Testing the functionality of precloacal secretions from both sexes in the South American lizard, *Liolaemus chiliensis*

Soledad Valdecantos[1,*], Antonieta Labra[2,3]

Abstract. The behavior of lizards can be highly influenced by chemical senses. The most studied pheromonal sources in lizards has been the femoral and precloacal gland secretions, although studies have been focused on male secretions, probably because these glands are usually only present in males or are poorly developed in females when they are present. Here, we aimed to study in *Liolaemus chiliensis*, one of the few *Liolaemus* species in which females have precloacal glands, if female precloacal secretions convey information. We recorded the response of both sexes to secretions from females and males, as well as to control (solvent). The lizards started to explore the secretions sooner than the control. Both sexes moved more when exposed to female secretions than to the control, and males, but not females, explored female secretions more than the other scents. These results suggest that volatile compounds of the secretions allow lizards to recognize the presence of conspecifics, and, at least for males, these trigger the exploration of non-volatile compounds of the secretions that may reveal the sex of the individual that deposited them. This is the first study that explores the response to female precloacal secretions in *Liolaemus*, and data indicate that the female secretions of *L. chiliensis* contain relevant information for social interactions.

Keywords: chemical signal, epidermal glands, Liolaemidae, sexual recognition, Squamata.

Introduction

Lizard social behavior is highly dependent on chemical sensory modality (Martín and López, 2014), the oldest sense shared by the whole animal kingdom involved in the perception of many types of scents, relevant for the individuals' fitness (Wyatt, 2014). During intrasexual interactions, scents allow male lizards, for example, to assess rival recognition (Carazo, Font and Desfilis, 2008), and the fighting abilities of the potential competitors (Labra, 2006). In intersexual interactions, females use male scents to assess their age (López, Aragón and Martín, 2003), quality (Martín and López, 2000), and local density (Martín and López, 2013). One important scent source are the specialized holocrine glands, the femoral and precloacal glands (Imparato et al., 2007; Khannoon, Dollahon and Bauer, 2013; Valdecantos, Martínez and Labra, 2014). Studies show that male secretions allow males, among others, self-recognition (Baird, McGee and York, 2015), and assess male familiarity (Alberts and Werner, 1993), while these secretions allow females to obtain information on males' dominance status (Moreira, López and Martín, 2006), and health or quality, which is relevant for mate choice (Martín and López, 2006).

Studies on the functionality of femoral and precloacal secretions are biased toward male secretions, and remarkably almost nothing has been learned about female secretions (for a review see Mayerl, Baeckens and Van Damme, 2015). This bias is striking when considering that these holocrine glands can be present in both sexes (Alberts, 1990; Martín et al., 2015), although they usually are less developed in females (Cole, 1966; Valdecantos, Martínez and

1 - Instituto de Biología y Geología del Noroeste Argentino (IBIGEO, Universidad Nacional de Salta-CONICET), Cátedra de Anatomía Comparada, Facultad de Ciencias Naturales, 9 de Julio 14, 4405 Rosario de Lerma, Salta, Argentina

2 - Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Casilla 70005, Correo 7, Santiago, Chile

3 - Centre for Ecological and Evolutionary Synthesis (CEES), Department of Biosciences, University of Oslo, PB 1066, 0316 Oslo, Norway

*Corresponding author; e-mail: solevaldecantosp@gmail.com

Labra, 2014). The few studies that have considered female secretions show sexual variation in the responses toward femoral secretions of both sexes (Cooper, López and Salvador, 1994; Khannoon et al., 2010), and that female secretions may be involved in triggering courtship behavior in males (Alberts, 1990). The sexual variation in the responses to secretions from both sexes may be partially a consequence of sexual differences in the chemical composition of the secretions, already reported in a few species (Alberts, 1990; Louw et al., 2007; Martín et al., 2015; García-Roa et al., 2016). In this context, our aim was to explore the functionality of females precloacal secretions in *Liolaemus chiliensis*, a species in which both sexes have precloacal glands (Lobo, 2001). In both sexes, glands are active as they produce secretions, although less abundantly in females (fig. 1; see below).

