonco Luan (Province of Neuquén) were considered as records of *Alsodes gargola neuquensis*, but later they were assigned to *A. neuquensis*, based on phylogenetic analyses by Blotto *et al*. (2013).

Since there are several records of this species in Argentina, *A. gargola* is listed as Least Concern species by the IUCN Red List of Threatened Species (IUCN 2021). However, given the few records in Chile, this species is considered an Endangered species by Reglamento de Clasificación de Especies of Ministerio del Medio Ambiente (RCE) (MMA 2015). The proximity to Lago Carlota National Reserve (9 km) in Chile, with a similar landscape, means that the species could be found in that protected area (Fig. 1). We suggest to carry out herpetological samplings in this reserve, in order to confirm its presence and the protection of the southernmost populations. Currently, *A. gargola* is protected in Argentina within Nahuel Huapi National Park, Lago Puelo National Park, Los Alerces National Park (Laguna Neira) and Nahuel Huapi National Park (Úbeda 2000, 2002, Úbeda *et al*. 2012). In Chile, it is protected only in Futaleufú National Reserve (Elgueta *et al*. 2006, as *A. verrucosus*).

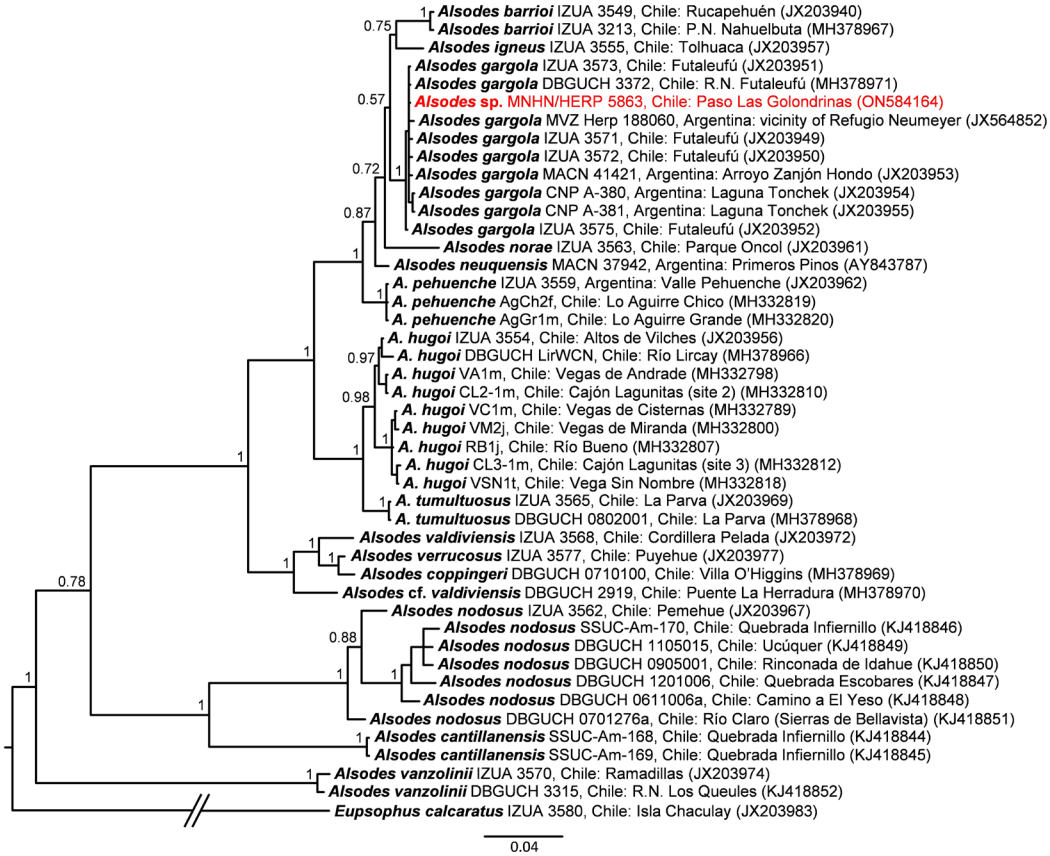

Figure 4. Bayesian consensus tree (50% majority-rule) of the fragment of the mitochondrial cytochrome b gene, showing the position of the subadult individual of *Alsodes* from Paso Las Golondrinas (red letters). Numbers next to the nodes correspond to posterior probabilities of the BI analysis. The scale bar below the tree represents the expected substitutions per site along the branches according to this analysis. For each specimen, the collection number or sample code, the locality and the GenBank accession number of the respective sequence (in parentheses) are specified. / Árbol de consenso bayesiano (regla de mayoría del 50%) del fragmento del gen citocromo b mitocondrial, que muestra la posición del individuo subadulto de *Alsodes* de Paso Las Golondrinas (letras rojas). Los números junto a los nodos corresponden a probabilidades posteriores del análisis BI. La barra de escala debajo del árbol representa las sustituciones esperadas por sitio a lo largo de las ramas según este análisis. Para cada espécimen, se especifican el número de colección o código de muestra, la localidad y el número de acceso de GenBank de la secuencia respectiva (entre paréntesis).

Our work and other recent studies, which extends the geographic distribution of Chilean herpetofaunal species, such as *Alsodes hugoi* (Correa *et al*. 2018), *Liolaemus kingii* (Mella *et al*. 2018, 2020), *Diplolaemus darwinii* (Mella *et al*. 2019), *Alsodes verrucosus* (Mella-Romero & Lamilla-Maulén 2019) and *Telmatobufo venustus* (Caro-Lagos & Charrier 2020, Díaz-Páez & Alveal 2021), emphasize the need of more extensive samplings, especially in Patagonian steppe and high Andean environments (Mella *et al*. 2018, Mella-Romero *et al*. 2020), to determine the real geographic distribution of a great number of Chilean amphibians and reptiles.

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