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A new species of *Alsodes* (Anura: Alsodidae) from Altos de Cantillana, central Chile

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Abstract

Based on morphological and molecular evidence (mitochondrial and nuclear sequences) we describe a new species of spiny-chest frog, *Alsodes cantillanensis*, from central Chile (around 34°S). The type locality, Quebrada Infiernillo, is located in the Coastal Range at approximately 65 km from Santiago (Metropolitan Region), the capital of Chile. The distribution of the new species is included entirely in that of *A. nodosus* (32–36°S approximately), which was identified as the sister taxon according to molecular phylogenetic analyses. Moreover, both species are sympatric in the type locality. The new species was found in a *Nothofagus macrocarpa* relict forest potentially threatened by gold mining activities. We identify other threats for its conservation and some biological data needed for understanding the evolution of this species. This discovery reveals the scarce knowledge about biogeography, evolution and ecology of spiny-chest frogs from central Chile.

Key words: spiny-chest frogs, microendemism, Chilean Mediterranean zone, *Nothofagus* forest, conservation

Introduction

The anuran genus *Alsodes* Bell currently is comprised of 18 species (Frost 2014), distributed in central-southern Chile (32–49°S) and in a narrow strip on the eastern slopes of the Andes in southwestern Argentina (36–44°S). *Alsodes* is the most diversified amphibian taxon present in Chile, where 17 species can be recognized (Correa *et al.* 2011; Blotto *et al.* 2013). Most of these species are microendemics inhabiting temperate forests from the Coastal and Andes ranges (Cuevas & Formas 2005), but there are four of them distributed in the Mediterranean zone (*A. nodosus* Duméril & Bibron, 32–36°S) or in high Andean environments (*A. montanus* Lataste, *A. pehuenche* Cei and *A. tumultuosus* Veloso, Iturra & Galleguillos, 33–36°S) from central Chile.

The taxonomy and systematics of *Alsodes* have been considered complex (Lynch 1978; Cei 1980; Díaz 1989; Wiens 1993; Blotto *et al.* 2013). Recently, Blotto *et al.* (2013) obtained the most complete phylogenetic hypothesis at date, which implies several changes in taxonomy and distribution: they identified a candidate species in Chile (related to *A. nodosus*), elevated to *A. neuquensis* Cei to full species status, and reported to *A. gargola* Gallardo for Chile. However, a thorough understanding of the phylogenetic relationships of this genus has not yet been achieved, because *A. australis* Formas, Úbeda, Cuevas & Nuñez (from the type locality), *A. montanus*, *A. kaweshkari* Formas, Cuevas & Nuñez, *A. monticola* Bell, and *A. vittatus* Philippi (the latter two have not been seen for over 100 years) were not included by Blotto *et al.* (2013). On the other hand, the geographic distribution, ecology, and natural history of most *Alsodes* species remain poorly known.

The genus has been diagnosed mainly by the absence of external tympanic ring and the presence of the following secondary sexual reproductive characters in males: bilateral spiny patches in the chest, thorny structures on fingers one and two, and thickened arms (Gallardo 1970; Cei 1980; Cuevas 2008). This last feature has earned some species the name of "Popeye frogs".

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In August 2011, during a field trip to Fundo San Juan de Piche, organized by the Ministerio del Medio Ambiente (Biodiversity and Natural Resources department) to explore an area that could be highly impacted by the installation of an open pit gold mine, we found a new species of *Alsodes*. This private area, declared a natural sanctuary in 2013, is located around 65 km southwest from Santiago, capital of Chile, in one of the administrative regions most impacted by human activity in the country. The type locality is a little stream surrounded by a pristine relict forest of *Nothofagus macrocarpa* trees. We obtained morphological and molecular evidence which support the distinction of the new taxon with respect to other species of central Chile. We also provide some future directions for the study of this new species and identify the main threats for its conservation.

Material and methods

Specimens. The description was based on two specimens, male and female, collected in the same stream of the type locality, Quebrada Infiernillo, about 850 m a.s.l. Animals were photographed alive, and notes of the habitat and color in life were taken in the field at the time of capture. Specimens used for the description were deposited in the Colección de Flora y Fauna Prof. Patricio Sánchez Reyes (SSUC) of the Ecology Department of the Pontificia Universidad Católica de Chile. Specimens were measured with a digital caliper to the nearest 0.1 mm. Abbreviations for the measurements are SVL (snout-vent length), HL (head length; posterior corner of jaw to tip of snout), HW (head width; from posterior corner of jaw), IOD (interorbital distance), ID (internarial distance), EL (eye length; from posterior to anterior corner), ThL (thigh length), TL (tibia length; knee to heel), and FL (foot length; proximal edge inner metatarsal tubercle to tip of fourth toe). These measurements were extracted of Wiens (1993).

