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SHORT COMMUNICATION

Mitochondrial variation of the Endangered *Pristidactylus* species (Squamata, Leiosauridae) from central Chile

Variación mitocondrial de las especies de *Pristidactylus* en peligro de extinción (Squamata, Leiosauridae) del centro de Chile

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ABSTRACT

Three endemic and threatened lizards of the genus *Pristidactylus* inhabit in central Chile, which have very limited distributions. Mitochondrial (cytochrome b) genetic variation was examined in two populations of each of these three species. The analyses suggest that they have large population sizes, high population structure and low gene flow between their populations. The implications of these results for the conservation of these species are discussed.

Keywords: conservation, control region, genetic distances, haplotypes, population structure.

RESUMEN

En Chile central habitan tres lagartos endémicos y amenazados del género *Pristidactylus*, que tienen distribuciones muy reducidas. Se examinó la variación genética mitocondrial (citocromo b) de dos poblaciones de cada una de estas tres especies. Los análisis sugieren que las tres tienen tamaños poblacionales grandes, alta estructura poblacional y bajo flujo génico entre sus poblaciones. Se discuten las implicaciones de estos resultados para la conservación de estas especies.

Palabras clave: conservación, distancias genéticas, estructura poblacional, haplotipos, región control.

Much of the territory of continental Chile makes up one of the 36 biodiversity hotspots worldwide, the Chilean winter rainfall-Valdivian forests, so its biota is characterized by high levels of endemism and threat (Arroyo *et al.* 2008). Among the vertebrates of this hotspot, amphibians and reptiles stand out. Although in Chile amphibians have more endemic higher taxa than reptiles (one family and four genera), a recent review indicates that the endemism of reptiles at the species level would be 60 %, slightly higher than that of amphibians (Ruiz de Gamboa 2020).

Until now, the only genus of reptiles endemic to Chile is *Garthia*, but recent molecular phylogenetic studies have shown that Chilean endemic representatives of the lizard genus *Pristidactylus* are not closely related to the congeneric species present east of the Andes (Argentina) (Morando *et al.* 2015; Femenias *et al.* 2020; Correa *et al.* 2022). This lineage, traditionally recognized as the Chilean group (Etheridge & Williams 1985; Cei *et al.* 2001, 2004), is composed of four species, three of which (*P. alvaroi* (Donoso-Barros, 1974), *P. valeriae* (Donoso-Barros, 1966) and *P. volcanensis* Lamborot and Díaz, 1987) have restricted and allopatric distributions in central Chile (32°55'-34°05' S). The fourth species, *P. torquatus* (Philippi, 1861), has a much broader distribution to the south (~34°30'-40° S). Phylogenetically, *P. volcanensis* and *P. valeriae* would be more related, forming the sister group of *P. alvaroi*; these three, in turn, would be the sister group of *P. torquatus* (Correa *et al.* 2022).

The three species from central Chile are classified as Endangered by IUCN (2023) and the Chilean legislation (Reglamento de Clasificación de Especies Silvestres) due to their limited distributions and threats to their habitats. Two of these species, *P. alvaroi* and *P. valeriae*, preferentially inhabit *Nothofagus* forests, while *P. volcanensis* is mainly found in Andean valleys covered by scrub vegetation (Garín *et al.* 2020). Among the factors that threaten these species are the loss and fragmentation of forests, forest fires, water scarcity, invasive species such as cats and dogs, and economic activities carried out in forests (livestock, soil leaf extraction) (Garín *et al.* 2020). This situation promoted the development of a government plan for the conservation of these three species (RECOGE), approved by the Ministerio del Medio Ambiente in 2021 (MMA 2020).

Recently, several new localities of *P. alvaroi* (Garín *et al.* 2020, Reyes-Olivares *et al.* 2021) and *P. volcanensis* (Gagliardi-Álvarez *et al.* 2023, Mella-Romero & Mella 2023) have been reported, expanding their distribution ranges and the types of environments they inhabit. These contributions have allowed us to improve knowledge about the geographical distribution of these species, but until now no studies have been carried out on their population genetics. Here, we use sequences of the mitochondrial cytochrome b gene to estimate the degree of intra- and interpopulation variation and genetic structure of the three *Pristidactylus* species endemic to central Chile.

