



Histological and histochemical study of the uropygial gland of chimango caracara (*Milvago chimango vieillot*, 1816)

MC Chiale^{1,2}, D Montalti^{1,2}, MA Flamini³, P Fernández⁴, E Gimeno^{2,4}, CG Barbeito^{2,3,4}

¹Ornithology, Vertebrate Zoology Division, Faculty of Natural Sciences and Museum, National University of La Plata, La Plata, ²CONICET, La Plata, ³Department of Histology and Embryology, Faculty of Veterinary Sciences, National University of La Plata, La Plata, and ⁴Department of Pathology, Faculty of Veterinary Sciences, National University of La Plata, La Plata, Buenos Aires, Argentina

Accepted June 30, 2015

Abstract

The uropygial glands of birds are sebaceous organs that contribute to the water-repellent properties of the feather coat. We studied the histological and histochemical characteristics of the uropygial gland of chimango caracara using hematoxylin and eosin (H & E), Gomori's trichrome, orcein, Gomori's reticulin, periodic acid-Schiff (PAS), Alcian blue (AB) and a variety of lectins. The gland is composed of two lobes and a papilla with 20 downy feathers. It is surrounded by a capsule of dense connective tissue that contains elastic, reticular and smooth muscle fibers. The papilla is delicate and has two excretory ducts. The gland mass relative to body mass was 0.143%. Both adenomer cells and their secretions were stained with Sudan IV, PAS and AB, and were positive for numerous lectins that indicated the presence of lipids and carbohydrates. Immunohistochemical techniques to detect PCNA confirmed cell proliferation in the basal stratum of the adenomer cells. The lipids and glycoconjugates secreted by the uropygial gland serve numerous functions including protection against microorganisms.

Key words: caracaras, Falconiformes, glycoconjugates, histology, preen gland

The uropygial gland is a dorsal compact glandular complex in the last caudal vertebral region of birds (Jacob and Ziswiler 1982). The uropygial secretion contributes to the water repellent and supple properties of the feather coat. Other functions have been postulated including pheromone production, control of plumage hygiene, thermal insulation and defense against bacteria and predators (Shawkey et al. 2003, Galván et al. 2008, Møller et al. 2009, Soler et al. 2012, Cziráj et al. 2013, Galván and Moller 2013).

The uropygial gland usually consists of two lobes and a papilla at its caudal end, where two or

more excretory ducts are located (Jacob and Ziswiler 1982). The ducts often are surrounded by a feather tuft (Johnston 1988, Chiale and Montalti 2013). The gland's shape and size vary considerably among species (Johnston 1988, Vincze et al. 2013). The morphology of the uropygial gland of some species of Falconiformes has been described including *Caracara plancus* (Polyborinae) and other species of the subfamily Falconinae (Johnston 1988).

The lobes of the uropygial gland consist of tubular or tubule-alveolar adenomers with a stratified epithelium (Wagner and Brood 1975); germinative, intermediate, secretory and degenerative layers can be identified. The uropygial secretion is a mixture of waxes (mono-, di- and tri-esters), triglyceride products of cell decomposition, sterols and hydrocarbons such as squalene and alkanols (Salibián and Montalti 2009, Zhang et al. 2010). Secretory cells also produce carbohydrates that can be demonstrated by periodic acid-Schiff (PAS) staining in several bird

Correspondence: María Cecilia Chiale, Sección Ornitología, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata. Paseo del Bosque s/n (B1900FWA), La Plata, Buenos Aires, Argentina. E-mail: ceciliachiale@fcnym.unlp.edu.ar

© 2015 The Biological Stain Commission

Biotechnic & Histochemistry 2015, Early Online: 1–8.

species (Cater and Lawrie 1951) and by lectin histochemical techniques in the rock dove, *Columba livia* (Montalti et al. 2001); immunohistochemical and cell proliferation investigations have not been reported.