Molecular data and phylogenetic analyses. For the phylogenetic analyses two specimens of the new species from the type locality (holotype and paratype), seven samples from seven localities of *A. nodosus* (including one male specimen from the type locality of the new species, SSUC-Am-170), one sample of each of three species of central Chile (32–36°S; *A. hugoi* Cuevas & Formas, *A. pehuenche*, and *A. tumultuosus*), and samples of other ten species of the genus (including sequences of the candidate species *Alsodes* sp. 1 of Blotto *et al.* 2013, here labeled as *A.* aff. *nodosus*) were used (Fig. 3 and Appendix I). Additionally, one specimen from R.N. Los Queules (36°S), here tentatively identified as *A.* cf. *vanzolinii* by its external characteristics, was included because it represents a distinct taxonomic entity from the coastal area immediately south of the distribution range of *A. nodosus*. Only five of the 18 species of the genus were not included: *A. australis*, *A. kaweshkari*, *A. montanus*, *A. monticola*, and *A. vittatus*. One species of the sister genus (*Eupsophus calcaratus* Günther), from Yaldad (Chiloé Island), was used as outgroup. DNA was extracted using a modification of the salt method of Jowett (1986) from thigh muscle. For the phylogenetic analyses, mitochondrial [a fragment of the gene cytochrome b (cytb) and other one which extends between ribosomal genes 12S and 16S, including the tRNA-Val (12S-16S)], and nuclear [fragments of rhodopsin exon 1 (rhod) and Seven in Absentia homolog I (SINA)] genes were used. Primers sequences and PCR conditions for amplifying the cytb (primers MVZ15-L and ControlP-H) and the fragment 12S-16S (16Sbr-H, 16Sar-L, 1216H, 1216LN, H1478, L1091) are found in Goebel *et al.* (1999), Correa *et al.* (2006), Correa *et al.* (2008a) and Correa *et al.* (2013). The primers for amplifying nuclear fragments are found in Bossuyt & Milinkovitch (2000) (rhod), and Bonacum *et al.* (2001) (SINA). PCR reaction mixture for rhod and SINA was (reaction volume of 30µL): 3.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.07 µM each primer, 1 U of Taq polymerase (Invitrogen), and 50–200 ng total DNA. PCR thermal profile for both genes was: 94° C for 1 min, followed by 38 cycles of 94° C for 45 sec, 55° C for 55 sec and 72° C for 50 sec, with a final extension at 72° C for 10 min. All mitochondrial and nuclear PCR products were sequenced in both directions in an ABI3730XL automatic sequencer. Sequences were edited with the program BioEdit v7.1.3 (Hall 1999) and deposited in GenBank under accession numbers KJ418844–KJ418852 (cytb), KJ418835–KJ418843 (fragment 12S-16S), KJ418853–KJ418861 (rhod), and KJ418862–KJ418870 (SINA). Edited sequences were aligned with ClustalX v2.1 (Larkin *et al.* 2007). For estimating phylogenetic relationships among *Alsodes* species, maximum parsimony (MP) and Bayesian inference (BI) analyses were performed with programs PAUP* v4.10b (Swofford 2002) and MrBayes v3.2.1 (Ronquist *et al.* 2012), respectively. For the MP analysis, a heuristic search was performed with one thousand replicates and random-addition of sequences, using the tree-bisection-reconnection branch-swapping algorithm. The statistical support for the nodes was calculated using 2000 seudoreplicates of non-parametric bootstrap with 20 random-addition-sequence replicates each, and a maximum of 200 trees saved per seudoreplicate. The gaps of the alignment of fragment 12S-16S were treated as a "fifth state" (an additional analysis was performed considering gaps as missing). For the BI analysis, 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 independently to each partition (one non-coding fragment, 12S-16S, and nine other partitions corresponding to codon positions of coding genes cytb, rhod and SINA). 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 1,000th generation. The first 10% of generations were 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 was assessed examining values of average standard deviation of split frequencies (ASDSF), and expected sampling sizes (ESS) and Potential Scale Reduction Factor (PSRF) for all parameters.

Results

Alsodes cantillanensis sp. nov.

Holotype. A male (SSUC-Am-168; Fig. 1A and Fig. 2) collected by Andrés Charrier and Alexander Baus in Quebrada Infiernillo, Fundo San Juan de Piche, Altos de Cantillana mountains (33°55'32.6"S, 71°02'34.6"W, 850 m a.s.l.; Fig. 3), on 3 August 2011.

Paratype. A female (SSUC-Am-169) collected by Andrés Charrier and Alexander Baus at the same place and date as the holotype.

Diagnosis. *Alsodes cantillanensis* can be distinguished from all other congeneric species for the following combination of characters: 1) thorny patches of the chest of males with spines uniformly distributed; 2) head with a triangle between the posterior border of the eyes and the snout tip, which contrasts with the background coloration; 3) superior part of the iris gold-yellowish brilliant, almost uniform; 4) postocular fold well-developed, extending beyond of the insertions of arms; 5) snout slightly truncated in dorsal and lateral profile; 6) dorsal skin smooth; 7) dorsal coloration ochre (holotype) or orange (paratype), with small and diffuse dark spots irregularly distributed; and 8) posterior legs slightly barred.