Liolaemus is the second most specious lizard genus of the world, after *Anolis* (Uetz and Hošek, 2017), and is distributed in a variety of environments in South America (Donoso Barros, 1966; Cei, 1986, 1993; Aparicio and Ocampo, 2010). Most *Liolaemus* species have precloacal pores (the external evidence of the precloacal glands; Valdecantos, Martínez and Labra, 2014), although these usually are only present in males (Etheridge, 1995; Lobo, 2001; Abdala, 2007). In some few species, however, females also have precloacal pores (Etheridge, 1993; Lobo, 2001; Abdala, 2007; Breitman, Morando and Avila, 2013), although their glands are smaller than those of males (Valdecantos, Martínez and Labra, 2014). The few studies in *Liolaemus* that have tested the functionality of precloacal secretions have considered just species in which only males have glands. Data show that male secretions are active by stimulating the vomeronasal organ in both sexes of *L. bellii* and *L. nigroviridis* (Labra, Brann and Fadool, 2005). In *L. tenuis*, male secretions induce in females more behavioral exploration than do male feces or skin secretions (Labra, 2008), but in males these secretions do not allow self-recognition (Labra et al., 2002).

Liolaemus chiliensis shows chemical recognition of conspecifics, behaving differentially in areas labeled with different scents (Labra and Hoare, 2014). Therefore, if female precloacal secretions convey information and if this differs from the information of male secretions, we expect that both sexes would exhibit differential
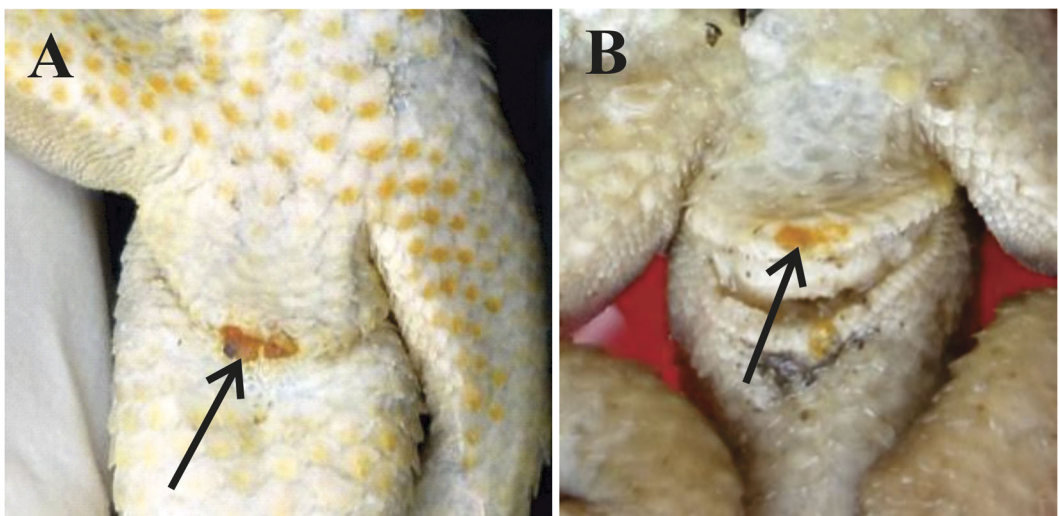

Figure 1. Ventral view of the cloacal area of an adult male (A) and a female (B) of *Liolaemus chiliensis* showing the precloacal pores (black arrow) with secretions. Note in (A) the thickening at the tail base where hemipenes are located. Pictures were taken in alive individuals in the lab.

responses to scents from females and a control, and that secretions from both sexes trigger different responses.

Materials and methods

Lizards and maintenance

In the spring of 2012, we collected in Melipilla, central Chile (33°41′S; 71°13′W), 25 adults of *L. chiliensis* (12♂, snout-vent length mean $\pm$ SE = 77.16 $\pm$ 4.63 mm and 13♀, snout-vent length mean $\pm$ SE = 88.66 $\pm$ 2.34 mm) in bushes, the most typical microhabitat of this species (Mella, 2005). Lizards were transported individually to the laboratory in cloth bags, where they were sexed, weighed, and measured. Thereafter, they were housed individually in a plastic enclosure (44.5 $\times$ 32 $\times$ 25 cm), which had two windows covered by plastic mesh, one in the front (10 $\times$ 5 cm) and the other in the lid, providing extra climbing surface, more light and good ventilation. Enclosures contained a sandy substrate, a clay pot to maintain water continuously, a wooden stick used as a perch, and an inverted clay pot used as shelter and basking place. Lizards were fed with mealworms dusted with vitamins for reptiles (SERA reptimineral C) three times per week. The enclosures were placed in an indoor vivarium with continuous ventilation, and maintained with similar conditions as a typical spring sunny day; a 13:11 light-dark cycle associated with temperatures ranging between 33 and 12°C, all provided by halogen lights. Lizards remained undisturbed (except for feeding) for at least one week before any trial to allow habituation to the experimental enclosures. At the end of all experiments, individuals were released in healthy conditions to their geo-referenced collecting points.

