Iguanian species-richness in the Andes of boreal Patagonia: Evidence for an additional new *Liolaemus* lizard from Argentina lacking precloacal glands (Iguania, Liolaeminae)

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Abstract

Most *Liolaemus* lizard species are characterized by the presence of precloacal glands in males. Only a few taxa lack these sexual signal emitter structures. Phylogenetic evidence suggests that those species are restricted to the clades *chiliensis* and *lineomaculatus*. Within the first lineage, *L. coeruleus*, *L. cristiani*, *L. flavipiceus*, *L. neuquensis* and *L. thermarum* lack precloacal glands, which have been considered as members of the *neuquensis* group. Whereas, in the second one, *L. periglacialis* (= *L. hatcheri*), *L. kolengh*, *L. lineomaculatus*, and *L. silvanae* exhibit this characteristic. In the present study we provide the description of *Liolaemus tregenzai*, an additional new species lacking precloacal glands in both sexes. This new taxon, member of the *chiliensis* clade, is so far known from the Copahue Volcano, Neuquén Province of Argentina, in boreal Patagonia. *Liolaemus tregenzai* differs from the remaining species of this clade in having a unique combination of morphologic and chromatic traits, such as a large body size, olive or chest-nut dorsal ground colour, with dark brown or blackish pigment on the flanks, green-bluish with intense black pigment on the ventral surface, and evident sexual dichromatism. The ecology of this new lizard is also remarkably, occurring in Andean antarctandic forests, and being common near thawing snows. Phylogenetic relationships of this species with other members of the *chiliensis* clade and with the taxa recognized as members of the *neuquensis* group are still unknown.

Key words: Precloacal glands, *Liolaemus*, *chiliensis* clade, *lineomaculatus* clade, *Liolaemus tregenzai*, Argentina

Resumen

La mayoría de las lagartijas del género *Liolaemus* se caracterizan por la presencia de glándulas precloacales en los machos. Sólo algunos escasos taxa carecen de estas estructuras responsables de la producción de señales sexuales. La evidencia filogenética sugiere que tales especies están restringidas a los clados *chiliensis* y *lineomaculatus*. Dentro del primer linaje carecen de estas estructuras *L. coeruleus*, *L. cristiani*, *L. flavipiceus*, *L. neuquensis* y *L. thermarum*, todos considerados como miembros del grupo *neuquensis*. Mientras que en el segundo conjunto *L. periglacialis* (= *L. hatcheri*), *L. kolengh*, *L. lineomaculatus* y *L. silvanae* exhiben esta caracteristica. En el presente estudio entregamos la descripción de *Liolaemus tregenzai*, una adicional nueva especie carente de glándulas precloacales en ambos sexos. Este nuevo taxón es conocido hasta ahora del Volcán Copahue, Provincia de Neuquén, Argentina, en la Patagonia boreal. *Liolaemus tregenzai* difiere de las demás especies de este clado debido a una combinación única de caracteres morfológicos y cromáticos, tales como un tamaño corporal grande, un colorido general verde oliva o castaño, con acumulaciones de pigmento café oscuro o negruzco sobre los flancos, un vientre verde-azulado con intensa pigmentación melánica, y evidente dicromatismo sexual. La ecología de esta nueva lagartija es también peculiar, habitando en bosques antartándicos andinos, donde resulta común en las proximidades de nieve en derretimiento. Las relaciones filogenéticas de esta especie con los demás miembros del grupo *chiliensis* y con los taxa reconocidos como miembros del grupo *neuquensis* son aún desconocidas.

Introduction

The development of recent evolutionary studies conducted in South American *Liolaemus* lizards has attempted to explain factors involved in its extraordinary diversification (Schulte *et al.* 2004; Espinoza *et al.* 2004). Consisting of more than 180 species, this genus represents an interesting opportunity to address questions concerning the role of selection and biogeography in the evolution of lineages (Etheridge & Espinoza 2000; Pincheira-Donoso & Ramirez 2005).

Recent molecular-based phylogenetic hypotheses have increased the knowledge about the specialization and conservatism of some traits in different *Liolaemus* groups. An interesting achievement is the recognition that the characteristics of precloacal sexual signal emitter glands (precloacal glands or pores), known in males of almost every species, can be predicted by phylogeny. It led to understand that the number of these structures is constrained by ancestry, and that natural selection appears not to play a major role in its evolution. Indeed, although some studies suggested that environmental factors affect specializations in this trait (e.g. Escobar *et al.* 2001), a recent analysis performed using phylogenetic comparative methods showed contrasting evidence. Pincheira-Donoso and Tregenza (in prep) observed that the influence of habitat structures and environmental temperatures do not predict variation in these glands, being actually constrained by shared ancestry. Nevertheless, the still scarce available information about *Liolaemus* lacking precloacal glands, mainly in relation to its species-richness and phylogeny, appears to be a fundamental handicap when developing conclusions in this field of research. Additional studies focused in these *Liolaemus* may allow, for instance, estimating the proportional frequency of clades lacking precloacal glands in relation to those exhibiting them, and how this trait, considered essential for sexual communication, can be involved in speciation.

During the last few years, different studies have contributed to increase the knowledge about the diversity, systematics and biogeography of *Liolaemus* lacking precloacal glands. The discovery of *L. cristiani* (Núñez *et al.* 1991), *L. kolengh* (Abdala & Lobo 2006), *L. thermarum* (Videla & Cei 1996) and *L. flavipiceus* (Cei & Videla 2003) suggests that these lizards are more diverse than previously thought (see Cei 1986). Also, the development of recent phenotypic and molecular phylogenetic hypotheses has led to the understanding that *Liolaemus* lacking precloacal glands belong to only two main lineages, the clades *chiliensis* and *lineomaculatus* (e.g. Schulte *et al.* 2000; Espinoza *et al.* 2004). Within the *chiliensis* clade, only *L. coeruleus*, *L. cristiani*, *L. flavipiceus*, *L. neuquensis* and *L. thermarum* lack these structures (which means less than 7% of the total number of species belonging to this lineage) (Videla & Cei 1996; Cei & Videla 2003; Pincheira-Donoso 2003; Pincheira-Donoso & Núñez 2005). Whereas, the entire clade *lineomaculatus*, consisting of *L. periglacialis* (= *L. hatcheri*), *L. kolengh*, *L. lineomaculatus* and *L. silvanae* exhibit absence of these glands (Etheridge 1995; Abdala & Lobo 2006).

