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New populations of two threatened species of *Alsodes* (Anura, Alsodidae) reveal the scarce biogeographic knowledge of the genus in the Andes of central Chile

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Abstract

High Andean environments of central Chile (32°–38°S) are inhabited by several endemic species of the genus *Alsodes*. Two of them, *A. pehuenche* and *A. hugoi*, have geographic distributions restricted to their type locality and surroundings. The Chilean government classifies *A. pehuenche* as Critically Endangered (like the IUCN) and *A. hugoi* as Vulnerable. In this study we report 16 new localities of *Alsodes*, corresponding to first order streams, located in the Andes of Chile between 35°58' and 36°32'S (1800–2470 m). In some of these sites, adults and juveniles morphologically similar to *A. pehuenche* and *A. hugoi* were observed, as well as specimens of *Alsodes* that could not be identified by their external morphology. A Bayesian phylogenetic analysis with mitochondrial sequences (cytochrome b) was performed to identify the new populations to species level. All populations around 36°S belong to *A. pehuenche*, while most of those located south of that area would be *A. hugoi*. The exception is Cajón de Plaza (36°23'S), where specimens with sequences of *A. hugoi* or *A. pehuenche* coexist, whose taxonomic status could not be determined. These findings imply not only a westward range extension of *A. pehuenche* in Chile of about 14.5 km and of *A. hugoi* about 100 km southward, but also that practically all the first order streams of the Andes of central Chile would be inhabited by populations of *Alsodes*. Both results demonstrate the scarce biogeographic knowledge of the genus in the Andes, which has important implications for its conservation at local and species levels.

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distribution extensions
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conservation
Critically Endangered
Vulnerable

Introduction

The amphibian fauna of the Andes of southern South America (Chile and Argentina) is relatively poor in comparison to tropical high-altitude environments (Duellman 1979, 1999). In fact, only three anuran genera, *Alsodes* Bell, 1843, *Rhinella* Fitzinger, 1826 and *Pleurodema* Tschudi, 1838, are represented in the Andes south of 30°S above 1500 m. *Alsodes* is the most diversified of these genera, with five species (*A. montanus* (Lataste, 1902), *A. tumultuosus* Veloso, Iturra & Galleguillos, 1979, *A. hugoi* Cuevas & Formas, 2001, *A. pehuenche* Cei, 1976 and *A. nodosus* (Duméril & Bibron, 1841)) present in the western slopes (Chile) between 33° and 36°S (Charrier et al. 2017). Four of these species are endemic to Chile, since *A. pehuenche* is also present in Argentina in a small area around the border between these countries (Valle Pehuenche, its type locality, 36°S) (Corbalán et al. 2010, Correa et al. 2013).

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* These authors contributed equally to this work.

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There are few published records of Andean populations of *Alsodes* in Chile and most of these were reported in the last decade. For instance, several reports since 2008 increased significantly the distribution ranges and number of known localities of *A. montanus* and *A. tumultuosus* from 33°20' to 35°S (Araya and Riveros 2008, Correa et al. 2008, Mora et al. 2015, Ramírez 2015, Correa 2017). Likewise, new records of *A. hugoi* (Araya and Cisternas 2008) and *A. pehuenche* (Corbalán et al. 2010, Correa et al. 2013) extended their distributions, but in both cases within a small area around their type localities. These last two studies are particularly relevant because they extended the distribution of *A. pehuenche* to Chile, a species previously known just from its type locality in Argentina. The fifth species, *A. nodosus*, also inhabits the western slopes of Andes, but it is not clear what altitude it reaches (e.g. Cuevas 2013). Despite this significant increase in the number of known localities of *Alsodes* in the Andes, there are still substantial distribution gaps. For example, there is a stretch of 380 linear kilometers where only *A. hugoi* and *A. pehuenche* are known, between the Tinguiririca River basin (34°55'S), the southern end of the distributions of *A. montanus* and *A. tumultuosus*, and San Ignacio de Pemehue, type locality of *A. vittatus* (Philippi, 1902) (38°05'S) (Fig. 1).

The distribution ranges of *A. pehuenche* and *A. hugoi* are extremely restricted. *Alsodes pehuenche* was first reported as *Telmatobius montanus* (now *A. montanus*) by Cei and Roig (1965) at two very close localities located on each side of the border of Chile and Argentina (36°S). The Argentinean locality (Valle Pehuenche) became the type locality when this species was formally described by Cei (1976), but the Chilean locality was subsequently ignored (see comment in Correa et al. 2013). For 45 years it was assumed that *A. pehuenche* inhabited only in its type locality, located in a drainage basin on the eastern slopes of the Andes (that ultimately flows into the Atlantic Ocean), until Corbalán et al. (2010) extended its distribution about 4 km to the southwest in Chile, in a contiguous drainage basin, but that drains to the west into the Pacific Ocean. Corbalán et al. (2010) also specified that on the Argentinian side the species only occupies five very close streams and indicated that attempts to find individuals in other nearby streams failed. Later, Correa et al. (2013) reported a second locality in Chile, which further extended the distribution approximately 3.4 km west. Similarly, *Alsodes hugoi* was known since its description only in its type locality (Altos de Vilches, 35°33'S, 900 m, Cuevas and Formas 2001) until Araya and Cisternas (2008) reported its presence in several sites around this locality. Although these new records extended the altitudinal range up to 2115 m, all sites are hydrologically connected to the Lircay River and the maximum distance between them does not exceed 10 km.

The known distribution ranges of these species have been fundamental in establishing their national and international conservation categories. The Chilean government, through its legal instrument Reglamento de Clasificación de Especies Silvestres (RCE, which applies similar criteria to those of IUCN), classifies *A. pehuenche* as Critically Endangered and *A. hugoi* as Vulnerable. These categories rest on the application of criterion B, which exclusively considers the extent of the geographical distribution. On the other hand, *A. pehuenche* is Critically Endangered and *A. hugoi* is Data Deficient according to the IUCN (2017). This last categorization of *A. pehuenche* is fundamentally based on estimates of the extent of occurrence (about 9 km^2) and area of occupancy (about 5 km^2) (IUCN SSC Amphibian Specialist Group 2013).

