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Comparative evidence for strong phylogenetic inertia in precloacal signalling glands in a species-rich lizard clade

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ABSTRACT

Background: The precloacal glands of lizards are responsible for the secretion of pheromones involved in chemical-based interactions, such as male territoriality and female mate choice. However, in spite of the significance of these structures for social and sexual communication, their evolution remains poorly studied. Previous research has suggested that the number of precloacal glands may reflect adaptive variation because a higher number of these organs increases the potential rate of secretion, compensating for the impact of extreme environmental conditions on the optimal quantity of secretions smeared on the substrate. Therefore, the number of precloacal glands may be expected to exhibit convergent evolution in response to similar environments. Nevertheless, the only available evidence testing this prediction ignored potential effects of shared phylogenetic history on the evolution of this trait.

Hypotheses: (1) Lizard precloacal gland number evolves adaptively in response to variation in environmental conditions, experiencing convergent patterns independent of phylogenetic relationships. (2) Species with a wider geographical distribution exhibit higher variance in the number of precloacal glands as a response to variation along environmental gradients.

Organisms: *Liolaemus* lizards, one of the largest and most ecologically diverse vertebrate genera.

Methods: Phylogenetic comparative methods. Regression analyses based on phylogenetic independent contrasts, and on raw data at intra-clade level. Historical estimates based on ancestral state reconstructions from explicit phylogenetic hypotheses.

Results: Precloacal glands are constrained by phylogenetic relationships. In contrast to previous work, we found no evidence for independent convergent events along the phylogenetic history of this lineage. Environmental conditions failed to predict the number of glands in species of the *Liolaemus* genus in both current and reconstructed ancestral states.

Conclusions: Our phylogenetically controlled comparative analysis fails to support the hypothesis that the number of precloacal emitter glands in lizards is the product of adaptive evolution.

Keywords: chemical communication, comparative method, *Liolaemus*, lizards, phylogenetic inertia, precloacal glands.

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INTRODUCTION

Communication between animals has fundamental implications for evolutionary processes driven by selection (Andersson, 1994; Fox *et al.*, 2003; Boul *et al.*, 2007; Puebla *et al.*, 2007). In general, animal interactions through visual and acoustic cues have received most attention (Bateson, 1983; Andersson, 1994; Hauser, 1997). How these signals rely on the correlation of morphological and behavioural traits, and their involvement in the evolution of biological communities, is now well understood (Nicholson *et al.*, 2007). In contrast, chemical communication has been studied mainly in relation to how environmental conditions affect the transmission and quality of signals (Endler, 1993; Escobar *et al.*, 2001; Vane-Wright and Boppré, 1993) and the mechanisms utilized by receptor individuals to process scents (Cooper, 1994; Cooper *et al.*, 1994; Labra *et al.*, 2001a, 2001b). However, in spite of their potential significance, the role of specializations in phenotypic traits involved in chemical interactions remains relatively under-explored (Bateson, 1983; Martín and López, 2000). Previous studies have found that, for example, the evolution of scent emitter glands in some Characidae fish are implicated in their diversification by sexual selection (Nelson, 1964), and that emitter glands in *Desmognathus* salamanders may correlate with sexually dimorphic teeth used to perforate the skin of females, where secretions are then deposited (Arnold and Houck, 1982).

Among vertebrates, lizards offer an excellent model to address hypotheses relating to divergence in chemical communication systems and its evolutionary significance. Lizards have evolved diverse and specialized strategies for sexual interactions through chemical scents (Simon, 1983; Labra and Niemeyer, 1999; Pough *et al.*, 2004). Almost every lizard species exhibits conspicuous epidermal glands, located on the anterior edge of the cloacae (precloacal glands) or on the ventral surface of the thigh (femoral glands) (Cole, 1966; Antoniazzi *et al.*, 1994; Martín and López, 2000). These structures are responsible for the production of complex chemical secretions (Donoso-Barros, 1966; Alberts, 1991, 1993; Mason, 1992). The more conspicuous development of precloacal and femoral glands in males, and their higher secretory activity during the breeding season, is indicative of their role in sexual communication (Alberts, 1993; Martín and López, 2000; López *et al.*, 2003; Pough *et al.*, 2004). Recent studies have shown that secretions produced by these glands convey fundamental information used to recognize mates, sexual competitors, and kin (Cooper and Vitt, 1986; Mason, 1992; Schwenk, 1995; Labra and Niemeyer, 1999; López *et al.*, 2003). Additionally, there is evidence that in some species female mate choice may be determined by chemical cues, rather than by visual traits (Olsson and Madsen, 1995; Tokarz, 1995; Martín and López, 2000; López *et al.*, 2003). For example, López *et al.* (2003) observed that female mate preferences in *Lacerta monticola* are not determined by visual cues (i.e. female choice on size or colour patterns), but by chemical signals. These authors suggested that chemical secretions may transmit detailed information about the genetic quality of a male to the female, providing the basis for female choice in lizards (Martín and López, 2000). Also, Cooper and Vitt (1986) found that males of the skink *Eumeces inexpectatus* react violently only towards conspecific males, but could be tricked into attacking heterospecific males if they were smeared with conspecific cloacal secretions. Finally, a recent study (Kratochvil and Frynta, 2002) of the relationships between male territorial behaviour and presence/absence of precloacal emitter glands in eublepharid geckos observed that species lacking these structures are less territorial.

