

## Comparative morphology of *Liolaemus* lizards precloacal glands

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**Abstract.** Liolaemid lizards and amphisbaenids have precloacal pores in the anterior border of the cloaca, where epidermal glands drain and expel pheromonal secretions. Precloacal glands occur usually only in males, but in those few species where both sexes have precloacal glands, these are larger in males. Only the morphology and/or histology of precloacal glands of amphisbaenids have been described, and it is unknown whether in lizards these glands differ across ages, sexes and/or species, and if the lack of pores is associated with a lack of glands. We investigated for the first time the morphology and histology of lizard precloacal glands, by studying three *Liolaemus* species that differ in the presence of pores in their cloaca: *L. irregularis*, in which adults and juveniles of both sexes have pores; *L. poecilochromus*, in which only adult males have pores, and *L. neuquensis*, in which the adults of both sexes lack pores. Results show that the number of pores varies among species and sexes, but not between ages of a species. Adults, but not juveniles, of *L. irregularis* have sexual dimorphism in pore sizes; these are larger in males than in females. In addition, pores are larger in adult males of *L. irregularis* than in *L. poecilochromus*. Glands are tubuloalveolar with holocrine secretion, having similar structure across individuals, although adult males have larger glands than females and juveniles. Finally, the structure of *Liolaemus* precloacal glands is very similar to those of the amphisbaenid precloacal glands and the femoral glands of other lizard species.

**Keywords.** Epidermal gland, histology, histochemistry, sexual dimorphism, ontogenetic variation.

### INTRODUCTION

Many lizard species have epidermal glands with secretions, which are a complex mix of lipids and proteins, involved in intraspecific chemical communication (Mason, 1992; Louw et al., 2007; Weldon et al., 2008; Martín and López, 2011; Louw et al., 2011). Externally, glands are recognized by patches of modified scales that allow the release of the secretions. One type, the generational glands, lacks a collecting portion and the associated scales have a glandular appearance through which secretions are expelled directly, without a pore (Van Wyk and Mouton, 1992). The other type of glands, the precloacal/femoral glands, have a unique and clear pore where the collect-

ing portion of the gland drains secretions to be expelled (Cole, 1966a; Maderson and Chiu, 1970; Baig and Böhme, 1991; Van Wyk and Mouton, 1992; Jared et al., 1999). The precloacal pores are situated on the anterior border of the cloaca, while the femoral ones are located on the ventral surface of the thighs (Alberts et al., 1992; Imparato et al., 2007; Labra, 2008). Pores are usually present only in adult males (Chauhan, 1986; Dujsebayaeva et al., 2009; Lobo et al., 2012). In species where both sexes have pores, these are larger in males (Cole, 1966b, Athavale et al., 1977, Ferreira, 2007), and if juveniles have pores, these are larger in adults than in juveniles (Cole, 1966b; Alberts et al., 1992). In a few species, both sexes have secondarily lost these pores (Darevsky and Szczerbak, 1997; Lobo, 2005).

The morphology of the femoral glands has received significant attention (e.g. Athavale et al., 1977; Chauhan, 1986; Ferreira, 2007; Imparato et al., 2007; Chamut et al., 2009), and they can be lobed (Antoniuzzi et al., 1993; Imparato et al., 2007) or tubular, simple (Chiu and Maderson, 1975), branched (Chiu and Maderson, 1975; Ferreira, 2007) or acinar (Khannoon et al., 2013). In contrast, the knowledge about the precloacal glands morphology is confused, because a variety of glands located in the cloacal area had been called precloacal glands, such as some generation glands and callose scales of several cordylid (e.g. Van Wyk and Mouton, 1992) and agamid lizards (e.g. Dujsebayaeva et al., 2007). The other group of glands, those traditionally called precloacal glands (Etheridge, 1995; Pinna et al., 2010), is located on the border of the cloaca, which have a recognizable secretory and collecting portion, and are found in amphisbaenids and liolaemid lizards. Focusing on these later precloacal glands, we can say that the knowledge on their morphology is limited to some studies on amphisbaenas (e.g. Antoniazzi et al., 1993). However, because the structure of the glands of liolaemid lizards is unknown, the understanding of the extent of morphological precloacal gland variation as compared to the femoral gland, it is not possible.

The lack of information regarding the morphology of precloacal glands and pores in *Liolaemus* lizards, the most diverse Liolaemidae genus (Lobo et al., 2010a), with more than 240 species (Uetz and Hošek, 2014), contrasts with the extensive use of precloacal pores as a taxonomic character, as well as for sex discrimination (Lobo, 2005). Gland secretions are involved in social recognition (Labra, 2008), and their lipid compositions differ among species and populations (Escobar et al., 2001; 2003). The glands apparently share a function across *Liolaemus*, but the variation in morphology among species, sexes or age classes (i.e., juveniles vs. adults) is largely unknown, and it is unclear if the lack of external pores is associated with a complete absence of these epidermal glands.

To shed some light on these questions, we studied the external, anterior ventral border of the cloaca in three *Liolaemus* species that differ in the occurrence of precloacal pores: 1- *L. irregularis*: adults and juveniles of males and females have pores (Valdecantos and Lobo, 2007); 2- *L. poecilochromus*: only adult males have pores (Laurent, 1986); and, 3- *L. neuquensis*: adults of both sexes lack pores (Scolaro et al., 2007).

## MATERIALS AND METHODS

### Animals

We processed individuals of the herpetological collection of the Museo de Ciencias Naturales of Universidad Nacional

de Salta (Argentina), in order to have an adequate sample size without affecting negatively the natural populations. Specimens were fixed in 10% buffered formalin solution and preserved in 70% alcohol, and were collected in consecutive summers (December-February of 2007, 2008, 2009), not in their mating season (Ramírez-Pinilla, 1991). We used 83 individuals of *L. irregularis*, 38 of *L. poecilochromus* and two of *L. neuquensis*. The description of the color of the secretions, however, was based on field observations of wild lizards.