Cei and Videla (2003) proposed the explicit hypothesis that the *Liolaemus* species of the *chiliensis* clade lacking precloacal glands would belong to the same evolutionary line. The first evidence had previously been provided by Videla and Cei (1996). Those authors observed that the phenotypic structure of *L. altissimus neuquensis* would relate this taxon to *L. coeruleus* and *L. thermarum*, rather than to *L. altissimus* from central Chile (see Donoso-Barros 1966, 1970). Later, Cei and Videla (2002, 2003) suggested that all these *Liolaemus* species should be included in a line they identified as *neuquensis* group. This hypothesis has been partially supported by a series of diagnostic traits common to *L. coeruleus*, *L. cristiani*, *L. flavipiceus*, *L. neuquensis* and *L. thermarum*, and absent from other similar groups, such as the *elongatus* and *kriegi* clades. However, it is well known for instance that some of the species related to the *elongatus* group may also exhibit reduction in the number of precloacal glands. For example, in some males of the Chilean species *L. leopardinus* and *L.*

valdesianus, precloacal glands can be almost completely absent (Pincheira-Donoso & Núñez 2005; see also Cei 1986). Therefore, it seems to be possible the existence of *Liolaemus* species lacking precloacal glands belonging to other groups.

In this paper we provide the results obtained from the study of an additional *Liolaemus* population coming from the Copahue Volcano in Argentina, lacking precloacal glands in both sexes. Our analyses conducted on this taxon suggest that it represents a new species from Andean ecosystems in the boreal Patagonia.

Material and methods

Studied species

We performed non-phylogenetic comparative analyses on different *Liolaemus* species, including the new *Liolaemus* taxon (*Liolaemus* **sp. nov.**). The studied material comprises species belonging to the *chiliensis* (*L. coeruleus*, *L. cristiani*, *L. flavipiceus*, *L. neuquensis* and *L. thermarum*; Table 1) and *lineomaculatus* (*L. periglacialis* [= *L. hatcheri*], *L. kolengh*, *L. lineomaculatus* and *L. silvanae*; Table 1) clades. The total sample consists of specimens with the following status: (i) alive individuals studied in the field and then released, (ii) specimens housed in scientific collections, with field information (e.g. locality), but without official institutional number (see appendix), and (iii) conserved specimens with official collection numbers and housed in the herpetological collections of the Museo Nacional de Historia Natural de Chile (MNHN), Zoological Museum, Universidad de Concepción, Chile (MZUC), Zoological Section, Museo de Historia Natural de Concepción, Chile (MHNC), Institute for Animal Biology, Universidad Nacional de Cuyo, Argentina (IBAUNC), Instituto Argentino de Investigaciones en Zonas Áridas, Argentina (IADIZA), Museo de La Plata, Argentina (MLP), Division of Reptiles and Amphibians, Natural History Museum of London, UK (NHML), and in the diagnostic collections of J. M. Cei (JMC-DC), J. A. Scolaro (JAS-DC) and D. Pincheira-Donoso (CHDPD). The samples detailed in the points (ii) and (iii) consisted of specimens conserved in ethanol (70%). The type series of the new *Liolaemus* species was initially preserved in 10% formalin and later transferred to 70% ethanol. Previously, muscle tissues were extracted to perform molecular analyses (currently in preparation). Since juvenile individuals tend to be phenotypically similar between closely related species (under the assumption that the new *Liolaemus* population may be related to at least one of the taxa included in the comparative analyses), non-mature specimens were only considered for descriptive purposes.

TABLE 1. Diversity and distribution in Argentina (Ar) and Chile (Ch) of the ten known *Liolaemus* species lacking precloacal glands, including the new taxa described herein.

Clade	Species	Country	Latitudinal range	Altitudinal range (m)
chiliensis	*L. coeruleus*[a, b]	Ar, Ch	38°38'–38°42'	1700–2100
	L. cristiani[a]	Ch	35°36'–35°38'	2436–2460
	L. flavipiceus[c]	Ar	35°58'–35°58'	2500–2500
	L. neuquensis[b]	Ar	37°47'–37°51'	2200–2200
	L. thermarum[d]	Ar	35°15'–35°15'	2400–2400
	L. tregenzai **sp.nov.**	Ar	37°50'–71°06'	2150–2150
lineomaculatus	*L. periglacialis*[b]	Ar	47°42'–48°02'	1000–1200
	L. kolengh[e]	Ar, Ch[f]	46°50'–47°01'	1000–1485
	L. lineomaculatus[a, b]	Ar, Ch	41°50'–51°30'	780–1500
	L. silvanae[b]	Ar	47°17'–47°23'	1500–1600

[a]See Pincheira-Donoso and Núñez (2005); [b]Cei (1986); [c]Cei and Videla (2003); [d]Videla and Cei (1996); [e]Abdala and Lobo (2006); [f]H. Núñez (pers. comm.).

Studied traits and data analysis

External and internal traits were comparatively studied. External traits include mainly allometric proportions and scale morphology. Measurements were taken using a digital calliper to the nearest 0.01 mm. Studied variables include snout-vent length (SVL), measured from the snout margin to the anterior edge of cloacae, forelimb length (from the axilla to the tip of the third finger), hind limb length (from the ingle to the tip of the fourth finger), tail length (measured from the anterior edge of cloacae to the tail tip), head length (from the tip of the snout to the inferior apex of the external auditory meatus), head width (measured across the widest region of the head, in the postocular zone), and head height (measured across the highest region of the head, in the parietal zone) (see Etheridge & Christie 2003; Núñez *et al.* 2003, 2004; Núñez & Pincheira-Donoso 2006). Scale morphology includes the classical traits detailed for Liolaeminae lizards by Cei (1986, 1993), Donoso-Barros (1966), Etheridge (1995, 2000), and Pincheira-Donoso and Núñez (2005). Scales around midbody were counted half-way between axilla and groin regions. Subdigital lamellae were counted from the most proximal scale wider than long, to the most distal one bordering the claw (see Etheridge & Christie 2003). Nomenclature used in the description of those traits follows the same authors. Internal traits studied include observations on the Meckel's groove and on the morphological characteristics of the tibia, as detailed by Etheridge (1995) and Núñez *et al.* (2004).