From the collected specimens, five males and two females were used only to extract precloacal secretions, following the protocol used by Escobar et al. (2001, 2003); lizards were placed backwards and pores were pressed gently with clean forceps. On average, we extracted 300 μg of secretions from each male and 150 μg from each female. Secretions were pooled by sex and placed in separate glass vials previously weighed. The vials were reweighed with the secretions and then dissolved in *n*-hexane, at equal concentration for both sexes, 75 μg/ml; vials were kept in a freezer at −20°C until use.

Treatments and experimental trials

Lizards were submitted only once to each three treatments using a partially counterbalanced design: the precloacal secretions of males, females and a control (*n*-hexane). We spread 400 μl of dissolved secretions or *n*-hexane over a clean round glass stone (4.0 cm of diameter $\times$ 1.0 cm of height). This nonporous surface ensured that the secretions would remain on the surface to be perceived by the lizards. Stones were prepared one day in advance to secure that no solvent traces would affect the responses of lizards. During the preparation, vials with the dissolved secretions were kept on ice to avoid the evaporation of *n*-hexane, and so, to

affect the concentration of the dissolved secretions, which could affect our results (Baeckens et al., 2017).

For the experiments, lizards were placed individually in an experimental enclosure, similar to those used for their maintenance. This enclosure had clean sand, and we placed the stone next to the front window. The test lizard was taken from its enclosure and placed in a cloth bag for 15 min (each lizard had its own bag) to reduce handling stress (Labra, 2011). Thereafter, the bag was carefully opened in the middle part of the experimental enclosure, allowing the lizard to move freely into the enclosure. Once the lizard was on the enclosure floor, the bag was removed and the enclosure was closed with a glass sheet (37 $\times$ 30 cm), instead of the plastic lid, to facilitate the filming. At 60 cm above the experimental enclosure, there was a video camera (Sony DCR-TRV 310 NTSC) that was connected to a TV. This facilitated the recording of the latency to the first tongue flick, i.e. the period since the enclosure was closed until the lizard made its first tongue flick to the air or to a substrate (except the stone). After this tongue flick, we started to film and then, we determined the latency to the first tongue flick to the stone, i.e. the period elapsed since the start of the recording until the first tongue flick made to the stone. If lizards did not tongue flick to the stone after 7 min, we finished the trial, and the lizard was placed back in its maintenance enclosure, remaining undisturbed (except for feeding) for at least three days before the next experiment. After the lizard tongue flicked the stone, we filmed it for 7 min more, after which the trial was ended. Thus, the maximum duration of a trail was 14 min. At the end of each trial, we checked the lizard body (cloacal) temperature to ensure that this was around the species mean selected body temperature, 35 $\pm$ 2°C (Labra, Pienaar and Hansen, 2009). The experimental enclosure and the stone were washed, and the sand was discarded.

Data analyses

Further than the two latencies recorded during the experiments, we registered from the videos: (1) Number of tongue flicks: number of times that lizards protruded and rapidly retracted the tongue, contacting the air or any substrate (excluding those directed at the stone). (2) Number of tongue flicks to the stone. (3) Total time in movement (s): for this proxy of behavioral exploration, we recorded the changes in position and movements of the whole body or just the head (Labra, Beltrán and Niemeyer, 2001). (4) Time spent performing escape behaviors (s): total time that lizards exhibited behaviors that allowed escaping, i.e. running and attempts to climb the enclosure walls (Labra, Reyes-Olivares and Weymann, 2016).

The total time that lizards were filmed varied and we standardized the number of tongue flicks, time in movement, and time spent performing escape behaviors by the total time of the trial. The number of tongue flicks to the stone was not standardized as this behavior could only be exhibited in the 7 min after the first latency to the tongue flick to the stone. We analyzed the effects of the treatment (scents), sex of the focal lizard and their interaction upon

the six recorded variables using two-way ANOVA for repeated measures (treatment), followed by post-hoc Fisher LSD' test. The residuals of the studied variables were normally distributed.