The chimango caracara (*Milvago chimango* Vieillot, 1816) is a diurnal raptor that belongs to the family Falconidae, subfamily Polyborinae (Fuchs et al. 2014). These birds feed on both carrion and live prey, including insects and small vertebrates (Bierregaard 1994). The bird inhabits almost every type of environment in southern South America, and ranges from Chile, eastern Bolivia, Argentina, Uruguay, Paraguay and the southeastern extreme of Brazil (Blake 1977). This species is abundant in rural areas (Bellocq et al. 2008), but is a common visitor to urban areas for foraging and nesting (Donázar et al. 1993).

The morphological and histochemical features of the uropygial gland of the chimango caracara have not been described and there has been no histochemical analysis of this gland in Falconidae species. Therefore, we investigated the histological and histochemical characteristics of the uropygial gland in chimango caracara and related them to its life habits.

Material and methods

Sample collection

Ten male and eight female adult chimango caracara were captured for study (permit N° 265/09 from Ministerio de Asuntos Agrarios de la Provincia de Buenos Aires) in autumn of 2009 near the city of La Plata. The body mass (BM) of each bird was measured using a spring balance (accuracy ± 5 g). The birds were sacrificed under mild anesthesia (halothane inhalation) and the uropygial glands were dissected according to the protocol of Montalti et al. (1998).

Feathers surrounding the gland were extracted manually and a straight, superficial skin incision was made around the uropygial gland without removing surrounding tissue. The caudal vessels and nerves were cut and the gland was removed completely.

The gland mass (GM) was measured to the nearest 0.001 g. These measurements were used to calculate the relative gland mass (RGM = (GM/BM) $\times$ 100) (Table 2).

Histology

Uropygial glands were fixed in 10% buffered formalin; some glands were cut transversely and others longitudinally. The tissue was processed

using standard techniques for embedment in paraffin. Sections were cut at 3 μ m.

A section of every sample was stained with one of the following: hematoxylin and eosin (H & E) for general histology; orcein for elastic fibers; Gomori's trichrome for collagen fibers; Gomori's reticulin for reticular fibers; Sudan IV for lipids (Bancroft 2008); Alcian blue-periodic acid-Schiff (AB-PAS) for glycogen, acid glycoconjugates and glycoconjugates with oxidizable vicinal diols; periodic acid-Schiff (PAS) for glycoconjugates with oxidizable vicinal diols including glycogen; PAS-diastase for glycogen; toluidine blue for acid glycoconjugates; Alcian blue (AB), pH 0.5, for sulfated glycoproteins; AB, pH 1, for glycoproteins with O-sulfated esters; and AB, pH 2.5, for glycoproteins with carboxyl groups and/or sulfated esters (Flamini et al. 2012, 2014). Sections of vizcacha (*Lagostomus maximus*) vagina (Flamini et al. 2012) were used as positive controls. The epithelial layers, tubule regions and papillae were classified according to Jacob and Ziswiler (1982).

Lectin histochemistry was performed using a standardized protocol from our laboratory (Sant'Ana et al. 2005, Zanuzzi et al. 2010). Sections were deparaffinized, then incubated with 0.03% H₂O₂ in methanol for 30 min to inhibit endogenous peroxidase. Sections were treated with 0.1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) for 30 min, then incubated with biotinylated lectins for 2 h at room temperature (Table 1). Sections were washed separately and incubated with

Table 1. Lectins and their acronyms and affinities.

Lectin	Acronym	Affinity
Group I		<i>Glc/Man</i>
<i>Concanavalina ensiformis</i>	Con A	β -D-Man; α -D-Glc
<i>Lens culinaris</i>	LCA	α -D-Man
Group II		<i>Glc/NAc</i>
<i>Triticum vulgare</i>	WGA	β -D-GlcNAc; NeuNAc
<i>Triticum vulgare</i>	sWGA	GlcNAc
<i>Lycopersicon esculentum</i>	LEA	β 1,4GlcNAc oligomers
Group III		<i>Gal/NAc/Gal</i>
<i>Glycine max</i>	SBA	α -D-GalNAc; β -D-GalNAc
<i>Ricinus communis</i>	RCA-I	β -Gal
<i>Bandeiraca simplicifolia-I</i>	BS-I	β -Gal
Group IV		L-Fuc
<i>Ulex europaeus-1</i>	UEA-I	L-Fuc