### Histology

We removed the external, anterior ventral border of the cloaca of 31 *L. irregularis* (♂: 7 juveniles, 8 adults; ♀: 9 juveniles, 7 adults), 22 *L. poecilochromus* (♂: 5 juveniles, 8 adults; ♀: 2 juveniles, 7 adults) and two *L. neuquensis* adults (1♂, 1♀), and dehydrated in graded series of ethanol solution, cleared in xylene and embedded in paraffin. Thereafter, paraffin sagittal serial sections (5-7 µm) were obtained with a rotary microtome (Reichter, Germany) and were stained with hematoxylin-eosin (H & E). Based on previous studies on sexual maturity (Valdecantos and Lobo, 2007), we considered juveniles of *L. irregularis* and *L. poecilochromus* individuals which snout-vent lengths ranged from 32.76 to 48.63 mm, without including newborns since they were not available in the collection. Adults of *L. irregularis* had sizes from 70.4 to 99.1 mm, and *L. poecilochromus*, from 62.14 to 75.03 mm. No age classes were studied in *L. neuquensis*, and the adult male measured 60.44 mm, while the adult female measured 59.25 mm.

Gland descriptions and their measurements were done on the three best central mid-sagittal sections (i.e., not on their borders) of the best-sectioned gland from each specimen. Descriptions follow the nomenclature of Cole (1966b), Geneser (2000), and Gartner and Hiatt (1994).

### Histochemistry

To identify neutral and acid mucosubstances, paraffin sections were treated separately with periodic acid Schiff's reagent (PAS) and alcian blue (AB) pH 2.5 (Martoja and Martoja-Pierson, 1970). Separately, we stained new slides with Bromophenol blue to identify proteins (Imparato et al., 2007). We did not search for lipids, because they solubilize in alcohol, which was used to preserve the lizards. Slides were observed under an Olympus BX40 microscope (Tokyo, Japan) and photographed with a digital camera Olympus DP25 (Tokyo, Japan).

### Scanning electron microscopy

We processed the external, anterior ventral cloacal border of four *L. irregularis*, one of each sex and age (juvenile, adult), and one adult male of *L. poecilochromus*. The tissues were dehydrated by critical point, dried with liquid carbon dioxide, sputtered with gold, and examined under a scanning electron microscopy (JEOL JSM-6480, Tokyo, Japan).

### Morphometry of preloacal pores and glands

We determined the number and diameter (mm) of the preloacal pores of *L. irregularis* and *L. poecilochromus* (see Table 1 for sample size). To obtain the pore diameters, we measured the length of the minor and major axes of each pore, using a binocular Arcano magnifying glass (HG 272931, Shanghai, China) with a graduated ocular lens. Each individual was characterized by the average value of the diameters of all its pores. Using a microscope Olympus magnifying glass (Tokyo, Japan) with a graduated ocular lens, and following Cole (1966b), we measured the maximum length of the glands from the anterior end to the posterior end of the basal layer of germinative epithelial cells. The maximum width was measured in the transverse axis of the preloacal gland (see Table 2 for sample size and Fig. 5 for indications of how these measurements were taken).

To remove the effect of body size in the statistical analyses of pores and glands sizes, we measured the snout-vent length of these lizards (see below).

### Statistics

The numbers of preloacal pores were not normally distributed, and comparisons were made with non-parametric Mann-Whitney tests. The  $\log_{10}$  transformed pore diameters and the  $\log_{10}$  length and width of the preloacal glands were normally distributed, and were compared using analysis of covariance (ANCOVA) with snout-vent length as covariate, followed by *a posteriori* Tukey tests (Zar, 1999).

## RESULTS

### Gross morphology and morphometry

Juveniles and adults of both sexes of *L. irregularis* (Figs. 1A, B, C, D), and all males of *L. poecilochromus* (Fig. 1E), had a line of preloacal pores, and each pore was located in a single modified scale. Two adult males of *L. poecilochromus* had, immediately posterior to the line of preloacal pores, a row of "secondary pores" (Fig. 1F), consisting of three to four pores, smaller than the primary ones. Because these "secondary pores" were uncommon, they were not considered in the final analyses of pore morphology. Three out of the seven adult females of *L. poecilochromus* had one or two preloacal pores (Fig. 1G), and one of these females had a gland that opened inside the cloaca, rather than with a pore in a scale. The remaining females did not have any trace of pores (Fig. 2F). Finally, three out of five juvenile males of *L. poecilochromus* had in the scales of the cloacal border, deep invaginations without openings, i.e. clefts (Fig. 1H). The inspection of the two *L. neuquensis* individuals did not reveal pores or clefts.

The secretions of living animals had an orange color (Fig. 1A). In adult males of *L. irregularis* and *L.*

