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Description of a proctodeal gland present in three South American *Liolaemus* lizards (Iguania: Liolaemidae)SOLEDAD VALDECANTOS¹, VIRGINIA MARTÍNEZ¹ & ANTONIETA LABRA^{2,3}¹IBIGEO – Instituto de Biología y Geología del NOA, Universidad Nacional de Salta, CONICET, Avenida Bolivia 5150, 4400, Salta, Argentina²Programa de Fisiología y Biofísica, Facultad de Medicina, Universidad de Chile, Casilla 70005, Correo 7, Santiago, Chile³University of Oslo, Department of Biosciences, Centre for Ecological and Evolutionary Synthesis, P.O. Box 1066 Blindern, 0316 Oslo, Norway

Corresponding author: SOLEDAD VALDECANTOS, e-mail: solevaldecantosp@gmail.com

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Vertebrates, with the exception of placental mammals and teleosteos, possess a cavity, the cloaca, internally divided into the urodaeum, coprodaeum, and proctodaeum, where the urogenital and digestive tracts lead (GADOW 1887). Thus, the cloaca is involved in defecation, elimination of nitrogenous debris, copulation, sperm transportation, and the passing of eggs or birth of offspring (GADOW 1887, SKADHAUGE 1974, MINNICH 1982, URIBE et al. 1998, KARDONG 2007, SÁNCHEZ-MARTÍNEZ et al. 2007). In squamates, additional functions have been suggested for the cloaca, such as conservation of water (BENTLEY 1976), precipitation of uric acid (MINNICH 1972, 1982), and scent production that allows identifying species or provide information about the sex and reproductive condition of the emitter of such scent (TRAUTH et al. 1987, URIBE et al. 1998). Regarding the morphology of the cloaca, different authors have reported and described glands in different cloacal regions of both sexes in all major groups of squamates (DISSELHORST 1904, GABE & SAINT GIRONS 1965, BURKOLDER & TANNER 1974, TRAUTH et al. 1987, COOPER & TRAUTH 1992, SÁNCHEZ-MARTÍNEZ et al. 2007). These glands may have various functions, but considering that in different lizard species, faeces are a source of pheromones (BULL et al. 1999a, 1999b, LABRA et al. 2002, MOREIRA et al. 2008, FENNER & BULL, 2011), their secretions and excretions might also be involved in chemical communication. So far, however, descriptions of glands in the urodeum and proctodeum regions that would be directly involved in scent production are limited to some scincid, cordylid, and sceloporid lizards (TRAUTH et al. 1987, COOPER & TRAUTH 1992).

Liolaemus WIEGMANN, 1834 (Liolaemidae) lizards use chemical information, among others, to assess self and

conspecific recognition (LABRA 2008), and faeces are one source of scents for both sexes (LABRA et al. 2002, VALDECANTOS et al. in prep.). Presently, there have only been descriptions of cloacal glands in females; crypt glands in the proctodaeum region of *Liolaemus albiceps* LOBO & LAURENT, 1995, and urodaeum glands in *Liolaemus quilmes* ETHERIDGE, 1993 (SÁNCHEZ-MARTÍNEZ et al. 2007). These glands may be associated with egg production, which does not exclude the possibility that their secretions could be involved in chemical communication, too. However, there have been no studies on the male cloaca, ignoring the possibility that they might also possess glands that can be involved in chemical communication.

Here we investigate the general characteristic of the ventral portion of the proctodeal region in individuals of both sexes of *Liolaemus irregularis* LAURENT, 1986, *L. poecilochromus* LAURENT, 1986 and *L. neuquensis* MÜLLER & HELLMICH, 1939, in order to search for glands that may contribute scents to faeces.

We processed well-preserved individuals from the herpetological collection of the Museo de Ciencias Naturales of Universidad Nacional de Salta (Argentina), collected in summer seasons, which had been fixed in 10% buffered formalin solution and preserved in 70% ethanol.