Only two sexual selection based hypotheses have been proposed to explain the evolution of morphological traits involved in chemical communication in these organisms, and in particular the substantial variation in the number of scent glands across taxa. One

prediction suggests that female preferences for males with a higher number and a low fluctuating asymmetry of femoral glands may lead to adaptive specializations in these structures (Martín and López, 2000; López *et al.*, 2003). The alternative hypothesis (Escobar *et al.*, 2001) is that precloacal glands in lizards experience convergent evolution under similar environmental conditions. Escobar *et al.* (2001) observed that these glands in *Liolaemus* species correlate with environmental temperature, wind, and atmospheric pressure. They postulated that harsh habitat conditions may select for the production of greater amounts of chemical secretions. This would compensate for the negative effects of environmental factors, such as extreme temperatures, on the secretions smeared on the substrates. Escobar *et al.* (2001) predicted that species living in warm and windy habitats would evolve a higher number of precloacal glands than species living in colder environments, because the amount of secretions may correlate positively with the number of glands (see also Escobar *et al.*, 2003). Nevertheless, in spite of the significance of these adaptive hypotheses, they remain almost totally untested. The first hypothesis (Martín and López, 2000; López *et al.*, 2003) has only been examined in a single lizard species (*Lacerta monticola*). The other hypothesis has only been addressed by Escobar *et al.* (2001), but they ignored phylogenetic relationships among species, and therefore their conclusions may have been biased by effects of shared ancestry (Felsenstein, 1985; Harvey and Pagel, 1991).

In this paper, we test the hypothesis that lizard species living in similar environmental conditions experience convergent evolution in the specialized precloacal glands responsible for the production of chemical scents (Escobar *et al.*, 2001). Additionally, we test the hypothesis that species experiencing a wider thermal range (as a result of their geographical range) exhibit greater variance in the number of precloacal glands. We investigated these predictions in South American *Liolaemus* lizards. The iguanian genus *Liolaemus* consists of more than 190 described species, belonging to at least six well-diagnosed and phylogenetically supported clades (Etheridge, 1995; Etheridge and Espinoza, 2000; Pincheira-Donoso and Núñez, 2005; Pincheira-Donoso *et al.*, 2007a) (see Table 1). This lineage inhabits the widest variety of environments recorded among living reptiles (Cei, 1993; Schulte *et al.*, 2004), occurring in the Atacama Desert (the driest place on earth) and in diverse habitats stretching to the most southerly area where reptiles have been reported, in Tierra del Fuego, and from sea level to more than 5000 m (Donoso-Barros, 1966; Cei, 1986; Pincheira-Donoso, 2005). In these lizards, precloacal glands are conspicuously developed in males of more than 95% of known species. They are very similar in size across taxa, and exhibit substantial variation in number between clades (Cei, 1986, 1993; Pincheira-Donoso and Núñez, 2005; Pincheira-Donoso and Scolaro, 2007).

METHODS

Study species

We studied 102 *Liolaemus* species distributed across almost the entire geographical range of the genus (Donoso-Barros, 1970; Cei, 1993; Etheridge, 2000). A sample of 3348 adult specimens from both sexes was used to construct geographical and morphological data sets. The total sample comprises specimens of three different types: (i) living individuals studied in the field and then released; (ii) preserved specimens without official collection numbers housed in different institutions (see Appendix); and (iii) preserved specimens with official collection numbers and housed in the institutions detailed in the Appendix. Specimen references can be found for most of the species in Pincheira-Donoso and Núñez (2005). Data for two species

Table 1. Species used in this study

Species	*n*	No. of precloacal glands		Latitudinal range (°S)	Altitudinal range (m)
		Mean ± standard error	Range		
chiliensis					
alticolor	21	3.3 ± 0.20	3–4	17°00′–21°35′	4000–4650
araucaniensis	9	3.5 ± 0.57	3–4	37°28′–38°50′	1400–1700
atacamensis	24	3.0 ± 0.58	2–4	23°55′–28°30′	0–2000
austromendocinus	7	2.9 ± 0.74	2–4	34°30′–36°20′	1000–2100
barbarae	14	3.9 ± 0.66	3–5	22°40′–23°13′	3050–4500
bellii	113	2.0 ± 0.00	2	33°11′–33°21′	2100–3000
bibronii	11	4.0 ± 0.67	3–5	32°00′–49°00′	0–3000
bisignatus	26	2.5 ± 0.52	2–3	26°20′–27°50′	0–500
buergeri	12	3.0 ± 0.57	2–4	36°00′–38°50′	1500–3000
ceii	6	4.0 ± 0.00	4	34°55′–38°48′	1000–2300
cf. elongatus	16	2.3 ± 0.52	2–3	34°17′–34°17′	1800–1800
chiliensis	92	2.5 ± 0.51	2–3	31°22′–39°24′	0–2100
chillanensis	13	4.0 ± 0.00	4	36°50′–39°27′	1500–2000
constanzae	132	3.5 ± 0.50	3–4	22°37′–23°55′	2200–2800
curicensis	65	2.4 ± 0.50	2–3	34°08′–35°03′	1520–1950
curis	49	1.7 ± 0.73	0–2	35°48′–35°48′	1520–1700
cyanogaster	14	2.4 ± 0.50	2–3	36°40′–41°45′	0–250
elongatus	13	3.4 ± 0.52	3–4	29°00′–46°00′	700–3000
fitzgeraldi	16	2.4 ± 0.49	2–3	32°46′–32°55′	2400–3200
fuscus	46	2.6 ± 0.50	2–3	30°30′–36°35′	1000–2100
gravenhorstii	12	2.5 ± 0.51	2–3	33°25′–33°35′	400–520
gununakuna	3	2.0 ± 0.69	1–3	37°55′–39°30′	500–1000
isabelae	19	2.4 ± 0.49	2–3	26°14′–26°26′	2850–3672
kriegi	27	3.5 ± 0.51	3–4	34°00′–42°04′	950–2000
lemniscatus	391	2.4 ± 0.49	2–3	30°26′–39°40′	0–1800
leopardinus	16	1.7 ± 0.62	0–2	33°15′–33°21′	2100–3000
lorenzmuelleri	23	3.0 ± 0.00	3	29°49′–30°13′	3200–3500
maldonadae	3	2.5 ± 0.70	2–3	30°43′–30°43′	2600–2800
melaniceps	2	3.0 ± 0.00	3	19°24′–19°24′	0–10
monticola	113	2.3 ± 0.47	2–3	33°11′–34°11′	1500–2500
moradoensis	54	2.0 ± 0.00	2	33°42′–33°45′	3300–3600
nigromaculatus	17	2.5 ± 0.51	2–3	23°50′–28°30′	0–250
nigroviridis	289	3.4 ± 0.50	3–4	32°58′–34°04′	1250–3370
nitidus	64	2.1 ± 0.35	2–3	28°15′–36°20′	0–2500
pagaburoi	6	3.6 ± 0.40	2–6	26°44′–27°30′	3000–4700
paulinae	35	4.3 ± 0.48	4–5	22°27′–22°28′	2200–2300
petrophilus	4	3.4 ± 0.92	2–5	41°20′–43°50′	600–1400
pictus	107	2.9 ± 0.63	2–4	35°27′–43°23′	0–1600
platei	41	3.0 ± 0.00	3	25°00′–31°38′	0–1050
pseudolemniscatus	7	2.4 ± 0.50	2–3	29°56′–32°10′	400–800
ramirezae	2	3.2 ± 0.60	3–5	24°20′–27°20′	2820–3300
ramonensis	8	3.4 ± 0.51	3–4	33°24′–33°30′	2500–3000
schroederi	118	2.3 ± 0.46	2–3	33°16′–36°37′	1800–2590
silvai	7	3.0 ± 0.00	3	29°05′–29°05′	140–150