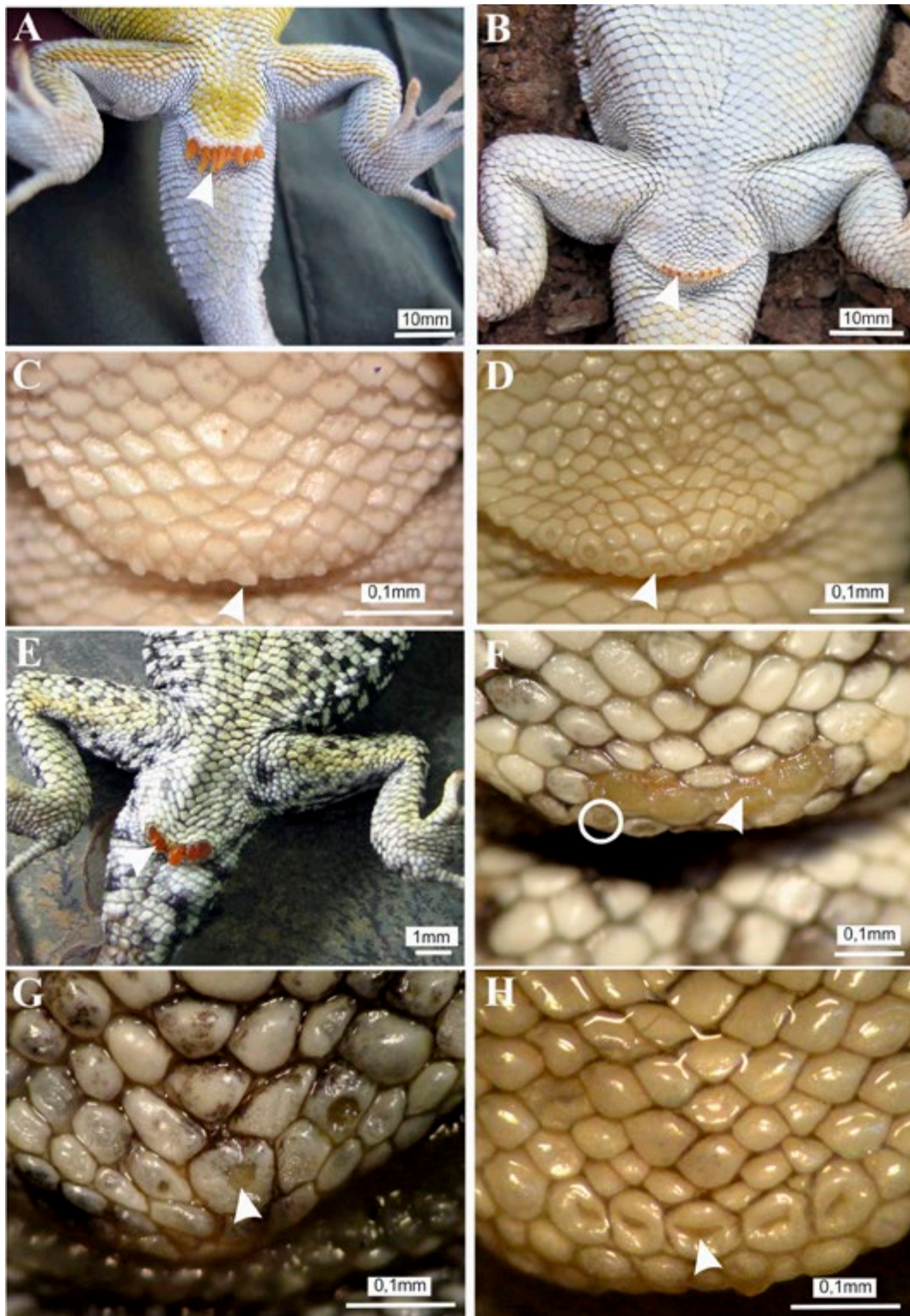
*poecilochromus* secretions were always visible as a solid and compact pack of dead squamous cells and secretory cells (Fig. 2A), which in most adult males emerged through the pores as long and rigid cylinders, resembling wax crayons (Figs. 1A, E). In contrast, in females and juveniles little of the glandular secretions emerged out of the pore (Figs. 1B, C, D).

The morphometry of preloacal pores is in Table 1. Males (adults and juveniles) of *L. irregularis* had more preloacal pores than females (adults:  $W = 338.50$ ,  $P = 0.0001$ ; juveniles:  $W = 25.00$ ,  $P = 0.004$ ). In addition, adult males had larger pores than adult and juvenile females (Figs. 2A, C), but there were no differences between juveniles of both sexes (Figs. 2B, D). Differences in pore size between ages were observed in females, but not in males ( $F = 26.51$ ,  $P < 0.0001$ ). Finally, *L. irregularis* had more ( $W = 122.00$ ,  $P < 0.0001$ , Figs. 1A, E, F) and larger preloacal pores ( $F = 9.14$ ,  $P = 0.0054$ , Figs. 2A, E) than *L. poecilochromus*.