Glc, glucose; Man, mannose; Gal, galactose; GalNAc, N-acetylgalactosamine; GlcNAc, N-acetylglucosamine; NeuNAc, N-acetylneuraminic acid; L-Fuc, L-fucose (Goldstein and Hayes 1978, Goldstein et al. 1980).

the avidin-biotin-peroxidase complex (ABC) (Vector Laboratories, Burlingame, CA); 0.02% diaminobenzidine (DAB) (Biogenex, San Ramón, CA) was used as the chromogen. Sections were counterstained with Mayer's hematoxylin. Staining intensity was graded using a semiquantitative scale: (–) negative reaction, (1) weak reaction, (2) moderate reaction, (3) strong reaction. Negative controls for lectin staining included exposure to horseradish peroxidase and substrate medium without lectin, and blocking by incubation with the appropriate blocking sugars (0.1–0.2 M in PBS).

Immunohistochemistry

Immunohistochemistry was performed according to previously described procedures (Eöry et al. 2012, Zanuzzi et al. 2012). Slides 3 µm thick from all samples were mounted on positively charged slides (HDAS001A; Biotraza microscope slides, Huida Medical Instruments Co., Jiangsu, China), deparaffinized, rehydrated, then incubated in 3% H₂O₂ in methanol for 30 min at room temperature after which they were rinsed in PBS. Antigen retrieval was performed using 750 W microwave irradiation, twice for 5 min each time, in pH 6.0 citrate buffer. Nonspecific binding sites were blocked with 2% bovine serum albumin (BSA) for 30 min in a humid chamber at 4° C followed by incubation with the primary antibody for 1 h at 37° C. Mouse monoclonal anti-PCNA was used as the primary antibody (clone PC 10, ascites fluid; Sigma Chemical Co., St. Louis, MO) diluted 1:3000 with bovine serum albumin (BSA) 0.1% in distilled water to determine cell proliferation, and anti-desmin (monoclonal anti-swine desmin, clone DE-R-11; Dako Glostrup, Denmark) was used to characterize muscle fibers. A system based on a polymer linked to secondary antibody and peroxidase (Envision system; DakoCytomation, Glostrup, Denmark) was used to recognize the primary antibody. Liquid DAB was used as the chromogen (DakoCytomation) and Gill's hematoxylin was used as counterstain.

Statistical analysis

Statistical analyses were performed using STATISTICA 7.0 program. Student's t-test was used for statistical analyses of the RGM. Values for $p \leq 0.05$ were considered significant.

Results

The uropygial gland of chimango caracara has two heart-shaped lobes and a short papilla; the ducts

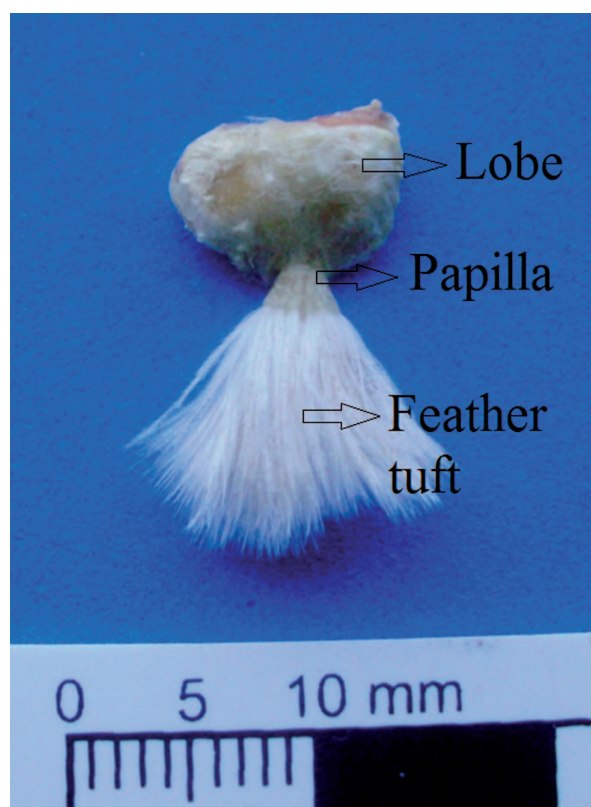


Fig. 1. General appearance of uropygial gland: two lobes and a short papilla with a downy feather tuft.

are surrounded by a tuft of 20 feathers (Fig. 1). The RGM in this species was 0.143%; differences between males and females were not statistically significant (Table 2).