In this study we report new Andean localities of *Alsodes* situated in Chile between 35°58' and 36°32'S. These localities were found during three field campaigns aimed to locate new populations of *A. pehuenche*, one in the surroundings of the known localities in Chile (around 36°S) and the other two south of the known distribution range of the species. We performed a phylogenetic analysis with mitochondrial cytochrome b sequences to identify most of the populations discovered to species level. We discuss the implications of these findings for the biogeography and conservation of the genus in Chilean Andes.

Material and methods

Field campaigns

We carried out three field campaigns in the western slopes of the Andes Range between 35°55' and 36°35'S to locate new populations of *A. pehuenche* in Chile (Fig. 1). The three areas explored are located almost entirely in the southeast part of the Maule River basin, except for the southernmost area that covers the northeast end of the Ñuble River basin. The explorations were focused on the surroundings and to the south of the known distribution of *A. pehuenche* in Chile (Paso Pehuenche and Laguna del Maule, around 36°S and 70°25'W; Correa et al. 2013). The longest campaign was carried out in 2016–2017 in the surroundings of Paso Pehuenche and Laguna del Maule (Fig. 1B), where a total of nine sites between 2200 and 2500 m were explored (Table 1). Two of these sites had previously been described (Corbalán et al. 2010, Correa et al. 2013). The other two campaigns were carried out in 2016, the first in the surroundings of Laguna El Dial (around 36°27'S; six days, five sites explored between 1830 and 2000 m; Fig. 1D), and the second in some eastern tributaries of the Guaiquivilo River (around 36°08'S; five days, five sites explored between 1800 and 2160 m; Fig. 1C) (Table 1). The last two areas were reached on horseback with the help of a guide and a muleteer. The searches focused on first order streams fed by melting snow, which are generally located in areas with a steep slope and are associated with flooded meadows, since this is the environment described for *A. pehuenche* (Cei and Roig 1965, Corbalán et al. 2008, 2010). When possible, we chose streams with continuous flow (presumably permanent throughout the year), since it was described that this species has a larval development that lasts several years (Corbalán et al. 2014). We consider as first order streams those that constitute the sources of the hydric systems and that do not have tributaries that feed them, following a top-down topological ordering. Each site