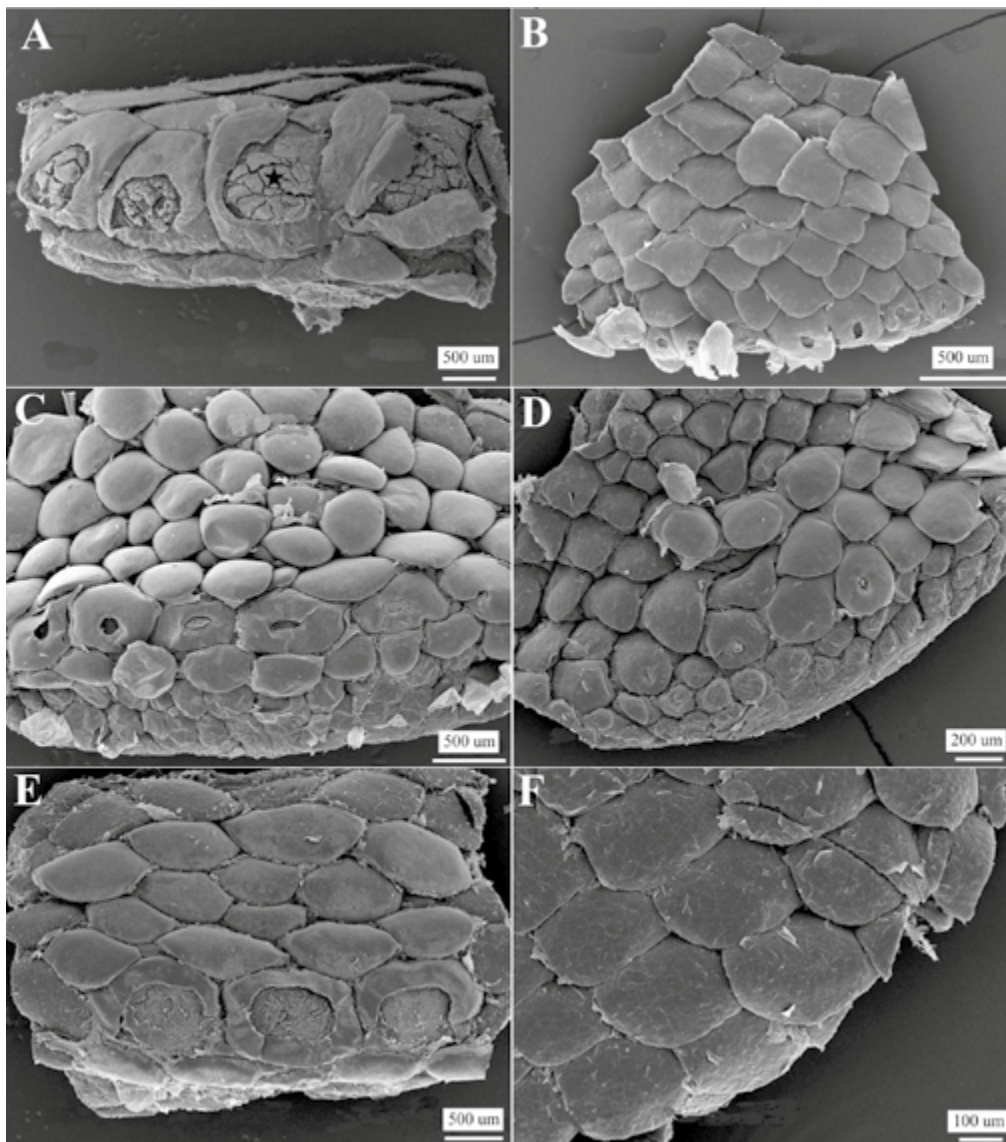
### Histology

Each pore was associated to a tubuloalveolar gland, compound in adult (Figs. 3A, E), simple in juveniles (Figs. 4A, C). Glands always had secretion in the lumen, independent of the age and sex of the individuals. The morphology, but not the size of these glands was similar between sexes and ages (Table 2; length:  $F = 9.16$ ,  $P = 0.0004$ ; width:  $F = 21.06$ ,  $P < 0.0001$ ). Adult males of *L. irregularis* had longer glands than adult females and juveniles, but there were no differences between juveniles of both sexes. The width of the glands was larger in adult males than in females, but no differences were found between age classes by sex. Finally, adult males of *L. irregularis* had longer ( $F = 5.93$ ,  $P = 0.0331$ ), but not wider ( $F = 0.0001$ ,  $P = 0.9910$ ), preloacal glands than *L. poecilochromus*. The size of the glands increased with the body size in adult males of *L. irregularis* (Figs. 5A, B), which are large enough to touch each other (Fig. 3A).

The following description corresponds to the glands of adult males of *L. irregularis* (Fig. 3A) and *L. poecilochromus* (Fig. 3E). The gland is surrounded by an envelope of a thin layer of connective tissue (Figs. 3B, E), which extends into the partitions that divide the gland into several elongated lobules (Figs. 3B, C, E). Each lobule has several tubules and alveoli that converge in a single and short central duct that collects the secretions, which finally reach the pore (Figs. 3A, E). Tubules and alveoli are internally coated by a layer of basal cells, the germinative epithelium (Figs. 3D, F). This layer consists of small, flat to cubic cells that have oval to spherical nuclei, with conspicuous nucleoli, which occupy the larg-



**Fig. 1.** Ventral view of adult and juvenile of *Liolaemus* lizards showing the cloacal area. *Liolaemus irregularis*: (A) adult male, (B) adult female, (C) juvenile male, (D) juvenile female. *L. poecilochromus*: (E) and (F) adult males, (G) adult female, (H) juvenile male. The white arrowhead from (A) to (G) shows the precloacal pores with secretions and in (H), the clefts. The white circle in (F) indicates a “secondary pore”. Figures (A), (B) and (E) were taken in wild lizards in the field, showing the normal condition of these secretions. The other pictures were taken from specimens from the herpetological collection.



**Fig. 2.** Scanning electron microscopy of the precloacal pores of *Liolaemus irregularis* (A to D) and *L. poecilochromus* (E and F). (A) adult male, (B) juvenile male, (C) adult female, (D) juvenile female, (E) adult male, (F) adult female. ★: pack of dead squamous cells and secretory cells.