Statistical comparisons were only developed between *Liolaemus* **sp. nov.**, and the species *L. coeruleus* and *L. neuquensis*, which appear morphologically (e.g. they lack precloacal glands) and biogeographically related to the new *Liolaemus* taxa. Comparisons with the remaining species lacking precloacal glands are detailed in the diagnosis. Variables included in these statistical analyses are SVL, head length, and number of scales around midbody (see Pincheira-Donoso & Núñez 2005). We did not conduct comparisons between allometric traits, which can exhibit more evident intraspecific phenotypic plasticity as a consequence of non-genetic ecological adaptations to particular habitat structures, such as proportional limb length or axilla groin length (e.g. Losos 2004; Losos *et al.* 2000).

All measurements were analyzed as millimetric values. One-way random effects-model analysis of variance (ANOVA) was applied to compare the variables detailed in the previous paragraph. Post-hoc analyses were performed using Tukey-Kramer test for multiple comparisons, to find out the relationships between the studied traits (Field 2006). In all cases, the mean value of each variable for each species was utilized, accompanied by standard error values.

Results

The statistical analyses revealed significant differences between *Liolaemus* **sp. nov.**, *L. coeruleus* and *L. neuquensis*. Analyses of variance supported significant morphological divergence in body size (ANOVA, $F = 34.75$, $P < 0.001$), head length (ANOVA, $F = 29.0$, $P < 0.001$), and the number of scales around midbody (ANOVA, $F = 23.47$, $P < 0.001$) between these taxa. Post-hoc Tukey-Kramer tests showed that such body size differences are observed when comparing *L. coeruleus* with *Liolaemus* **sp. nov.** ($P < 0.001$) and *L. neuquensis* with *Liolaemus* **sp. nov.** ($P < 0.001$). However, body size in *L. coeruleus* and *L. neuquensis* does not differ significantly ($P = 0.960$). In relation to head length, significant differences were observed when comparing *L. coeruleus* with *Liolaemus* **sp. nov.** ($P < 0.001$), and *L. neuquensis* with *Liolaemus* **sp. nov.** ($P < 0.001$), whereas *L. coeruleus* and *L. neuquensis* do not differ significantly in this trait ($P = 0.710$). The post-hoc analysis revealed that the number of scales around midbody does not exhibit significant differences between *L. neuquensis* and *Liolaemus* **sp. nov.** ($P = 0.099$). Conversely, this trait differs significantly when comparing *L. coeruleus* with *L. neuquensis* ($P = 0.004$) and with *Liolaemus* **sp. nov.** ($P < 0.001$).

Our comparative analysis conducted on coloration traits also suggest that *Liolaemus* **sp. nov.** represents an unknown species (see diagnosis), which therefore we describe below.

***Liolaemus tregenzai* sp. nov.**
Figs. 1–2

Type Material. *Holotype.* MLP.R. 5274, adult male. Collected in Copahue Volcano slopes (37°50'S; 71°06'W), 2150 m, near Termas del Copahue, Departamento de Ñorquín, Neuquén Province, Argentina, by J.A. Scolaro, N. Scolaro, G. Scolaro and F. Tavera, February 6–10, 2006.

Allotype. MLP.R. 5275, adult female. The same data as holotype.

Paratypes. MLP.R. 5276-5277, both adult females. The same data detailed for the holotype and allotype. CHDPD 01098, adult male, CHDPD 01099, adult female. The same data detailed for the holotype and allotype. JAS-DC 806, adult male, JAS-DC 807, adult female. The same data detailed for the holotype and allotype.
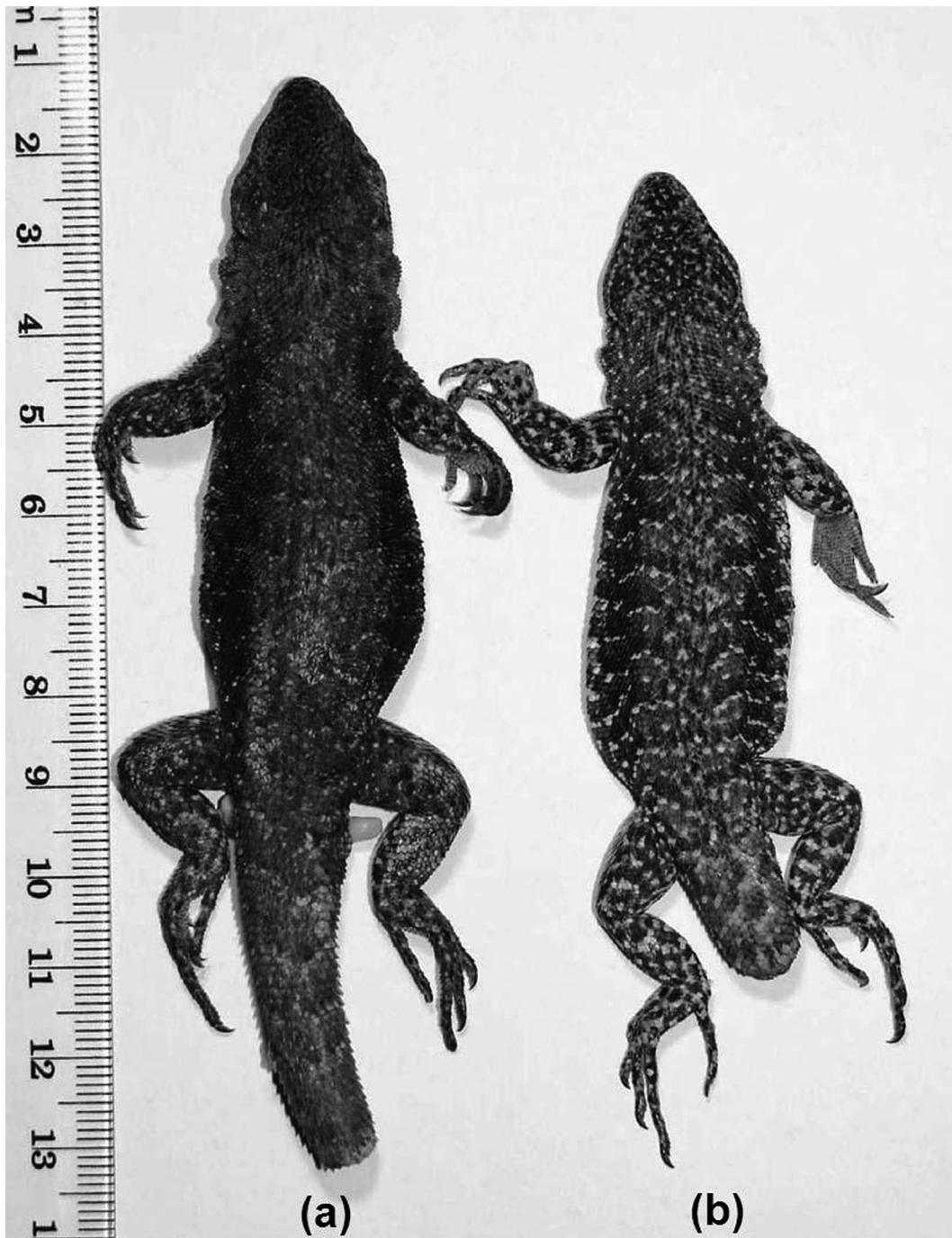