Results

The mean value of the latency to the first tongue flick to the stone, the number of tongue flicks, and the time spent performing escape behaviors are given in table 1; none of these variables were affected by the studied factors (table 2). In contrast, latency to the first tongue flick was affected by treatment (table 2); lizards had shorter latencies when they were exposed to the precloacal secretions than to the control (LSD test, $p = 0.011$♂ and $p = 0.0013$♀; table 1, fig. 2A). The number of tongue flicks

Table 1. Mean (±SE) of three variables displayed by both sexes of *Liolaemus chiliensis* when they were exposed to three experimental conditions: precloacal secretions of males, females and a control. The values of the number of tongue flicks and the time spent performing escape behaviors are standardized by the total time of the trial.

	Latency to first tongue flick to the stone (s)	N° of tongue flicks	Time spent performing escape (s)
Male precloacal secretions	305.33 ± 36.13	3.04 ± 0.43	0.32 ± 0.123
Female precloacal secretions	338.90 ± 32.76	2.51 ± 0.34	0.63 ± 0.232
Control	359.87 ± 28.89	2.66 ± 0.38	0.44 ± 0.251

to the stone was affected by the interaction between the treatment and the sex of the focal individual (table 2; fig. 2B); males tongue flicked more to the stones with female secretions than to any other scent (LSD test, $p < 0.05$; table 2). Finally, the time in movement was also affected by the treatment (table 2), as lizards, independently of their sex, were more active in presence of female secretions than with the control (LSD test, $p = 0.009$; fig. 2C).

A few individuals exhibited some displays, but their low frequency of exhibition precluded statistical analysis. We observed in four occasions eye-bulging (Labra, Reyes-Olivares and Weymann, 2016); one male performed this when it was exposed to male secretions and the other three lizards (2♂ and 1♀) did it in the control. In nine trails lizards displayed face rubbing against the enclosure walls (Labra, 2006); six lizards (1♂ and 5♀) did it with female secretions, one (♀) with male secretions, and two (♀) with the control. Finally, three lizards (1♂ and 2♀) defecated, one (♀) with male secretions, and the other individuals did it with the control.

Discussion

This is the first study in *Liolaemus* that tests the functionality of the secretions from female precloacal glands. We found that in *L. chiliensis* these secretions seem to encode information, particularly relevant for males as they exhibited more tongue flicks towards the female

Table 2. Results of two-way ANOVAs with repeated measures, to determine the effect of the sex of the focal individual (male vs. female), the treatment (secretions of male, female and a control) and their interactions upon the six behavioral variables recorded in *Liolaemus chiliensis*. The number of tongue flicks, the time in movement, and the time spent performing escape behaviors were the standardized values of the variables. The test statistics (F) and its P value are indicated. Results with $P < 0.05$ are given in bold.