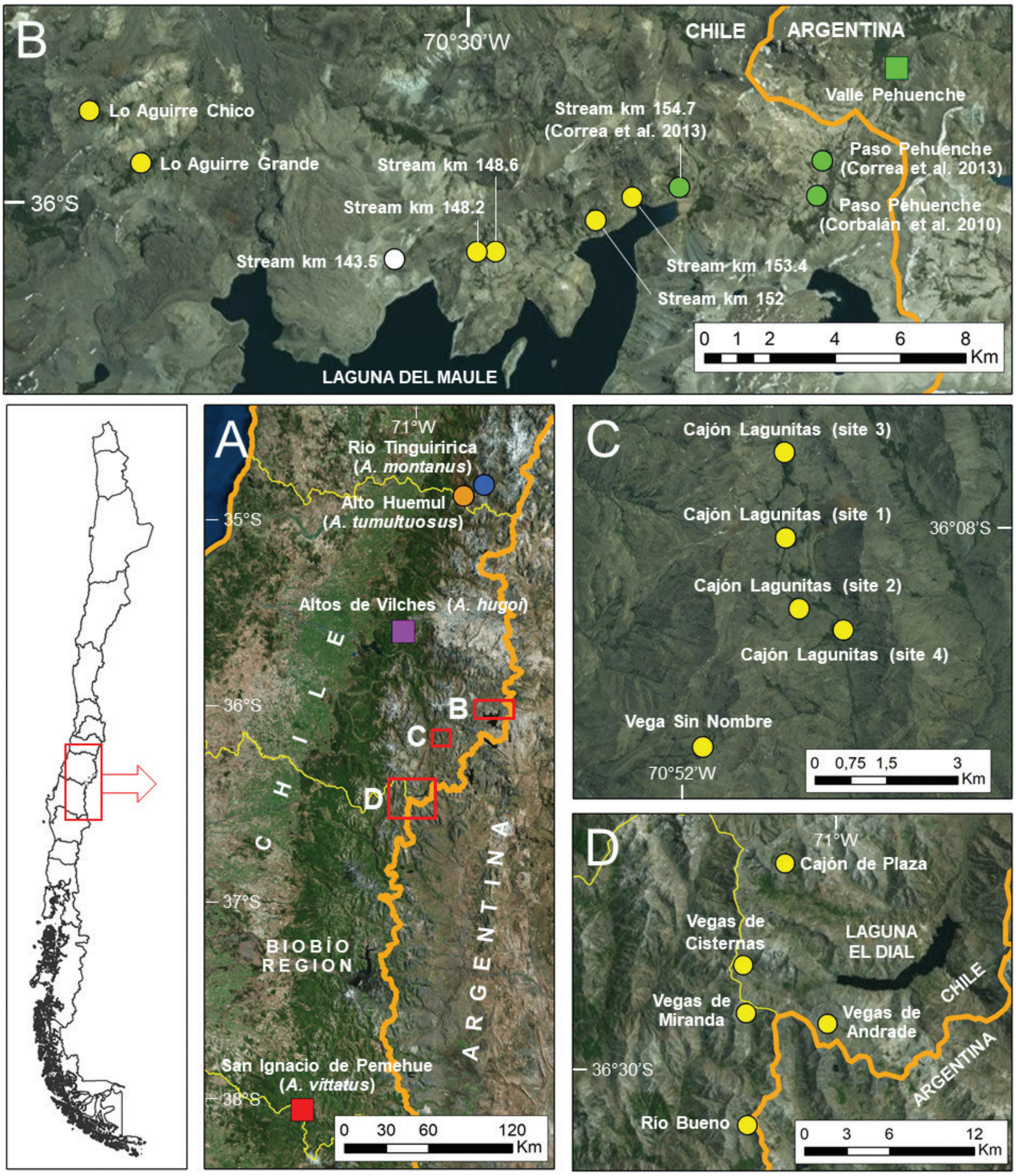

Figure 1. New and literature records of *Alsodes* from the Andes Range between 34°50' and 38°05'S. Yellow circles represent the new localities reported in this study; squares represent type localities. **A.** Andean localities of *Alsodes* of the literature between 34°50' and 38°05'S: the southernmost localities of *A. montanus* and *A. tumultuosus*, the type locality of *A. hugoi*, localities of *A. pehuenche* (within red box B, see map B) and the type locality of *A. vittatus*. There is a record of a putative new species related to *A. nodosus* in Pemehue (*Alsodes* sp. 1 of Blotto et al. 2013; not included in the map), presumably the same type locality of *A. vittatus*. Red boxes correspond to the three explored areas described in this study (maps B, C and D). **B.** Area explored during the first field campaign (Paso Pehuenche, Laguna del Maule and surroundings). All colored symbols correspond to localities of *A. pehuenche*: yellow circles correspond to new records; green circles and the square are all previously known localities of the species. The white circle is the place where no amphibian was found. **C.** Area and sites explored during the third field campaign (tributaries of the Guaiquivilo River). **D.** Area and sites explored during the second field campaign (surroundings of Laguna El Dial). See details of the localities and the populations discovered in Table 1. Orange lines represent the boundary between Chile and Argentina; thinner yellow lines indicate the boundaries of the administrative regions of Chile.

was visited once and was explored by three or four people for between one and three hours, using visual encounter and refugia search (cavities between rocks under water) surveys. Most of sites were explored at night, except for one small stream that flows into a tributary of the Guaiquivilo River (Cajón Lagunitas, site 4; Table 1).

Table 1. Geographic data and information about the specimens observed in the 19 localities of *Alsodes* surveyed in the western slopes of Andes (Chile, 35°58’–36°32’S). Localities are grouped by explored area, ordered from north to south (Fig. 1). Two of the sites explored had previously been described in the literature (citations in parentheses). The small streams that flow into the northern edge of the Laguna del Maule (“Stream km xxx”) were named according to their location along the international road CH-115. Stream km 154.7 corresponds to the second locality of Correa et al. (2013). The relative number of observed tadpoles is based on a gross estimation of more (numerous) or less (a few) than 100 specimens approximately. Phenotypes refer exclusively to adults and/or juveniles (some adult males are depicted in Fig. 2). The codes of buccal mucosa or tail fin samples per locality used for the phylogenetic analysis are indicated (m: male; f: female; j: juvenil; t: tadpole).

Locality	Latitude (S)	Longitude (W)	Altitude (m a.s.l.)	Specimens observed	Phenotypes	Sample codes
Paso Pehuenche (Corbalán et al. 2010)	36°00’01”	70°24’14”	2463	> 25 adults; many juveniles; numerous tadpoles of different sizes	*A. pehuenche*	-
Stream km 154.7 (Correa et al. 2013)	35°59’55”	70°26’31”	2215	> 20 adults; numerous tadpoles of different sizes	*A. pehuenche*	DBGUCH1203027 (sequence from GenBank)
Stream km 153.4	36°00’02”	70°27’18”	2225	Eight adults, one juvenile; a few tadpoles of medium size	*A. pehuenche*	-
Stream km 152	36°00’20”	70°27’54”	2270	Four adults; a few tadpoles of medium and small size	*A. pehuenche*	-
Stream km 148.6	36°00’46”	70°29’33”	2375	Seven adults; a few tadpoles of medium and small size	*A. pehuenche*	-
Stream km 148.2	36°00’46”	70°29’51”	2418	10 adults, two juveniles; a few tadpoles of medium size	*A. pehuenche*	-
Stream km 143.5	36°00’52”	70°31’12”	2372	No amphibian was observed		
Lo Aguirre Grande	35°59’35”	70°35’24”	2260	> 25 adults; many juveniles; numerous tadpoles of different sizes	*A. pehuenche*	AgGr1m, AgGr19m
Lo Aguirre Chico	35°58’53”	70°36’14”	2316	> 25 adults; many juveniles; numerous tadpoles of different sizes	*A. pehuenche* (Fig. 2F)	AgCh2f
Cajón Lagunitas (site 1)	36°07’58”	70°50’52”	2026	Two adult males and two juveniles; numerous tadpoles of great size	similar to *A. hugoi*	-
Cajón Lagunitas (site 2)	36°08’37”	70°50’43”	1913	Two adult males and one female; numerous tadpoles of different sizes	similar to *A. hugoi*	CL2-1m, CL2-2m
Cajón Lagunitas (site 3)	36°07’11”	70°50’53”	2157	> 10 adult males and females; a few tadpoles of medium and great size	similar to *A. hugoi* (Fig. 2E)	CL3-1m, CL3-2f, CL3-3m, CL3-4m, CL3-5m, CL3-6f
Cajón Lagunitas (site 4)	36°08’48”	70°50’13”	1805	A few tadpoles of different sizes	Undetermined	-
Vega Sin Nombre	36°09’52”	70°51’49”	2046	A few tadpoles of great size	Undetermined	VSN1t
Vegas de Cisternas	36°26’47”	71°03’27”	1967	One adult male; numerous tadpoles of different sizes	similar to *A. hugoi* (Fig. 2A)	VC1m, VC2t
Cajón de Plaza	36°23’40”	71°01’52”	1966	Seven adults, four juveniles; a few tadpoles of great size	similar to *A. pehuenche* (Fig. 2B)	CP1f, CP2m, CP4m, CP5m, CP9m, CP10m, CP11f
Vegas de Andrade	36°28’36”	71°00’15”	1998	One adult male; numerous tadpoles of different sizes	similar to *A. hugoi* (Fig. 2C)	VA1m, VA2t
Vegas de Miranda	36°28’16”	71°03’21”	1972	Two adults, five juveniles; a few tadpoles of great size	Undetermined (most) (Fig. 2D) and *A. hugoi* (one juvenile)	VM2j, VM3j, VM4m, VM5m, VM6m, VM7j, VM10j
Río Bueno	36°31’41”	71°03’19”	1836	One juvenile; a few tadpoles of great size	similar to *A. hugoi*	RB1j, RB2t, RB3t