FIGURE 1. Holotype (a; MLP.R. 5274) and female paratype (b; MLP.R. 5276) of *Liolaemus tregenzai* in dorsal view.

FIGURE 2. Male (a) and female (b) of *Liolaemus tregenzai* in life.

Etymology. This new lizard species is named *Liolaemus tregenzai* after Tom Tregenza (Royal Society Research Fellow, University of Exeter), in recognition of his contributions to evolutionary biology. This dedication is also a personal recognition for the time spent guiding DP-D through a doctoral period of research in the United Kingdom.

Diagnosis. A large *Liolaemus* species belonging to the *chiliensis* clade diagnosed by Etheridge (1995), because it has a low number (4-4) of narrow supralabial scales, the last one elongated and upturned in the posterior margin, without a bladelike process on the posterior distal tibia, without femoral patch of enlarged scales, without tridentate dorsal scales, and with Meckel's groove fused (see also Laurent 1992; Schulte *et al.* 2000; Lobo 2001; Pincheira-Donoso & Núñez 2005). All these traits differentiate *L. tregenzai* from *L. periglacialis, L. kolengh, L. lineomaculatus* and *L. silvanae*, which exhibit the diagnostic traits detailed for the *lineomaculatus* clade (see Donoso-Barros & Cei 1971; Cei 1986; Etheridge 1995). Etheridge (1995) recognized four or fewer precloacal glands in males of most of the species belonging to the *chiliensis* clade. In *Liolaemus tregenzai* these glands are entirely absents, which differentiate this species from almost every taxa into this clade, except from *L. coeruleus, L. cristiani, L. flavipiceus, L. neuquensis,* and *L. thermarum,* which do not exhibit precloacal glands. *Liolaemus tregenzai* differs from *L. cristiani* in having a Patagonian distribution, and green-bluish ventral coloration with intense black pigment on the throat, chest and abdomen, whereas *L. cristiani* occurs in the Andes of central Chile, and exhibit a brown-yellowish or copper-yellowish ventral coloration, with total absence of intense black pigmentation in both sexes. Also, in *L. tregenzai* sexual dichromatism is evident, being absent in *L. cristiani*. Significant differences in the colour pattern differentiate *L. tregenzai* from *L. flavipiceus*. The olive, brown-blackish or chest-nut dorsal coloration and the green-bluish ventral colour with intense black pigmentation characteristics of *L. tregenzai*, contrast with the yellow dorsal background, black on the head, flanks, throat and chest, and with the greyish or reddish ventral colour with scattered orange or reddish spots observed in *L. flavipiceus*. *Liolaemus tregenzai* also differs from *L. thermarum*, because this last species does not exhibit black pigmentation on the ventral surface, which is whitish with conspicuous pinkish shades. The *Liolaemus* species lacking precloacal glands that exhibit more phenotypic and geographical similitude with *L. tregenzai* are *L. coeruleus*, from Zapala and Pino Hachado (Cei 1986; Pincheira-Donoso 2003) and *L. neuquensis*, from Copahue (Cei 1986; Videla & Cei 1996), the same area where the new species occurs (see above). *Liolaemus tregenzai* differs from *L. coeruleus* in having a body size ranging between 63.4–90.2 mm (see below for details) which contrasts significantly (see above) with the 44.7–60.2 mm in *L. coeruleus*. Also, the number of scales around the midbody (and therefore, the proportional size of body scales in relation to body size) is significantly different between these species, having *L. tregenzai* a mean value of 80.63, whereas the mean value in *L. coeruleus* is 63.4. In addition, while *L. tregenzai* exhibits a green-bluish ventral coloration, the abdominal and pectoral background in *L. coeruleus* is blue or sky-blue. Finally, *L. tregenzai* differs from *L. neuquensis* in having a significantly larger body size (55.3–61.1 mm in *L. neuquensis*; see above), and because *L. neuquensis* shows a prominent hemigular fold, completely absent in *L. tregenzai*. The same ventral differences detailed for *L. coeruleus* in relation to *L. tregenzai*, are valid for *L. neuquensis*.

Description of the Holotype (Fig. 1). Adult male, snout-vent length 82.5 mm, forelimb length 32.3 mm, hind limb length 47.9 mm. Tail broken. Head length 18.0 mm, head width 16.5 mm, head height 9.2 mm. Stout body, with prominent head. Hind limb adpressed reaches the shoulder. Neck wider than head. Lateral cervical folds, postauricaular, supra-auricular, longitudinal neck, oblique neck, and antehumeral, prominent, forming a V behind the external auditory meatus. Deep prehumeral pocket. Lateral nuchal scales small, granular, convex, nonoverlapping, with numerous tiny granules in the interstitial spaces. Hemigular fold entirely absent.

Upper head scales smooth, pitted with numerous sensitive organs mainly on snout. Rostral shield pentagonal, about four times as wide as high, and as wide as mental, directly surrounded by six scales (two supralabials, two lorilabials and two postrostrals). Two postrostrals, together with anterior supralabials almost separate nasals from rostral. Nasal shields longer than wide, as large as postrostrals, surrounded by six scales.