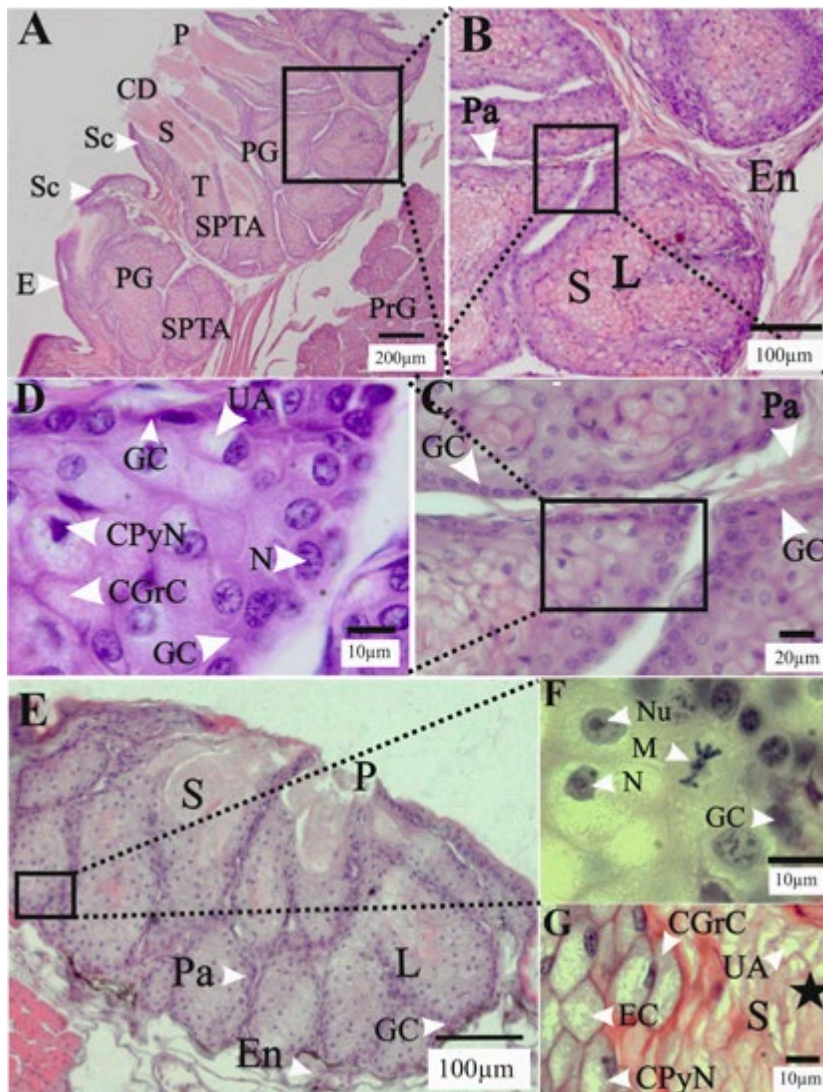
est part of the cells (Figs. 3E, F). Mitoses are observed in the germinative stratum (Fig. 3F), indicating a continuous proliferation of cells that replenish the dead ones. As cells proliferate, they move toward the periphery, and during this migration, they enlarge, increasing their cytoplasmic content with granules (Figs. 3D, G), which show a weak PAS positive reaction (i.e., pink tones; Fig. 6A). Towards the center of the tubules and alveoli, most free/luminal cells are degenerated as evidenced by the gradual pyknosis of their nuclei or because they are enucleated, with a disaggregated cytoplasm (Figs. 3D, G). Inside the

central duct, the structural integrity of the cells is not recognizable any more (Fig. 3G). The final secretion appears as a mixture of secretory granules and cell debris, corresponding to a holocrine secretion (Figs. 3G, 4B, D).

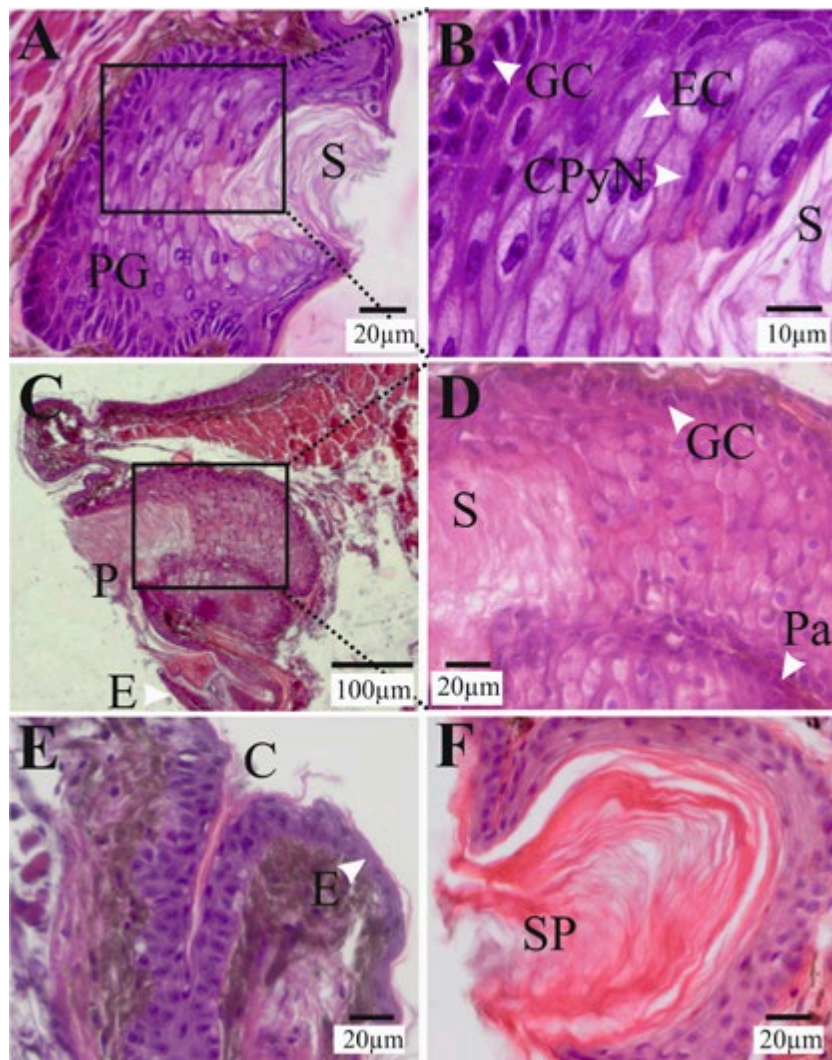
Three out of seven adult females of *L. irregularis* had precloacal glands with similar structure as those of adult males. The glands of the remaining adult females (n=4), those of the adult females of *L. poecilochromus* (n=3) with precloacal pores, and those of juveniles of both sexes of *L. irregularis* had fewer or non-existent partitions and alveoli, although they had secretions (Figs. 4A, B, C, D).