Molecular data and phylogenetic analysis

For extracting DNA, we used mainly buccal mucosa of adults and juveniles obtained with Copan 516CS01 swabs (immediately dried with silica gel). Individuals were released at the same capture site after being measured and photographed. A few unidentified tadpoles were also sampled. A small piece of the end of the tail fin from these individuals (3 mm × 3 mm, approximately) was excised for DNA extraction. For this procedure, the tadpoles were anesthetized with buffered MS222 (tricaine methanesulfonate, 0.2%; Mitchell 2009) and then released after being submerged in fresh water for about half an hour to remove the anesthetic. DNA was extracted with the Promega Wizard SV Genomic DNA Purification System kit. A fragment of the mitochondrial cytochrome b gene (cytb) was used for the phylogenetic analysis. Information about primers (MVZ15-L and ControlP-H) and PCR conditions for amplifying this fragment are found in Correa et al. (2013). PCR products were sequenced in both directions in an ABI3730XL automatic sequencer (Macrogen Inc., Seoul, South Korea). Sequences were edited and aligned manually with the program BIOEDIT v7.1.3 (Hall 1999). Substitution saturation of the sequences was assessed with DAMBE v6.4.29 (Xia 2017). Sequences were deposited in GenBank (MH332789–MH332821 and MH378965–MH378971).

We performed a phylogenetic analysis to assess the specific identity of the new *Alsodes* populations. In this analysis 14 of the 19 species of *Alsodes* were represented, including specimens from the type localities of *A. pehuenche* and *A. hugoi*. This set of specimens represents the maximum number of species of the genus that can be included by combining all published sequences (Blotto et al. 2013, Correa et al. 2013, Charrier et al. 2015). Most of the sequences of Blotto et al. (2013) are shorter (about one third) than those of the other sources, so we included additional specimens of some species to evaluate possible topological artifacts due to the unequal length of the sequences. We included specimens from 11 of the 17 new localities (excluding streams very close to each other or sites located in the same water system). Phylogenetic relationships were estimated using a Bayesian inference (BI) method, performed with the program MRBAYES v3.2.6 (Ronquist et al. 2012). A reversible-jump Markov Chain Monte Carlo (MCMC) method for exploring the space of all General Time Reversible sub-models, plus gamma and proportion of invariable sites parameters, was applied to the entire cytb fragment. Two independent analyses (each consisting of two groups of four chains that run independently) applying that method were run for 20 million generations, sampling every 1000 generations. The first 25% of generations was conservatively discarded as burn-in after observing the stationarity of ln-likelihoods of trees in TRACER v1.6 (Rambaut et al. 2014). Convergence and mixing of chains were assessed by examining values of average standard deviation of split frequencies (ASDSF), and expected sampling sizes (ESS) and Potential Scale Reduction Factor (PSRF) for all parameters. Trees were rooted with one specimen of *Eupsophus emiliopugini* Formas, 1989, a representative of the sister genus of *Alsodes* (Blotto et al. 2013).

Results

Considering the three field campaigns we found tadpoles of *Alsodes* in 18 of the 19 sites explored (Fig. 1, Table 1). In 16 of those 18 sites we also observed adults and/or juveniles (see their tentative identifications in Table 1). The only site where no specimen was observed was a relatively short and narrow stream located north of Laguna del Maule (Stream km 143.5, Fig. 1), which at the sampling date (January) had an intermittent flow. Most of the other streams had a continuous flow, except Vegas de Miranda and Vega Sin Nombre. In both sites we observed large tadpoles in isolated remnant pools located in dry sectors of the streambeds. Tadpoles could not be identified to species by their external characteristics, so their identification relied exclusively on the phylogenetic analysis. Some of the adults and juveniles were tentatively identified as *A. pehuenche* or *A. hugoi* by their external characters (Table 1), but most specimens from Vegas de Miranda could not be identified to species level (Fig. 2D).

We obtained an alignment of the cytochrome b of 933 nucleotide sites, although this matrix included a few sequences of Blotto et al. (2013) of 318 base pairs. We did not observe effects of saturation or stop codons in the cytb sequences. The phylogenetic analysis shows that all the new populations are related to *A. pehuenche* or *A. hugoi* (most) (Fig. 3). All specimens from Laguna del Maule and surroundings comprise one well-supported clade together with the reference samples of *A. pehuenche* (from the type locality, Valle Pehuenche, Blotto et al. 2013, and from Paso Pehuenche and Stream km 154.7, Correa et al. 2013). The geographic distribution of this lineage implies a range extension of this species of 14.5 km to the west (Lo Aguirre Chico and Lo Aguirre Grande, Fig. 1B). Within this clade also four specimens from Cajón de Plaza (Figs 1 and 2) are included (see comment below). The genetic divergence among the specimens within this group is extremely low, including some that have identical sequences coming from localities separated by more than 60 km (Lo Aguirre Grande and Cajón de Plaza, Fig. 1). On the other hand, almost all samples coming from the ten southern sites (field campaigns two and three, Figs 1C and 1D), which are genetically and phenotypically more variable (Fig. 2), comprise two well-supported sister clades in the Bayesian consensus tree (Fig. 3). One of these clades includes the representative of the type locality of *A. hugoi* (Altos de Vilches). These clades do not exactly match the two groups of geographically closest localities, since specimens of some localities are distributed into the two clades, so we consider them as conspecific with that species. Although the specimens that constitute these two groups come from localities separated by up to 100 km they have very low genetic distances, which reinforces the hypothesis of their conspecificity. The population of Cajón de Plaza (Fig. 1D), represents a special case, since there specimens with sequences of the *A. pehuenche* and *A. hugoi* type are mixed, but they all resemble *A. pehuenche* phenotypically (Fig. 2B).