Nostril rounded, slightly over one half length of nasal, posterior in position. Four internasal scales, the medial pair as large as postrostrals. Two irregularly shaped azygous forming a longitudinal row on the snout, the anterior twice as large. The posterior in direct contact with a prefrontal azygous. A frontal azygous, slightly larger than interparietal. Two postfrontal shields, smaller than interparietal. Interparietal shield small, as large as nasals, pentagonal, slightly enlarged, flanked posterolaterally by a pair of irregularly shaped parietal scales relatively larger than frontal azygous, and anteriorly in direct contact with postfrontals. Parietal eye small and conspicuous, posterior in position. Supratemporal scales relatively convexes, smooth, irregularly shaped and exhibiting high variation in size. Temporal scales more regulars, weakly keeled and subimbricate or juxtaposed. Circum orbitalis conspicuous, formed by 13-13 small and often hexagonal scales. Supraoculars 6-4, strongly hexagonals and transversally expanded, up to eight times larger than circum orbitalis scales. Three rows of smaller scales disposed between supraorbitals and superciliars. Superciliar scales longitudinally expanded, imbricate. Canthal margin inconspicuous. Loreal region weakly deep, covered by 5-5 irregularly shaped scales which exhibit high variability in size. Ciliar scales do not form palpebral "combs". Upper ciliar scales 15-15, lower ciliars 11-10. A single and longitudinally enlarged subocular shield. Supralabial scales 4-4, between two and three times longer than high, the fourth scale is evidently upturned on the posterior margin. Between subocular and supralabials there is a single row of lorilabial scales whose size is similar or slightly smaller than supralabials. External auditory meatus as large as the eye surface, oval, and higher than wide. Tympanic scales 3-3, convex, slightly projected backwards over external auditory meatus. Auricular scales 2-2, weakly convexes, as large as tympanics.

Mental shield about twice as wide as high, surrounded by the anterior infralabials and the anterior postmentals, but not in contact with anterior sublabials. Two divergent and decreasing rows of 5-5 postmental scales. Infralabial shields 5-5, between two and three times wider than high, whose surface is similar or slightly larger than supralabials. A single row of sublabial scales between the anterior infralabials and the anterior postmentals. Gular scales smooth, flat, imbricate, with rounded margin.

Dorsal body scales small, whose surface represents 45–60% of the interparietal shield, subtriangular, subimbricate, with a conspicuous but blunt keel, and without mucrons. There are scarce tiny and granular scales in the interstitial spaces. Dorsal scales grade laterally into slightly smaller scales, being fundamentally similar, but with abundant tiny granules in the interstices. Scales surrounding forelimb insertion small, convex, granular and juxtaposed. Ventral body scales 1.5–2 larger than dorsal scales, rounded, smooth, flat, and imbricate. Scales around midbody 81.

Forelimb scales as large as the dorsal body scales, subtriangular, imbricate and slightly keeled, becoming smooth on the dorsal surface of the hand. Lower humeral region covered by small, convex, granular, and juxtaposed, becoming larger, rounded, subimbricate and weakly keeled on the forearm. Several scales on this zone exhibit a tridentate margin. Infracarpal scales smaller, strongly imbricate, with well developed keels, and often with a tridentate margin. Subdigital lamellae laterally expanded, with three prominent keels, often with three conspicuous mucrons in the margin, although several of them can exhibit up to five mucrons. Posterior surface of thighs with tiny, granular, convex and juxtaposed scales. Upper hind limb scales slightly larger than dorsal scales, subtriangular, subimbricate, and weakly keeled on the femoral area, grading into more rounded, more keeled and subimbricate-juxtaposed on the tibial area. Supratarsal scales almost smooth, triangular and imbricate. On the lower femoral surface (infrafemorals), scales are smooth, flat, rounded and imbricate, grading into slightly larger scales on the tibial zone (infratibials). Infratarsal scales small, strongly imbricate, with well developed keels, and with a tridentate margin. Subdigital lamellae laterally expanded, with 3–4 strong keels, and with up to six prominent mucrons on the margin. Subdigital lamellae on the fourth toe of the left foot 26 (non-measurable on the right foot). Upper caudals larger than dorsal body scales, keeled and imbricate. On the ventral surface, scales are triangular, slightly keeled and imbricate. Without precloacal glands.

Coloration. Based in the whole type series, including males and females. A sexually dichromatic species (Figs. 1 and 2). Although the coloration pattern is fundamentally similar in both sexes, pigments in males are more conspicuous.

Males. Background coloration of dorsum and upper head surface dark green olive, with disperse light green scales. Some specimens can exhibit a brown-blackish general colour on the upper head scales. On the back sides the ground colour becomes darker, forming a kind of wide lateral band, which can be brown-blackish or almost completely black in some individuals. The lateral fusion of some small blackish spots can form transversal and fine stripes which contact the dark band of the flanks. Upper surface of limbs ranging from green olive to dark brown, with abundant, contrasting and small light green spots irregularly dispersed. Lower surface of limbs black. Dorsal surface of tail with dark green semilunar spots surrounded by inconspicuous light green shades, black on the lower surface. Background colour of ventral surface light and shiny green-blueish. This coloration reaches the lower flanks. On the throat, chest, and medial surface of abdomen completely and intensely black.

Females. Dorsal head surface dark green, with a few small light green spots. Dorsal background colour dark chest-nut, with abundant and irregularly dispersed light green scales and irregularly shaped dark brown or blackish spots. On the anterior back sides there is a relatively wide dark brown or blackish lateral band. Background colour of ventral surface pale green-bluish, with abundant and irregularly dispersed light brownish or brown-copper, sometimes forming incipient transversal stripes. The medial colorations of throat, chest and abdomen are dark grey or greyish, never black as in males. Upper surface of limbs and tail colour dark chest-nut with abundant light green and blackish small spots. In juveniles the coloration is more similar to females, with dark greyish ventral surface.