We cut the ventral cloacal border from 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♀). Tissues were dehydrated in graded series of ethanol solution, cleared in xylene and embedded in paraffin. Serial sections (5–7 µm) were obtained with a rotary microtome and stained with hematoxylin-eosin (HE). Based on previous

studies on sexual maturity of these species (VALDECANTOS & LOBO 2007), we regarded as juveniles those individuals with snout–vent lengths ranging from 32.8 to 48.6 mm, while adults of *L. irregularis* were from 70.4 to 99.1 mm, and those of *L. poecilochromus* were from 62.1 to 75.0 mm. No age classes were studied in *L. neuquensis*, and the adult male measured 60.4 mm, while the adult female measured 59.3 mm.

Subsequent descriptions of the glands and histological descriptions are based on the three best central mid-sagittal planes of the best-sectioned gland from each specimen. Descriptions follow the nomenclature used by GENESER (2002) and GARTNER & HIATT (1994).

To identify neutral and acidic mucosubstances, paraffin sections were treated with the periodic acid Schiff's reagent (PAS), hematoxylin (H), and Alcian-blue (AB) pH 2.5 (MARTOJA & MARTOJA-PIERSON 1970). We also used Bromophenol-blue to identify proteins. We did not search for lipids, because they are soluble in the alcohol that was used to preserve the lizards. Slides were inspected under a microscope (Olympus BX40; Tokyo, Japan) and photographed with a digital camera (Olympus DP25).

In the three *Liolaemus* species, an integument with epidermal scales externally coats the borders of the cloaca, which internally is continuous with the mucosa of the cloaca. Between the integument and the mucosa, there is a striated muscle. The epidermis is a keratinised stratified epithelium that forms the integument with epidermic scales (Figs 1A, B, C), and the dermis is composed of a dense, modelling connective tissue.

Males, both adult and juvenile, of all three species have a gland immersed between the cloacal mucosa and the musculature, and since it opens directly to the proctodaeum, we named it proctodeal gland (Figs 1A, B). It is located anterior to the precloacal glands in species that have these glands, i.e., *L. irregularis* (Fig. 1B) and *L. poecilochromus*. We did not find any trace of proctodeal glands in females of the three species of *Liolaemus* examined (Fig. 1C).

The proctodeal gland is a compound, tubuloacinar gland with an apocrine secretion. It is divided into lobes that are organized in lobules, and each lobule has several acini (Figs 1D, E, F), the basic functional unit of the gland. Cell secretion is delivered into secondary ducts, which end in a main duct that opens into the cloaca (Figs 1D, E, F). The acini cells have large and round nuclei (Fig. 1D) with conspicuous nucleoli and loose chromatin that are typical of cells synthesizing proteins. Based on the morphology of the acini, glands were divided into three stages: S1 – Highly active glands, with enlarged secretory lobes that reach the basal region of the integument (Fig. 1B). The acini are formed by enlarged and pyramidal cells with basal nuclei, containing abundant accumulations of cytoplasmic secretory vesicles, visible as secretory granules in the apical cytoplasm of the cell, and the ducts are full of secretions (Fig. 2A). S2 – Acini are formed by cubic and/or pyramidal cells with central nuclei, the cytoplasm has fewer secretory vesicles, and the ducts have little or no secretion (Fig. 2B). S3 – Acini are formed by cubic cells with central nuclei, the

cytoplasm has no secretory granules, and the ducts are empty (Fig. 2C).

All adults of *L. irregularis* had proctodeal glands in S1, while only half of the adults of *L. poecilochromus* had glands in S1, and the other half had them in S2. Three out of seven juveniles of *L. irregularis* and one out of five juveniles of *L. poecilochromus* had glands in S2 and all others in S3. The only male of *L. neuquensis* had the gland in S2.