Description of the holotype. Male, SVL 45.9 mm. Head 1.24 times wider than long (Figs. 2A and 2B). HL 34.4% the SVL. Snout slightly truncated in lateral view (Fig. 2C). Nostrils with scarce relief and dorsolateral orientation, located midway between the tip of the snout and the anterior border of the eye. ID a quarter of the HW. EL 1.4 times the ID. Well-developed canthus rostralis, with a dark coloration on its underside, extending beyond the insertions of arms. Tympanic ring absent. Supratympanic fold well developed, without granulations. Chest with two clearly delimited bilateral nuptial round thorny patches. Spines of the chest patches minute, whitish and uniformly distributed (Fig. 2B). White thorny excrescences on the interior of the first and second fingers (Fig. 2D). Dorsum, arms, legs and ventral surface of body smooth. Granular flanks. Hands without webbing. Fingers with white rounded tips, in order of increasing length: II < I < IV < III (Figs. 2D and 2E). Outer metacarpal tubercle rounded, and bigger than the others tubercles (Fig. 2E). Inner metacarpal tubercle ovoid, smaller than the outer, but also well developed. One subarticular tubercle on digits I and II, and two on digits II and IV (Fig. 2E). Posterior legs with slightly marked dark transversal bars. Feet without webbing. Toes long, with white rounded tips, without lateral fringes, in order of increasing length: I < II < V < III < IV (Fig. 2F). Inner and outer metatarsal tubercles present, similar in size, the inner ovoid and the outer rounded. General dorsal coloration ochre, with a lighter triangle on the head between the posterior border of the eyes and the snout tip. A very thin yellowish vertebral line in the posterior half of the dorsum. Ventral coloration whitish. Measurements of the holotype (in mm) are, SVL: 45.9; HL: 15.8; HW: 19.6; IOD: 5.6; ID: 5.1; EL: 6.9; ThL: 24.6; TL: 24.1; FL: 41.4.

Comparison with congeneric species. *Alsodes cantillanensis* can be distinguished from *A. nodosus*, its sister species, for the following characters (Fig. 1) (characters of *A. nodosus* in parentheses): superior part of the iris gold-yellowish, almost uniform (black, with small yellow spots), a light triangle on the head (absent), dorsal skin smooth (granular), and a regular arrangement of spines in the chest patches (spines arranged in at least nine small clusters in each chest patch) (see description of *A. nodosus* in Cuevas 2013). Externally, *A. cantillanensis* resembles more *A. vanzolinii* Donoso-Barros: both taxa have dorsal smooth skin, a triangle of lighter color on the head (yellowish and much more marked in *A. vanzolinii*, where it contrasts with the lateral dark coloration of the head), and the upper part of the iris yellowish (although in *A. vanzolinii* the iris coloration is more discontinuous; see Fig. 1 of Rabanal & Alarcón 2010). However, *A. vanzolinii* has the dorsum dark brownish with yellowish spots and the posterior extremities strongly barred (Donoso-Barros 1974; Formas 1980; see Fig. 1 of Rabanal & Alarcón 2010). Furthermore, the phylogenetic analyses show that *A. cantillanensis* is closer to *A. nodosus* than to *A. vanzolinii*; these three species constitute one of the two main clades in which the genus is divided (Fig. 4). *Alsodes cantillanensis* also can be distinguished from the species of the other main clade by other characters. For example, the new taxon has no webbing toes, unlike *A. gargola*, *A. montanus*, *A. pehuenche*, and *A. tumultuosus*; it lacks of a black triangular zone on top of head extending backwards from space between the eyes, present in *A. hugoi*, *A. igneus* Cuevas & Formas, *A. barrioi* Veloso, Díaz, Iturra & Penna, and *A. norae* Cuevas; and it also differs of *A. monticola*, which has a green-grass dorsal color, of *A. verrucosus* Philippi which has the dorsal skin granular, and of *A. vittatus*, which has a well-defined vertebral line extending from the tip of the snout to the vent. Finally, *A. cantillanensis* lacks of folds of loose skin on the lateral areas of the body like *A. australis*, *A. coppingeri*, and *A. kaweshkari*. Furthermore, all these species have allopatric distributions with respect to *A. cantillanensis* (see Cuevas & Formas 2005).

FIGURE 1. Specimens of *Alsodes cantillanensis* sp. nov. (**A**, holotype, SSUC-Am-168) and *A. nodosus* (**B**, adult male, not captured) from Quebrada Infiernillo. **C** and **D** show details of the irises of *A. cantillanensis* and *A. nodosus*, respectively.

Etymology. The specific name of the new taxon is the genitive Latin form of Cantillana, part of the name (Altos de Cantillana) of a mountain system with foothills covered by forests close to Santiago (65 km).

Variation. Outside of the obvious differences due to the sex of the specimens of the type series (patches of spines on the chest and arms thicker in the male), the most remarkable difference between them is the dorsum background coloration: the holotype is ochre, with a lighter triangle on the head, and the paratype is orange, with the triangle reddish-orange. The holotype and other individuals observed in the type locality and in Quebrada Lisboa (see below) had a thin yellow vertebral line in the posterior part of the dorsum. These individuals exhibited an overall ochre coloration, but with different tones. Measurements of the paratype (in mm) are, SVL: 44.2; HL: 14.9; HW: 18.7; IOD: 5.3; ID: 4.7; EL: 6.4; ThL: 24.6; TL: 25.3; FL: 39.6.

