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ORIGINAL ARTICLE

The uropygial gland of the Eared Dove and its evolutionary history within the Columbiformes (Aves)

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Abstract

The uropygial (preen) gland is a holocrine organ unique of Aves. Although several studies have been performed on the uropygial gland of different bird species, knowledge about this gland in Columbiformes is scarce. In order to fill this gap, we analysed in detail the external morphology and the histological and histochemical features of the uropygial gland of the Eared Dove (*Zenaida auriculata*) in a comparative context. The uropygial gland of the Eared Dove is characterized by its pear-like shape composed of two lobes, conical and naked papilla, tubule-alveolar adenomers, a large primary storage chamber (a feature also present in other terrestrial avian species), and reticular and elastic fibres in the capsule and connective tissue surrounding the adenomers. The histochemistry showed a positive reaction to periodic acid-Schiff, Alcian Blue 2.5 and several lectins, evidencing the presence of diverse glycoconjugates in this organ. Since the uropygial gland may be independently present or absent within Columbiformes, we also used character mapping on a molecular phylogeny to infer the character states of this gland at ancestral nodes to understand its evolutionary history. The analysis shows that the presence of the uropygial gland is the ancestral state for Columbiformes and that its loss occurred more than once independently.

Keywords Preen gland · *Zenaida auriculata* · Histology · Histochemistry · Lectin · Ancestral state reconstruction

Zusammenfassung

Die Bürzeldrüse der Ohrflecktaube (*Zenaida auriculata*) und ihre evolutionsbiologische Geschichte innerhalb der Taubenvögel

Die Bürzeldrüse ist eine nur bei Vögeln vorkommende holokrine Drüse. Obwohl es einige Untersuchungen dieser Drüse bei unterschiedlichen Vogelarten gibt, wissen wir nicht viel über sie bei Tauben. Um diese Lücke zu schließen, analysierten wir vergleichend und im Detail die äußerlichen morphologischen sowie die histologischen und histochemischen Eigenschaften der Bürzeldrüse von Ohrflecktauben (*Zenaida auriculata*). Ihre Bürzeldrüse ist durch ihre Birnenform charakterisiert und setzt sich zusammen aus zwei Lappen, einer konischen und nackten Papille, tubulealveolaren Adenomen, einer großen primären Speicherkapsel (die es auch bei anderen Landvögeln gibt), retikulären und elastischen Fasern in der Kapsel und Bindegewebe um die Adenomen herum. Die Histochemie zeigte positive Reaktionen auf PAS, Alcian-Blau 2,5 und mehrere Lektine, was auf das Vorhandensein von diversen Glykokonjugaten in diesem Organ hinweist. Da die Bürzeldrüse bei Tauben unabhängig voneinander vorhanden sein oder fehlen kann, betrachteten wir ihr Vorhandensein innerhalb des molekularen Stammbaums der Tauben, um die evolutionsbiologische Geschichte der Drüse zu verstehen. Die Analyse zeigt, dass das Vorhandensein der Bürzeldrüse bei Tauben entwicklungsgeschichtlich der Normalfall war und sie mehr als einmal unabhängig voneinander verlorenging.

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Introduction

The uropygial (preen) gland is a compact sebaceous organ unique to many species of birds (Jacob and Ziswiler 1982). It is generally composed of two lobes and a papilla in its caudal end, where the external ducts are situated, and may be surrounded by a feather tuft (Jacob and Ziswiler 1982; Johnston 1988). Histologically, it is a holocrine gland surrounded by a capsule of dense connective tissue. From this capsule, thin connective trabeculae originate that support the organ internally. The functional portions are composed of tubule-alveolar secretory unities (adenomers) covered by stratified epithelium: the basal or germinative stratum, the intermediate stratum, the secretory stratum and the degenerative stratum (Lucas and Stettenheim 1972; Jacob and Ziswiler 1982). The tubular system converges in secondary chambers and, eventually, in a primary storage chamber (Jacob and Ziswiler 1982; Kozlu et al. 2011; Chiale et al. 2014, 2015). According to Lucas and Stettenheim (1972), the adenomers can be divided into three zones depending on their epithelial height and lumen width: zone I (the outermost zone), zone II (the middle zone) and zone III (the innermost zone). In the papilla region, the external ducts are surrounded by dense connective tissue, feather follicles (when a tuft is present), blood vessels and nerves (Chiale et al. 2015, 2016).

Functions attributed to uropygial gland secretions include: water-repellent properties of feathers (Jacob and Ziswiler 1982), involvement in sexual behaviour through the production of pheromones (Hirao et al. 2009; Zhang et al. 2010) and mate choice (Amo et al. 2012), plumage hygiene and defence against microorganisms (Shawkey et al. 2003; Galvan et al. 2008; Møller et al. 2009; Soler et al. 2012; Czirjak et al. 2013). The uropygial secretion consists of a complex mixture of lipids (Jacob 1976), although some biochemical and histochemical studies found a variety of glycoconjugates in different bird species (Bhattacharyya 1972; Kamiya et al. 1986; Chiale et al. 2015, 2016), including the Rock Dove (*Columba livia*) (Montalti et al. 2001, 2005).