Table 1.—*continued*

Species	*n*	No. of precloacal glands: Mean ± standard error	No. of precloacal glands: Range	Latitudinal range (°S)	Altitudinal range (m)
tenuis	264	2.6 ± 0.58	2–4	32°01′–41°44′	0–1800
valdesianus	17	1.7 ± 0.77	0–2	33°47′–33°56′	1800–2800
velosoi	42	2.5 ± 0.52	2–3	26°23′–27°23′	0–750
zapallarensis	110	3.5 ± 0.50	3–4	30°00′–33°00′	0–800
archeforus-kingii					
archeforus	8	6.5 ± 0.25	5–8	46°38′–47°10′	610–1600
escarchadosi	3	6.6 ± 0.22	5–8	50°30′–50°40′	800–1100
kingii	12	7.6 ± 0.50	7–8	43°00′–51°40′	0–1000
sarmientoi	9	6.5 ± 1.04	5–8	52°00′–52°15′	85–250
scolaroi	32	8.3 ± 0.23	7–10	46°49′–46°52′	850–920
tari	2	7.0 ± 0.81	6–8	49°12′–49°16′	280–350
tristis	2	8.0 ± 0.18	6–10	46°50′–47°00′	700–1000
zullyi	14	7.4 ± 0.98	6–9	46°45′–47°00′	820–1400
montanus					
andinus	47	7.9 ± 0.65	7–9	22°43′–26°00′	4100–4900
eleodori	51	5.0 ± 0.25	3–7	29°06′–29°10′	2500–3500
erguetae	2	5.5 ± 0.57	5–6	22°00′–22°25′	4300–4570
fabiani	30	5.6 ± 0.51	5–6	22°55′–23°45′	2300–2450
famatinae	10	5.5 ± 0.17	2–7	28°45′–28°55′	3700–4200
filiorum	4	6.0 ± 0.57	5–7	22°00′–22°15′	2600–3100
foxi	19	5.1 ± 0.73	4–6	22°41′–22°44′	3200–3600
hajeki	23	6.4 ± 0.51	6–7	21°19′–22°20′	3500–3900
jamesi	16	3.9 ± 0.71	3–5	17°00′–20°55′	3500–4600
multicolor	7	6.4 ± 0.50	6–7	21°40′–23°05′	3600–4200
nigriceps	19	5.9 ± 0.66	5–7	24°00′–28°42′	3200–5100
pantherinus	24	5.4 ± 0.51	5–6	16°23′–21°42′	4000–4600
patriciaiturrae	31	5.6 ± 0.51	5–6	26°14′–26°26′	2850–3500
pleopholis	5	5.5 ± 0.50	5–6	18°12′–18°12′	4240–4400
puritamensis	16	6.5 ± 0.51	6–7	22°55′–22°55′	2400–2500
robertoi	20	5.5 ± 0.50	5–6	29°47′–30°28′	2400–3700
rosenmanni	58	7.0 ± 0.65	6–8	26°27′–28°42′	1960–4200
ruibali	161	5.1 ± 0.62	4–6	32°27′–32°55′	2370–3000
signifer	6	5.5 ± 0.51	5–6	16°35′–22°47′	4000–4500
stolzmanni	4	6.5 ± 0.51	6–7	21°29′–22°50′	3700–4300
vallecurensis	12	6.0 ± 0.66	5–7	29°34′–29°39′	2050–2200
fitzingerii					
abaucan	4	5.5 ± 0.15	4–6	27°19′–27°47′	1200–1900
albiceps	12	7.9 ± 0.31	6–9	23°30′–24°26′	3060–4020
boulengerii	13	7.3 ± 0.29	5–7	34°00′–42°00′	0–2000
darwinii	19	6.2 ± 0.09	4–8	28°28′–42°55′	800–3000
enigmaticus	1	6.0 ± 0.00	6	18°13′–18°13′	4650–4650
hermannunezi	8	8.0 ± 0.77	7–9	37°30′–37°32′	1428–1521