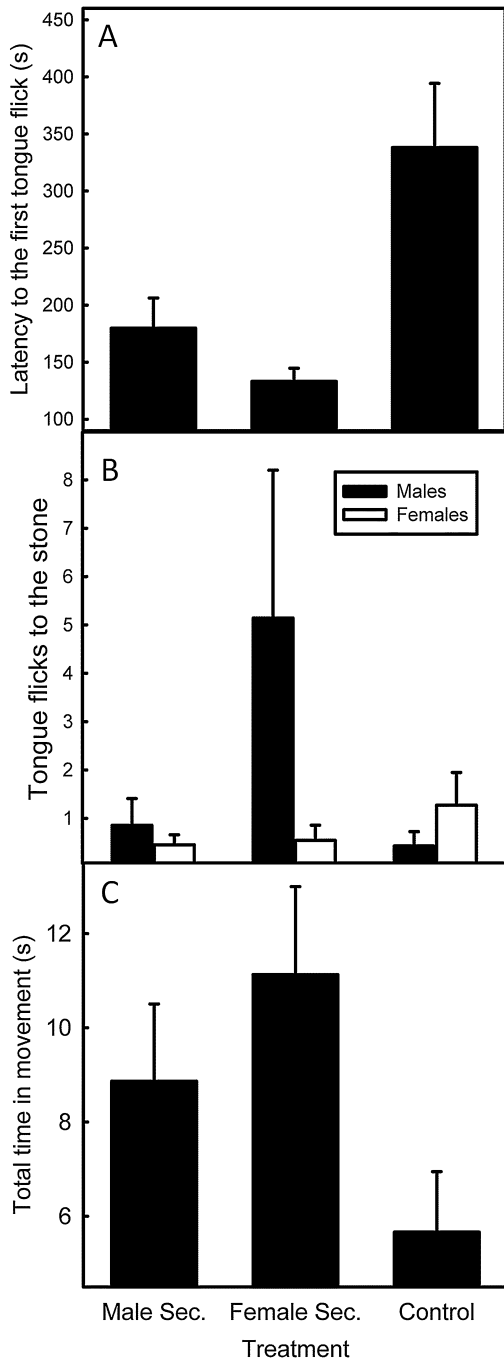|	Degree freedom	N° tongue flicks	N° tongue flicks to the stone	Lat. first tongue flick	Lat. first tongue flick to the stone	Time in movement	Time spent performing escape
		$F(P)$	$F(P)$	$F(P)$	$F(P)$	$F(P)$	$F(P)$
Sex	1, 16	1.11 (0.31)	2.47 (0.14)	0.34 (0.57)	0.15 (0.7)	0.54 (0.47)	2.67 (0.12)
Treatment	2, 32	0.24 (0.79)	2.54 (0.09)	**6.38 (0.005)**	1.06 (0.36)	**4.58 (0.018)**	0.48 (0.62)
Sex × Treatment	2, 32	1.01 (0.37)	**3.52 (0.04)**	0.01 (0.99)	0.59 (0.56)	1.72 (0.19)	0.87 (0.43)

Figure 2. Mean (+SE) of three behaviors recorded in *Liolaemus chiliensis*, in three experimental conditions: precloacal secretions of males, females and a control. (A) Latency to the first tongue flick. (B) Number of tongue flicks to the stone. (C) Time in movement, standardized by the total time of the trial.

secretions, as was reported in *Blanus cinereus* (Cooper, López and Salvador, 1994). The fact that female secretions of *L. chiliensis* produced attraction or interest in males, however, does not preclude that they can use other female-scent sources, as occurs in those *Liolaemus* species in which females lack precloacal glands (Labra and Niemeyer, 1999; Labra, Beltrán and Niemeyer, 2001). In fact, it may be possible that males require the information of other scent sources to initiate an active search for females, considering that the increased chemical exploration toward female secretions was not accompanied by an increased behavioral search; males did not moved more in the presence of female secretions. On the other hand, the differential response to the secretions of both sexes suggests that sexes differ in the chemical composition and/or in the proportion of some compounds of the secretions, as has been shown in a few species (see Mayerl, Baeckens and Van Damme, 2015; García-Roa et al., 2016).

Males of *L. chiliensis* showed similar chemical exploration to the stones with male secretions and the control, which contrasts with previous results in *Liolaemus* (Labra et al., 2002) and other Squamata. For example, males of *Acanthodactylus boskianus* tongue flicked more to femoral secretions than to a control, and showed discrimination between secretions, as they only directed aggressions towards male secretions (Khannoon et al., 2010). *Liolaemus chiliensis* displays very little during social interactions (Bozzo, 2014), or predation risk (Labra and Hoare, 2014), and in the present study, the absence of aggressive or other displays toward male secretions reduces the possibility to unravel if males discriminate between male secretions and control.

Females of *L. chiliensis* showed similar chemical exploration towards the different scents, as females of *Liolaemus pacha* (Vicente and Halloy, 2016) and *A. boskianus* (Khannoon et al., 2010). This similar response to the different scents not necessarily means a lack of discrimination (Cooper, 1998), and here females

displayed more "extra behaviors" than males, particularly face rubbing (i.e. a marking behavior; Labra, 2008), when they were exposed to female secretions.

Lizards had shorter latency to the first tongue flick in presence of secretions than with the control. This suggests that secretions have volatile compounds, probably perceived by the olfactory system, which trigger tongue flicking. This response provides non-volatile compounds to the vomeronasal organ, probably with a more detailed information of the scent owner (Cowles and Phelan, 1958), as was proposed for *Iguana iguana* (Alberts and Werner, 1993). *Liolaemus chiliensis*, however, showed similar latency with secretions from both sexes which suggests that volatile compounds may only inform on the conspecific presence, and not on the sex of the scent owner, as was reported in other squamate species (Cooper, López and Salvador, 1994). *Liolaemus chiliensis* lives in shrubby environments (Mella, 2005), where a poor visibility makes visual displays less effective for communication, but probably, a strong selective pressure for volatile scents to fulfill this task.