Variation. Based on the entire type series. Variation in allometric proportions revealed the existence of sexual dimorphism in most of the studied variables. Snout-vent length differs significantly between both sexes (*t*-test, $t_9 = 2.978$, $P = 0.016$), being 75.7–90.2 mm (mean = 82.6 mm; SD = 5.94) in males, and 63.4–80.8 mm (mean = 71.41 mm, SD = 6.02) in females. Head length shows also significant sexual differences (*t*-test, $t_9 = 2.884$, $P = 0.018$), ranging between 17.3–20.5 mm (mean = 18.83 mm, SD = 1.45) in males, and between 14.5–18.9 mm (mean = 16.29 mm, SD = 1.38) in females. Head weight differs significantly between sexes (*t*-test, $t_9 = 2.828$, $P = 0.02$), being 13.1–16.5 mm (mean = 15.2 mm, SD = 1.6) in males, and 12.4–14.4 mm (mean = 13.2 mm, SD = 0.79) in females. Sexes differ in head height (*t*-test, $t_9 = 2.414$, $P = 0.039$), ranging between 9.1–12.2 mm (mean = 10.2 mm, SD = 1.44) in males, and between 7.1–9.3 mm (mean = 8.51 mm, SD = 0.91) in females. Conversely, tail and limb lengths do not show sexual dimorphism. Tail length in males is 8.46 mm (only one specimen with the entire tail), and 93.5–105.4 mm (mean = 95.1 mm, SD = 1.69) in females. Forelimb length in males is 27.8–32.3 mm (mean = 29.38 mm, SD = 2.05) in males, and 23.8–30.8 mm (mean = 26.62 mm, SD = 2.8) in females ($P = 0.123$). Hind limb length is 41.8–47.9 mm (mean = 44.58 mm; SD = 2.73) in males, and 35.9–46.0 mm (mean = 40.56 mm, SD = 3.35) in females ($P = 0.073$). When contrasting the derived variables forelimb length/snout-vent length (*t*-test, $P = 0.351$; 0.36 ± 0.03 in males, versus 0.37 ± 0.02 in females) and hind limb length/snout-vent length (*t*-test, $P = 0.256$; 0.54 ± 0.04 in males, versus 0.57 ± 0.03 in females) they do not show significant differences between sexes.

Variation in scale characteristics shown indistinctly for both males and females. Number of supraocular scales 5–6 (mean = 5.33, SD = 0.52). Scales forming circum orbitalis 13–14 (mean = 13.33, SD = 0.51). Upper ciliar scales 13–15 (mean = 13.83, SD = 0.75). Lower ciliar scales 10–14 (mean = 11.67, SD = 1.63). Number of loreal scales 5–9 (mean = 6.71, SD = 1.38). Number of supralabial scales 4–4 in the whole sample. Number of tympanic scales 2–4 (mean = 3.0, SD = 0.63). Number of auricular scales 2–3 (mean = 2.33, SD = 0.51). Number of postmental scales 4–6 (mean = 4.83, SD = 0.75). Number of infralabial scales 5–7 (mean = 6.0, SD = 0.63). Subdigital lamellae on the fourth toe of the left foot 24–26 (mean = 24.75, SD = 0.96; we counted lamellae on the left food because in the holotype the right one is damaged). Variation in dorsal body scales is observed in form and disposition, ranging from rhomboidal to subtriangular (Fig. 3a, b), being juxtaposed-subimbricate to subimbricate-imbricate. Ventral body scales do not exhibit remarkable variation in relation to the holotype (Fig. 3c, d). Number of scales around the midbody 71–85 (mean = 80.63, SD = 4.27). Precloacal glands are absent in males and females of the whole sample (Fig. 3e, f).
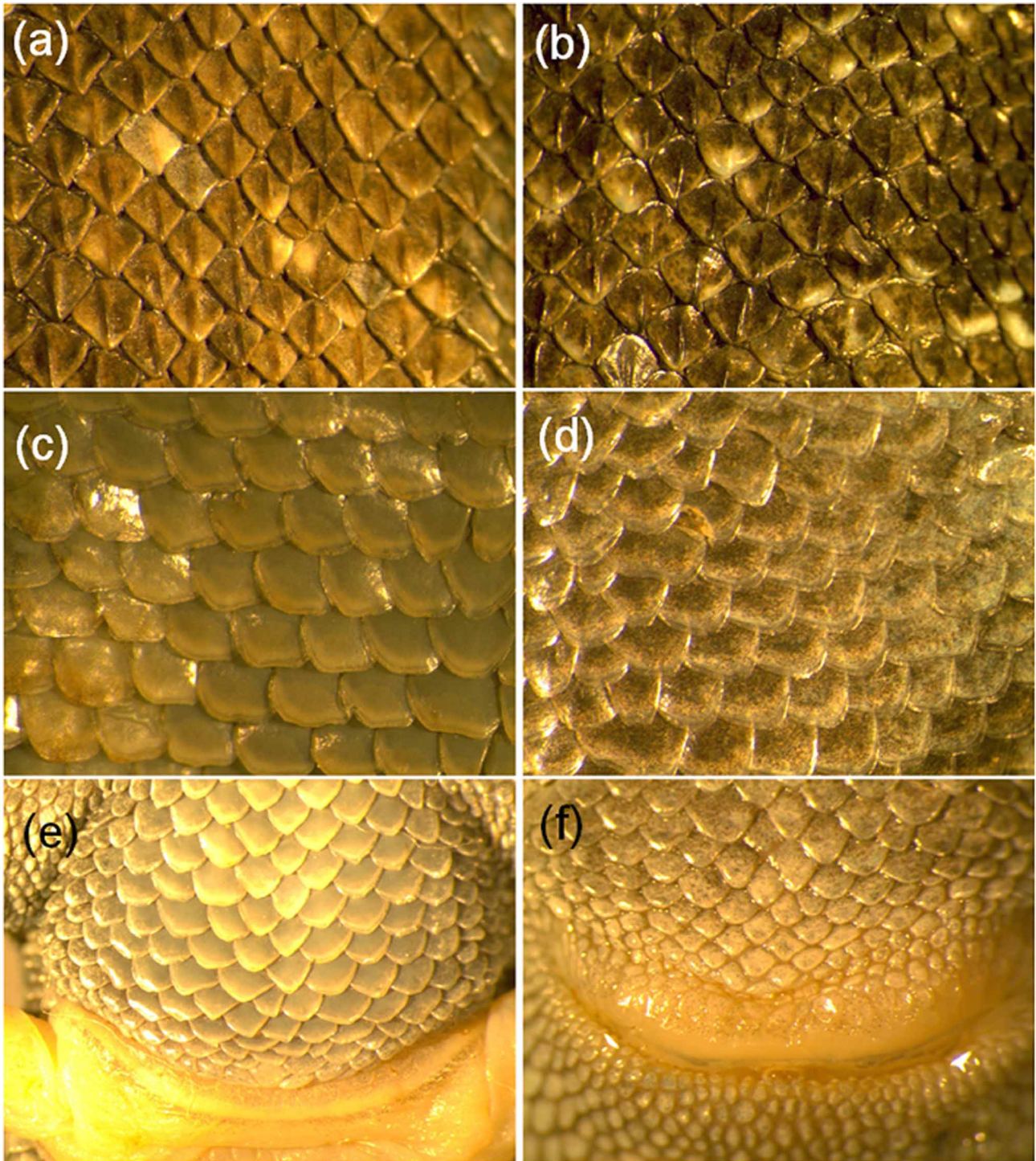
FIGURE 3. Variation in morphological traits in the holotype male (a, c, e) and allotype female (b, d, f) of *Liolaemus tregenzai*. Dorsal body scales in the holotype (a) and in the allotype, ventral body scales in the holotype (c) and in the allotype, and cloacal region in the holotype (e) and in the allotype (f).