The Columbiformes (pigeons and doves) are a very uniform and distinctive avian order, consisting of about 316 species within a single extant family, the Columbidae, and are distributed throughout the world (Gibbs et al. 2010). Morphologically, they are characterized by a stout body of variable size, small head, short beak with a fleshy cere at its base, naked and colourful periorbital rings, and short hind limbs (Gibbs et al. 2010). Columbiformes are commonly divided into granivorous species, usually terrestrial with dull plumage, and frugivorous species, usually arboreal with brightly coloured plumage (Gibbs et al. 2010; Lapiedra et al. 2013). Moreover, within Columbiformes, the uropygial gland may be independently present or absent; when it is present it is naked (i.e. the feather tuft is absent) (Johnston 1988).

Even though several studies have been performed on the uropygial gland of different bird species to determine its size, morphology, histology, morphogenesis, secretion and function (e.g. Lucas and Stettenheim 1972; Bride and Gomot 1978; Jacob and Ziswiler 1982; Johnston 1988; Fukui 1997; Montalti and Salibian 2000; Salibian and Montalti 2009; Chiale and Montalti 2013; Chiale et al. 2014, 2015, 2016; Rehorek et al. 2017; Moreno-Rueda 2017), only a few of these studies have focussed on the Columbiformes. More recent studies describe very generally the histology of only four species of columbids, *Columba palumbus*, *Streptopelia turtur*, *Drepanoptila holosericea* and *Ducula goliath* (Jacob and Ziswiler 1982), the chemical composition of the uropygial gland secretion of the Rock Dove (Montalti et al. 2001, 2005), and the presence/absence of the uropygial gland in ca. 34% of the species included in the order Columbiformes (Johnston 1988).

As a consequence of our poor knowledge about the uropygial gland in this speciose group of birds, the main objective of the present study is to analyse in detail and in a comparative context the external morphology and histological and histochemical features of the uropygial gland of the Eared Dove (*Zenaida auriculata*). This species is a member of the Columbinae subfamily whose capture has no restrictions because it is considered an agricultural pest (Gibbs et al. 2010), thus there is an abundant supply of specimens. It is a terrestrial and mainly granivorous dove, found throughout South America in open habitats, woodland and urban areas (Baptista et al. 1997). To the best of our knowledge, this is the first thorough investigation of the uropygial gland of the Eared Dove and the first in-depth study of the uropygial gland of any columbiform. The results obtained in the present study are expected to increase our knowledge of the uropygial gland of birds in general, and of Columbiformes in particular, and to serve as an empirical basis for future studies. Also, taking into account the variability of the presence/absence of the uropygial gland within Columbiformes, we used character mapping on a molecular phylogeny to infer the character states at ancestral nodes to understand its evolutionary history.

Materials and methods

Eight Eared Dove adults, four males and four females, were captured for this study (permit no. 23128/13; Ministerio de Asuntos Agrarios, Buenos Aires province, Argentina), in the environs of La Plata (Argentina) during the non-reproductive period. The body mass (BM) of each bird was assessed by a spring balance (accuracy ± 5 g), and then the uropygial gland was dissected following the protocol in Montalti

et al. (1998). Gland mass (GM; including both lobes) was measured to ± 0.001 g. These values were used to calculate the relative GM (RGM; RGM = GM/BM × 100). We used a *t*test to compare the BM and RGM of males and females to determine if there was a difference between the sexes. *p* values < 0.01 were considered highly significant and $p < 0.05$ as significant. Statistical analyses were performed with STATISTICA 7.0.

Histological techniques

Uropygial glands were fixed in 10% buffered formalin and transversal and longitudinal cuts were made and processed for their storage in paraffin. Samples were serially cut (5 µm) and stained with haematoxylin–eosin for the general histological description (12 sections per bird), orcein for elastic fibres, Gomori's trichrome for collagen fibres, and Gomori's reticulin for reticular fibres (three sections per bird for each technique) (Suvarna et al. 2012). The diverse epithelial layers, adenomers sectors and papillae were classified according to Jacob and Ziswiler (1982).

Histochemical and lectin-histochemical techniques

Another set of samples (three sections per bird for each technique) was stained using periodic acid-Schiff (PAS) for glycoconjugates with oxidizable vicinal diols including glycogen. Alcian Blue (AB) pH 0.5 was used for sulphated glycoproteins, AB pH 1 for glycoproteins with *O*-sulphated esters, AB pH 2.5 for glycoproteins with carboxyl groups and/or with sulphated esters, and AB-PAS for glycogen, acid glycoconjugates and glycoconjugates with oxidizable vicinal diols (Suvarna et al. 2012). Slices of Plains Viscacha (*Lagostomus maximus*) vagina previously tested positive for AB, PAS and different lectins (Flamini et al. 2012) were used as positive controls.