Table 1.—*continued*

Species	*n*	No. of precloacal glands		Latitudinal range (°S)	Altitudinal range (m)
		Mean ± standard error	Range		
irregularis	10	8.9 ± 0.16	8–10	23°55′–24°11′	3060–5000
koslowsky	4	5.7 ± 0.12	4–7	27°11′–29°18′	800–2450
laurenti	3	7.1 ± 0.10	5–9	28°10′–30°12′	800–1100
melanops	5	8.5 ± 0.26	6–10	36°26′–43°00′	900–2070
morenoi	3	9.0 ± 0.92	8–10	38°47′–41°06′	740–1023
olongasta	3	6.7 ± 0.17	5–9	28°38′–31°14′	900–1770
ornatus	24	7.7 ± 0.17	6–10	22°00′–24°00′	3500–4800
quilmes	4	5.7 ± 0.08	4–9	24°43′–27°03′	1600–3000
rothi	18	9.2 ± 0.35	6–12	38°50′–41°25′	500–1903
sagei	3	8.3 ± 0.22	7–10	39°01′–40°17′	931–1355
uspallatensis	35	6.0 ± 0.23	4–7	32°32′–32°40′	1830–2200
wiegmannii					
lutzae	3	5.8 ± 0.19	5–8	22°53′–23°53′	50–1200
multimaculatus	4	8.1 ± 0.16	5–11	35°00′–41°01′	0–1000
occipitalis	4	8.7 ± 0.19	8–10	27°02′–33°11′	0–250
rabinoi	2	7.9 ± 0.34	7–9	35°00′–35°05′	1800–1800
riojanus	1	8.4 ± 0.25	7–10	29°00′–32°00′	500–1000
salinicola	3	7.9 ± 0.15	6–10	27°00′–32°07′	0–2050
scapularis	3	7.3 ± 0.13	5–10	23°00′–32°00′	1000–2100
wiegmannii	12	6.1 ± 0.17	3–9	17°17′–40°50′	0–2600

Note: Taxa for which phylogenetic information was available are indicated in **bold**.

of the *archeforus-kingii* clade (*L. tari* and *L. tristis*) were obtained mainly from J.M. Cei. Species identification criteria follow the latest available studies (Scolaro and Cei, 1997; Etheridge, 2000; Etheridge and Espinoza, 2000; Pincheira-Donoso, 2005; Pincheira-Donoso and Núñez, 2005, 2007).

Precloacal glands in the *Liolaemus* genus are almost completely restricted to males and are clearly developed and easily counted (Donoso-Barros, 1966; Cei, 1993). Conversely, in females, although it is possible to observe some of these structures, they are scarce, restricted to a few individuals in some species, and are frequently hard to identify because of their limited development (Etheridge, 2000; Pincheira-Donoso and Núñez, 2005). Therefore, to conduct analyses of precloacal glands we only studied males, which were represented by a sample of 1817 specimens.

Environmental estimations

Temperature, humidity, and wind have been recognized as the primary environmental factors affecting chemical communication in terrestrial habitats (Alberts, 1992). Therefore, we evaluated variation in precloacal gland number in relation to latitude and elevation. These parameters capture much of the variation in factors such as temperature, humidity, and wind intensity (Donoso-Barros, 1966; Lutgens and Tarbuck, 1998; Escobar *et al.*, 2001; Ashton, 2002; Ramírez and Pincheira-Donoso, 2005). To test the effect of variation in environmental conditions on

patterns of precloacal gland variation in *Liolaemus* lizards, we performed both phylogenetic and non-phylogenetic regression analyses. First, to test the hypothesis that the mean number of glands correlates with latitude and altitude (Hypothesis 1 in the abstract), we performed bivariate regression analyses between the mean number of precloacal glands per species and its spatial position. Since environmental temperatures decrease with increasing latitude and elevation, we calculated the adjusted latitudinal midpoint (ALM) recently calibrated by Cruz *et al.* (2005). This approach and other similar versions that combine latitude and elevation under the same scale have been used extensively to provide an estimate of the temperature experienced by a taxon for use in multi-species studies (Espinoza *et al.*, 2004; Cruz *et al.*, 2005; Pincheira-Donoso *et al.*, 2007b; Wiens *et al.*, 2007). The ALM assumes that environmental temperature declines 0.65°C each 100 m of increased altitude (Lutgens and Tarbuck, 1998; Cruz *et al.*, 2005). Cruz *et al.* (2005) obtained a corrected latitudinal value for latitudinal and altitudinal thermal covariation using the formula $y = 0.009x - 6.2627$, where x represents the altitudinal midpoint for each species. Then, the result of this formula (y) is added to the latitudinal midpoint for each species (the details of this formula were provided personally by F.B. Cruz, as they are not published in the same format in Cruz *et al.*). The final value is referred to as the adjusted latitudinal midpoint for the South American areas where *Liolaemus* occurs (Cruz *et al.*, 2005).

Second, to test the hypothesis that thermal range predicts precloacal gland variance (Hypothesis 2), we performed regression analyses on the observed range of precloacal glands per species and the thermal range that each species occupies, calculated from the same covariation assumptions of temperature and latitude–altitude gradients previously described (0.65°C for every 100 m rise in altitude). We obtained this thermal range between the coldest and warmest locations identified for each species based on the lowest combination of latitude and altitude at the extreme points in the range of each taxon. While the adjusted latitudinal midpoint represents the distributional location of every species, and therefore the thermal midpoint of their distributional range (Blackburn *et al.*, 1999; Cruz *et al.*, 2005), the thermal range reflects the thermal variation experienced by each taxon.

Phylogenetic control and data analysis

Phenotypic traits in closely related lineages are not phylogenetically independent (Cheverud *et al.*, 1985; Felsenstein, 1985). To control for potential effects of shared ancestry, we analysed the data set in two different ways (e.g. Harvey and Rambaut, 2000; Schluter, 2000; Carvalho *et al.*, 2006). First, based on the raw data, we examined the influence of environmental factors separately for each of the five main clades recognized within the genus. We identified these clades as *chiliensis*, *archeforus-kingii*, *montanus*, *fitzingerii*, and *wiegmannii* following the latest nomenclature (Etheridge, 2000; Espinoza *et al.*, 2004; Cruz *et al.*, 2005; Pincheira-Donoso and Núñez, 2005) (see Fig. 1). Although this approach does not transform measured variables into phylogenetically controlled values, it allows for the control of the statistical bias that trait values from other clades may introduce into the model (e.g. Felsenstein, 1985; Harvey and Pagel, 1991). Second, we analysed the data set calculating phylogenetic independent contrasts (Felsenstein, 1985; Garland *et al.*, 1993) as implemented in COMPARE version 4.6b (Martins, 2004). We examined the interaction of variables following Espinoza and colleagues' (2004) phylogenetic hypothesis. Because this phylogeny is based on both molecular and morphological data, we performed analyses under a speciational Brownian motion model of evolutionary change with branch lengths set equal to 1.0 (Garland *et al.*, 1993; Espinoza *et al.*, 2004; Martins, 2004; Cruz *et al.*, 2005). The ancestral states