In summary, the phylogenetic analysis indicates that all sampled individuals belong to only two previously known species in the Andes Range between 35°30’ and 36°S, *A. pehuenche* or *A. hugoi*. These results imply the extension of the distribution range of *A. pehuenche* 14.5 km to the west in Chile, and of *A. hugoi* of 100 km to the south. Also, the

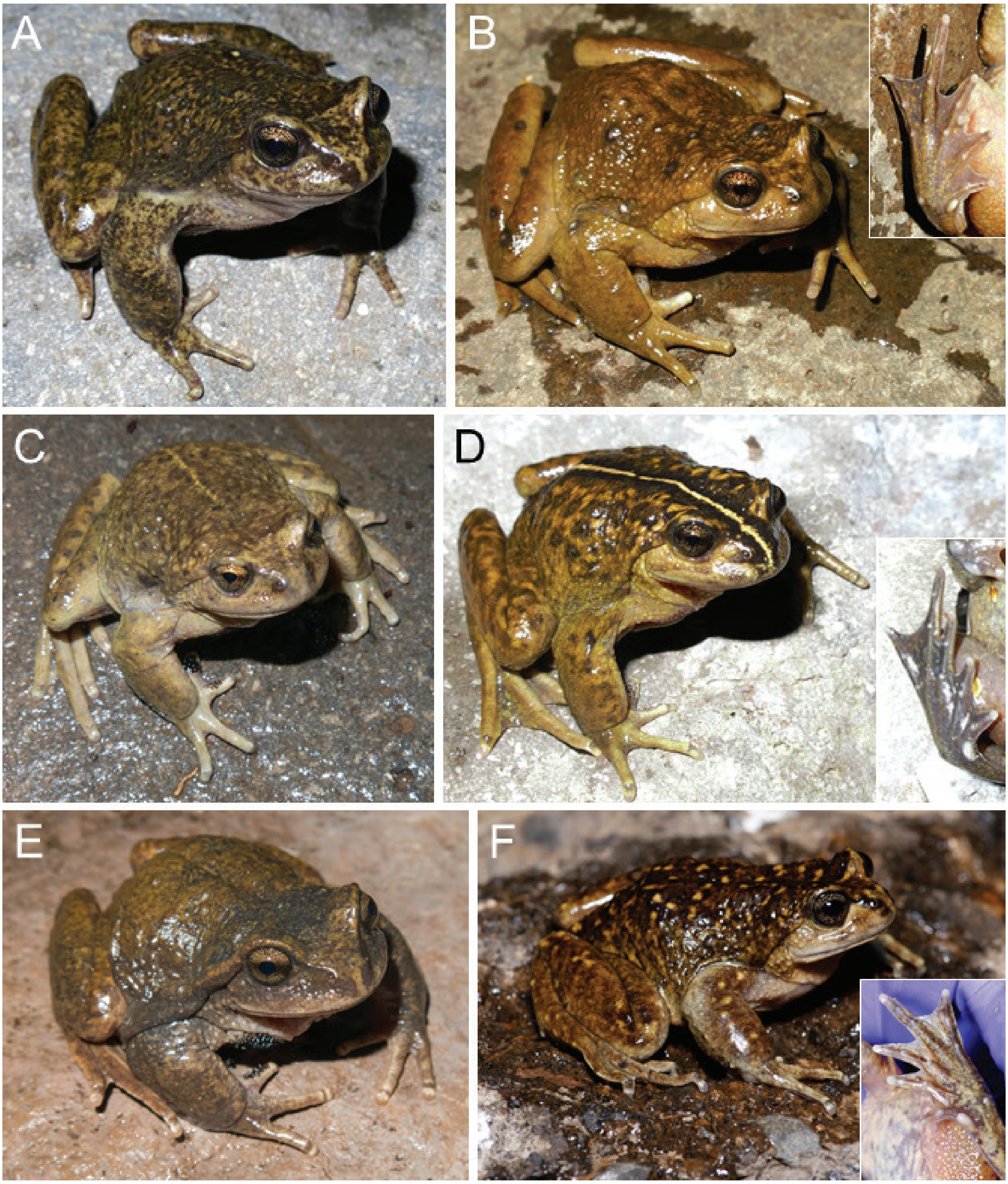

Figure 2. Adult males of *Alsodes* from the new discovered localities. In parentheses the specific identification according to the phylogenetic analysis (Fig. 3), the snout-vent length (SVL) and code of the respective buccal mucosa sample are indicated. **A.** Vegas de Cisternas (*A. hugoi*, SVL = 68.3 mm, VC1m). **B.** Cajón de Plaza (undetermined, SVL = 54.8 mm, CP5m). **C.** Vegas de Andrade (*A. hugoi*, SVL = 57.2 mm, VA1m). **D.** Vegas de Miranda (*A. hugoi*, SVL = 56.3 mm, VM6m). **E.** Cajón Lagunitas (site 3) (*A. hugoi*, SVL = 71.7 mm, CLP3-5m). **F.** Lo Aguirre Chico (*A. pehuenche*, SVL = 52.0 mm, AgCh4m). Some populations are characterized by well-developed interdigital webbing in the hind feet (shown in the insets).

altitudinal range of *A. hugoi* was slightly increased since 2115 m (Araya and Cisternas 2008) to 2157 m (Table 1). We do not recognize Cajón de Plaza as a new population of *A. pehuenche* because even though all the specimens we observed resemble this species externally (Fig. 1B), some of them have mitochondrial sequences of the *A. hugoi* type. More phenotypic and genetic data are needed to determine the taxonomic status of this population.