We used three sections per bird for the histochemistry of each lectin, following a standardized protocol frequently used in our laboratory (Zanuzzi et al. 2010). Paraffin-embedded sections were deparaffinized, then incubated with 0.03% hydrogen peroxide in methanol for 30 min to inhibit the endogenous peroxidase. Tissue slices were then treated with 0.1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) pH 7 for 30 min, and incubated with biotinylated lectins (Table 1) for 2 h at room temperature (Table 1). The optimal lectin concentration was 30 mg/ml in PBS for all lectins, except for peanut agglutinin, which was applied at a concentration of 10 mg/ml. Slices were washed separately and incubated with the avidin–biotin–peroxidase complex (Vector Laboratories, Burlingame, CA); diaminobenzidine 0.02% (Biogenex, San Ramón, CA) was used as the chromogen. Staining intensity was graded according to a semi-quantitative range: negative reaction (−), weak reaction (+), moderate reaction (++), strong reaction (+++).

Previously established negative controls for lectin staining include 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).

Table 1 Lectins used for histochemical analysis of the uropygial gland of the Eared Dove (*Zenaida auriculata*), their abbreviations and affinities (Goldstein and Hayes 1978; Goldstein et al. 1980)

Lectin	Abbreviation	Affinity	Blocking sugar
Group I		Glc/Man	
Concanavalia ensiformis agglutin	Con A	β-D-Man; α-D-Glc	α-D-Methylmannose
Group II		Glc/NAc	
Triticum vulgaris agglutin	WGA	β-D-GlcNAc; NeuNAc	*N*-acetylglucosamine
Triticum vulgaris agglutin	sWGA	GlcNAc	*N*-acetylglucosamine
Lycopersicon esculentum agglutin	LEA	β1,4GlcNAc oligomers	*N*-acetylglucosamine
Group III		GalNAc/Gal	
Glycine maximus agglutin	SBA	α-D-GalNAc; β-D-GalNAc	*N*-acetylgalactosamine
Ricinus communis agglutin	RCA-I	β-Gal	M D-Galactose
Arachis hypogea agglutin	PNA	β-Gal	M D-Galactose
Group IV		L-Fuc	
Ulex europaeus-1 agglutin	UEA-I	L-Fuc	M α-L-Fucose

Con A Concanavalin A, *WGA* wheat germ agglutin, *sWGA* succinylated WGA, soybean agglutinin, *PNA* peanut agglutinin, *Glc* glucose, *Man* mannose, *Gal* galactose, *GalNAc N*-acetylgalactosamine, *GlcNAc N*-acetylglucosamine, *NeuNAc N*-acetylneuraminic acid, *L-Fuc* L-fucose

Ancestral state reconstruction

We mapped the presence/absence of the uropygial gland on a molecular phylogeny of Columbiformes and reconstructed the ancestral states using parsimony and likelihood analyses conducted in Mesquite version 3.5 (Maddison and Maddison 2018). Information on the character states of different species was retrieved from Johnston (1988) and from our own observations on the Eared Dove. The tree used is based on the taxon-rich phylogeny of Heupink et al. (2014).

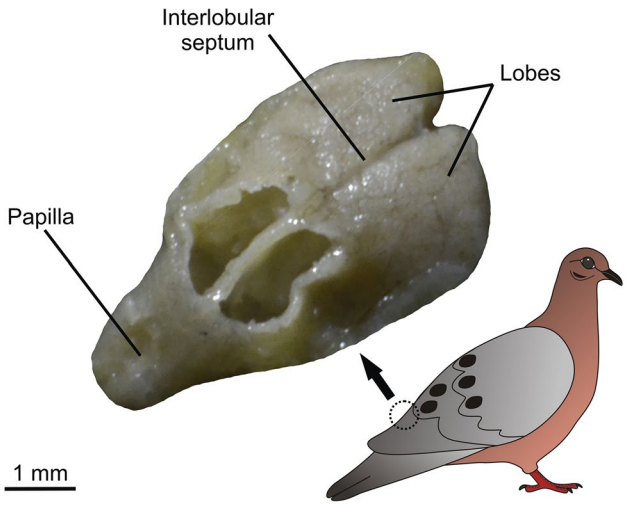

Fig. 1 Macroscopic view of the uropygial gland of the Eared Dove (*Zenaida auriculata*)

Results

Morphology and histological structure

The uropygial gland of the Eared Dove has a pear-like shape and is composed of two lobes and a conical papilla with a rectilinear direction in relation to the lobes; it is characterized by the absence of a feather tuft (Fig. 1).

There was no difference in BM between males (135.03 ± 12.11) and females (129.21 ± 7.62) ($p > 0.05$). The RGM for this species was 0.025 ± 0.007, and there was no difference in the RGM between males and females ($p > 0.01$).

The uropygial gland is surrounded by a capsule of dense connective tissue (Fig. 2a). The capsule, the medial septum and the branches of connective tissue around the adenomeres are rich in collagen fibres with abundant reticular (Fig. 2b) and elastic fibres (Fig. 2c), but have no muscular fibres.

The adenomers are tubule-alveolar and there is no clear division of the different zones in which the lobules of the uropygial gland can be divided (sensu Lucas and Stettenheim 1972). The uropygial gland of this species is characterized by the presence of a large primary storage chamber (Fig. 2a). The epithelium of the adenomeres has one layer of basal or germinative stratum, one or two layers of intermediate stratum, three or four layers of secretory stratum and one or two layers of degenerative stratum.