**Table 1.** Descriptive statistics (mean  $\pm$  SD) of snout-vent length (SVL, mm) of the individuals of *L. irregularis* and *L. poecilochromus* measured to characterize the precloacal pores, considering separately both sexes and age classes. %IP: percentage of individuals with precloacal pores from the total number on individuals examined. The characteristics of the precloacal pores of these two *Liolaemus* species are: N°P: number and PD: diameter (mm) of pores. In parentheses, the minimum and maximum values. n: sample size.

	<i>L. irregularis</i>				<i>L. poecilochromus</i>
	Juveniles		Adults		Adults
	Females n = 9	Males n = 8	Females n = 16	Males n = 15	Males n = 15
SVL	36.6 $\pm$ 3.4 (31.7–43.9)	38.9 $\pm$ 2.6 (36.7–45.0)	73.5 $\pm$ 6.1 (62.0–85.0)	82.0 $\pm$ 11.25 (66.3–97.6)	68.2 $\pm$ 5.3 (58.3–76.1)
% IP	67	100	100	100	100
N°P	4.1 $\pm$ 3.2 (0–8)	8.4 $\pm$ 1.5 (7–11)	5.9 $\pm$ 1.9 (1–8)	8.4 $\pm$ 1.1 (7–10)	4.0 $\pm$ 1.2 (3–7)
PD	0.08 $\pm$ 0.06 (0.09–0.15)	0.15 $\pm$ 0.04 (0.12–0.23)	0.22 $\pm$ 0.05 (0.16–0.33)	0.54 $\pm$ 0.19 (0.25–0.81)	0.49 $\pm$ 0.14 (0.18–0.75)



**Fig. 3.** Histological sections of the anterior cloacal borders of an adult male of *Liolaemus irregularis* (A to D and G) and an adult male of *L. poecilochromus* (E and F). (A) General view of the precloacal glands draining, each one, in a single scale. (B) Magnification of the box in (A), showing details of the envelope surrounding the gland, a thin layer of connective tissue, and the division of glands in lobules. (C) Magnification of the box in (B), showing details of the partitions that divide the gland into several elongated lobules and the germinative epithelium that coated tubules and alveoli, which consists of small, flat to cubic cells, with oval to spherical nuclei. (D) Magnification of the box in (C), with details of the different cell types. (E) General view of precloacal glands. (F) Details with immersion of the box shown in (E), showing germinative cells with spherical nuclei and conspicuous nucleoli and mitoses in the germinative stratum. (G) Details with immersion of a basal portion of alveoli of precloacal gland where the different cell types and degenerated cells can be appreciated. Degenerated cells are enucleated with a disaggregated cytoplasm where cytoplasmic membranes cannot be recognized anymore. Abbreviations: CD: central duct, CGrC: cell with granular cytoplasm, CPyN: cell with pyknotic nucleus, E: epidermis, EC: enucleated cells, En: envelope, GC: germinative cells, L: lobule, M: mitosis, N: nucleus, Nu: nucleolus, P: pore, Pa: partition, PG: precloacal gland, PrG: Proctodeal gland, S: secretion, Sc: scale, SPTA: secretory portion tubule alveolar, T: tubule, UA: unstained area; ★: enucleated cells with a disaggregated cytoplasm and degenerated cells. Stain: Hemalum-Eosin.



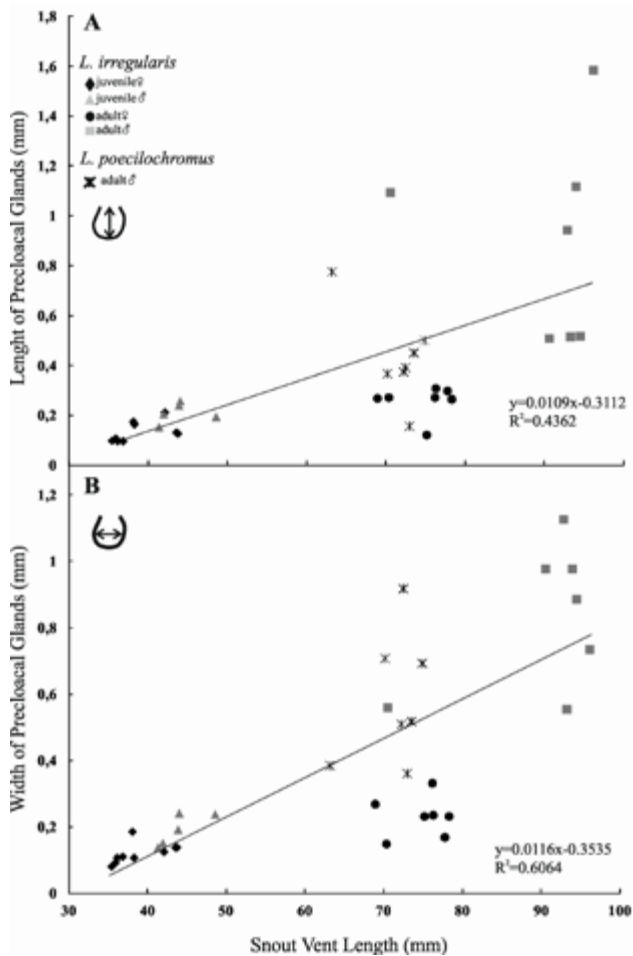
**Fig. 4.** Histological section of the cloacal border of *L. irregularis* (A to D) and *L. poecilochromus* (E and F). (A) Precloacal gland of a juvenile female showing the precloacal gland. (B) Magnification of the box in (A), with details of the different cell types. (C) Precloacal gland of a juvenile male with a single partition. (D) Magnification of the box in (C), showing details of partition, germinative cells and secretion. (E) Cleft of a juvenile male. (F): “Secondary pore” of an adult male. Abbreviations: C: cleft, CPyN: cell with pyknotic nucleus, E: epidermis, EC: enucleated cells, GC: germinative cells, Pa: partition, PG: precloacal gland, S: secretion, SP: secondary pore. Stain: Hemalum-Eosin.

