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SHORT COMMUNICATION

Phylogenetic relationships of three endemic and endangered lizard species of *Pristidactylus* from central Chile based on the cytochrome b

Relaciones filogenéticas de tres especies de lagartos *Pristidactylus* endémicos y En Peligro del centro de Chile basadas en el citocromo b

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ABSTRACT

Lizards of the genus *Pristidactylus* distribute in southern South America (Chile and Argentina). In Chile, there are four endemic species (*P. alvaroi*, *P. torquatus*, *P. valeriae* and *P. volcanensis*) that distribute in the central-south zone of the country. We performed a Bayesian analysis with the mitochondrial gene cytochrome b to assess the phylogenetic position of these four species, which shows that they comprise a high supported clade that is separated from the other species of the genus.

Keywords: Argentina, Chile, leiosaurids, polyphyly.

RESUMEN

Los lagartos del género *Pristidactylus* se distribuyen en el sur de América del Sur (Chile y Argentina). En Chile existen cuatro especies endémicas (*P. alvaroi*, *P. torquatus*, *P. valeriae* y *P. volcanensis*) que se distribuyen en la zona centro-sur del país. Realizamos un análisis bayesiano con el gen mitocondrial citocromo b para evaluar la posición filogenética de estas cuatro especies, el que muestra que ellas comprenden un clado altamente soportado que se separa de las otras especies del género.

Palabras clave: Argentina, Chile, leiosáuridos, polifilia.

Chile has a high level of endemism of reptiles, which currently reaches 60% (Ruiz de Gamboa 2020). Most of the endemic species correspond to lizards of the genus *Liolaemus* Wiegmann, 1834 (one of the most diverse genus of reptiles of the world; Abdala *et al.* 2021), but there are also representatives of other genera shared with other South American countries. Among them is the genus *Pristidactylus* Fitzinger, 1843, which currently comprises 10 species distributed in south-central Chile (Garín *et al.* 2020) and Argentina (Morando *et al.* 2015).

Like other genera of the same family (Leiosauridae), lizards of the genus *Pristidactylus* are characterized by stout bodies and legs, robust heads, and short tails that usually do not autotomize (Cei 1986). Traditionally, the species of this genus have been divided into two groups based on morphological and ecological (habitats) characteristics and geographic distribution: the Chilean group (four species) and the Argentine one (six species) (Etheridge & Williams 1985; Cei *et al.* 2001, 2004).

There are several phylogenetic studies, using morphological and/or molecular characters, where *Pristidactylus* and other genera of leiosaurids have been included (reviewed in Morando *et al.* 2015). These studies have included a variable number of species of the genus and have recovered it as monophyletic (Frost *et al.* 2001; Abdala *et al.* 2009, in some of their morphological analysis) or polyphyletic (Abdala *et al.* 2009, in their supertree combining morphological and molecular data; Pyron *et al.* 2013; Morando *et al.* 2015).

The most complete phylogenetic studies of the genus to date, in terms of species and number of genes (mitochondrial and nuclear), are Morando *et al.* (2015) and Femenias *et al.* (2020). One of the main results of those studies is that *P. torquatus* (Philippi, 1861) does not group with the rest of the species of the genus. However, both studies included almost exclusively species of *Pristidactylus* from Argentina and only included *P. torquatus* among the Chilean representatives. Among the four species from Chile, *P. torquatus* is the one with the widest geographic distribution; the other three species, *P. alvaroi* (Donoso Barros, 1974), *P. valeriae* (Donoso Barros, 1966) and *P. volcanensis* Lamborot and Díaz 1987, have much more restricted distributions in central Chile (Núñez & Urra 2016; Garín *et al.* 2020) (Fig. 1). In addition, the quality of the habitats has decreased or deteriorated, which is why these three species are considered Endangered by Chilean legislation (Garín *et al.* 2020).

