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The skin structure of greater rhea (Rheidae, Palaeognathae)

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Abstract

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The aim of this study was to describe the histological structure of the skin of greater rhea (*Rhea americana*), a ratite bird native to South America. Skin samples were taken from three regions of the trunk (alar, dorsal and pelvic) in 14 specimens which ages ranged from 7 days to adulthood. Serial sections were obtained and subjected to different staining procedures (haematoxylin and eosin, orcein, Masson's trichrome and Gomori), and a morphometric analysis was carried out on stained slides. In general, both epidermis and dermis showed increased thickness of its layers with age. Some differences between regions can be detected both in epidermis and in dermis; for example in adults and 7-day-old birds, the *stratum corneum* of the alar region was thicker than of the dorsal region. In general, the skin of greater rhea was similar to that described in ratites and other birds (a thin epidermis compared to dermis, dermis with scarce elastic fibres, a slender and vascularized stratum superficiale, collagen fibres arranged in three directions). The scarcity of elastic fibres and the general cross-weaved arrangement of the collagen fibres in the dermis of the adult greater rhea provide strength and flexibility to the dermis, two important features in leather industry.

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Introduction

The integument of birds is characterized by being thin, by the absence of glands, their ability to generate keratin and lipids (Stettenheim 2000 and literature cited herein) and the presence of feathers. These are the most conspicuous features of integumentary structure and numerous studies have focused on different aspects of the feathers such as their arrangement (e.g. Nitzsch 1867), morphogenesis and evolutionary origin (e.g. Homberger and de Silva 2000; Sawyer and Knapp 2003; Alibardi 2007). The histological structure and properties of bird skin, although it is known for several species (e.g. Ahmed *et al.* 1968; Matoltsy 1969; Lucas and Stettenheim 1972; Hodges 1974; Menon *et al.* 1996; Weir and Lunam 2004, 2011), have received less attention than feathers. To date, the skin histology of many birds species like the greater rhea (*Rhea americana*) has not been explored. The greater rhea is a bird native to South America which conforms the taxon Ratitae together with emu (*Dromaius)*, cassowary (*Casuarius*), ostrich (*Struthio*) and kiwi (*Apteryx*). These birds are flightless and, with the exception of the kiwi, they show large body sizes (between 1.00 and 2.00 m in height depending on species, Folch 1992). The Ratitae plus Tinamidae (Tinamous), comprises the Palaeognathae, a basal and monophyletic group with respect to the remaining birds, the Neognathae (e.g. Livezey and Zusi 2007; Hackett *et al.* 2008; Johnston 2011). The study of Palaeognathae birds is of interest because of their relevance in the evolution and systematics of modern birds. Also, species like greater rhea, ostrich and emu have economic importance and worldwide farming has been developing since the mid-1980s (Glatz and Miao 2008), with meat, leather and oil being the main products of commercial interest (Cooper 2001; Engelbrecht *et al.* 2009; Cloete *et al.* 2012). Despite the importance of Palaeognathae birds, updated studies on its anatomy are uncommon. The histological structure of Ratitae skin has only been studied in the emu and ostrich (Menon *et al.* 1996; Weir and Lunam 2004, 2011), whereas in the Neognathae, skin histology was studied in both domestic and wild birds (Greschik 1915; Ahmed *et al.* 1968; Lucas and Stettenheim 1972; Hodges 1974; Weir and Lunam 2004 and literature cited herein, Picasso *et al.* 2014).

The aim of this work was to describe the skin histology of adult and immature specimens of greater rhea by light microscopy and to compare them with the patterns reported for other birds in general and Ratitae species in particular. This information will be useful in two main aspects: (i) in studies that compare the skin of birds with different phylogenetic and ecological affinities and (ii) in the leather industry, because the basic structural aspects of the skin of greater rhea have not been described yet. (Luvisutti Suárez and Bernard 2011).