The primary chamber epithelium does not show secretory characteristics and is cornified in aspect. The excretory

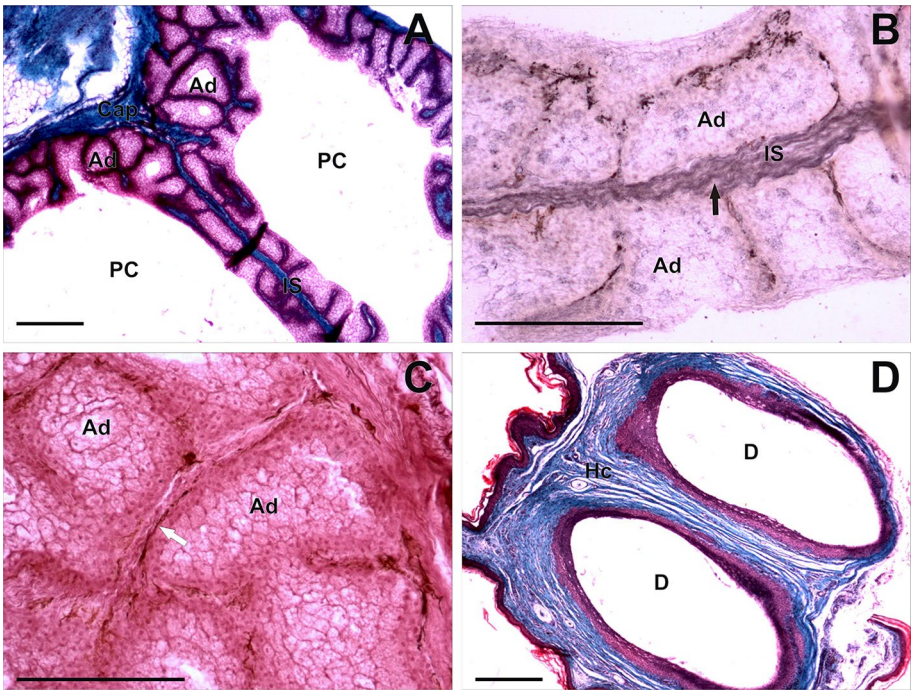

Fig. 2 Histology of the uropygial gland (**a**–**c**) and papilla (**d**) of the Eared Dove. **a**, **d** Gomori's trichrome ×4, **b** Gomori's reticulin ×20, **c** orcein ×20; black arrow reticular fibres, white arrow elastic fibres. *Ad* Adenomeres, *Cap* capsule, *D* external ducts, *Hc* Herbst corpuscles, *IS* interlobular septum, *PC* primary chamber. Scale bar 500 µm

ducts have their origin in this chamber, and the number of the ducts is one per lobe. The papilla have abundant connective tissue, blood vessels, nerves and Herbst corpuscles; no muscular fibres are found (Fig. 2d).

There were no histological differences between males and females.

Histochemistry and lectin histochemistry

The results of the histochemistry and lectin histochemistry are summarized in Tables 2 and 3, respectively.

The germinative stratum, basal membrane and secretions of the uropygial gland of the Eared Dove reacted positively with PAS (Fig. 3a). AB 2.5 positivity was observed in the germinative and degenerative strata (Fig. 3b). Other histochemical results were negative.

The different strata of the adenomers and the secretion of the uropygial gland of this species showed a strong positive reaction to the lectins RCA-I and PNA (Fig. 3c, d) and a medium reaction to WGA. They also showed a weak reaction to Con A and LEA. Reactions to the other lectins tested in this study were negative.

Evolutionary history of the uropygial gland within the Columbiformes

The ancestral character state reconstruction yielded no conflicts between the parsimony and likelihood models. The analysis showed that the presence of the uropygial gland is a plesiomorphic feature for Columbiformes (Fig. 4). This state is preserved in almost 74% of the species included in the analysis. The loss of the uropygial gland evolved independently four times in (1) the subfamily Gourinae (absence 0; 0.95 probability), (2) the Tooth-billed Pigeon (*Didunculus strigirostris*) (subfamily Didinculinae), (3) the Zoe Imperial Pigeon (*Ducula zoeae*) (subfamily Treroninae), and (4) the clade including the genera *Treron*, *Ptilinopus* and *Alectroenas* within the subfamily Treroninae (absence 0; 0.85 probability) (Fig. 4). In this last clade, reversals (i.e. the presence of the uropygial gland) occurred in the Madagascar Blue Pigeon (*Alectroenas madagascariensis*), Rose-crowned Fruit Dove (*Ptilinopus regina*), Eastern Superb Fruit Dove (*Ptilinopus superbus*) and Yellow-breasted Fruit Dove (*Ptilinopus occipitalis*) (Fig. 4).