**Table 2.** Descriptive statistics (mean ± SD) of the length (LG; mm) and width (WG; mm) of precloacal glands of *L. irregularis* and *L. poecilochromus*, considering separately both sexes and age classes. n: sample size.

	<i>L. irregularis</i>				<i>L. poecilochromus</i>	
	Juveniles		Adults		Adults	
	Females n = 9	Males n = 5	Females n = 7	Males n = 7	Females n = 3	Males n = 7
LG	0.13 ± 0.04	0.21 ± 0.04	0.26 ± 0.06	0.90 ± 0.41	0.16 ± 0.05	0.43 ± 0.19
WG	0.12 ± 0.03	0.19 ± 0.05	0.23 ± 0.06	0.83 ± 0.22	0.20 ± 0.08	0.58 ± 0.20

The clefts of the juvenile males of *L. poecilochromus* are invaginations of the epidermis on the cloacal border (Fig. 4E), without secretions, which are located in the same position where the large pores of adults are observed. The “secondary pores” found in two adult males of *L.*

*poecilochromus* were morphologically different from the precloacal glands, as they are a sac filled with a material whose nature probably is secretion (Fig. 4F). No traces of precloacal pores or clefts were observed in either sex of *L. neuquensis*.



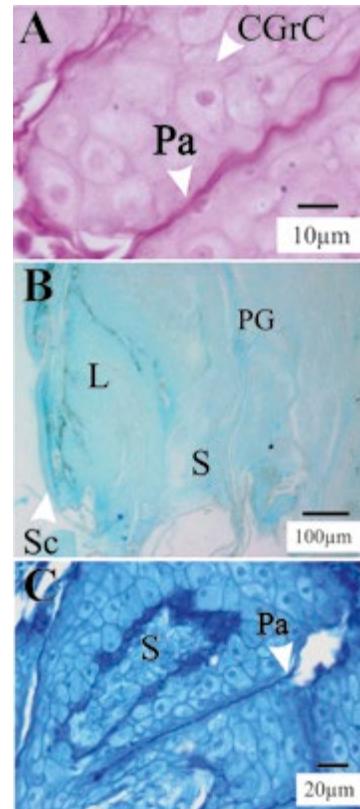
**Fig. 5.** Length (A) and width (B) of preloacal glands in relation to the snout-vent length of *L. irregularis* and *L. poecilochromus*. Each point represents an individual. The regression values were obtained including all the individuals measured in this study, independent of the species. Each graph has an insert showing how glands were measured.

### Histochemical Observations

The secretion in the glandular lumen showed positive reaction to PAS (i.e. pink tones, Fig. 6A) and AB (i.e., light blue tones, Fig. 6B). Intracytoplasmic granules and secretions were positive to Bromophenol blue (Fig. 6C). The cytoplasm of many cells showed unstained areas, independent of the stain used (Figs. 3D, G).

### DISCUSSION

Each preloacal pore in *Liolaemus* is located in a unique scale, without modification, such as those observed in teiids, where the scales resemble a rosette (e.g.



**Fig. 6.** Histological section of preloacal glands of adult males with different stains. (A) Details of a partition and cells with granular cytoplasm of *L. poecilochromus*, stained with PAS. The pink cytoplasm indicates a weak positive reaction. (B) Details of the secretion and glands of *L. irregularis* stained with AB. The light blue color indicates a weak positive reaction. (C) Details of a partition and cells with granular cytoplasm and secretion of *L. poecilochromus*, stained with Bromophenol blue. The intense blue tone indicates a strong positive reaction. Abbreviations: CGrC: cell with granular cytoplasm, L: lobule, Pa: partition, PG: preloacal gland, S: secretion.

Imparato et al., 2007). Each pore has an associated epidermal gland, and if glands are present, they are always functional, i.e. they have secretions in the lumen, independent of the sex and age of the individual. In *L. irregularis*, there is sexual dimorphism in the size and number of these pores, biased towards males. In the case of *L. poecilochromus*, we found “secondary pores” in some adult males, and juvenile males had clefts, which would represent the origin of the adult preloacal pores, as what was reported for the femoral pores of *Crotaphytus* (Cole, 1966b). To our knowledge, we are describing for the first time in *Liolaemus* these clefts, likely because they are not easily detected by the naked eye. The “secondary pores” had been described in some other *Liolaemus* species, which are used as a taxonomic character (e.g. Lobo et al., 2010b),



although their function is unknown, and so, if their product might be involved in chemical communication.