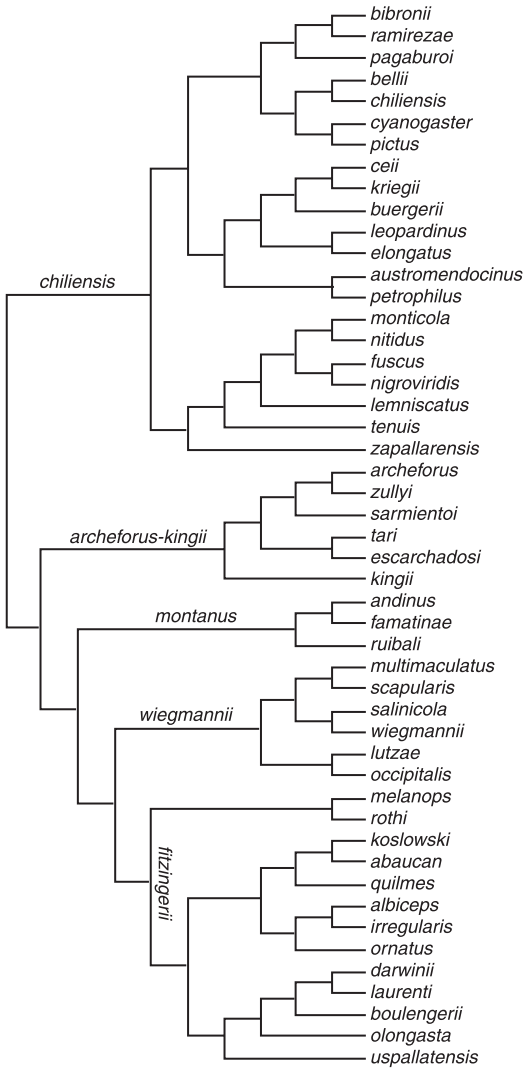

Fig. 1. Phylogenetic relationships between the five clades and 49 of the 102 species included in this study.

for precloacal glands and environmental predictors (ALM and thermal range) were reconstructed using the same software (COMPARE v. 4.6b) and the same branch length assumptions (Martins, 2004). This approach estimates ancestral variable values from sets of interspecific data using a generalized least squares (GLS) procedure (Martins and Hansen, 1997).

Of the 102 species studied, phylogenetic information was available for 49 (Espinoza *et al.*, 2004) (Fig. 1, Table 1). To ensure that the reduction of species sample size (from 102 to 49 species) did not affect our conclusions based on the models, we performed the same regression analyses for separate clades, but using only the 49 species included in the phylogeny.

RESULTS

Our results do not differ qualitatively when using the entire data set ($N = 102$) or when using the 49 species included in the phylogeny (Fig. 1) (F-range = 0.88–5.24, P-range = 0.127–0.431; see below).

Effects of latitude and elevation

Bivariate regression analyses showed that the adjusted latitudinal midpoint (ALM) does not reliably predict variation in precloacal glands. This environmental variable explained low proportions of variance in the clades *chiliensis* ($R^2 = 0.001$, $F_{1,47} = 0.04$, $P = 0.85$), *montanus* ($R^2 = 0.01$, $F_{1,20} = 0.21$, $P = 0.65$), *fitzingerii* ($R^2 = 0.13$, $F_{1,16} = 2.19$, $P = 0.16$), and *wiegmannii* ($R^2 = 0.14$, $F_{1,7} = 0.99$, $P = 0.36$). In contrast, ALM significantly predicted the number of precloacal glands only in the *archeforus-kingii* clade ($R^2 = 0.62$, $F_{1,7} = 9.69$, $P = 0.02$), where it was negatively correlated with the number of precloacal glands (Fig. 2). In this clade, species living in colder environments tend to have fewer precloacal glands than species distributed in warmer climates. Phylogenetic analysis combining data for the entire genus using independent contrasts revealed no significant influence of ALM on the number of precloacal glands across species ($R^2 = 0.01$, $F_{1,47} = 0.35$, $P = 0.56$) (Fig. 3).

Precloacal gland variance and thermal range

Bivariate regression analyses performed on raw (separately for each clade) and phylogenetically controlled data revealed that a wider geographical distribution is not associated with a greater variance in the number of precloacal glands in most of the studied clades.

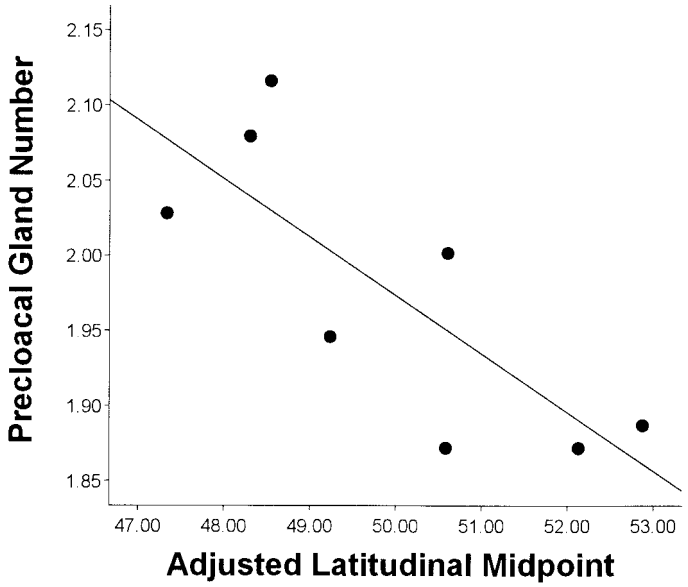

Fig. 2. Regression analysis of adjusted latitudinal midpoint (ALM) and precloacal gland number in the clade *archeforus-kingii* using raw data ($R^2 = 0.62$, $F_{1,8} = 9.69$, $P = 0.02$).

