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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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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