Table 2 Cell layers of the uropygial gland of the Eared Dove presenting positive reactions during histochemical incubation

	Germinative stratum	Intermediate and secretory strata	Degenerative stratum	Secretion
PAS	+	–	+	+
AB 2.5	+	–	+	+
AB 1	–	–	–	–
AB 0.5	–	–	–	–
AB-PAS	+	–	++	+

– Negative reaction, + weak reaction, ++ moderate reaction

PAS Periodic acid-Schiff, *AB* Alcian Blue

Table 3 Summary of lectin-binding patterns in different zones of the uropygial gland of the Eared Dove

Lectins	Germinative stratum	Intermediate and secretory strata	Degenerative stratum	Secretion
Con A	+	+	+	+
WGA	++	++	++	++
sWGA	–	–	–	–
LEA	+	+	+	+
SBA	–	–	–	–
PNA	+++	+++	+++	+++
RCA-I	+++	+++	+++	+++
UEA-I	–	–	–	–

+++ Strong reaction; for abbreviations and other symbols, see Tables 1 and 2

Discussion

Comparison of the morphology and histology of the uropygial gland of the Eared Dove with other Columbiformes and other bird species

The uropygial gland varies in shape and size among different bird species (Jacob and Ziswiler 1982; Johnston 1988), even among closely related ones. In the case of the Eared Dove, the shape of the gland is pear-like, contrary to that described for other columbids [i.e. heart shaped in *C. palumbus*, *S. turtur*, *Drepanoptila holosericea* and *Ducula goliath* (Jacob and Ziswiler 1982)]; the papilla is conical and naked and aligned rectilinearly in relation to the lobes, as reported for other columbids (Jacob and Ziswiler 1982).

There were no differences in the BM or RGM of the Eared Dove between the sexes (RGM: 0.025 ± 0.007), as also recorded by Salibian and Montalti (2009) for this species (RGM: 0.026 ± 0.010). However, Salibian and Montalti (2009) did find differences in these characteristics between the sexes in the Rock Dove (RGM: males 0.026 ± 0.011, females 0.036 ± 0.016) and postulated that some functions such as pheromone production can affect the mass of this gland. A more detailed study that includes capture at different periods (reproductive vs. non reproductive) is necessary to determine if the RGM of the Eared Dove does show variation between the sexes that can be linked to pheromone production (Zhang et al. 2010).

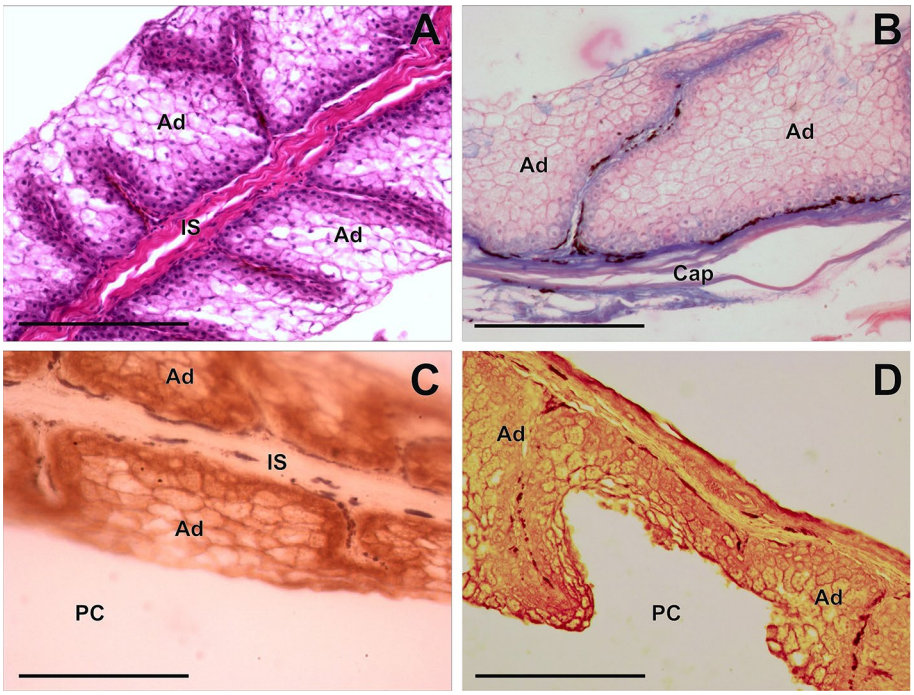

Fig. 3 Positive histochemical and lectin-histochemical reactions of the uropygial gland of the Eared Dove. **a** Periodic acid-Schiff ×20, **b** Alcian Blue pH 2.5 ×20, **c** PNA ×20, **d** RCA-I ×20. For other abbreviations, see Fig. 2. Scale bar 500 µm

We found a large primary chamber in the uropygial gland of the Eared Dove that comprises most of the gland surface and is similar to that described for other columbids (e.g. *C. palumbus* and *D. goliath*) (Jacob and Ziswiler 1982). A large primary chamber has also been found in the uropygial gland of other terrestrial bird species such as White Stork (Ciconiiformes) and Osprey (*Pandion haliaetus*; Accipitriformes) (Jacob and Ziswiler 1982; Sawad 2006; Harem et al. 2010;), and caracaras (Chiale et al. 2015, 2016). It has been postulated that this chamber functions in the storage of uropygial secretions, since terrestrial species secrete from the uropygial gland less frequently than aquatic species (Chiale et al. 2014). Our results in the Eared Dove support this hypothesis.