Buccal mucosa samples were taken from 31 individuals of the three species of *Pristidactylus* from central Chile: *P. alvaroi*, *P. valeriae* and *P. volcanensis* (Figs. 1B-1D). Table 1 indicates the coordinates of each locality (two per species) and the number of individuals sampled per locality. Fig. 1A shows the location of the six localities. Mucosal samples were taken with Copan 516CS01 swabs, gently scraping the inside of the mouth. The individuals were handled for the shortest possible time, photographed, measured and released at the same capture site. DNA was extracted with the ReliaPrepTM gDNA Tissue Miniprep System kit (Promega, Madison, WI), following the manufacturer's instructions.

A fragment of the mitochondrial cytochrome b gene was sequenced with the primers GluDGL (5'-TGACTTGAARAACCAYCGTTG-3') and Cyt-b 3 (5'-GGCAAATAGGAARTATCATTC-3') (Palumbi *et al.* 1991), using the PCR protocol of Morando *et al.* (2015) and references therein. Sequences were edited with Bioedit v7.1.3 (Hall 1999) and aligned with Muscle (Edgar 2004). The sequences published in GenBank of the three species (ON787825-ON787826 and ON787829-ON787832) were used, to which new ones were added (accession numbers pending).

Several indices of population diversity were calculated (number of haplotypes, haplotypic diversity, nucleotide diversity), grouping the samples by species due to the low number of samples from some localities. In addition, a haplotype network was constructed with the median-joining method using the program PopART (Leigh & Bryant 2015). Haplotypes for this analysis were previously obtained in DnaSP v6.12.03 (Rozas *et al.* 2017). Finally, to quantify divergence patterns between populations, the average Tamura-Nei genetic distances were calculated with the program Mega 11 (Tamura *et al.* 2021).

Sixteen haplotypes were obtained among the 31 sequenced samples (Table 2). The highest values of haplotypic and nucleotide diversity were observed in *P. valeriae*, probably due to the greater number of individuals sampled. However, the three species showed similar values of nucleotide diversity due to a relatively high divergence between some haplotypes of the same species (Fig. 1E).

The haplotype network shows three groups of haplotypes corresponding to each of the species (Fig. 1E), separated by the longest branches (highest number of mutational steps). Another relevant aspect of the haplotype network is that none of the six sampled localities share haplotypes, which can be interpreted as a high mitochondrial genetic structure in the three species.

Table 1. Geographical origin of the individuals of *Pristidactylus* included in this study. The asterisk indicates type locality. / Origen geográfico de los individuos de *Pristidactylus* incluidos en este estudio. El asterisco indica localidad tipo.

Species	Locality	Latitude (S)	Longitude (W)	Number of individuals
Pristidactylus alvaroi	Cerro El Roble*	32°59'39.9"	71°01'37.6"	1
Pristidactylus alvaroi	Cerro Chicauma	33°11'40.5"	70°58'26.3"	3
Pristidactylus volcanensis	Río Clarillo	33°42'34.3"	70°27'15.1"	2
Pristidactylus volcanensis	El Volcán*	33°48'40.0"	70°10'10.3"	6
Pristidactylus valeriae	Piche	33°54'48.4"	71°03'27.6"	13
Pristidactylus valeriae	Alhué*	34°01'24.1"	70°59'16.6"	6