Individuals of *L. irregularis* had functional precloacal glands from early in the ontogeny (i.e. juveniles), which experience a marked ontogenetic change in males; the number of tubules, alveoli and the size of glands increase from juveniles to adults. Even though we did not include newborns of *L. irregularis* (individuals with ~ 27 mm of snout-vent length), the fact that all juveniles have functional secreting glands, suggests that individuals are born with precloacal glands. The observed ontogenetic variation of the *L. irregularis* glands is similar to what was reported for the femoral glands; in *Crotaphytus collaris* there is an increase in the size and complexity of glands after birth in males, but not in females (Cole, 1966b). In *Cordylus polyzonus polyzonus*, the variation of the males' gland size is correlated with spermatogenic activity (Van Wyk, 1990), and in *Iguana iguana*, the activity of the glands varies across the reproductive cycle, and they are always larger in males than in females (Ferreira, 2007). Unlike *L. irregularis*, the precloacal glands of *L. poecilochromus* are absent in juveniles, which only have invaginations of the epidermis, in the same position where pores are located in adults, which suggests that glands start to develop when males are juveniles, reaching maturity in adults. All together, observations suggest that gland development would be associated with the individual's sexual maturity, as was reported for other *Liolaemus* species (e.g. Valdecantos and Lobo, 2007).

The *Liolaemus* precloacal secretions contain acid mucosubstance, which contrast to the neutral mucosubstances found in other species of lizards and amphisbaenids (Antoniuzzi et al., 1993; Imparato et al., 2007). Unfortunately, there are no studies exploring the role played by these mucosubstances. The precloacal secretions also contain proteins, as has been reported for the femoral/precloacal gland secretions of other species (Antoniuzzi et al., 1993; Imparato et al., 2007). It was proposed that proteins of the femoral secretions of *Iguana iguana* might act as a barrier to reduce lipid evaporation (Alberts, 1991). In addition, the observed intra and inter-specific variability in the protein profiles may provide significant information about the identity of the owner (Alberts et al., 1993). On the other hand, it is well known that femoral/precloacal secretions contain lipids (Imparato et al., 2007; Martín and López, 2011), also described in the precloacal secretions of *Liolaemus* species (Escobar et al., 2001; 2003). In this study, we could not explore the presence of lipids because specimens were preserved in alcohol, but the unstained areas observed in the glands suggest that the content would be of lipidic nature.

The secretions are expelled as rigid cylinders, in opposition to the flexible condition of femoral secretions (e.g. "paste-like secretions") reported by Chauhan (1986). Probably precloacal secretions are delivered passively in thin layers while lizards perform their daily activities. In fact, we never observed pieces of these cylinders on lizard perches in the field, nor individuals with broken cylinders of secretion. A passive delivery is facilitated by the position of these pores, as has been suggested for *Amphisbaena alba* (Jared et al., 1999). Moreover, secretions may be adhered to faeces during defecation, contributing to their pheromonal properties (Labra, 2008). This does not exclude, however, the possibility that secretions may be more actively delivered by dragging the cloaca in the substrate (Labra et al., 2002).

The precloacal glands of these *Liolaemus* species have a general histological structure similar to the precloacal glands of amphisbaenas (Antoniuzzi et al., 1993; Jared et al., 1999), and to the femoral glands of lizards from different families (Cole, 1966b; Imparato et al., 2007). Thus, it may be possible to consider that the femoral and precloacal glands are homologous; both are holocrine with pheromonal secretions (Martín and López, 2011) and have the same embryological origin (ectoderm). It is intriguing however, what determines variation in the area where glands appear (cloaca vs. thighs).

In *Liolaemus*, there is a significant intra and inter-specific variation in the number of precloacal pores, and moreover, some few species have secondarily lost them (Etheridge, 1995; Lobo, 2005). In most species (> 90%) pores are only present in males, and it is unclear which factors determine the loss (e.g. in some species) or gain (e.g. in females) of these glands, or their occurrence early in the ontogeny (e.g. *L. irregularis*). In Cordylidae, the presence of generational glands correlated with the environment temperature; females, and in few cases males, of species that inhabit at high altitude lack these glands (Cordes et al., 1995). In contrast, Escobar et al. (2001) found that *Liolaemus* species that live at higher altitude have more pores than those from lower altitude, which was explained as a need to produce more secretions due to a faster degradation of these at high altitude. As for the species studied here, altitude/environment temperature cannot explain the difference in the number of precloacal pores, as both species with pores, *L. irregularis* and *L. poecilochromus*, inhabit high elevations and the presence of precloacal pores in females differed between these two species. Moreover, *L. neuquensis* is one of the few species of the subgenus *Liolaemus* (Lobo et al., 2010a) that lacks precloacal pores although it lives at low elevations (< 500 masl), while other species in this *Liolaemus* subgenus that inhabit at high elevations, have precloacal pores (e.g.

Martínez Oliver and Lobo, 2002). Potentially, phylogenetic constraints may explain the interspecific difference between *L. irregularis* and *L. poecilochromus* that belong to distinct subclades of *Eulaemus* (Schulte et al., 2000; Pincheira-Donoso et al., 2008; Lobo et al., 2010a).

Further than phylogenetic constraints upon the occurrence of the precloacal pores, their evolution may also be tightly related to selective forces upon chemical communication, considering that precloacal secretions are involved in social communication (Labra, 2008). If so, the lack of precloacal glands (i.e. *L. neuquensis*) may be related to a reduction in the use of chemical communication, unless other pheromonal sources fulfill the function of these glands. In contrast, *L. irregularis*, the species in which pores are present in all individuals, may use significantly more chemical communication, and precloacal secretions may encode important information for different aspects of their social communication, while secretions in *L. poecilochromus* would play only a key role in the reproductive success of adult males. It is unclear, however, why some females of *L. poecilochromus* develop these glands. It seems intuitive that this species is experiencing selective forces to use more the precloacal secretions in chemical communication, which also may explain the occurrence of “secondary pores” in some males, and precloacal glands in some females.

In summary, this first morphological study on lizard precloacal glands shows that in *Liolaemus* their presence varies across sexes and species, although, they are always functional, producing secretions. Future studies will unravel the selective forces that determine gains and losses of these glands across species, sexes, and ages.

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