Regarding its general histological description, the gland of the Eared Dove has similar features to those of other bird species (Lucas and Stettenheim 1972; Jacob and Ziswiler 1982; Montalti et al. 2001; Sawad 2006; Harem et al. 2010), but the adenomers do not show a clear division of the lobules into different zones [depending on the epithelial height and lumen width of the adenomeres, sensu Lucas and Stettenheim (1972)] as in other species like penguins, skuas, storm petrels (Chiale et al. 2014) and caracaras (Chiale et al. 2015, 2016). The absence of different zones could be related to the presence of the large primary chamber, which is not found in aquatic species (Chiale et al. 2014).

The uropygial gland of the Eared Dove has both elastic and reticular fibres comprising part of the connective tissue surrounding the gland, the connective tissue of the medial septum, among the adenomeres, and in the papilla region. Elastic fibres in the uropygial gland were also found by Jacob and Ziswiler (1982), Chiale et al. (2014, 2015, 2016), but not by Hou (1928). This type of fibre is expected in an organ with the ability to distend according to its functional state, such as the uropygial gland. Reticular fibres have been reported as absent by Kozlu et al. (2011) in the uropygial gland of the White Stork *Ciconia ciconia* (Ciconiiformes), but these fibres have been found in the uropygial gland of other species like ducks, herons, doves, some passerines (Bhattacharyya 1972), penguins, skuas, storm petrels (Chiale et al. 2014) and caracaras (Chiale et al. 2015, 2016). Also, Herbst corpuscles have been found in the papillae within the connective tissue, as in other bird species (Paris 1912; Chiale et al. 2016).

Comparative histochemistry and lectin histochemistry of the uropygial gland of the Eared Dove

Like the sebaceous glands present in the skin of mammals, the uropygial gland mainly produces a lipidic secretion (Jacob and Ziswiler 1982; Salibian and Montalti 2009). However, it has long been known that this gland shows a positive reaction to stains that characterize carbohydrates. Cater and Lawrie (1950) divided the lobes of the gland histochemically into two regions: a peripheral or sebaceous region, and a central or glycogenic region, the latter positive to PAS. While the PAS assay identifies glycogen, it also identifies numerous carbohydrates or saccharides that form glycoconjugates (glycolipids and glycoproteins). Glycoconjugates comprise a very high variety of saccharides that can be differentiated by various techniques, including