The lobes consist of tubular-alveolar adenomers (Fig. 2) that are branched profusely near the margins of the gland and slightly branched toward the inner portions of the lobes. The gland is surrounded by a capsule of dense connective tissue that contains collagen, muscular, reticular and elastic fibers (Fig. 3A, B). The medial septum and interalveolar tissue are thin and have abundant reticular (Fig. 3B) and elastic fibers.

The papilla is a delicate type according to the morphological classification of Jacob and Ziswiler (1982) and it is composed of wide ducts separated

Table 2. BM, GM and RGM of chimango caracara males and females.

	BM	GM	RGM (%)
Males	282.21 ± 13.68	0.419 ± 0.11	0.149 ± 0.04
Females	305.62 ± 10.81	0.418 ± 0.08	0.137 ± 0.026
t-test			T = 0.78 * $p > 0.05$

Values are means ± SD in grams.

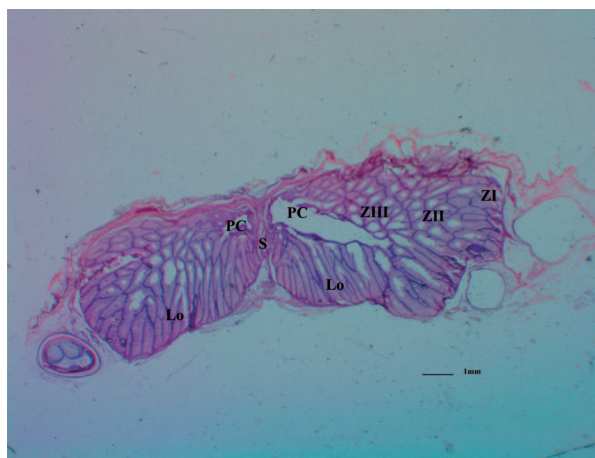


Fig. 2. Histology of the uropygial gland. H & E. Lo, lobe; PC, primary chamber; S, septum; ZI, zone I; ZII, zone 2; ZIII: zone 3. Scale bar = 1 mm.

from each other by slender connective tissue septa; it also contains abundant dense connective tissue with collagen and reticular fibers, some smooth muscular fibers and lymphoid tissue. There are two excretory ducts, one per lobe, surrounded by feather follicles, with some feathers among them (Fig. 4). The muscle fibers appear to be associated with the feather tuft follicles. No evidence of a muscular sphincter was found around the excretory ducts.

The adenomers have a stratified epithelium with four distinct layers. Depending on the height and lumen width, the adenomers may be divided into three distinct zones: zone 1 contains adenomers with a very small lumen and very tall epithelium (Fig. 5A), zone 2 adenomers have a wide lumen and relatively tall epithelium (Fig. 5B), and zone 3 adenomers have a very wide lumen and very thin epithelium (Fig. 5C). Zone 1 consists of a basal stratum, 1–2 layers of intermediate stratum, 3–4 layers of a secretory stratum, and 1–2 layers of a degenerative stratum. Adenomers in zone 2 typically have a single basal stratum, 1–2 intermediate cell layers, 2–3 secretory layers, and 1–2 layers of a degenerative stratum. In zone 3, the basal stratum may

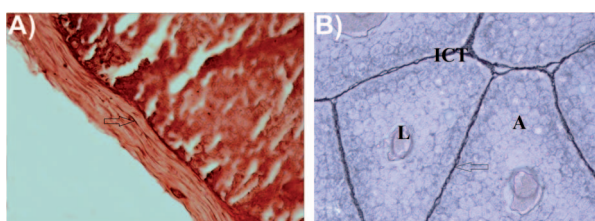


Fig. 3. A) Elastic fibers (arrow) in the capsule. Orcein 20 × B) Reticular fibers (arrow) in the interalveolar connective tissue. Gomori's reticulin 20 ×. A, adenomer; ICT, interalveolar connective tissue; L, lumen; PC, primary chamber; bs, basal stratum; ds, degenerative stratum; is, intermediate stratum; ss, secretory stratum.