Distribution and natural history. The new species is only known from the type locality and one nearby site, Quebrada Lisboa (33°55'33.6"S, 71°01'05.3"W, 729 m a.s.l), both within the Natural Sanctuary San Juan de Piche, in the western slopes of the Altos de Cantillana mountains (Fig. 3). Altos de Cantillana mountains have a maximum height of 2281 m a.s.l. and are located in an area with Mediterranean climate that has less than 600 mm of rain per year and an average temperature of 10.7°C (Costa 2002). *Alsodes cantillanensis* inhabits mixed old grown forest of *Nothofagus macrocarpa*, *Drymis winteri*, *Mirtacea* sp., and vains of the genus *Proustia*. Specimens of the type series were found under rocks under the water during spring in the morning. Others (not collected) were found under a thick cover of litter fall near the stream (30 cm) and under logs close to the water. Additional specimens were observed at night walking along the stream of Quebrada Lisboa. In the creek at the type locality, the amphibians *Alsodes nodosus* ("sapo arriero") and *Pleurodema thaul* (four-eyed frog) also were found.

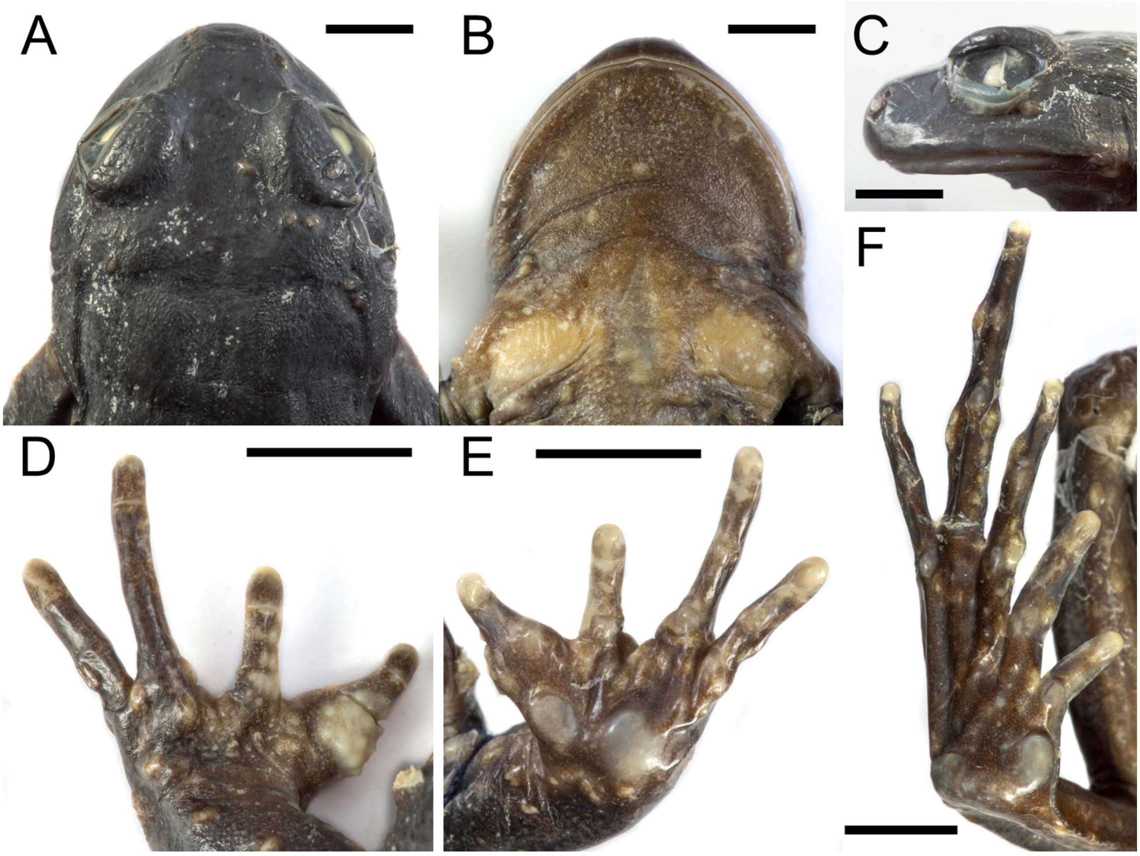

FIGURE 2. Morphological details of the holotype of *Alsodes cantillanensis* sp. nov. (SSUC-Am-168). **A**. Dorsal view of the head. **B**. Ventral view of the head. Note the spines patches on the chest. **C**. Lateral view of the head. **D**. Dorsal view of the left hand. Note the nuptial pads on fingers one and two, formed by small whitish spines. **E**. Ventral view of the left hand. **F**. Ventral view of the right foot. Bars equal 5 mm.