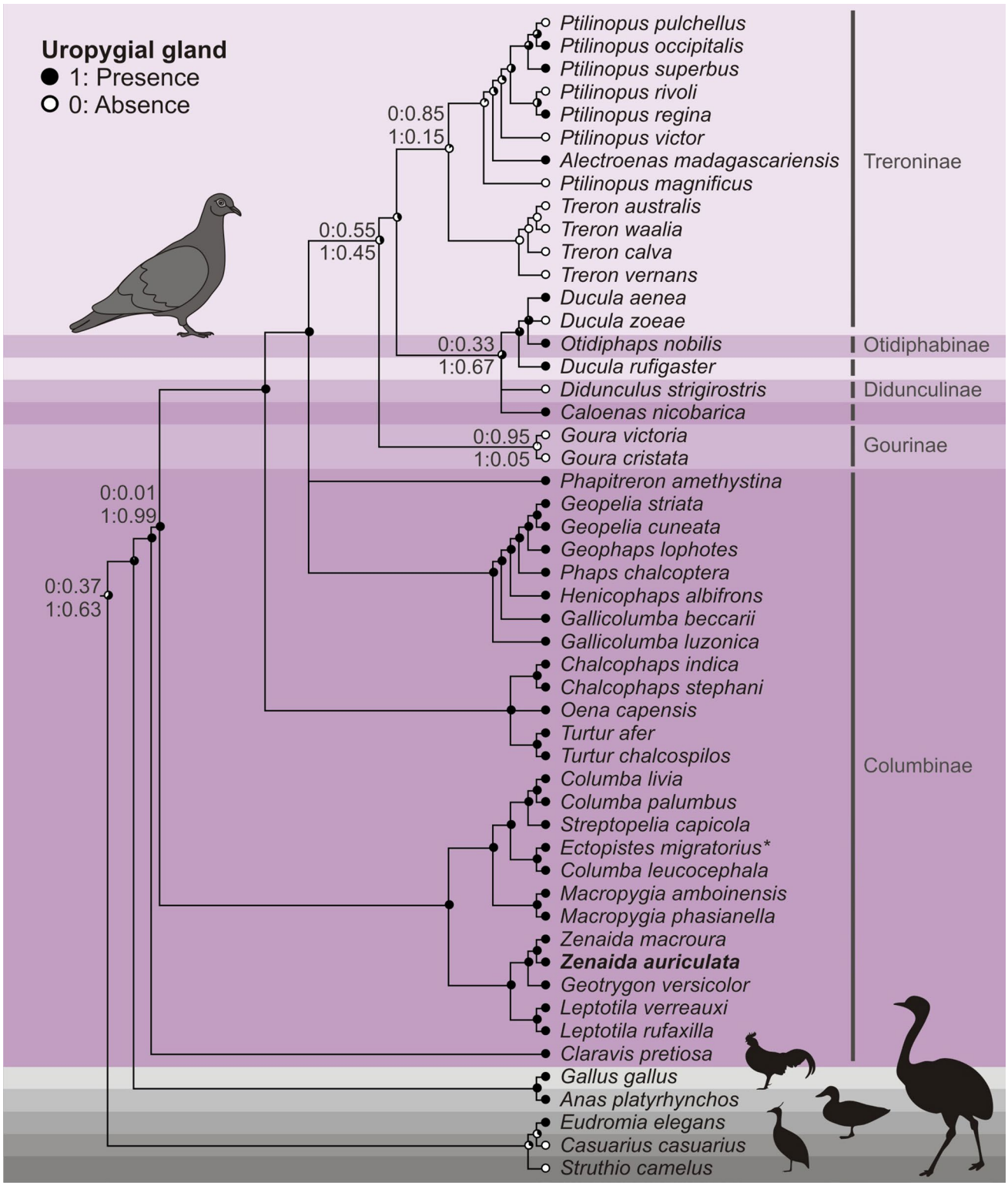

Fig. 4 Ancestral-state reconstruction of the presence/absence of the uropygial gland in Columbiformes based on likelihood analyses. Phylogenetic proposal from Heupink et al. (2014). Asterisk indicates extinction

modified PAS and AB assays, and lectin histochemistry. The carbohydrate components of the glycoconjugates have very important biological functions that differ from the energy reservoir function of glycogen, and are involved in signalling mechanisms related to cell proliferation, cell death and immune response, among other processes (Gabius 2000).

Recent studies have found that different regions of the uropygial gland do not show different reactivity to PAS, although all the secretory regions of the gland are positive to other stains like AB, and lectin histochemistry, demonstrating that other carbohydrates are present in the gland (Chiale et al. 2015, 2016). Similar results were found in the Eared Dove.

The uropygial gland of the Eared Dove reacted positively to PAS and AB pH 2.5, as does that of the Rock Dove (Montalti et al. 2001), which specifically reveals the presence of glycoconjugates with oxidizable vicinal diols including glycogen and glycoconjugates with carboxyl groups and/or with sulphated esters. These types of glycoconjugates are associated with protective functions in different organs and in several species of birds (Yashpal et al. 2014; Díaz et al. 2008), and probably also play a role in the uropygial gland of the Eared Dove. However, the uropygial gland of the Eared Dove did not react to AB pH 1, as also seen in other birds species such as ducks and caracaras (Kamiya et al. 1986; Chiale et al. 2015, 2016). It did not react to AB 0.5 either, as also reported for caracaras (Chiale et al. 2015, 2016). These results show there is a small variety of carbohydrates in the uropygial gland of the Eared Dove.

The lectin-binding pattern of the uropygial gland of the Eared Dove is similar to that of the Rock Dove, and their AB binding is also similar (Montalti et al. 2001). The uropygial gland of species such as caracaras (Chiale et al. 2015, 2016) showed positive reactions to more lectins than the Eared Dove, and many investigations have found positive reactions to different lectins for other sebaceous glands (Ookusa et al. 1983; Atoji et al. 1989; Iwamoto et al. 1998). The glycan residues in glycoconjugates secreted by cutaneous glands are linked to antimicrobial functions (Yasui et al. 2005; Meyer et al. 2007), and one of these glycans is proposed to comprise part of an antimicrobial substance, α-fucose (Yasui et al. 2005), which has been detected in the sebaceous glands of rats (Iwamoto et al. 1998) and goats (Meyer et al. 2007) by lectin UEA-1, as well as in the uropygial glands of the caracaras (Chiale et al. 2015, 2016), but not in the Rock Dove (Montalti et al. 2001) or Eared Dove (this paper). However, the lectin–histochemical pattern in doves demonstrates the presence of other residues proposed as components of antimicrobial substances such as α-D-mannose, *N*-acetyl-β-D-glucosamine, α-D-glucose, *N*-acetyl-D-galactosamine, β-D-galactose, α-D-galactose (Yasui et al. 2005), which have also been found in the sebaceous glands of mammals (Yasui et al. 2005; Meyer et al. 2007) and in the uropygial gland of the caracaras (Chiale et al. 2015, 2016).

A possible explanation for the negative response to AB 0.5 and AB 1 and the minor lectin affinity of the uropygial gland in the Eared Dove compared to that of the caracaras could be related to the different feeding habits of these species. Caracaras feed on carrion and may be more exposed to microorganisms as a consequence of this, whereas the Eared Dove feeds on the seeds of cultivated plants such as sorghum, wheat and maize, and possibly small fruit and insects (Gibbs et al. 2010), which may explain why the diversity of antimicrobial substances in their sebaceous secretions is small.

Finally, the similar structure and pattern of lectin binding between the Eared Dove and the Rock Dove (Montalti et al. 2001) could be related to their phylogenetic proximity and/or to similar feeding habits. Future studies on other distantly related terrestrial species with dissimilar diets would help elucidate if the presence of glycoconjugates is linked to phylogenetic distance or to similar feeding habits.