In non-ophidian squamate males there is a disparity in the presence/absence of epidermal glands and these may have been absent in the common ancestor of this group (Mayerl, Baeckens and Van Damme, 2015). In Liolaemidae however, these glands are present in all three genera (Frost and Etheridge, 1989), and may be an ancestral character, at least in males (Pincheira-Donoso, Hodgson and Tregenza, 2008). Females of a few Liolaemidae species, and in fact only from *Liolaemus*, have precloacal pores with secretions (Valdecantos, Martínez and Labra, 2014). It is likely, that the precloacal glands of both sexes are homologues structures encoded by the same genes present in the *Liolaemus* ancestor. However, because few studies have documented correctly these structures in females, as many of them have mistaken pits without secretions for precloacal pores (pers. obs.), presently, it is not possible to track the evolution of the female glands in *Liolaemus*. Therefore, it is unclear why only in some few species females develop precloacal glands. For *L. chiliensis*, the poor visibility to assess displays in its microhabitat may have driven the evolution of female precloacal glands (but see Baeckens et al., 2015). Low visibility, however, may not be the only selective pressure involved in the evolution of female glands, considering that in places such as the Puna in Argentina, a desert habitat with scarce bushes (Cabrera, 1994), there are several *Liolaemus* species whose females have precloacal pores (Laurent, 1986; Lobo and Laurent, 1995; Martínez Oliver and Lobo, 2002).

References

Abdala, C.S. (2007): Phylogeny of the boulengeri group (Iguania: Liolaemidae, *Liolaemus*) based on morphological and molecular characters. Zootaxa **1538**: 1-84.

Alberts, A.C. (1990): Chemical properties of femoral gland secretions in the desert iguana, *Dipsosaurus dorsalis*. J. Chem. Ecol. **16**: 13-25.

Alberts, A.C., Werner, D.I. (1993): Chemical recognition of unfamiliar conspecifics by green iguanas: functional significance of different signals components. Anim. Behav. **46**: 197-199.

Aparicio, J., Ocampo, M. (2010): *Liolaemus* group montanus Etheridge, 1995 (Iguania-Liolaemidae). Cuad. Herpetol. **24**: 133-135.

Baeckens, S., Edwards, S., Huyghe, K., Van Damme, R. (2015): Chemical signalling in lizards: an interspecific comparison of femoral pore numbers in Lacertidae. Biol. J. Linn. Soc. **114**: 44-57.

Baeckens, S., Huyghe, K., Palme, R., Van Damme, R. (2017): Chemical communication in the lacertid lizard *Podarcis muralis*: the functional significance of testosterone. Acta Zool. **98**: 94-103.

Baird, T.A., McGee, A.A., York, J.R. (2015): Responses to femoral gland secretions by visually adept male and female collared lizards. Ethology **121**: 513-519.

Bozzo, V. (2014): Interacciones sociales en el lagarto llorón (*Liolaemus chiliensis*). Thesis for the Degree of Veterinarian Physician. Facultad de Ciencias Silvoagropecuarias, Escuela Medicina Veterinaria, p. 35. Universidad Mayor, Santiago.

Breitman, M.F., Morando, M., Avila, L.J. (2013): Past and present taxonomy of the *Liolaemus lineomaculatus* section (Liolaemidae): is the morphological arrangement hypothesis valid? Zootaxa **168**: 612-668.

Cabrera, A.L. (1994): Regiones Fitogeográficas Argentinas. Acme S.A.C.I., Buenos Aires.

Carazo, P., Font, E., Desfilis, E. (2008): Beyond 'nasty neighbours' and 'dear enemies'? Individual recognition by scent marks in a lizard (*Podarcis hispanica*). Anim. Behav. **76**: 1953-1963.

Cei, J.M. (1986): Reptiles del Centro, Centro-Oeste y Sur de la Argentina. Herpetofauna de las Zonas Aridas y Semiaridas. Turin, Museo Regionale di Scienze Naturali Monografie.

Cei, J.M. (1993): Reptiles del Noroeste, Nordeste y Este de la Argentina. Herpetofauna de las Selvas Subtropicales, Puna y Pampa. Turin, Museo Regionale di Scienze Naturali Monografie.