Molecular data and phylogenetic relationships. The combined alignment of the four fragments had 3024 nucleotide sites in length (318 of cytb, 1999 of the fragment 12S-16S, 310 of rhod, and 397 of SINA). Only the fragment 12S-16S showed gaps (36 sites, including the outgroup). MP analysis including the sites with gaps was based on 359 parsimony-informative characters. Thirteen most parsimonious trees were found, with a length of 878 steps, which differ only in the relative position of *A. gargola*, *A. igneus*, *A. neuquensis*, *A. norae*, and *A. pehuenche* (data not shown; see consensus tree in Fig. S1). MP analysis treating gaps as missing also produced 13 trees (829 steps, 339 parsimony-informative characters) differing only in the relative position of the same taxa (data not shown). Regarding the BI, the post burn-in mean ln-likelihood values of the two independent analyses were -8296.26 and -8297.08. Convergence and mixing of chains were achieved according to ASDSF (< 0.002), ESS (> 200), and PSRF (0.9999–1.0001) values. Results of these analyses are shown as the 50% majority-rule consensus tree (Fig. 4), where bootstrap values of MP analysis including sites with gaps were added. Topologies of MP and BI analyses were highly congruent, showing two main clades for the genus with high statistical support (Fig. 4), which agrees with the hypothesis of Blotto *et al.* (2013). One clade is comprised by *A. nodosus*, *A. cantillanensis*, and *A. vanzolinii*, including the specimen identified a priori as *A.* cf. *vanzolinii*, and the other by the rest of *Alsodes* species. Within this main clade, two groups can be recognized also with high MP bootstrap support and Bayesian posterior probability: one comprised by all samples of *A. nodosus* (including one specimen from Pemehue, here labeled as *A.* aff. *nodosus*, a candidate species according to Blotto *et al.* 2013) and the two samples of the new species, and the other by the specimen of *A. vanzolinii* from Ramadillas (type locality) and the specimen of *A.* cf. *vanzolinii* from R.N. Los Queules. Within the second main clade, the group comprised by *A. coppingeri*, *A. valdiviensis*, and *A. verrucosus* diverges first, followed successively by *A. hugoi* plus *A. tumultuosus*, and *A. barrioi* (Fig. 4). The rest of species, *A. gargola*, *A. igneus*, *A. neuquensis*, *A. norae*, and *A. pehuenche*, form a politomy in the consensus tree. In summary, MP and BI analyses show that all samples of *A. nodosus* group with high support values and constitutes the sister group of the new species, ratifying the morphological distinction between both taxa (Fig. 1). However, the more compelling evidence to support the specific status of *A. cantillanensis* is that is sympatric and clearly diagnosable of *A. nodosus* at Quebrada Infiernillo.