Evolutionary history of the loss of the uropygial gland within the Columbiformes

The uropygial gland is a unique organ of the clade Aves and probably evolved once in their phylogeny (Jacob and Ziswiler 1982). Our ancestral states reconstruction reinforces this hypothesis (Fig. 4). The uropygial gland is present in most avian species, but is absent in ostriches (Struthionidae), rheas (Rheidae), cassowaries (Casuariidae), emus (Dromaiidae), mesites (Mesitornithidae), some woodpeckers (Picidae), bustards (Otidae), frogmouths (Podargidae), some parrots (Psittaciformes) and some columbids (Columbiformes) (Jacob and Ziswiler 1982; Johnston 1988).

This is the first study to examine the presence/absence of the uropygial gland in a phylogenetic context. The ancestral states reconstruction suggest that the presence of the uropygial gland is the ancestral condition of Columbiformes (Fig. 4). This character state is maintained in almost 74% of columbiform species, including all the members of the basal subfamily Columbinae, while in the more derived subfamily Treroninae it has a heterogeneous distribution.

The loss of the uropygial gland occurred independently more than once in the order Columbiformes according the ancestral states reconstruction (Fig. 4). The uropygial gland is absent in (1) the subfamily Gourinae (i.e. *Goura victoria* and *Goura cristata*), (2) the only member of the subfamily Didinculinae (i.e. *Didunculus strigirostris*), (3) *Ducula zoeae*, and (4) the clade including the genera *Treron*, *Ptilinopus* and *Alectroenas* within the subfamily Treroninae, which in turn show four reversals to the ancestral state (i.e. the presence of the uropygial gland in *A. madagascariensis*, *P. regina*, *P. superbus* and *P. occipitalis*).

All species of Columbiformes that lack an uropygial gland inhabit forests and rainforests, are mostly distributed in eastern Asia and Australasia (except for *Treron australis*, *Treron waalia* and *Treron calva* with an Afrotropical distribution), have a frugivorous diet and are arboreal (except for *Goura victoria* and *Goura cristata*, which are mainly granivorous and terrestrial) (Gibbs et al. 2010). Since columbiformes lacking the uropygial gland do not have a homogeneous diet or habit, and furthermore the uropygial gland may be indistinct present or absent in Columbiformes that do have a similar diet and habit, we cannot currently give functional explanations for the loss of this gland.

During morphogenesis, the uropygial gland starts as a pair of epidermal placodes that invaginate into the mesenchyme and form a pair of glandular lumen; this is followed by the formation of the papilla as an elevation of the skin around the uropygial ducts (Fukui 1997). In the presence of epidermal growth factor, the formation of the uropygial papilla and glandular lumen is inhibited (Fukui 1997). In mammals, the molecular aspects of sebaceous gland development involve several signal cascades and transcription factors (reviewed in Niemann and Horsley 2012). However, the molecular basis for the development of the uropygial gland, or which genes are activated in the epidermis for its formation, are still unknown. Sebaceous glands are absent from some species of mammals (including hirsute ones such as the primate *Pongo abelii*), which is associated, in various clades, to *MC5R* gene inactivation; this gene is involved in sebocyte differentiation (Springer and Gatesy 2018). *MC5R* has been found in birds (Thomas et al. 2018), but its expression during the development of the uropygial gland has yet to be analysed. Complete loss or inactivation of *MC5R* occurs in multiple placental lineages that lack sebaceous glands (Springer and Gatesy 2018). We hypothesize that this could also be the case in some taxa of birds, and that Columbiformes might have a greater predisposition for the loss or inactivation of this gene. The possibility that the inactivation of a single gene through genetic or epigenetic mechanisms could inhibit the development of the sebaceous glands could explain the reversals of the loss of the uropygial gland that have occurred in several species of the subfamily Treroninae. Future studies on this subject are expected to clarify the mechanisms of the predisposition of Columbiformes to the secondary loss of the uropygial gland, and reversal to the ancestral character state in some members of the order.

Conclusion

This study represents the first thorough investigation of the uropygial gland of the Eared Dove and the first in-depth study of the uropygial gland of any Columbiformes. We demonstrate that the uropygial gland of the Eared Dove has many of the general characteristics of this gland in other birds, such as the presence of two lobes, the sebaceous secretion of its adenomeres and the presence of a connective capsule with elastic and reticular fibres. As in many other Columbiformes, but in contrast to many other bird taxa, there is no feather tuft on the uropygial gland's papilla in the Eared Dove. The primary storage chamber, which is the site of accumulation of the gland's secretion, is very large, as in other terrestrial avian species, and there is no zonation in the structure of its lobes. The histochemistry and lectin histochemistry show that carbohydrates are important components of the secretions of the uropygial gland. Ancestral state reconstruction shows that the presence of the uropygial gland is the ancestral character state of Columbiformes, and that the loss of the gland has occurred independently several times throughout the evolution of the clade, as cases of reversal to the ancestral character state have been identified. We hypothesize that this may be related to a process of activation and inactivation of genes such as *MC5R*, which is involved in the formation of glands.

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