Distribution. *Liolaemus tregenzai* is known from Copahue Volcano slopes (37°50'S; 71°06'W), 2150 m, near Termas del Copahue, Departamento de Ñorquín, Neuquén Province, in north-western Patagonia of Argentina (Fig. 4).

Natural History. *Liolaemus tregenzai* is a Patagonian and diurnal species. Occurs in the Andean ecotonal zones between open antarctandic forests formed by Araucaria (*Araucaria araucana*) and areas recovered by *Chasquea* sp. It is a common species in areas characterized by clay sediments on the slopes of the Copahue

volcano. Remarkably, *L. tregenzai* has often been found in the neighbourhoods of snow deposits, and close to thawing snow, which frequently form streams.

The diet of *L. tregenzai* is almost completely herbivorous. One of the studied specimens kept in captivity during more than nine months, was exclusively fed with lettuce. Nothing is known about other aspects of its natural history, remaining a mystery its reproductive mode.
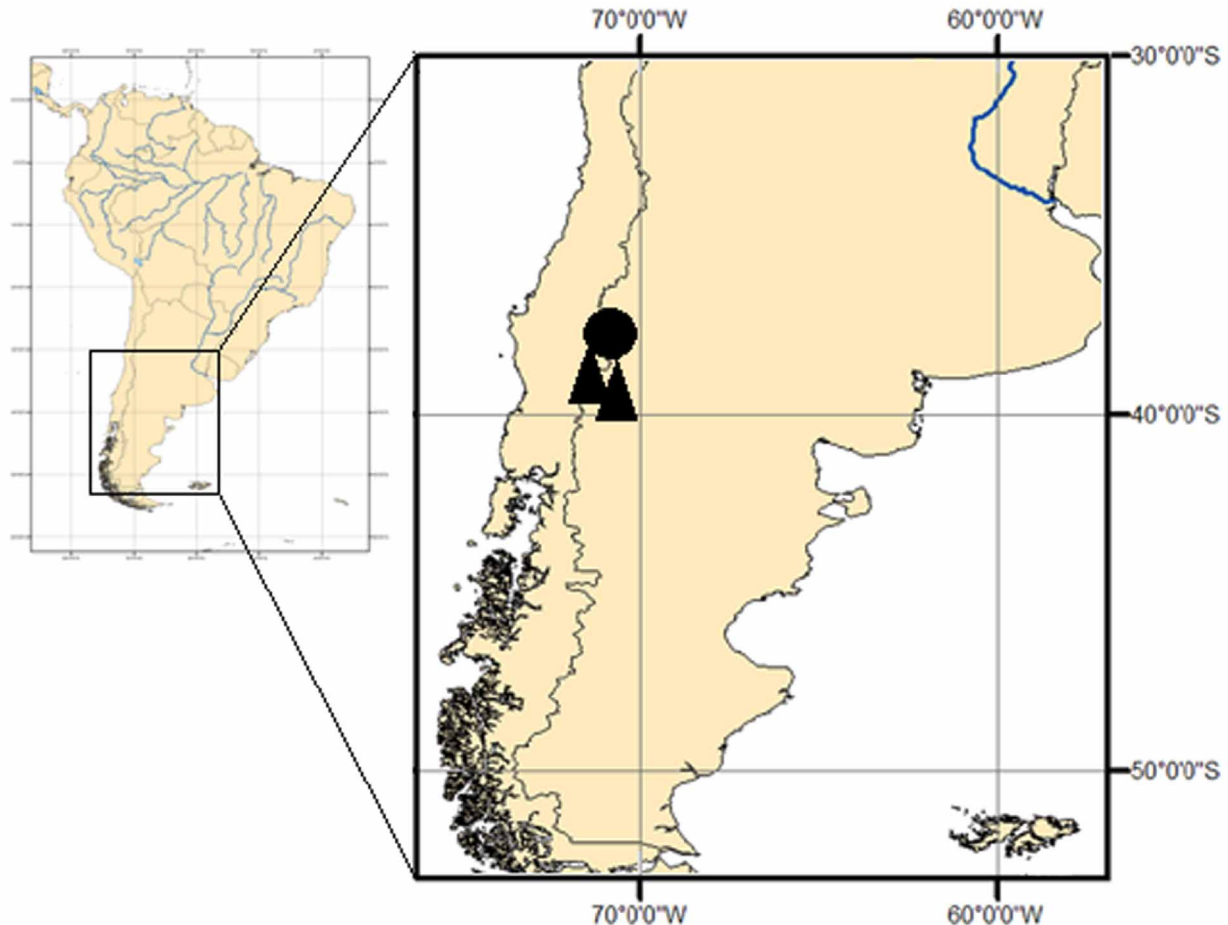

FIGURE 4. Map of the North-Western Patagonia of Argentina and Chile, showing the distribution of *Liolaemus tregenzai* and *L. neuquensis* (black circle; Copahue Volcano) and *L. coeruleus* (both black triangles; Pino Hachado, Primeros Pinos)

Discussion

Our results support the hypothesis that the species herein recognized as *Liolaemus tregenzai* is a new taxon, different from the remaining *Liolaemus* species lacking precloacal glands. And more fundamentally, from *L. coeruleus* and *L. neuquensis*, both members of the *chiliensis* clade, and also distributed in the boreal Patagonia of Argentina and Chile (Cei 1986; Videla & Cei 1996; Pincheira-Donoso 2003).