Cole, C.J. (1966): Femoral glands of the lizard, *Crotaphytus collaris*. J. Morph. **118**: 119-135.

Cooper, W.E.J. (1998): Evaluation of swab and related tests as a bioassay for assessing responses by Squamate reptiles to chemical stimuli. J. Chem. Ecol. **24**: 841-866.

Cooper, W.E.J., López, P., Salvador, A. (1994): Pheromones detection in amphisbaenian. Anim. Behav. **47**: 1401-1411.

Cowles, R.B., Phelan, R.L. (1958): Olfaction in rattlesnakes. Copeia **1958**: 77-83.

Donoso Barros, R. (1966): Reptiles de Chile. Universidad de Chile, Santiago.

Escobar, C.A., Labra, A., Niemeyer, H.M. (2001): Chemical composition of precloacal secretions of *Liolaemus* lizards. J. Chem. Ecol. **27**: 1677-1690.

Escobar, C.M., Escobar, C.A., Labra, A., Niemeyer, H.M. (2003): Chemical composition of precloacal secretions of two *Liolaemus fabiani* populations: are they different? J. Chem. Ecol. **29**: 629-638.

Etheridge, R. (1993): Lizards of the *Liolaemus darwinii* complex (Squamata: Iguania: Tropiduridae) in northern Argentina. Mus. Reg. Sci. Nat. Bull. **11**: 137-199.

Etheridge, R. (1995): Redescription of *Ctenoblepharys adspersa* Tschudi, 1845, and the taxonomy of Liolaeminae (Reptilia: Squamata: Tropiduridae). Am. Mus. Novit. **3142**: 1-34.

Frost, D.R., Etheridge, R. (1989): A phylogenetic analysis and taxonomy of iguanian lizards (Reptilia: Squamata). U. Kansas Mus. Nat. Hist. Mis. Publ. **81**: 1-65.

García-Roa, R., Carreira, S., López, P., Martín, J. (2016): Genders matters: sexual differences in chemical signals of *Liolaemus wiegmannii* lizards (Iguania, Liolaemidae). Biochem. Syst. Ecol. **69**: 108-114.

Imparato, B.A., Antoniazzi, M.M., Rodrigues, M.T., Jared, C. (2007): Morphology of the femoral glands in the lizard *Ameiva ameiva* (Teiidae) and their possible role in semiochemical dispersion. J. Morph. **268**: 636-648.

Khannoon, E., Breithaupt, T., El-Gendy, A., Hardege, J.D. (2010): Sexual differences in behavioural response to femoral gland pheromones of *Acanthodactylus boskianus*. Herpetol. J. **20**: 225-229.

Khannoon, E.R.R., Dollahon, N.R., Bauer, A.M. (2013): Comparative study of the pheromone-manufacturing femoral glands in two sympatric species of lacertid lizards (*Acanthodactylus*). Zool. Sci. **30**: 110-117.

Labra, A. (2006): Chemoreception and the assessment of fighting abilities in the lizard *Liolaemus monticola*. Ethology **112**: 993-999.

Labra, A. (2008): Multi-contextual use of chemosignals by *Liolaemus* lizards. In: Chemical Signals in Vertebrates 11, p. 357-365. Hurst, J., Beynon, R., Roberts, S., Wyatt, T., Eds, Springer, New York.

Labra, A. (2011): Chemical stimuli and species recognition in *Liolaemus* lizards. J. Zool. **285**: 215-221.

Labra, A., Niemeyer, H.M. (1999): Intraspecific chemical recognition in the lizard *Liolaemus tenuis*. J. Chem. Ecol. **25**: 1799-1811.

Labra, A., Hoare, M. (2014): Chemical recognition in a snake-lizard predator-prey system. Acta. Ethol. **18**: 173-179.

Labra, A., Beltrán, S., Niemeyer, H.M. (2001): Chemical exploratory behavior in the lizard *Liolaemus bellii*. J. Herpetol. **35**: 51-55.

Labra, A., Brann, J.H., Fadool, D.A. (2005): Heterogeneity of voltage- and chemosignal-activated response profiles in vomeronasal sensory neurons. J. Neurophysiol. **94**: 2535-2548.

Labra, A., Pienaar, J., Hansen, T.F. (2009): Evolution of thermal physiology in *Liolaemus* lizards: adaptation, phylogenetic inertia, and niche tracking. Am. Nat. **174**: 204-220.