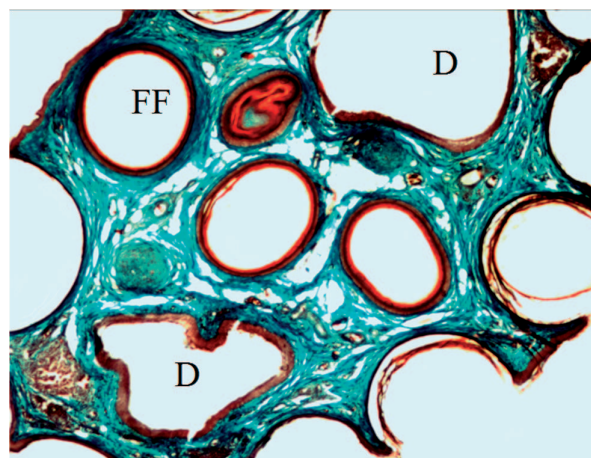


Fig. 4. Papilla of uropygial gland. Gomori's trichrome 4 ×. FF, feather follicles; D, external ducts.

contain 1–2 cell layers, a single intermediate layer, 1–2 secretory layers, and 1–2 degenerative layers. Beyond zone 3, the ends of the adenomers form collecting tubules or secondary chambers that end in a primary storage chamber; the epithelium of this chamber does not show secretory characteristics.

Histochemistry

The basal stratum of the adenomer, its basal membrane and its secretion were PAS-positive in every zone. PAS positivity also was demonstrated using the PAS-diacetate technique, which indicated that this reaction was not due to glycogen.

AB staining was performed at different pHs. We found cells in different strata of every region that

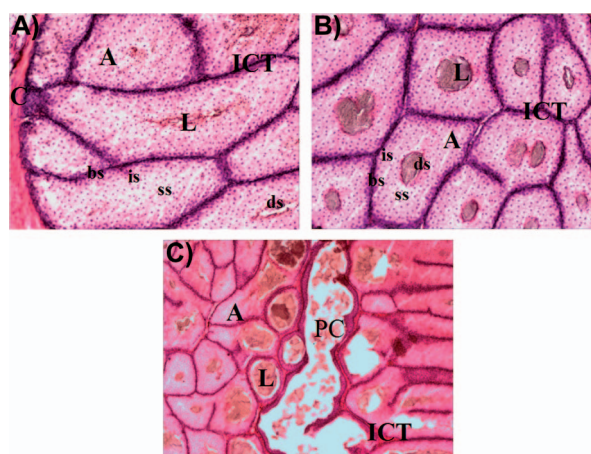


Fig. 5. Three zones of the uropygial gland. A) zone 1; B) zone 2; C) zone 3, H & E. 4 ×. A, adenomer; C, capsule; ICT, interalveolar connective tissue; L, lumen; PC, primary chamber; bs, basal stratum; ds, degenerative stratum; is, intermediate stratum; ss, secretory stratum.

Table 3. Staining of cell-layers of the uropygial gland of chimango caracara.

	Basal cells	Adenomer cells	Degenerative stratum	Secretion
PAS	1	—	0–1	1
AB 2.5	2	2	2	3
AB 1	1	1	2	2
AB 0.5	0–1	0–1	1	2
AB-PAS	2	2 (AB)	2–3	3
AT 5.6	—	—	—	—
AT 4.2	—	—	—	—
Sudan	—	3	—	3

(—) negative, (1) weak reaction, (2) moderate reaction, (3) strong reaction.