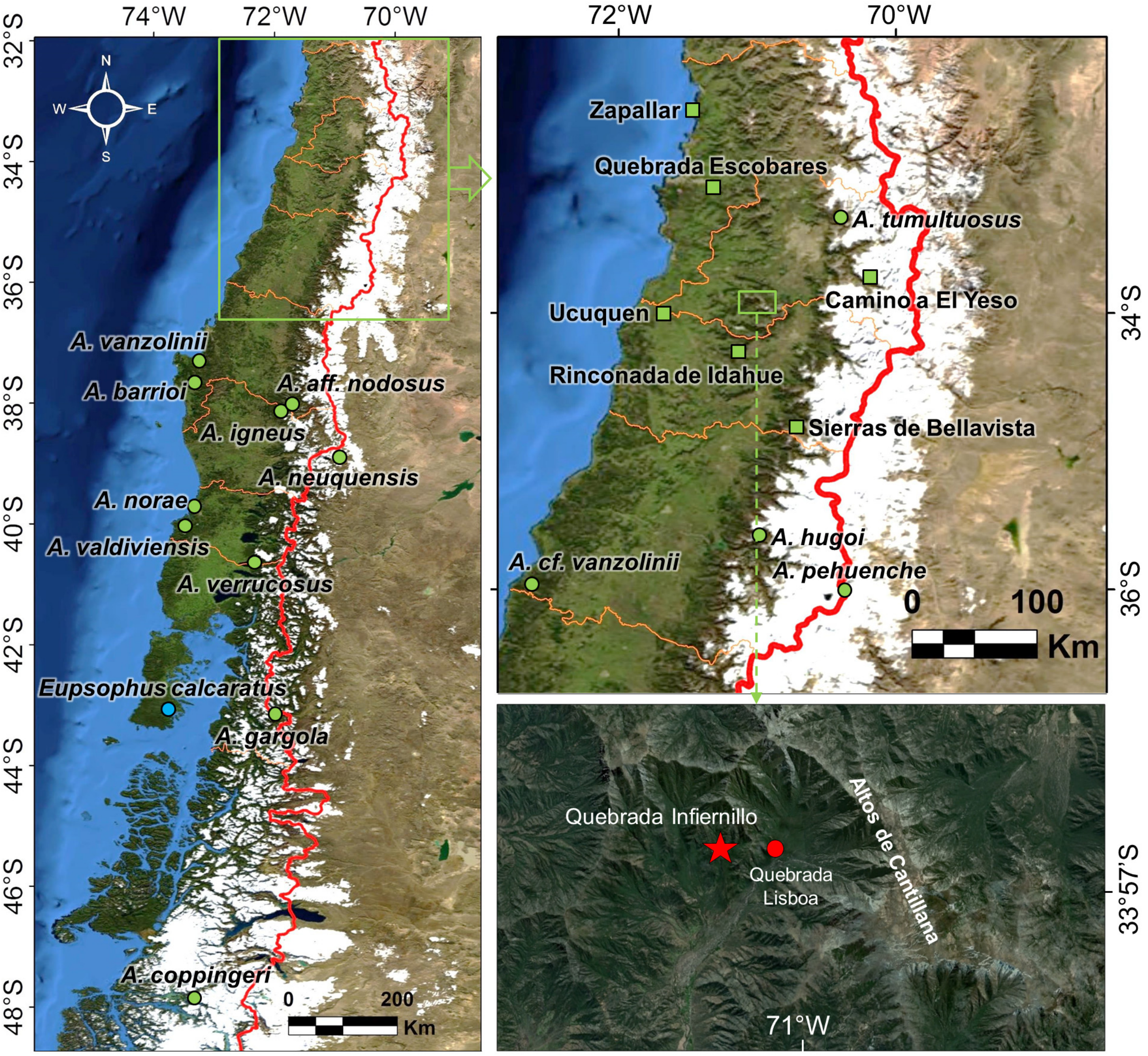

FIGURE 3. Localities of *Alsodes* and *Eupsophus* (outgroup) samples included in the phylogenetic analyses. Circles indicate localities of all species of *Alsodes*, except for those of *A. nodosus* (squares, right map). The map of the right inferior corner shows the area of Altos de Cantillana mountains where the type locality (red star) and the other known site of *A. cantillanensis* sp. nov. are located.