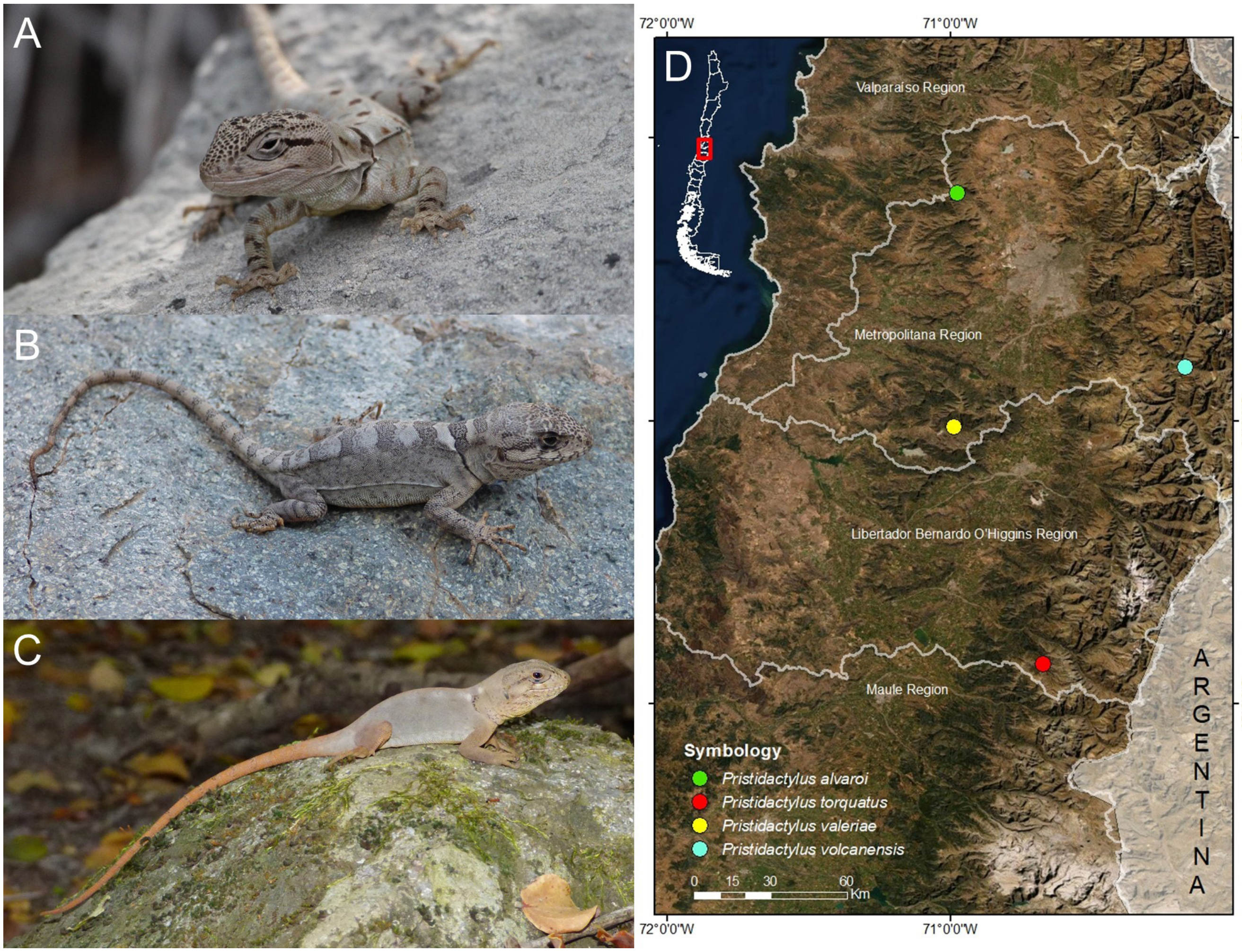

Figure 1. Adult individuals of the *Pristidactylus* species from central Chile and sampling sites. A. *Pristidactylus alvaroi*. B. *Pristidactylus volcanensis*. C. *Pristidactylus valeriae*. D. Location of sampling sites (see details in Table 1). / Individuos adultos de las especies de *Pristidactylus* de Chile central y sitios de muestreo. A. *Pristidactylus alvaroi*. B. *Pristidactylus volcanensis*. C. *Pristidactylus valeriae*. D. Ubicación de los sitios de muestreo (ver detalles en la Tabla 1).

The results of Morando *et al.* (2015) and Femenias *et al.* (2020) ratify that the genus *Pristidactylus* is polyphyletic and suggest that the Chilean and Argentine species correspond to lineages that have evolved separately, on opposite sides of the Andes. Previous phylogenetic studies that have included part or all of the Chilean species (using only morphological characters for the three from central Chile) have not recovered the reciprocal monophyly of these two lineages (Frost *et al.* 2001; Abdala *et al.* 2009). Therefore, the objective of this study is to assess the phylogenetic position of the four Chilean species of *Pristidactylus* with sequences of the mitochondrial gene cytochrome b.

We sampled two individuals from one locality of each of the four Chilean species: *P. alvaroi*, *P. torquatus*, *P. valeriae* and *P. volcanensis* (Fig. 1; Table 1). In the case of these last two species, the individuals come from their respective type localities. Oral mucosa samples were obtained with swabs Copan 516CS01 to extract DNA. The sampled individuals were photographed and released at the same capture site. DNA was extracted with the kit ReliaPrep^{TM} gDNA Tissue Miniprep System (Promega, Madison, WI), following the manufacturer's instructions. A fragment of the mitochondrial gene cytochrome b (cytb) was obtained. The primers and PCR protocols to obtain that fragment are found in Morando *et al.* (2015) and references therein. Sequences were edited with Bioedit v7.1.3 (Hall 1999) and then aligned with Muscle (Edgar 2004). The eight sequences of the four species were deposited in GenBank under the numbers ON787825-ON787832.