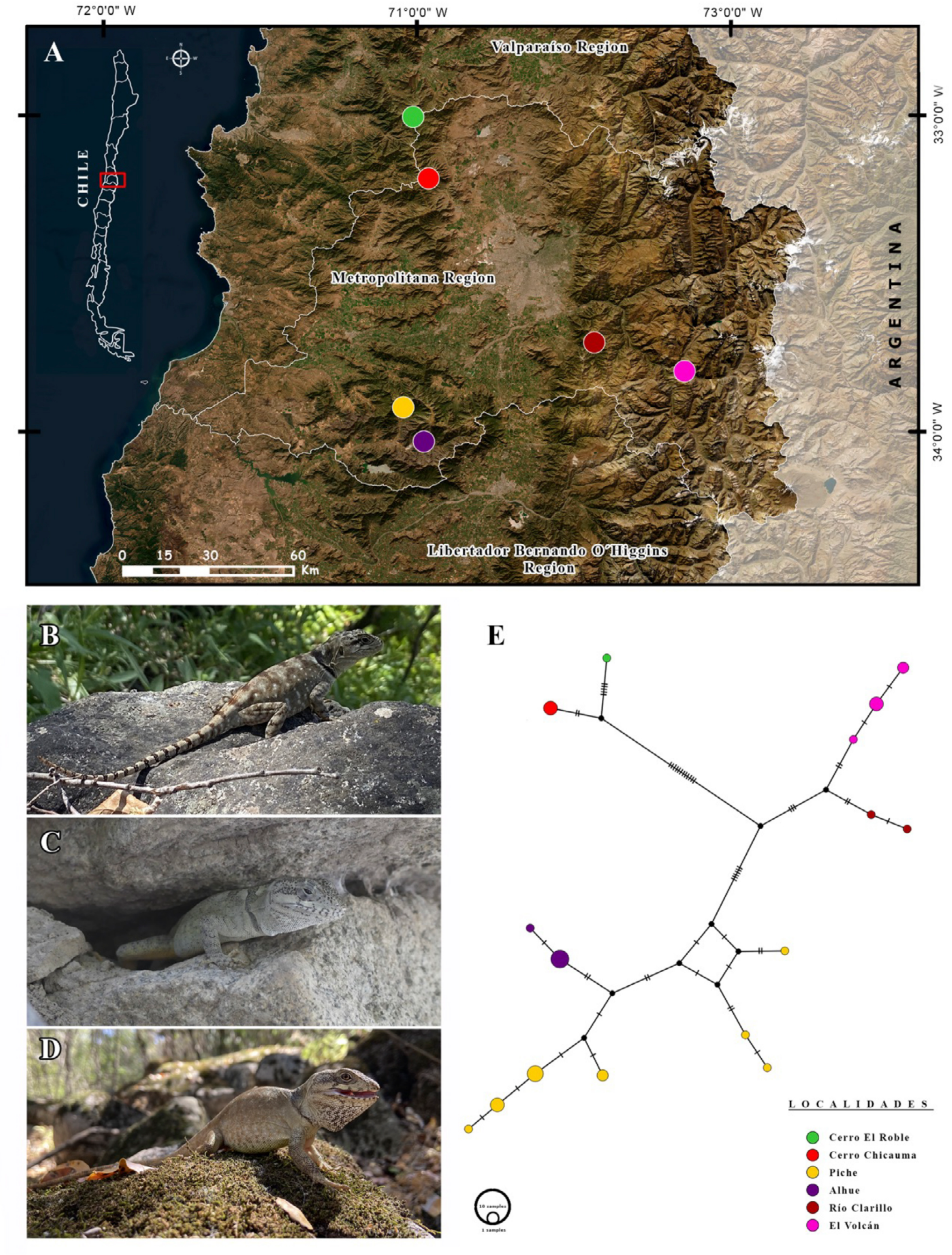

Figure 1. Location of the localities, adult individuals and haplotype network of the *Pristidactylus* populations included in this study. A. Location of sampling sites (see details in Table 1). The localities are colored according to the colors of the haplotype network of (E). B. Adult of *Pristidactylus alvaroi*. C. Adult of *Pristidactylus volcanensis*. D. Adult of *Pristidactylus valeriae*. E. Haplotype network. The size of the circles (haplotypes) is proportional to the frequency with which they were observed in the total samples. The short lines perpendicular to the branches joining the haplotypes represent the mutational steps. The haplotypes are colored according to their locality of origin (see map of A): Cerro El Roble and Cerro Chicauma (*P. alvaroi*); Piche and Alhué (*P. valeriae*); Río Clarillo and El Volcán (*P. volcanensis*). / Ubicación de las localidades, individuos adultos y red de haplotipos de las poblaciones de *Pristidactylus* incluidas en este estudio. A. Ubicación de los sitios de muestreo (ver detalles en la Tabla 1). Las localidades están coloreadas de acuerdo a los colores de la red de haplotipos de (E). B. Adulto de *Pristidactylus alvaroi*. C. Adulto de *Pristidactylus volcanensis*. D. Adulto de *Pristidactylus valeriae*. E. Red de haplotipos. El tamaño de los círculos (haplotipos) es proporcional a la frecuencia con que se observaron en el total de las muestras. Las líneas cortas perpendiculares a las ramas que unen los haplotipos representan los pasos mutacionales. Los haplotipos están coloreados de acuerdo a su localidad de origen (ver mapa de A): Cerro El Roble y Cerro Chicauma (*P. alvaroi*); Piche y Alhué (*P. valeriae*); Río Clarillo y El Volcán (*P. volcanensis*).