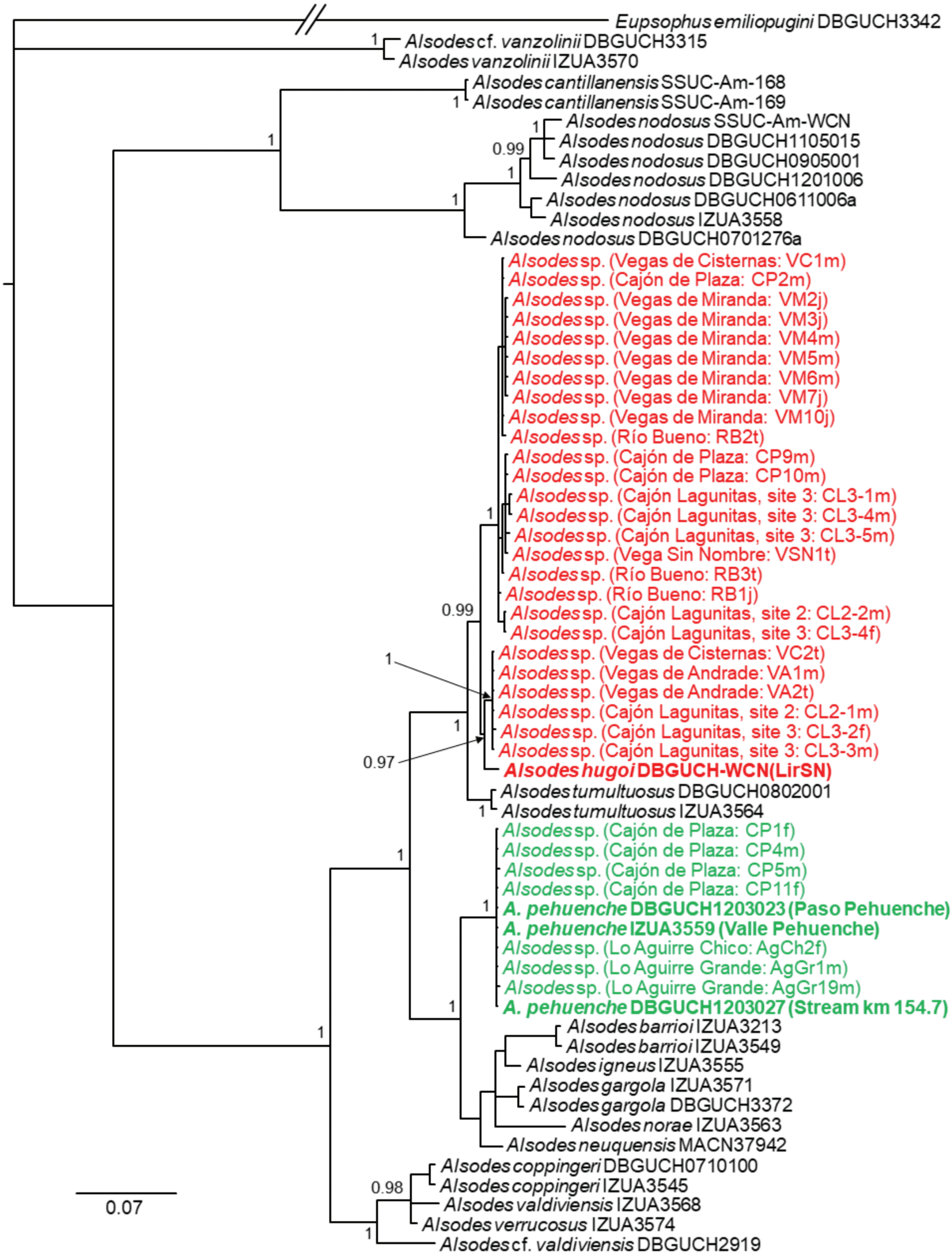

Figure 3. Bayesian consensus tree (50% majority-rule) showing the relationships of the new Andean populations of *Alsodes*. Representatives of the new populations are labeled in green (related to *A. pehuenche*) and red (related to *A. hugoi*) (see details of the new localities in Table 1). Note that specimens from Cajón de Plaza are distributed in both the red and green clades. Reference sequences of the type localities of *A. pehuenche* and *A. hugoi* are in bold. Numbers next to the nodes correspond to posterior probabilities (only values ≥ 0.95 of the more internal nodes are shown). The scale bar in the lower left corner represents the expected substitutions per site along the branches.

Discussion

This study reports new localities of two species of *Alsodes* endemic to the Andes Range, whose geographic distributions were considered extremely restricted. These findings add to a series of Andean populations of *Alsodes* reported in the last decade between 33°25' and 36°S in Chile (Araya and Cisternas 2008, Araya and Riveros 2008, Correa et al.

2008, Corbalán et al. 2010, Correa et al. 2013, Mora et al. 2015, Ramírez 2015, Correa 2017), which have expanded considerably our knowledge about the geographic distribution of the genus. Below we discuss the implications of these discoveries for the biogeography and conservation of the species involved and the genus.

The localities of *A. pehuenche* reported here constitute the third and widest distribution extension since the species was described (Cei 1976), more than doubling the known distribution until 2013 (Correa et al. 2013). Moreover, the occurrence of this species is extended to another hydric system in Chile (Lo Aguirre Grande and Lo Aguirre Chico), not connected directly to Laguna del Maule (Fig. 1). However, the area of occupancy is still very small as this species is restricted to watercourses (see comment below). Although all known sites in Chile are found within the hydrographic basin of the Maule River, it is possible that currently populations from Lo Aguirre Grande and Lo Aguirre Chico are demographically disconnected from those streams that discharge directly into the Laguna del Maule. The new localities also allow us to discard the presence of *A. montanus* around Laguna del Maule mentioned by Cei and Roig (1965). We could not confirm the presence of *A. pehuenche* south of 36°S. Although in Cajón de Plaza we found specimens phenotypically similar to *A. pehuenche*, even with mitochondrial sequences identical to those found in the type locality, they coexist with individuals with sequences of *A. hugoi*. Currently, we have no additional data to identify the possible causes of this mixture of mitochondrial sequences, so we consider the taxonomic status of this population as uncertain. The distribution extension of *A. hugoi* reported here is much more significant, reaching the northeastern end of the Ñuble River basin about 100 km south of the type locality (which is in the Maule river basin). Moreover, this species presents high levels of geographical variation in attributes such as body coloration and development of interdigital webbing, which had been described so far only in another species of the genus, *A. gargola* (Blotto et al. 2013).