Table 1. Geographical origin of the individuals of the Chilean species of *Pristidactylus* added in this study. The asterisk indicates type locality. / Origen geográfico de los individuos de las especies chilenas de *Pristidactylus* agregados en este estudio. El asterisco indica localidad tipo.

Species	Locality	Latitude (S)	Longitude (W)	Codes of individuals
Pristidactylus alvaroi	Cerro Chicauma	33°11'40.5"	70°58'26.3"	AL1, AL2
Pristidactylus volcanensis	El Volcán*	33°48'40.0"	70°10'10.3"	VOL1, VOL2
Pristidactylus valeriae	Alhué*	34°01'24.1"	70°59'16.6"	VAL1, VAL2
Pristidactylus torquatus	Alto Huemul	34°51'42.9"	70°40'15.6"	TOR1, TOR2

We inferred the phylogenetic relationships of the four Chilean species of *Pristidactylus* in relation to the Argentine species of the genus and the genera most closely related to *Pristidactylus* (*Diplolaemus* Bell, 1843 and *Leiosaurus* Duméril & Bibron, 1837) (Morando *et al.* 2015; Femenias *et al.* 2020). Therefore, we included representatives of all species of genera *Diplolaemus* and *Leiosaurus* and of five of the six species of *Pristidactylus* from Argentina. Also, we included one specimen of *P. torquatus* from Cordillera de Nahuelbuta (Morando *et al.* 2015). We performed a Bayesian Inference (BI) analysis with the program MrBayes v3.2.7a (Ronquist *et al.* 2012). A reversible-jump method to explore the space of all General Time Reversible sub-models, plus gamma and proportion of invariable sites parameters was applied independently to each codon position (three partitions). 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 after observing the stationarity of ln-likelihoods of trees in Tracer v1.7.1 (Rambaut *et al.* 2018). 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 a representative of the genus *Urostrophus* Duméril & Bibron, 1837 following the most recent phylogenetic studies of these leiosaurid genera (Morando *et al.* 2015; Femenias *et al.* 2020).

We obtained an alignment of 809 nucleotide sites for the cytb. We recovered four main clades, two corresponding to the genera *Diplolaemus* and *Leiosaurus*, and other two made up of the Chilean and Argentine species of *Pristidactylus* (Fig. 2). According to our results, the Chilean clade of *Pristidactylus* is the sister group of *Leiosaurus*, but this relationship is supported by a low posterior probability (pp = 0.84), whereas the Argentine species of *Pristidactylus* comprise the sister group of the genus *Diplolaemus*, also with a low support (pp = 0.70). The four Chilean species of *Pristidactylus* comprise a highly supported clade (pp = 0.99), where *P. torquatus* is the first to diverge, followed by *P. alvaroi*, which corresponds to the sister taxon of *P. valeriae* + *P. volcanensis* (this last relationship with low support, pp = 0.80).