The phenotypic traits studied in *L. tregenzai* exhibit significant differences from the traits observed in *L. coeruleus* and *L. neuquensis*. Indeed, although *L. tregenzai* and *L. neuquensis* occur in the same area (the Copahue volcano in the Neuquén Province of Argentina, see above), a more evident similitude in phenotypic traits was observed between *L. neuquensis* and *L. coeruleus*. The number of scales around the midbody was found as the only variable which differs significantly (a mean value of 72.3 in *L. neuquensis*, versus 63.5 in *L. coeruleus*). Also, coloration traits are strongly overlapped in these two taxa. Such findings reinforce the idea

that a more detailed study is needed to establish the species boundaries of *L. coeruleus* and *L. neuquensis*, both still poorly known taxa in terms of trait variation and biogeographical distribution. Our observations suggest that *L. coeruleus* and *L. neuquensis* might be conspecific taxa. Therefore, the conduction of additional statistical and phylogenetic studies on these species would represent a primary step to clarify the relationships of *Liolaemus* populations lacking precloacal glands currently recognized as different taxa. In fact, in a lineage as *Liolaemus*, in which the number of known species and the distributional and ecological recorded ranges are one of the most extraordinary observed among vertebrates (see e.g. Etheridge 1995; Schulte *et al.* 2004), the clarification of such aspects appears to be fundamental. For example, being a clade consisting of more than 180 named species, *Liolaemus* lizards may be used as a promising model system to address questions concerning speciation. Nevertheless, to perform this kind of evolutionary research, it is necessary to make sure that the studied taxa are indeed well supported species, and not artefacts of taxonomical bias, in order to avoid misleading interpretations of the observed macroevolutionary and macroecological patterns (May 1988; Alroy 2003; Isaac *et al.* 2004). And of course, it is also necessary the development of accurate phylogenetic hypotheses. Particularly in the case of *Liolaemus* lacking precloacal glands, the species belonging to the *lineomaculatus* clade are quite well known phylogenetically (e.g. Espinoza *et al.* 2004), while those belonging to the *chiliensis* clade are known on the basis of a single species, *L. coeruleus* (Schulte *et al.* 2000; Espinoza *et al.* 2004).

In spite of the limited phylogenetic information on *Liolaemus* species of the *chiliensis* clade lacking precloacal glands, our phenotypic and biogeographical observations may support Cei and Videla's (2003) hypothesis that at least *L. coeruleus* and *L. neuquensis* belong to the *neuquensis* group (see introduction for details; also Cei & Videla 2002). Nevertheless, additional information is needed to clarify the evolutionary relationships of *L. tregenzai* with Patagonian *Liolaemus* lineages. On the basis of the available phenotypic evidence, it is possible to establish that this species may be related to the large Andean-Patagonian complex *elongatus-kriegi*. The species of this lineage are commonly characterized by large and stout body sizes, similar colour pattern and scutelation in body, legs and head. Future studies will provide additional evidence to increase the knowledge on the origin and evolution of *Liolaemus* species lacking precloacal glands.

Acknowledgements

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Appendix 1. Specimens examined and localities. Studied series are from Argentina and Chile. Museum symbolic codes as detailed in methods

Liolaemus coeruleus.—Paso Pino Hachado, Lonquimay Valley, Araucania Region, Chile, MZUC 23918-23927; Paso Pino Hachado, Lonquimay Valley, Araucania Region, Chile, MZUC 23904-23917; nr Zapala, Neuquén Province, Argentina, 28 JAS-DC specimens.

Liolaemus cristiani.– Cerro El Peine, Altos de Vilches, Maule Region, Chile, MNHN 1957-1961, paratypes.

Liolaemus flavipiceus.— Near Pehuenche Pass, Malargüe Department, Mendoza Province, Argentina, MLP-S 2158, holotype; Near Pehuenche Pass, Malargüe Department, Mendoza Province, Argentina, MLP-S 2159, paratype; Near Pehuenche Pass, Malargüe Department, Mendoza Province, Argentina, IADIZA 307-310, paratypes.

Liolaemus kolengh.– National Reserve Jeinimeni, Aysén Region of Chile, seven MNHN without official museum number.

Liolaemus periglacialis (= *L. hatcheri*).—6 km E Lago Belgrano, Santa Cruz Province, Argentina, JMC-DC 506-512, paratypes *L. periglacialis*; North shore of Río Belgrano, Santa Cruz Province, Argentina, JMC-DC 514-516, paratypes *L. periglacialis*; Cerro Beltza, 10 km E Lago Belgrano, Santa Cruz Province, Argentina, JMC-DC 517-519, paratypes *L. periglacialis*; near Lago Guitarra, Meseta Asador-Aguila, Santa Cruz Province, Argentina, JMC-DC 521-525, paratypes *L. periglacialis*; Lago Belgrano, Santa Cruz Province, Argentina, CHDPD 00814, paratype *L. periglacialis*; Meseta Asador-Aguila, Santa Cruz Province, Argentina, CHDPD 00815, paratype *L. periglacialis*.

Liolaemus lineomaculatus.—Laguna Amarga, Magallanes Region, Chile, MNHN 1681-1682; Dos Lagunas, Coyhaique, Aysén Region, Chile, MNHN 3430-3436; Portezuelo, N Lago Buenos Aires, Santa Cruz Province, Argentina, CHDPD 00812-00813; Balmaceda, Aysén Region, Chile, CHDPD 00831; "Patagonia", BMNH xxii.90.RR.1946.8.5.72-75, type series.

Liolaemus neuquensis.—Copahue Volcano, Departamento de Ñorquín, Neuquén Province, Argentina, 26 JAS-DC specimens.

Liolaemus silvanae.—Puesto Lebrún, Meseta Lago Buenos Aires, Santa Cruz Province, Argentina, IBAUNC 519-1, holotype; Puesto Lebrún, Meseta Lago Buenos Aires, Santa Cruz Province, Argentina, IBAUNC 519-2, paratype; Laguna del Sello, Santa Cruz Province, Argentina, IBAUNC 519-4, 520-1, 520-2, 534, paratypes.

Liolaemus thermarum.– Baños del Azufre, 10 km from Peteroa Volcano, Malargüe Department, Mendoza Province, Argentina, four JMC-DC specimens.

Liolaemus tregenzai.— See results for details.