These findings have important implications for the biogeography of the genus at different spatial scales. The new localities discovered south of 36°S are in a stretch of the western slopes of the Andes of about 250 km where no populations of *Alsodes* were known. However, an apparent discontinuity in the distribution of the genus of approximately 180 km still persists, covering almost completely the mountainous zones of the Administrative Region of Biobío (Fig. 1A). The relief, altitude, climatic conditions and types of vegetation in this area of the Andes are similar to those found where the new populations of *A. hugoi* were discovered, so this distribution gap is probably due to a lack of exploration. The new localities of *Alsodes* recently reported in the Andes Range (see references above), several of them located in areas of difficult access, support this idea. One of the issues that remains to be clarified is the reciprocal distribution limits between *A. hugoi* and *A. pehuenche*, since *A. hugoi* is to the south and north of the Maule River, whereas *A. pehuenche* is restricted to the head of that river at an intermediate latitude. Although there seems to be an altitudinal segregation between them (*A. hugoi* is between 900 and 2157 m, *A. pehuenche* between 2215 and 2463 m; Table 1), the data are very scarce and fragmentary to establish if one species replaces the other along the water systems. The mixture of mitochondrial sequences at Cajón de Plaza is compatible with the syntopy of both species, but this issue must be assessed with more phenotypic and genetic data.

At a smaller spatial scale, the effectiveness of our searches, together with previous antecedents, suggest that the genus is present in practically all first order watercourses of the western slopes of the Andes. We found tadpoles in 18 of the 19 sites explored (adults and/or juveniles in 16), with a sampling effort of up to three hours per day per site. Similar patterns have been reported for species of the same genus in the Andes. For example, Araya and Cisternas (2008) found reproductive specimens of *A. hugoi* in the 10 sites that they explored, expanding the altitudinal range and the type of environments where this species inhabits (although they did not specify the time spent in the searches). Further north, at Potrero Grande (Metropolitan Region), Correa (2017) reported three localities of *A. montanus* and *A. tumultuosus* discovered in a three-day field campaign, two of them first order streams. Moreover, the relative ease of finding individuals in various stages of development in most localities reported here (for example, Paso Pehuenche, Lo Aguirre Grande and Lo Aguirre Chico; see Table 1) suggests high local abundance. It is important to note that these observations were made at night, when the activity of these species is greater. In many cases the tadpoles were observed immediately at the beginning of the surveys (at sunset) and the first adults or juveniles in less than half an hour. The presence of numerous tadpoles, and juveniles in some cases, indicates that first order streams are reproduction and recruitment sites that could harbor abundant populations. Although we did not quantify exactly the number of larvae, our estimates are compatible with the number of specimens and densities reported for the type locality of *A. pehuenche* (Corbalán et al. 2008, 2014). This last study showed that *A. pehuenche* has a prolonged larval development that lasts at least four years, a strategy that could also occur in other Andean species of the genus (see references in Corbalán et al. 2014). This development strategy would require hydric systems with permanent flow, so our observations of tadpoles in two intermittent watercourses suggest that adverse environmental conditions, such as a drought (Corbalán et al. 2014), could affect the recruitment.

The distribution range extensions of *A. pehuenche* and *A. hugoi* have the potential to change their conservation categories, as happened with *A. montanus* and *A. tumultuosus*, which recently were down-listed from Critically Endangered to Vulnerable due to significant range extensions (IUCN 2017). However, the known range of *A. pehuenche* remains extremely restricted and is not represented in any protected area. Moreover, it is unclear whether more than one subpopulation (as defined by the IUCN) can be considered to exist. This species, due to its aquatic habits, only occupies streams and contiguous swamps (Corbalán et al. 2008, 2010; C. Correa, D. Vásquez, personal observations), so its area of occupancy (AOO) is probably much less than its extent of occurrence (EOO). The estimates of the IUCN (2017) reflect these observations, considering an EOO of 9 km^2 and an AOO of about 5 km^2 (although this last figure would be an overestimation as it is based on lower resolution satellite images). In addition, it remains to be assessed whether the localities of the western Andean slopes (Chile) are genetically and/or demographically separated from those located on the eastern slopes (Argentina), since they belong to different drainage basins. However, the limit of the drainage basins, corresponding to the border crossing between both countries, does currently not seem to be an important geographical barrier since the nearest streams of both basins are less than 50 m apart. Preliminary mitochondrial data are consistent with the absence of a barrier, suggesting that the localities from both sides of the border can be considered as inhabited by one population (Correa et al. 2013; unpublished data). Some of the threats identified by Corbalán et al. (2010) (habitat alteration, contamination, aquatic invasive species, livestock, natural floods, climate change) and Ghirardi et al. (2014) (chytrid fungus) also might affect the new described localities. Until now we have only been able to verify the effects of livestock and the presence of garbage, which are particularly intense at sites close to the border crossing (here called Paso Pehuenche), but all sites may be potentially threatened by environmental factors such as natural floods and climate change. In the case of *A. hugoi*, its distribution is currently composed of three highly disconnected areas separated by 60 and 40 km approximately, so they surely represent at least three populations, only one of which is present in a protected area (the type locality). This species is threatened by forest fires and tourism in its type locality (IUCN 2017), but we detected the effects of transhumant livestock activities on all discovered sites.

The observations made in this study add to a series of antecedents that show that high Andean environments where *Alsodes* species inhabit are threatened by natural and anthropogenic causes (Araya and Riveros 2008, Corbalán et al. 2010, Charrier et al. 2017). In this context, the geographic data obtained in this study acquire a special relevance since they suggest that the genus is widely distributed in the Andes, so any type of anthropic intervention (for example, mining projects, hydroelectric plants, mountain recreational centers, intensive livestock) has the potential to affect some population. Therefore, although the knowledge of the biogeography of Andean *Alsodes* species has improved, several aspects must be considered to reassess their conservation status. First, the few known populations seem to be threatened by several natural and anthropogenic factors. Second, their known distribution ranges are highly fragmented so their estimated AOOs would be extremely small. And third, there is no information on population connectivity, abundance or demographic trends, so we recommend maintaining their categories until more population and distribution data are obtained.