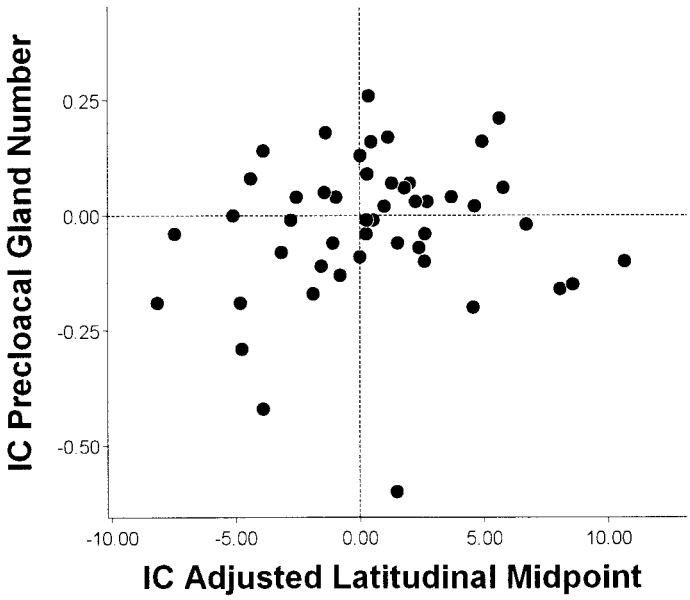

Fig. 3. Regression analysis of phylogenetic independent contrasts (through the origin) of precloacal gland number and adjusted latitudinal midpoint (ALM) performed on the total sample of *Liolaemus* species included in this study.

Using raw data, the range of precloacal gland number does not vary predictably with thermal range (and hence with wider geographical distribution) in the clades *archeforus-kingii* ($R^2 = 0.09$, $F_{1,7} = 0.66$, $P = 0.45$), *montanus* ($R^2 = 0.05$, $F_{1,20} = 1.06$, $P = 0.32$), *fitzingerii* ($R^2 = 0.1$, $F_{1,19} = 2.25$, $P = 0.15$), and *wiegmannii* ($R^2 = 0.42$, $F_{1,7} = 4.34$, $P = 0.08$). In contrast, although thermal range explained a low proportion of precloacal gland variance in the *chiliensis* clade, a significant relationship was observed in this lineage ($R^2 = 0.14$, $F_{1,47} = 7.8$, $P = 0.007$) (Fig. 4). After removing two observed outliers (standardized residuals with values higher than 2, outliers were only found in this clade), the observed relationship was still statistically significant ($R^2 = 0.16$, $F_{1,45} = 8.83$, $P = 0.005$). Regression analyses conducted on phylogenetic independent contrasts (regressed against the origin), controlled for the entire genus *Liolaemus*, also revealed that thermal range does not predict variance of precloacal glands ($R^2 = 0.003$, $F_{1,48} = 0.13$, $P = 0.72$) (Fig. 5).

Effect of phylogenetic history

In contrast to environmental variables, phylogenetic history in the genus *Liolaemus* is a powerful predictor of the number of precloacal glands. Each one of the five main clades (i.e. *chiliensis*, *archeforus-kingii*, *montanus*, *fitzingerii*, and *wiegmannii*) exhibits a narrow range of precloacal gland number (Fig. 6). Comparison of the five clades using analysis of variance (ANOVA) revealed significant differences ($F_{4,101} = 123.7$, $P < 0.0001$) (Fig. 6). A *post-hoc* Games-Howell test revealed that the mean number of precloacal glands only failed to differ significantly between the clades *archeforus-kingii* (7.24 ± 0.25), *fitzingerii* (7.28 ± 0.31), and *wiegmannii* (7.53 ± 0.37) (*archeforus-kingii*/*fitzingerii*: $P = 0.999$; *archeforus-kingii*/*wiegmannii*: $P = 0.981$; *fitzingerii*/*wiegmannii*: $P = 0.980$), and was highly

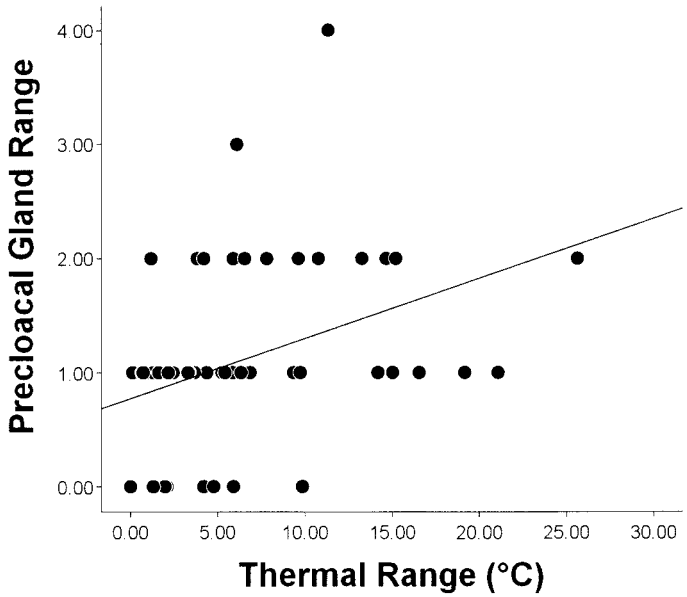

Fig. 4. Regression analysis of precloacal gland range and thermal range in the clade *chiliensis* ($R^2 = 0.14$, $F_{1,47} = 7.8$, $P = 0.007$).