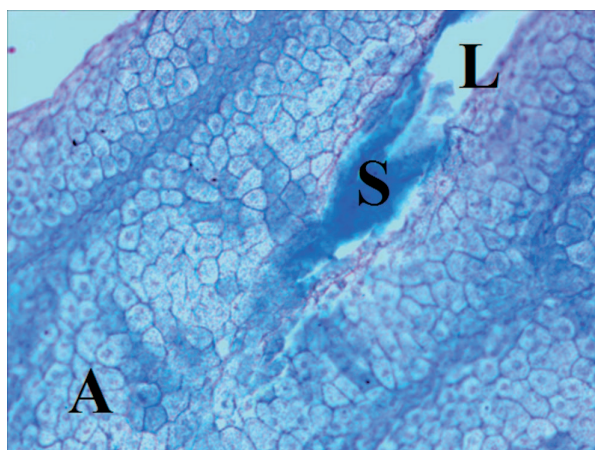
was stained using these techniques (Table 3), which indicated the presence of carbohydrates (Fig. 6).

The presence of intracellular lipids in the adenomers of every region and in their secretion was confirmed by Sudan IV staining.

No structures were stained by the toluidine blue technique.

Lectin histochemistry

Table 4 summarizes the lectin binding pattern. Strong lectin staining of the secretory cells in various strata and the secretion was observed (Fig. 7A, B). There were no differences among the adenomers of the several glandular regions for most of the samples. Some lectins strongly stained the glycocalyx on the intercellular boundaries in all regions of the adenomers. The secretion in the chambers was stained using several lectins that had shown affinity for the adenomer secretory cells, such as

**Fig. 6.** Carbohydrate producing cells in the uropygial gland. Alcian blue, pH 2.5. 20 ×. A, adenomer; L, lumen; S, secretion.**Table 4.** Lectin-binding patterns in different zones of the uropygial gland of chimango caracara.

Lectins	Zone I	Zone II	Zone III	Secretion
CON-A	2	2	C2 (DS3)	3
LCA	3	2–3 (BS3)	3	3
WGA	—	—	—	—
sWGA	2–1 (SS/DS1)	2–1 (SS/DS1)	2–1 (SS/DS1)	2
LEA	1	1	1	1
SBA	1–2 (BS1)	1–2 (BS1)	1–2 (BS1)	2 (IS3)
PNA	1	1	C1 (DS2)	2
RCA-I	3	3	3	3 (IS3)
BS-I	1	1	1	2
UEA-I	1	1	1	2

(—) negative, (1) weak reaction, (2) moderate reaction, (3) strong reaction. BS, basal stratum; IS, intermediate stratum; SS, secretory stratum; DS, degenerative stratum.

CON-A, LCA, sWGA, LEA, SBA, PNA, RCA-I, BSI and UEA-I. The cells of the chamber were stained only in the glycocalyx area.

Immunohistochemistry

Anti-desmin antibody confirmed the presence of muscle fibers in the capsule of the gland. The PCNA technique showed staining in one or two cell layers of the basal stratum in all regions of the lobules (Fig. 8).

Discussion

The morphology and size of uropygial glands vary among bird species (Montalti and Salibián 2000). In chimango caracara, the gland exhibits characteristics similar to other Falconidae. It is heart-shaped and has two well-differentiated lobes like the Eurasian kestrel, *Falco tinnunculus* (Jacob and Ziswiler 1982) and the southern caracara, *Caracara plancus* (Johnston 1988). The papilla has a tuft of 20 downy feathers, more than other species of the same family, such as *Falco tinnunculus* (17 feathers) (Jacob and Ziswiler 1982) and *F. sparverius* (10 feathers) (Chiale and Montalti 2013). Like other Falconidae (e.g., *F. tinnunculus*) (Jacob and Ziswiler 1982), the gland

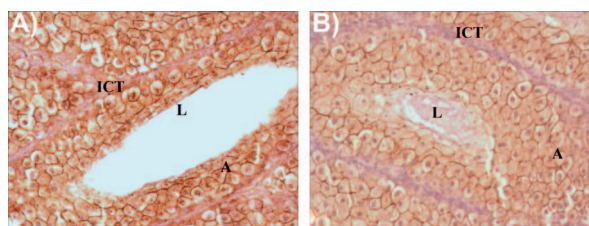
**Fig. 7.** Lectin secretory cells in the uropygial gland. A) RCA-I lectin. 20 ×. B) SBA lectin. 20 ×. A, adenomer; ICT, interalveolar connective tissue; L, lumen; S, secretion.