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References

Araya S, Cisternas J (2008) Antecedentes sobre el hábitat, localización y anuros simpátricos a *Alsodes hugoi* (Amphibia, Cycloramphidae) en la localidad tipo. Boletín del Museo Nacional de Historia Natural 57: 127–131.

Araya S, Riveros E (2008) Ampliación del rango de distribución geográfica de *Alsodes montanus* (Amphibia, Leptodactylidae) a la Región de O'Higgins. Boletín del Museo Nacional de Historia Natural 57: 117–123.

Blotto BL, Nuñez JJ, Basso NG, Úbeda CA, Wheeler WC, Faivovich J (2013) Phylogenetic relationships of a Patagonian frog radiation, the *Alsodes* + *Eupsophus* clade (Anura: Alsodidae), with comments on the supposed paraphyly of *Eupsophus*. Cladistics 29(2): 113–131. https://doi.org/10.1111/j.1096-0031.2012.00417.x

Cei JM (1976) Remarks on some neotropical amphibians of the genus *Alsodes* from southern Argentina. Atti della Società Italiana di Scienze Naturali e del Museo Civico di Storia Naturale di Milano 117(3–4): 159–164.

Cei JM, Roig VG (1965) The systematic status and biology of *Telmatobius montanus* Lataste (Amphibia: Leptodactylidae). Copeia 1965 (4): 421–425. http://www.jstor.org/stable/1440989

Charrier A, Correa C, Castro C, Méndez MA (2015) A new species of *Alsodes* (Anura: Alsodidae) from Altos de Cantillana, central Chile. Zootaxa 3915(4): 540–550. http://dx.doi.org/10.11646/zootaxa.3915.4.5

Charrier A, Mora M, Correa C, Palma E (2017) Monitoreo y Conservación de Anfibios Alto Andinos de la Región Metropolitana. Imprenta América Ltda., Valdivia, 79 pp.

Corbalán V, Debandi G, Úbeda CA (2008) *Alsodes pehuenche* (NCN). Larval biology. Herpetological Review 39(4): 457–458.

Corbalán V, Debandi G, Martínez F (2010) *Alsodes pehuenche* (Anura: Cycloramphidae): Past, present and future. Cuadernos de Herpetología 24(1): 17–23. http://www.scielo.org.ar/scielo.php?script=sci_arttext&pid=S1852-57682010000100002

Corbalán V, Debandi G, Martínez F, Úbeda C (2014) Prolonged larval development in the Critically Endangered Pehuenche's frog *Alsodes pehuenche*: implications for conservation. Amphibia-Reptilia 35: 283–292. https://doi.org/10.1163/15685381-00002951

Correa C (2017) Refuting the revalidation of *Telmatobius laevis* Philippi 1902. Herpetological Journal 27(1): 69–78.

Correa C, Pastenes L, Iturra P, Calderón P, Vásquez D, Lam N, Salinas H, Méndez MA (2013) Confirmation of the presence of *Alsodes pehuenche* Cei, 1976 (Anura, Cycloramphidae) in Chile: morphological, chromosomal and molecular evidence. Gayana 77(2): 117–123. https://doi.org/10.4067/S0717-65382013000200006

Correa C, Sallaberry M, Iturra P, Collado G, Méndez MA (2008) Amphibia, Anura, Cycloramphidae, *Alsodes montanus*: New record and geographic distribution map. Check List 4(4): 467–471. https://doi.org/10.15560/4.4.467

Cuevas CC (2013) The identity of the Chilean frog *Alsodes laevis* (Philippi 1902) (Cycloramphidae): Synonymy and generic partitioning of the type series. Herpetological Journal 23(3): 145–152.

Cuevas CC, Formas JR (2001) A new species of *Alsodes* (Amphibia, Anura, Leptodactylidae) from Central Chile. Amphibia-Reptilia 22(2): 187–198. https://doi.org/10.1163/15685380152030418

Duellman WE (1979) The Herpetofauna of the Andes: Patterns of Distribution, Origin, Differentiation and Present Communities. In: Duellman WE (Ed.). The South American herpetofauna: its origin, evolution, and dispersal. Monograph of the Museum of Natural History, The University of Kansas 7: 371–459.

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

Ghirardi R, Levy MG, López JA, Corbalán V, Steciow MM, Perotti MG (2014) Endangered amphibians infected with the chytrid fungus *Batrachochytrium dendrobatidis* in Austral temperate wetlands from Argentina. Herpetological Journal 24(2): 129–133.

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

IUCN (2017) The IUCN Red List of Threatened Species. Version 2017-3. http://www.iucnredlist.org [accessed: March 10, 2018]

IUCN SSC Amphibian Specialist Group (2013) *Alsodes pehuenche*. The IUCN Red List of Threatened Species 2013: e.T56319A18136036. http://dx.doi.org/10.2305/IUCN.UK.2013-1.RLTS.T56319A18136036.en [Downloaded on 16 October 2017]

Mitchell MA (2009) Anesthetic considerations for amphibians. Journal of Exotic Pet Medicine 18(1): 40–49. https://doi.org/10.1053/j.jepm.2008.11.006

Mora M, Horta I, Soffia F, Charrier A, Palma E, Correa C (2015) Estudios para la conservación de los anfibios altoandinos de Chile central. Boletín Chileno de Herpetología 2: 8–11.

Rambaut A, Suchard MA, Xie D, Drummond AJ (2014) Tracer v1.6. http://beast.bio.ed.ac.uk/Tracer

Ramírez D (2015) Nueva localidad para *Alsodes tumultuosus* Veloso, Iturra & Galleguillos, 1979 (Amphibia, Alsodidae) en la Cordillera de los Andes, Región de O'Higgins, Chile. Biodiversity and Natural History 1: 1–5.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

Xia X (2017) DAMBE6: New tools for microbial genomics, phylogenetics and molecular evolution. Journal of Heredity 108 (4): 431–437. https://doi.org/10.1093/jhered/esx033

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