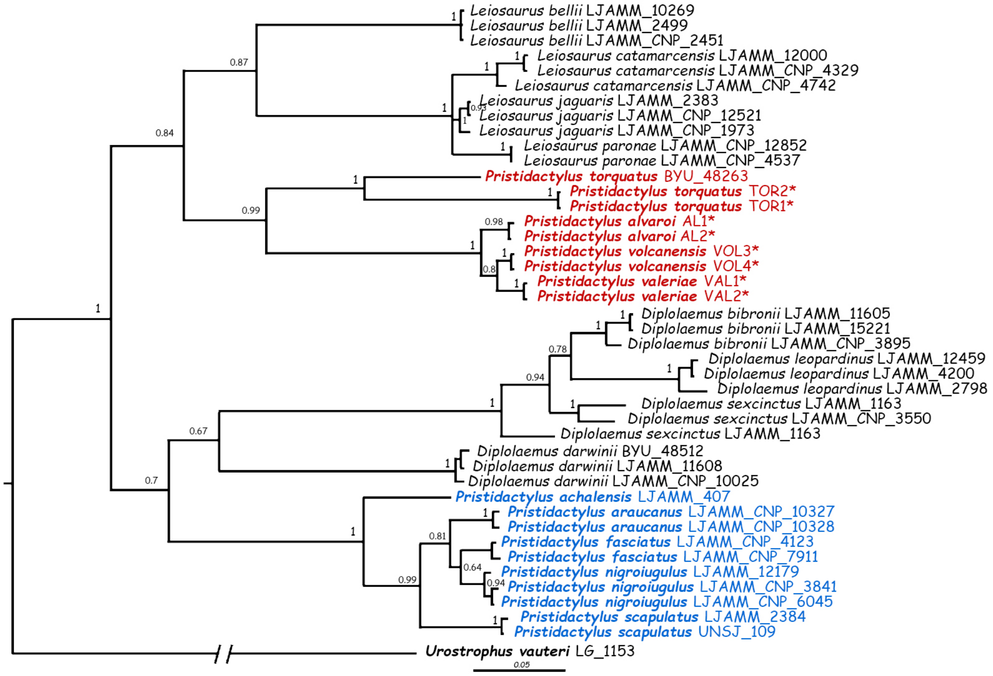

FIGURE 2. Bayesian consensus tree (50% majority-rule) of the fragment of the cytochrome b, showing the relationships of *Pristidactylus*, *Diplolaemus* and *Leiosaurus*. Specimens of the two *Pristidactylus* lineages are shown with different colors: Chilean (red) and Argentine (blue). The values next to the nodes correspond to posterior probabilities and the scale bar below the tree represents the expected substitutions per site along the branches. / Árbol de consenso bayesiano (regla de la mayoría del 50%) del fragmento del citocromo b, mostrando las relaciones de *Pristidactylus*, *Diplolaemus* y *Leiosaurus*. Los dos linajes de *Pristidactylus* se muestran con diferentes colores: chileno (rojo) y argentino (azul). Los valores junto a los nodos corresponden a las probabilidades posteriores y la barra de escala debajo del árbol representa las sustituciones esperadas por sitio a lo largo de las ramas.

In the last two decades, several studies have been conducted to elucidate the phylogenetic relationships and evolution of the leiosaurid genera *Pristidactylus*, *Diplolaemus* and *Leiosaurus* (reviewed in Morando *et al.* 2015). The most recent and complete phylogenetic studies of these genera (Morando *et al.* 2015; Femenias *et al.* 2020) have been consolidating the position that *Diplolaemus* and *Leiosaurus* are monophyletic, whereas *Pristidactylus* is polyphyletic. Here, we obtained similar results, recovering the same four main groups of leiosaurids, but there are some differences from those studies.

We obtained low support values (pp < 0.95) for the clades corresponding to the genera *Diplolaemus* and *Leiosaurus* and a relationship between *Diplolaemus* and the Argentine species of *Pristidactylus* (also with low support) (Fig. 2). Instead, Morando *et al.* (2015) (in their species tree) obtained high levels of support for the genera *Diplolaemus* and *Leiosaurus* and the clade formed by the Argentine species of *Pristidactylus*. However, like Morando *et al.* (2015), we recovered a relationship between *P. torquatus* and *Leiosaurus*, although with low support. On the other hand, Femenias *et al.* (2020) (in their species tree) also obtained high support for *Diplolaemus*, *Leiosaurus* and the Argentine species of *Pristidactylus*, but recovered to *P. torquatus* as sister of these three clades. These differences can be attributed to the fact that we used only one mitochondrial marker, but it should be noted that despite the fact that Morando *et al.* (2015) and Femenias *et al.* (2020) used several genes (two mitochondrial and eight nuclear), in general they did not obtain high support values for the relationships among genera.

Both Morando *et al.* (2015) and Femenias *et al.* (2020) recovered to *P. torquatus* separated from the Argentine species of the genus, although in different positions. Here, we obtained the same result, but including all species of *Pristidactylus* from Chile, which grouped with high support; moreover, a division is observed between *P. torquatus* and the three species from central Chile, also with a high support (Fig. 2). The relationship of *P. torquatus* as sister to the other species from Chile had already been obtained in previous studies, for example, by Frost *et al.* (2001) (in their analysis of morphological and molecular data combined, where the Argentine species *P. fasciatus* is closely related to the Chilean species) and Abdala *et al.* (2009) (in their morphological analysis, where *P. alvaroi* was not included). Among the three species from central Chile, we recovered *P. valeriae* and *P. volcanensis* as sister species (Fig. 2), a result also obtained by Abdala *et al.* (2009) in their morphological analysis.

In summary, despite using only one mitochondrial gene, we confirmed previous studies that recovered *Pristidactylus* as a polyphyletic genus (Morando *et al.* 2015; Femenias *et al.* 2020). Our analysis also shows that the four endemic species of Chile make up a well-supported lineage that has evolved separately from their counterparts on the other side of the Andes. This is consistent with the morphological and ecological differences that have traditionally been used to distinguish the two groups of species within the genus. However, a larger number of samples from different populations and the inclusion of nuclear markers are required to better understand the evolutionary history and establish the taxonomic status of the *Pristidactylus* lineage that is restricted to the west of the Andes in Chilean territory.

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