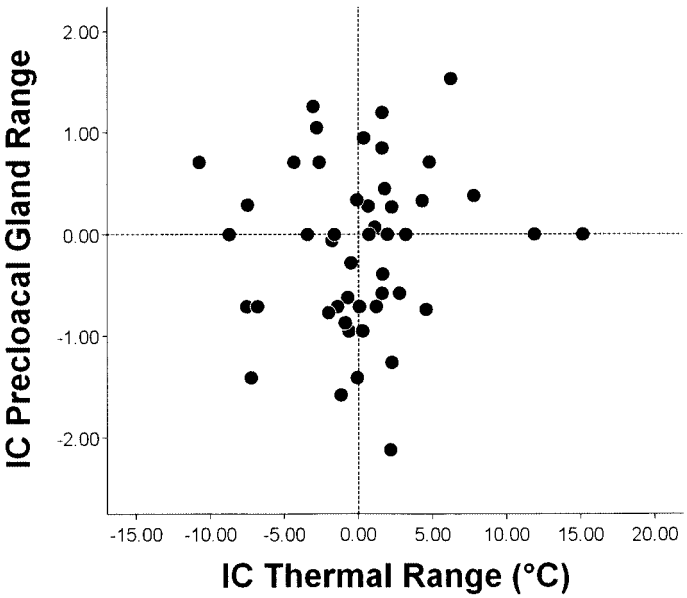

Fig. 5. Regression analysis of phylogenetic independent contrasts (through the origin) of precloacal gland range and thermal range for the entire *Liolaemus* genus.

significant for all remaining pair comparisons ($P < 0.007$). Within-clade comparisons conducted using ANOVA showed non-significant differences between the sub-clades within the *chiliensis* ($F_{2,20} = 0.715$, $P = 0.503$) and *fitzingerii* ($F_{1,10} = 0.040$, $P = 0.85$) clades. The lack of

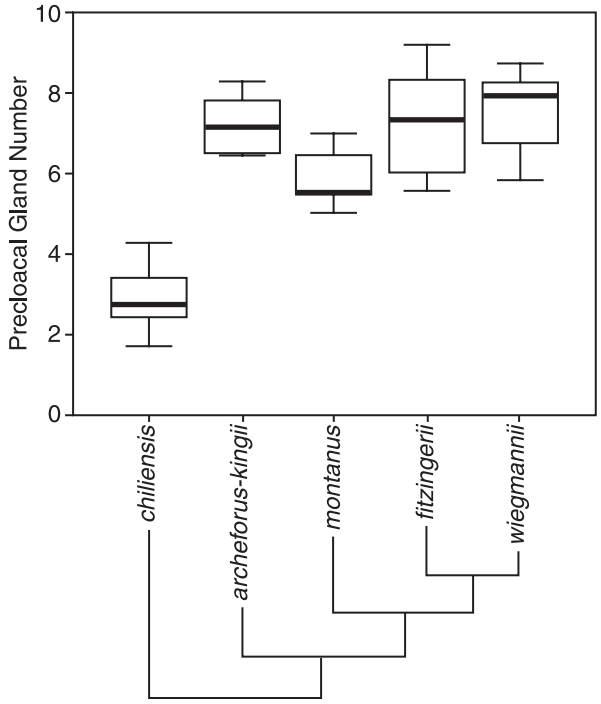

Fig. 6. Phylogenetic distribution of precloacal gland number (mean ± 1 standard error) among the five main clades of *Liolaemus* included in this study.

sufficient phylogenetic information for the remaining three clades meant it was not possible to conduct such within-clade analyses in these lineages (see Fig. 1).

Ancestral state reconstructions of precloacal glands

Ancestral state reconstructions reveal that the common ancestor of the *Liolaemus* genus had more precloacal glands than any of the 48 studied species of the *chiliensis* clade, and ranged between the mean values observed for the four remaining clades (reconstructed mean number 5.03 ± 0.97 for 49 taxa). The diversification of this ancestor shows substantial divergence in the number of precloacal glands, leading to 4.00 ± 0.31 glands (reconstructed for 21 taxa) in the ancestor of the *chiliensis* lineage, and 6.05 ± 0.32 glands (reconstructed for 28 taxa) in the ancestral node for the clades *archeforus-kingii*, *montanus*, *fitzingerii*, and *wiegmannii*. All of the lineages (i.e. sub-clades, species) originating from the *chiliensis* clade ancestor retained a mean number of precloacal glands between 1.7 and 4.0, while in its sister lineage (comprising the four remaining main *Liolaemus* clades) the mean number of precloacal glands ranged between 3.9 and 9.2 (Table 1).

Reconstruction of ancestral states for ALM and thermal range suggest that environmental factors have not significantly affected variation in precloacal glands on an evolutionary time scale. Similar numbers of glands are found in clades originating in different environmental conditions (according to reconstructed ALM) if they share the same ancestor, and clades with significantly different mean values of precloacal glands

appear to have originated in similar environmental conditions (Table 1). Also, reconstructed ancestral thermal range values do not predict reconstructed variance in the number of glands across the phylogenetic history of the genus *Liolaemus* ($R^2 = 0.02$, $F_{1,47} = 0.7$, $P = 0.41$, $N = 48$).

DISCUSSION

This study provides the first phylogenetic approach to exploring the hypothesis that organs responsible for chemical communication in a wide range of lizard lineages (i.e. precloacal glands) experience convergent adaptations in response to similar environmental conditions (Escobar *et al.*, 2001). Our results fail to support this prediction. These scent glands appear to be highly constrained by shared ancestry, and do not vary predictably in relation to environments in most of the clades studied. Also, in four of the five clades studied, species experiencing wider geographical ranges do not exhibit higher intraspecific variation in the number of precloacal glands than species with more restricted distributions.