The secretion accumulated in the glandular lumen proved PAS positive, indicating the presence of mucosubstances. The intracytoplasmic granules and secretions were positive to Bromophenol-blue, indicating the presence of protein (Fig. 2A). The Alcian-blue pH 2.5 was negative for glandular structures, indicating that the mucosubstances were neutral. The staining shows intracytoplasmic granules in the pyramidal cells and the secretions of the ducts.

Males, juveniles, and adults of the three *Liolaemus* species possess a proctodeal gland, which previously remained unreported in this genus. Different glands have been described from the proctodeal region of both sexes of different lizard species (GABE & SAINT-GIRONS 1965, BURKHOLDER & TANNER 1974, TRAUTH et al. 1987, COOPER & TRAUTH 1992, SÁNCHEZ-MARTÍNEZ et al. 2007). From these, the gland found in *Liolaemus* has a morphology and position similar to the one described in males of *Sceloporus graciosus* BAIRD & GIRARD, 1852, whose secretion was proposed to serve as a lubricant for the hemipenis during copulation (BURKHOLDER & TANNER 1974). It is unknown whether the gland in *Liolaemus* has the same function as in *S. graciosus*, but the fact that only adults of *Liolaemus* males have glands with secretions suggests that it may have the same function as has been proposed for *Sceloporus*.

The position of the *Liolaemus* proctodeal gland, however, is also similar to the ventral portion of the glandular complex described for *Plestiodon* (DUMÉRIL & BIBRON, 1839) and *Gerrhosaurus* WIEGMANN, 1828 (TRAUTH et al. 1987, COOPER & TRAUTH 1992). We did not extract the whole cloaca, thus we cannot rule out the possibility that the proctodeal gland of these *Liolaemus* males is part of a glandular complex such as the one described for *Plestiodon laticeps* (SCHNEIDER, 1801) (Scincidae) and *Gerrhosaurus nigrolineatus* HALLOWELL, 1857 (Gerrhosauridae) (TRAUTH et al. 1987, COOPER & TRAUTH 1992), specifically the portion closest to the cloacal opening. On the other hand, the glandular complex was much less active and developed in females of *G. nigrolineatus* than in males (COOPER & TRAUTH 1992), and the urodeal glands of female *Lacerta agilis* LINNAEUS, 1758 (Lacertidae) only show a high degree of development during the reproductive season (REGAMEY 1935 in TRAUTH et al. 1987). In *P. laticeps* and *L. agilis*, high secretory activity takes place during the breeding season, and after that, the gland suffers a regression in females (REGAMEY 1935 in TRAUTH et al. 1987). Thus, we cannot rule out the possibility that females of these *Liolaemus* species have a more reduced gland complex than males, with urodeal glands.

We know that *Liolaemus* species show self-recognition through their faeces (LABRA et al. 2002), supporting the

hypothesis that the cloaca could have glands with scenting activity. In addition, the seasonal change reported in this recognition (LABRA et al. 2001) can be ascribed, among others, to changes in the scent production and/or composition in relation to the reproductive season like those described by TRAUTH et al. (1987) in females of *P. laticeps*.

No other glands were found in the females studied here. Future studies including the dorsal area of the proctodeal region will help to clarify if the proctodeal gland of *Lio-laemus* is the same as the one described by BURKHOLDER & TANNER (1974) or part of the glandular complex described by TRAUTH et al. (1987). Finally, although there are no hints