Fig. 8. One cell layer (arrow) within the basal stratum of the uropygial gland showing positive PCNA staining. 10 ×. A, adenomer; L, lumen.

has one excretory duct per lobe. The chimango caracara has a larger RGM than other Falconidae (Montalti and Salibián 2000).

In general, the histology of the uropygial gland of the chimango caracara is similar to other birds (Sandilands et al. 2004, Sawad 2006, Harem et al. 2010, Chiale et al. 2014). The adenomer epithelium is composed of diverse cellular strata like most birds (Lucas and Stettenheim 1972, Jacob and Ziswiler 1982, Montalti et al. 2001, Chiale et al. 2014). The capsule consists of dense connective tissue with smooth muscle, and reticular and elastic fibers like other birds (Jacob and Ziswiler 1982). The papilla is delicate like other species of Falconiformes (Jacob and Ziswiler 1982), but lacks a muscular sphincter around the excretory ducts.

PCNA immunostaining demonstrated dividing cells in the basal stratum; cells with proliferative capacity are located in equivalent sites in mammalian holocrine glands, such as mouse preputial glands (Barbeito et al. 2009).

The adenomer epithelial cells typically were rich in lipids (Montalti et al. 2005, Salibián and Montalti 2009); these lipids are the major component of the uropygial secretion in chimango caracara.

The literature contains no reports of histochemical studies of the uropygial glands of other Falconidae species. We investigated the carbohydrate moieties of the secretion of the uropygial gland. Carter and Lawrie (1950) divided the uropygial gland of fowl into two regions based on the histochemical characteristics of the adenomers: a sebaceous outer zone with a richly lipid secretion and a glycogenic inner region that was PAS-positive. We found PAS staining in the adenomers of the three glandular zones. The PAS technique is

used to demonstrate the presence of carbohydrates by staining mainly glycogen, but this method also can stain amino or alkaminos that substitute 1, 2 glycols and may produce aldehydes when oxidized by periodic acid (Hotchkiss 1948, Bancroft 2008). Diastase treatment destroys glycogen. When used with the PAS technique we demonstrated that glycogen was not present in significant quantities in the secretion, because diastase treatment did not alter the PAS staining. Consistent with our results, Atoji et al. (1989), using electronic microscopy, found no glycogen in the secretory cells of the preputial gland of goats. PAS and AB staining enabled us to demonstrate the presence of both neutral and acidic glycoconjugates in the uropygial secretion; the acidic glycoconjugates were present in higher concentration. Montalti et al. (2001) reported PAS and AB staining in the uropygial gland of the rock dove, *Columba livia*.

Lectin histochemistry enabled us to identify a variety of glycan residues in the uropygial secretion. The lectin staining pattern was different from that in *Columba livia* in which only PNA, WGA and RCA-1 lectins were detected in the germinative stratum; CON-A lectin was detected in the degenerative stratum and the secretion was positive only for WGA (Montalti et al. 2001). Glycoconjugates in gland secretions may serve as protectants by blocking the adhesion of microorganisms and their later entrance into the epidermis (Ookusa et al. 1983, Atoji 1989, Iwamoto et al. 1998). Other investigators have reported the antimicrobial function of the uropygial gland secretion (Czirják et al. 2013, Møller et al. 2009, 2010); accordingly, we propose that the variety of polysaccharides present in the gland may be associated with this function. The mucosal cells of fish and amphibian epidermis, and certain cutaneous glands in mammals also produce antimicrobial substances (Meyer et al. 2007).

The difference between the scarce polysaccharides in the rock dove, *Columba livia* (Montalti et al. 2005), and the abundant polysaccharides in the chimango caracara could be related to the type of diet, because a carrion bird presumably would have more opportunities for contact with bacteria owing to its dietary habits.

Carbohydrates in the basal strata of the uropygial gland adenomers of chimango caracara suggest that these cells likely are a source of the glycomplexes that are released with lipids during holocrine secretion. This interpretation of our results is consistent with observations using electronic microscopy (Wagner and Brood 1975) in which the most superficial cells showed degenerative changes that preclude glycoconjugate synthesis *de novo*.