Labra, A., Reyes-Olivares, C., Weymann, M. (2016): Asymmetric response to heterotypic distress calls in the lizard *Liolaemus chiliensis*. Ethology **122**: 758-768.

Labra, A., Escobar, C.A., Aguilar, M.P., Niemeyer, H.M. (2002): Sources of pheromones in the lizard *Liolaemus tenuis*. Rev. Chil. Hist. Nat. **75**: 141-147.

Laurent, R.F. (1986): Description of new Iguanidae of the genus *Liolaemus*. Acta Zool. Lilloana **38**: 87-106.

Lobo, F. (2001): A phylogenetic analysis of lizards of the *Liolaemus chiliensis* group (Iguania: Tropiduridae). Herpetol. J. **11**: 137-150.

Lobo, F., Laurent, R.F. (1995): Un nouveau *Liolaemus* Andin (Tropiduridae). Rev. Fr. d'Aquariol.-Herpetol. **22**: 107-116.

López, P., Aragón, P., Martín, J. (2003): Responses of female lizards, *Lacerta monticola*, to males' chemical cues reflect their mating preference for older males. Behav. Ecol. Sociobiol. **55**: 73-79.

Louw, S., Burger, B.V., Le Roux, M., Van Wyk, J.H. (2007): Lizard epidermal gland secretions I: chemical characterization of the femoral gland secretion of the sungazer, *Cordylus giganteus*. J. Chem. Ecol. **33**: 1806-1818.

Martín, J., López, P. (2000): Chemoreception, symmetry and mate choice in lizards. Proc. R. Soc. Lond. B **267**: 1265-1269.

Martín, J., López, P. (2006): Links between male quality, male chemical signals, and female mate choice in Iberian rock lizards. Funct. Ecol. **20**: 1087-1096.

Martín, J., López, P. (2013): Responses of female rock lizards to multiple scent marks of males: effects of male age, male density and scent over-marking. Behav. Process. **94**: 109-114.

Martín, J., López, P. (2014): Pheromones and chemical communication in lizards. In: Reproductive Biology and Phylogeny of Lizards and Tuatara, p. 43-77. Rheubertustin, J., Siegel, D., Trauth, S., Eds, CRC Press, Boca Raton.

Martín, J., Martínez-Silvestre, A., López, P., Ibáñez, A., Rodríguez-Domínguez, M.A., Verdaguer, I. (2015): Lipophilic compounds in femoral secretions of males and females of the El Hierro giant lizard *Gallotia simonyi* (Lacertidae). Biochem. Syst. Ecol. **61**: 286-292.

Martínez Oliver, I., Lobo, F. (2002): Una nueva especie de *Liolaemus* del grupo alticolor (Iguania: Liolaemidae) de la Puna salteña, Argentina. Cuad. Herpetol. **16**: 47-64.

Mayerl, C., Baeckens, S., Van Damme, R. (2015): Evolution and role of the follicular epidermal gland system in non-ophidian squamates. Amphibia-Reptilia **36**: 185-206.

Mella, J.E. (2005): Guía de Campo Reptiles de Chile: Zona Central. Centro de Ecología Aplicada Ltda, Santiago.

Moreira, P.L., López, P., Martín, J. (2006): Femoral secretions and copulatory plugs convey chemical information about male identity and dominance status in Iberian rock lizards (*Lacerta monticola*). Behav. Ecol. Sociobiol. **60**: 166-174.

Pincheira-Donoso, D., Hodgson, D.J., Tregenza, T. (2008): Comparative evidence for strong phylogenetic inertia in precloacal signalling glands in a species-rich lizard clade. Evol. Ecol. Res. **10**: 11-28.

Uetz, P., Hošek, J. (2017): The reptile database. http://www.reptile-database.org.

Valdecantos, S., Martínez, V., Labra, A. (2014): Comparative morphology of *Liolaemus* lizards precloacal glands. Acta Herpetol. **9**: 147-158.

Vicente, N.S., Halloy, M. (2016): Chemical recognition of conspecifics in a Neotropical lizard, *Liolaemus pacha* (Iguania: Liolaemidae): relation to visual displays, season and sex. J. Ethol. **34**: 329-335.

Wyatt, T.D. (2014): Pheromones and Animal Behavior. Chemical Signals and Signature Mixes. Cambridge University Press, New York.