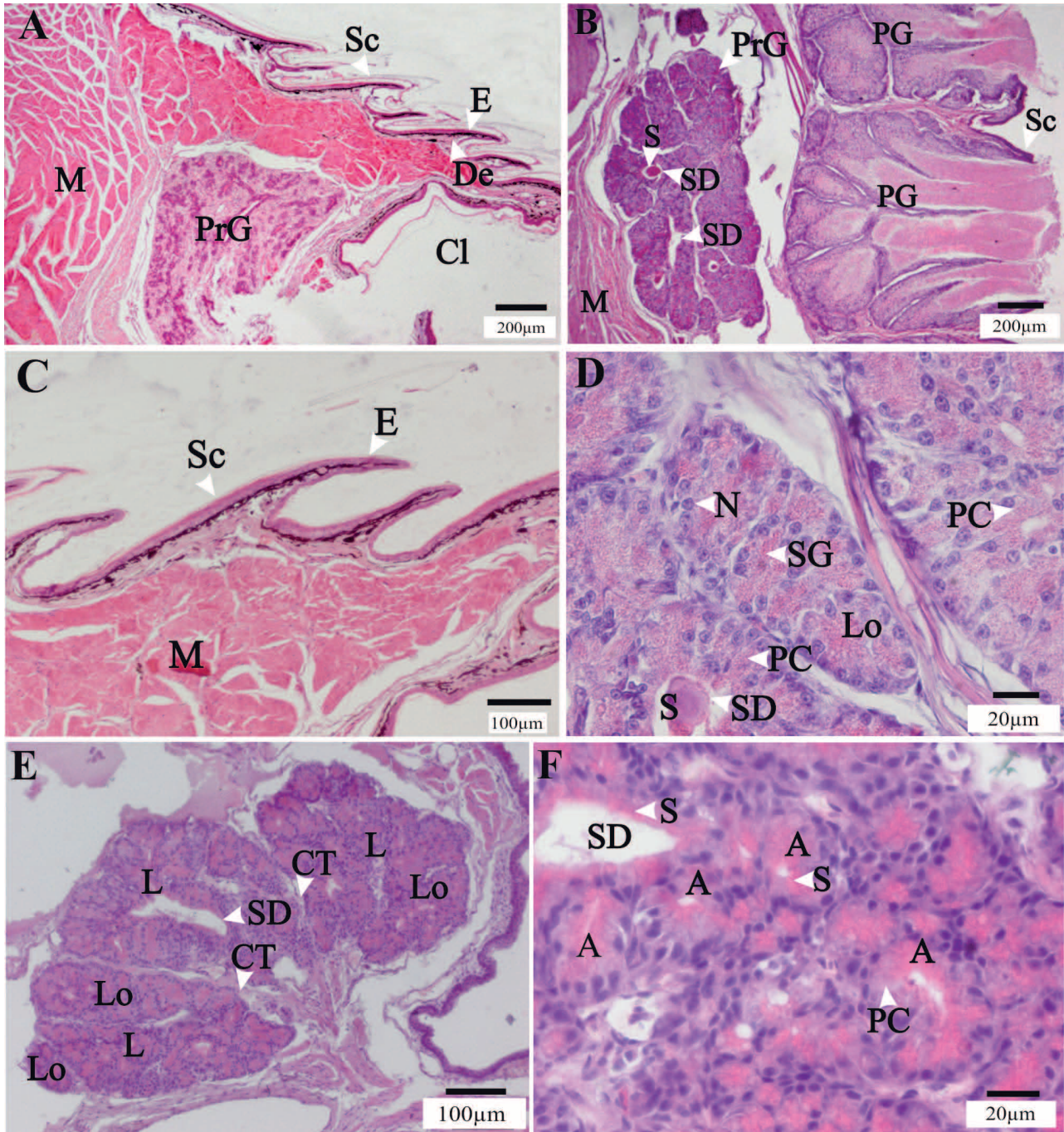


Figure 1. The proctodeal gland in adults of *L. neuquensis* A) male and C) female; *L. irregularis*: B, D) male, E, F) male of *L. poecilochromus*. Abbreviations: A – acinus; Cl – cloaca; CT – connective tissue; De – dermis; E – epidermis; L – lobes; Lo – lobules; M – musculature; N – nuclei; PC – pyramidal cell; PG – precloacal gland; PrG – proctodeal gland; S – secretions; Sc – scale; SD – secondary duct; SG – secretory granules. Stains: Hematoxylin-Eosin.