Adaptive hypotheses and phylogenetic constraints

The phylogenetic evidence reported here shows that species living in similar environments exhibit significant differences in precloacal gland number if they are phylogenetically unrelated (Table 1). However, phylogenetically related species that occur in totally different environments (e.g. Atacama Desert and austral Patagonia, see above) exhibit similar numbers of precloacal glands if they share a recent ancestor (Table 1). Although Escobar and colleagues' (2001) hypothesis was formulated on the basis of observations carried out on the same model system used in this study, the discordance between our conclusions can be explained entirely by the incorporation of phylogenetic information. In the absence of phylogenetic evidence, we expect to find a higher number of precloacal glands in most of the high Andean species (i.e. > 5) because they are mainly members of the *montanus* clade, characterized by a high number of such glands (Laurent, 1992; Cei, 1993; Etheridge, 1995; Pincheira-Donoso and Núñez, 2005) (Table 1). Conversely, those species living in Chilean lowlands [i.e. most of the non-*montanus* species studied by Escobar *et al.* (2001)] have a lower number of precloacal glands (i.e. often ≤ 4), because all of them belong to the *chiliensis* clade, characterized by a low number of glands (Laurent, 1992; Etheridge, 1995; Pincheira-Donoso and Núñez, 2005) (Table 1). This implies some clade-level assortment of gland number, not reflected in more recent divergence and invasion of new regions. Therefore, the evolutionary outcome proposed by these authors is most parsimoniously explained as a result of shared ancestry than as the result of phylogenetically independent adaptive events. It is also worth noting that Escobar *et al.* (2001) elaborated their hypothesis on the assumption that species that occur in habitats at high elevations experience higher temperatures. However, several studies (Conrad and Pollak, 1950; Lutgens and Tarbuck, 1998; Blackburn and Ruggiero, 2001; Ashton, 2002; Espinoza *et al.*, 2004; Cruz *et al.*, 2005) have shown that environmental temperatures decrease with increasing elevation and latitude. Consequently, some of the limitations of this hypothesis may relate to it being based on potentially flawed assumptions.

The strong effect of phylogenetic constraints on the patterns of precloacal gland variation is also apparent when testing the hypothesis that species with larger geographical ranges exhibit higher variance in the number of these structures. If the number of precloacal glands were determined by adaptive responses to local environmental factors, we

might expect to see more inter-population variation in species with large latitudinal or altitudinal ranges. However, our analyses conducted on phylogenetically controlled data and separately for each clade revealed that a broader range of environmental thermal variation experienced by a species does not substantially affect variation in the number of glands in most of the lineages studied (Fig. 5).

Similarly, variation in environmental conditions appears not to have caused a significant effect on the variation of these emitter glands along the phylogenetic history of the genus *Liolaemus*. Analyses of reconstructed ancestral trait values indicate that precloacal glands in ancestors of current *Liolaemus* clades did not vary predictably with environmental variation. Similar numbers of precloacal glands are conserved in clades originating in different environmental conditions if they are phylogenetically related, while unrelated clades that occur in similar environments differ significantly in the number of these structures (Table 1). Therefore, environmental conditions not only fail to predict evolution of emitter glands for chemical communication in current species, but also through the evolutionary history of this lineage.

Although our study rejects the general hypothesis that precloacal gland number is most parsimoniously explained as the result of adaptations to environmental conditions, the ultimate explanation for the observed patterns of variation in this trait remains unknown. The answer may lie in one of three possible alternatives. First, the pattern of variation in precloacal glands and the existence of species-specific chemical communication in these lizards (e.g. Labra and Niemeyer, 1999; Labra *et al.*, 2001a, 2001b) is the result of sexual selection, which has led to the evolution of some highly conserved aspects of the signal–receiver relationship either between males and females or between competing males, such that speciation events have occurred but the number of precloacal glands has been conserved among closely related species. Second, an alternative possibility is that the chemical structure of secreted compounds does differ among closely related species, but that this occurs without significant differentiation in the morphological expression of emitter glands. Previous studies have revealed that the great diversity of specialized pheromones observed among closely related and morphologically similar moth species may evolve by means of major shifts restricted to the molecular structure of these scents (Roelofs *et al.*, 2002; Roelofs and Rooney, 2003). Although information for lizards is still very limited, a molecular mechanism similar to that reported in moths may explain why the evolution of chemical communication in *Liolaemus* lizards (e.g. Labra *et al.*, 2001b) does not correlate with predictable variation in the emitter glands. Third, we recognize the caveat of statistical power. It remains possible that the signal of convergent evolution of gland number to environment is too weak to detect using our sample sizes. However, we sampled a large proportion of this genus (Etheridge and Espinoza, 2000; Pincheira-Donoso and Núñez, 2005), and sampled intensively within species to tighten our estimates of species-level means. Hence, any signal that may exist will probably remain undetected. Determining the extent to which each of these three alternatives explains the observed pattern will be a challenging task, which might be aided by a better understanding of how related species evolve specialized chemical scents for intraspecific communication (Labra and Niemeyer, 1999; Escobar *et al.*, 2003; Labra *et al.*, 2001a, 2001b, 2002). The study of this topic offers more questions than answers, but hopefully better resolved and more comprehensive phylogenies of reptile groups, and further analyses focused on the molecular characteristics of their scents for chemical communication, will lead to progress.

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APPENDIX

The studied material is housed in the herpetological collections of the following institutions. Collections identified with an asterisk (*) indicate the existence of specimens with recollection data, but without an official collection number at the moment of our study: Division of Reptiles and Amphibians, Museo Nacional de Historia Natural de Chile (MNHN*); Zoological Museum, Facultad de Ciencias Naturales y Oceanograficas, Universidad de Concepcion, Chile (MZUC*); Division of Zoology, Museo de Historia Natural de Concepcion, Chile (MHNC*); Department of Cell Biology and Genetics, Facultad de Medicina, Universidad de Chile (DBCGUCH*); Instituto de Biologia Animal, Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Argentina (IBAUNC*); Instituto Argentino de Investigaciones de las Zonas Aridas, CRICYT, Argentina (IADIZA); Natural History Museum of London (NHML); Jose Miguel Cei Diagnostic Collection (JMC-DC); Jose Alejandro Scolaro Diagnostic Collection (JAS-DC); and the Herpetological Collection of the senior author, D. Pincheira-Donoso (CHDPD*).