Table 2. Diversity indices of cytochrome b sequences by species. / Índices de diversidad de las secuencias del citocromo b por especie.

Species	Number of sequences	Number of haplotypes	Haplotype diversity (H)	Nucleotide diversity (π)
P. alvaroi	4	2	0,500	0,0042
P. valeriae	19	9	0,883	0,0053
P. volcanensis	8	5	0,857	0,0035

Table 3. Mean Tamura-Nei genetic distances (below the diagonal) between the populations (localities) of the three *Pristidactylus* species. The numbers on the diagonal are the standard errors. / Distancias genéticas promedio de Tamura-Nei (bajo la diagonal) entre las poblaciones (localidades) de las tres especies de *Pristidactylus*. Los números sobre la diagonal son los errores estándar.

	Cerro Chicauma	Cerro El Roble	Alhué	Piche	El Volcán	Río Clarillo
Cerro Chicauma		0,0034	0,0061	0,0058	0,0059	0,0058
Cerro El Roble	0,0085		0,0062	0,0058	0,0063	0,0060
Alhué	0,0313	0,0326		0,0021	0,0046	0,0044
Piche	0,0274	0,0282	0,0066		0,0043	0,0041
El Volcán	0,0251	0,0288	0,0213	0,0196		0,0026
Río Clarillo	0,0229	0,0267	0,0205	0,0188	0,0069	

The genetic distance values (Table 3) reflect the patterns observed in the haplotype network. Thus, the distances between the populations of each species are smaller (less than half) than the distances between species. Among the interspecific distances, the lowest values correspond to *P. valeriae*-*P. volcanensis*, while the largest correspond to *P. alvaroi* with respect to the other two species. This result is consistent with the phylogenetic relationships inferred between these three species by Correa *et al.* (2022) ((*P. valeriae* + *P. volcanensis*) + *P. alvaroi*).

Despite the low number of individuals sampled and the inequality of samples by locality, the analyzes reveal some patterns of genetic variation that are relevant to understanding the evolutionary history of these populations and contributing to their conservation.

Firstly, the patterns of intra- and interspecific variation agree with the current taxonomy of *Pristidactylus* in the central zone of Chile. The three genetic groups recovered in the haplotype network (Fig. 1E) correspond to the nominal species, but it must be considered that the sampling only included two localities per species, in all cases very close to each other. Taking into account the range expansions of the three species that have been reported in the last decade, more extensive sampling is required, including intermediate localities, to reevaluate and more precisely define the spatial limits between them.

Secondly, there are no localities that share haplotypes. This may be due in part to the low number of individuals sampled in some localities, but this segregation is also observed in *P. valeriae*, the species with the largest number of samples and whose localities are closest (~13.7 km in a straight line). This suggests high genetic structuring and low levels of gene flow (at least for females) within the three species. This has implications for their conservation, since, if this pattern is maintained throughout the entire distribution of these species, multiple conservation units could be defined considering these high levels of genetic variation.

Finally, relatively high divergence was observed between some haplotypes within species, particularly in *P. alvaroi* and *P. valeriae* (Fig. 1E). This suggests that the population sizes of these species may be large and/or that their populations have not experienced drastic declines in size. Some populations of these species appear to be low in abundance (judging by the high sampling effort to locate a few specimens; M. Mora, C. Garín, C. Correa, personal observations), but these are anecdotal observations and so far there are no studies on abundance or demographic trends in any of them.

In summary, this preliminary examination of mitochondrial genetic variation suggests that *Pristidactylus* populations from central Chile have large sizes, high genetic structure, and low levels of gene flow. All these aspects are relevant for the conservation of these threatened species, which should be re-evaluated with higher resolution nuclear markers (for example, SNPs). Furthermore, future genetic analyzes should include as many localities as possible to better cover their distributions and more individuals per locality. These genetic studies should be complemented with field studies of abundance, connectivity and demographic dynamics that inform and support the conservation measures contemplated in the plan promoted by the government of Chile.

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