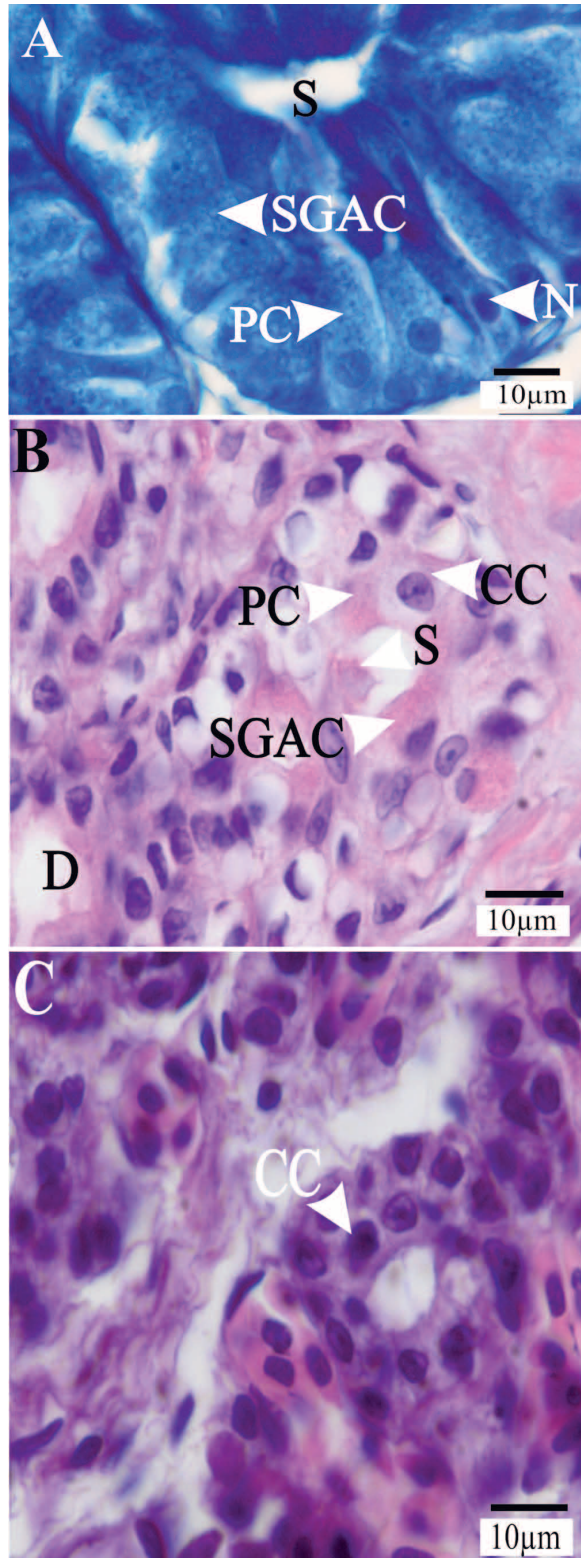


Figure 2. The three stages of proctodeal glands. A) acinus at stage 1; B) stage 2 and C) stage 3. Abbreviations: CC – cubic cell; D – duct; N – nuclei; PC – pyramidal cell; S – secretion; SGAC – secretory granules in apical cytoplasm PAS positive. Stains: A) Bromophenol-blue; B, C) Hematoxylin-Eosin.

on the function of the proctodeal glands in *Liolaemus*, nor of the role of the different compounds found in these glands, we postulate that their secretions may be involved in reproductive aspects, such as in *Sceloporus* or *Plestiodon* (BURKHOLDER & TANNER 1974, TRAUTH et al. 1987, COOPER & TRAUTH 1992), considering that active glands were observed only in adults. Alternatively, the activity of the proctodeal gland could be related to some social characteristics of individuals, such as a dominant status (e.g., ALBERTS et al. 1992), since, for example, males of *L. poecilo-chromus* with highly active glands, in S₁ and others in S₂, were found within the same season.

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