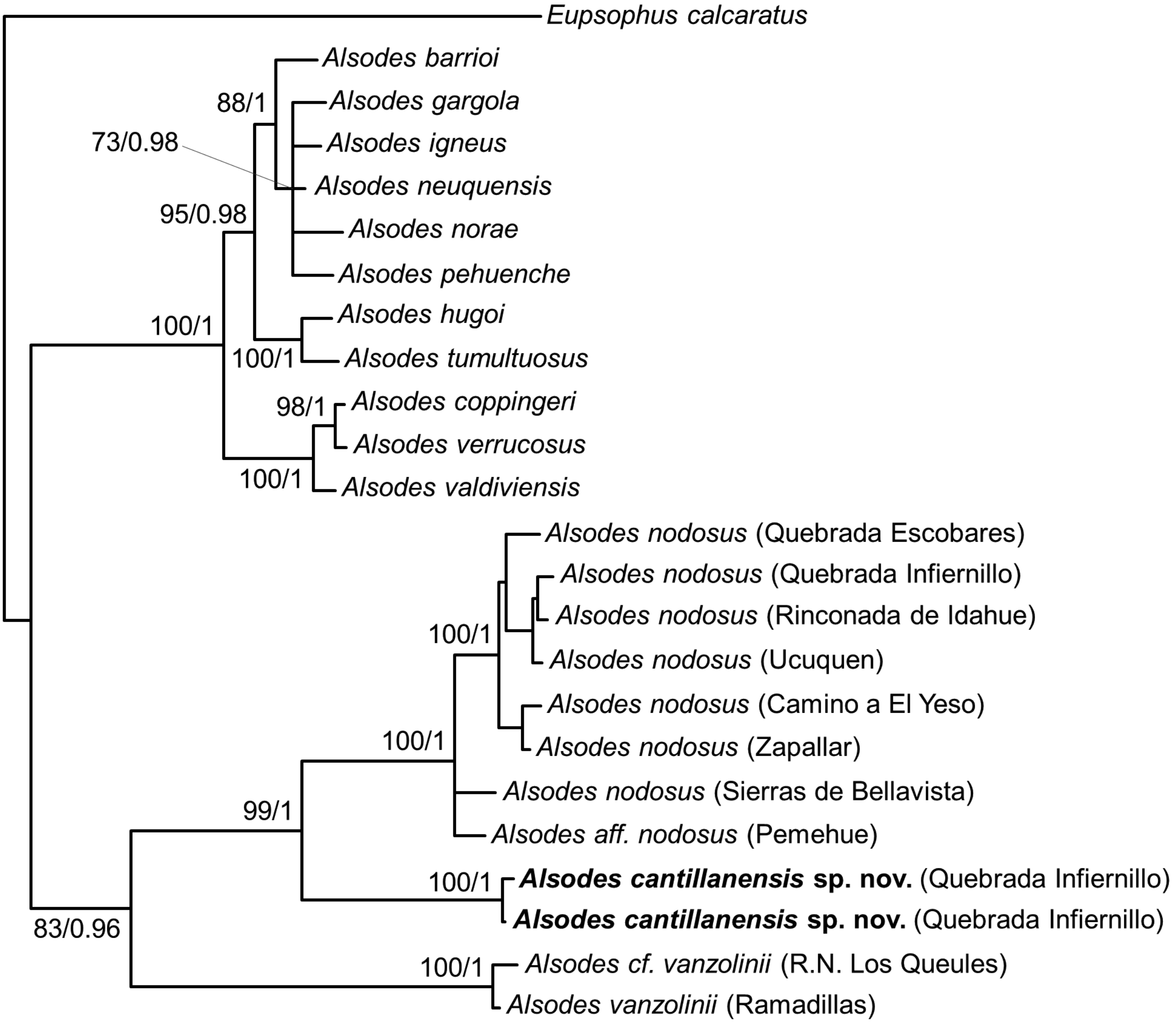

FIGURE 4. Bayesian inference consensus tree (50% majority-rule) showing the position of the new species, *Alsodes cantillanensis* (in bold), within the genus. Numbers next to the nodes, separated by slashes, correspond to bootstrap values of maximum parsimony analysis, treating gaps as a fifth state, and posterior probability of Bayesian inference, respectively.

Discussion

Morphological characteristics and molecular evidence show that the new taxon belongs to the genus *Alsodes*. The holotype and the paratype show two diagnostic characters of the genus: bilateral thorny patches in the chest of males (only in the holotype and apparently not completely developed) and absence of external tympanic ring (Gallardo 1970; Cuevas 2008). The molecular evidence shows that the new species groups with two other endemic species from central Chile, *A. nodosus* and *A. vanzolinii*, distributed between 32 and 38°S, and it is not related directly with the Andean species *A. hugoi* and *A. tumultuosus*, distributed between 33 and 36°S.

According to the phylogenetic analyses, *A. cantillanensis* and *A. nodosus* are sister species. Nevertheless, this relationship is not accompanied by a morphological similarity between both species. In fact, some characteristics of the new species, like the continuous distribution of the spines of the chest patches, the golden-yellowish coloration of the superior part of the iris, the triangle on the head and the smooth skin, are observed in some species from southern Chile and Argentina (37–49°S), but not shared with *A. nodosus*. These two species also show an unusual geographic distribution pattern within the genus, which is mainly comprised by allopatric species (Cuevas & Formas 2005). The type locality of *A. cantillanensis* is completely surrounded by localities of *A. nodosus*, where a population of this last species also was found. In fact, both species inhabit the same creek and reproduce apparently in the same ponds.

This finding demonstrates the poor scientific knowledge we have about biogeography and evolution of the genus *Alsodes* in Chile. For example, in recent years a species of the Coastal Range from Chile was described (Cuevas 2008) and new localities from Los Andes Range were reported (Araya & Riveros 2008; Correa *et al.* 2008b; Corbalán *et al.* 2010). More recently, Blotto *et al.* (2013) obtained the most complete phylogenetic hypothesis for the genus at date, proposing taxonomic changes, identifying a candidate species for Chile, and reporting to *A. gargola* for this country. Our phylogenetic analyses recovered the main subdivisions of the genus obtained by Blotto *et al.* (2013), showing that the new species is related to *A. nodosus* and *A. vanzolinii*, both species with significant differences in morphology and chromosome number. The combination of external characters of *A. cantillanensis*, more similar to *A. vanzolinii*, and its distribution, entirely included within that of *A. nodosus*, reveal an interesting aspect of the evolution of *Alsodes* from central Chile. So, future research directions could be the potential mechanisms that would maintain the reproductive isolation between both species (number of chromosomes, differences in mating call, niche segregation) and the extension of overlap of their distribution ranges.

Quebrada Infiernillo, the type locality, and the other creek where *A. cantillanensis* was observed are located in a private property, within of the recently formed Natural Sanctuary San Juan de Piche, thus the species is now territorially protected. However, the area near Quebrada Infiernillo historically has been impacted by charcoal industry and now it is threatened by the potential construction of an open pit gold mine (according to the Chilean legislation the Natural Sanctuary status does not exclude the possibility for developing mining projects). An additional threat during the summer are the forest fires, such as that occurred in January 2012. In a wider geographical context, all the area surrounding Altos de Cantillana mountains, where Quebrada Infiernillo is located, is highly impacted by agriculture, livestock and exotic trees plantations. In fact, historically this is one of the most impacted zones of central Chile by human occupation and land use change (Armesto *et al.* 2010; Schulz *et al.* 2010).

The discovery of a new amphibian species in Altos de Cantillana ratifies the high biodiversity richness of this and other mountain systems located near Santiago. Currently, these systems can be considered islands of biodiversity, surrounded completely by land of human use, so we hope this discovery draw attention of the need to preserve them and stimulate new studies for describing the biodiversity components of these mountains. Finally, the new taxon should be considered critically endangered due to its restricted distribution, and the high degree of threat represented by possible fires and gold mining activities.

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APPENDIX I. Localities, collection numbers and GenBank accession numbers of the previously available sequences included in the phylogenetic analyses. In this study, *Alsodes* sp. is labeled *A.* aff. *nodosus*. All localities are in Chile, except that of *A. neuquensis*, which is in Argentina.

Species	Locality	Collection Number	cytb	12S-16S	rhod	SINA
Alsodes barrioi	Rucapehuén	IZUA 3549	JX203940	JX204153	JX204089	JX204224
Alsodes coppingeri	Puerto Río Frío	IZUA 3545	JX203943	JX204156	JX204092	JX204227
Alsodes gargola	Futaleufú	IZUA 3571	JX203949	JX204162	JX204098	JX204233
Alsodes hugoi	Altos de Vilches	IZUA 3554	JX203956	JX204169	JX204102	JX204237
Alsodes igneus	Tolhuaca	IZUA 3555	JX203957	JX204157	JX204103	JX204238
Alsodes neuquensis	10 km O Primeros Pinos	MACN 37942	AY843787	AY843565	AY844539	AY844767
Alsodes nodosus	Zapallar	IZUA 3558	JX203960	JX204174	JX204107	JX204241
Alsodes norae	Parque Oncol	IZUA 3563	JX203961	JX204175	JX204108	JX204242
Alsodes pehuenche	Valle Pehuenche	IZUA 3559	JX203962	JX204176	JX204109	JX204243
Alsodes sp.	Pemehue	IZUA 3543	JX203965	JX204180	JX204112	JX204245
Alsodes tumultuosus	La Parva	IZUA 3564	JX203968	JX204183	JX204115	JX204248
Alsodes valdiviensis	Cordillera Pelada	IZUA 3568	JX203972	JX204187	JX204119	JX204251
Alsodes vanzolinii	Ramadillas	IZUA 3570	JX203974	JX204189	JX204121	JX204253
Alsodes verrucosus	Puyehue	IZUA 3574	JX203975	JX204190	JX204122	JX204254
Eupsophus calcaratus	Yaldad	IZUA 3578	JX203982	JX204197	JX204128	JX204261

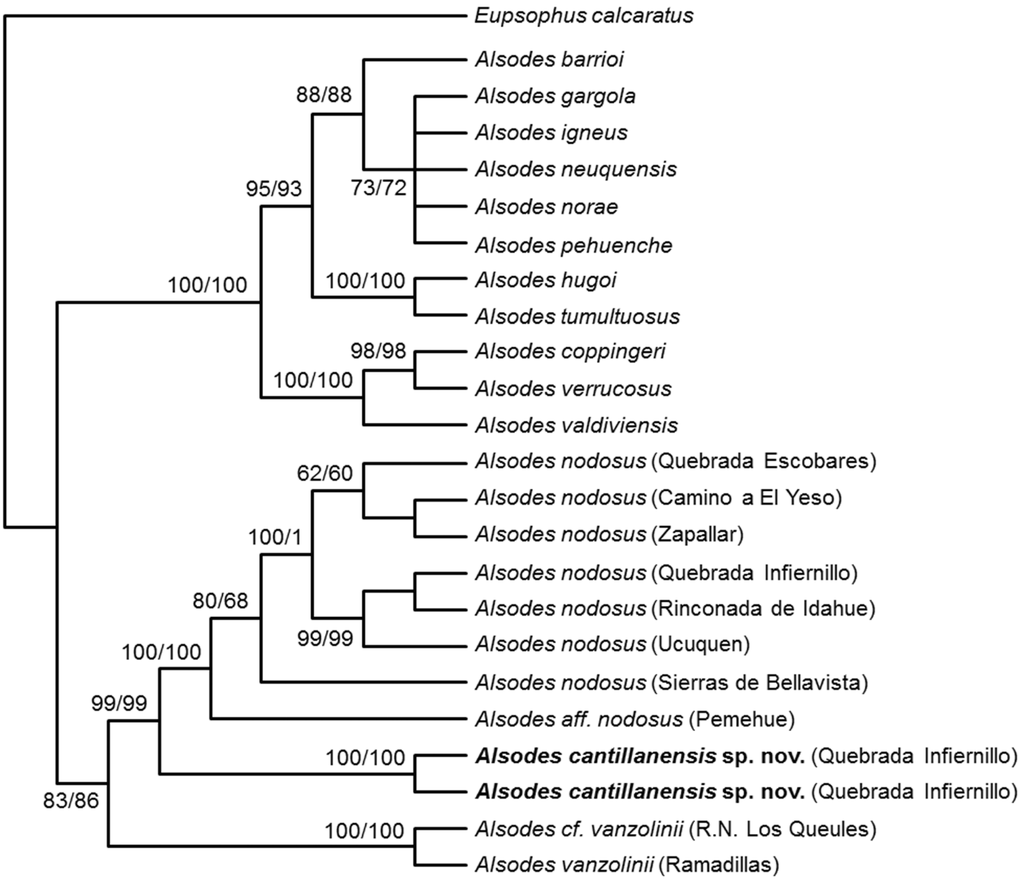

FIGURE S1. Bootstrap 50% majority-rule consensus tree of Maximum Parsimony analyses showing the position of the new species, *Alsodes cantillanensis* (in bold), within the genus. Consensus trees of analyses treating gaps as a fifth state or treating them as missing, show the same topology. Numbers next to the nodes, separated by slashes, correspond to bootstrap values of the analysis with gaps treated as a fifth state and treated as missing, respectively.