Materials and Methods

Rhea americana specimens of different ages were obtained from a commercial farm located in Buenos Aires province, Argentina. Fourteen specimens of three ontogenetic stages were used: adulthood (2-year-olds, three males and two females); 5 month old (four specimens of unknown sex) and 7-day-olds (four specimens of unknown sex). Selected birds were healthy and reared in semi-extensive conditions in accordance with Argentinean regulations for greater rhea farming. Birds were obtained and sacrificed (by cervical dislocation or electrical stunning) at a commercial abattoir. Skin samples (1 × 1 cm) were taken from three regions of the trunk (Fig. 1): alar region, which is an apteria, and the feathered dorsal and pelvic regions. These regions were chosen because they are located in the body regions where the skin is extracted for the leather manufacture. Samples were fixed in a 10% formalin solution and processed for embedding in paraffin wax. A total of 12 (3 μm) serial sections were taken from each sample. To determine the direction of connective tissue fibres in relation to body axis, each sample was oriented following the cranial–caudal direction of the bird body. The following stains were performed: haematoxylin and eosin for general histological description, orcein for elastic fibres, Masson's trichrome (using fast green or aniline blue to stain collagen fibres) and Gomori for reticular fibres. Also to locate muscle, an immunohistochemistry technique to locate desmin was performed according to the protocol by Ortega *et al.* (2007).

For the inspection of tissue structure, an Olympus CH30 light microscope was used. The thickness of each layer was measured with Image Pro-Plus 6 (Media Cybernetics, Silver Spring, MA, USA) software and using a 40× objective.

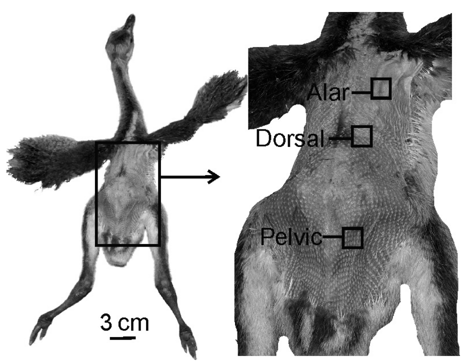

Fig. 1—Dorsal view of a 7-day-old chick showing the skin regions that were studied.

To minimize bias in the region to be measured, we used a protocol similar to Weir and Lunam (2004), but measures were taken at 2, 4 and 6 mm from the left-hand edge of the section and only those slides without artefacts or damage were used. For each age and region, the mean and the standard deviation of the thickness of each layer were measured. Also, the percentage that each layer occupied in relation to the total thickness of dermis or epidermis was calculated and, for this, the cellular layers of epidermis, that is the *stratum basale* and the *stratum intermedium* were considered together.

Differences among regions and ages were analysed with ANOVA, and *P*-values < 0.05 were taken as significant. A preliminary analysis on adult specimens showed that there were no differences between sexes; therefore, male and female specimens were included in the same age group. Anatomical terminology follows Baumel *et al.* (1993).

Results

Adult skin

According to our observations, the *epidermis* was thinner than the dermis (Fig. 2A). This consisted of a acellular cornified *stratum corneum* and a cellular layer with a *stratum intermedium* formed by one or two layers of cells and by a *stratum basale* with one layer of cuboidal cells (Fig. 2B).

The *dermis* was composed of the typical four layers known for birds: *stratum superficiale*, *stratum compactum*, *stratum laxum* and *lamina elástica* (Fig. 2A,C). The *stratum superficiale* (Fig. 2A,C), of loose connective tissue, was characterized for being thin and by the irregular arrangement of the collagen fibres. Profuse elastic and reticular fibres were present near to the basal membrane (Fig. 3A). This stratum showed abundant blood vessels close to the *stratum compactum* (Fig. 3B). The *stratum compactum* of dense connective tissue was broad (Fig. 2A) and showed thicker collagen fibres in comparison with those of the *stratum superficiale* (Fig. 3C). In the alar and dorsal regions, these fibres were oriented parallel to the skin surface (Fig. 3D) and occasionally perpendicular fibres were observed. In contrast, in the pelvic region (Fig. 3C), collagen bundles ran in three directions: perpendicular and parallel to the skin surface, both cranio-caudal and latero-medial direction. This arrangement became more irregular in the surroundings of the feather follicles. Also, this layer showed scattered elastic and reticular fibres. While the elastic fibres were observed in association with collagen bundles of the more superficial region of this stratum (Fig. 2C), reticular fibres were located mainly around blood vessels (Fig. 3E), which were more abundant at the interface with the *stratum laxum*.

The *stratum laxum* (Fig. 2A) consisted of collagen and elastic fibres and adipose tissue that supported blood vessels, nerves and feather follicles.

Feathers and feather muscles, the latter recognized specifically with antidesmin immunohistochemistry technique, were present in the *stratum compactum* and *stratum laxum* of the

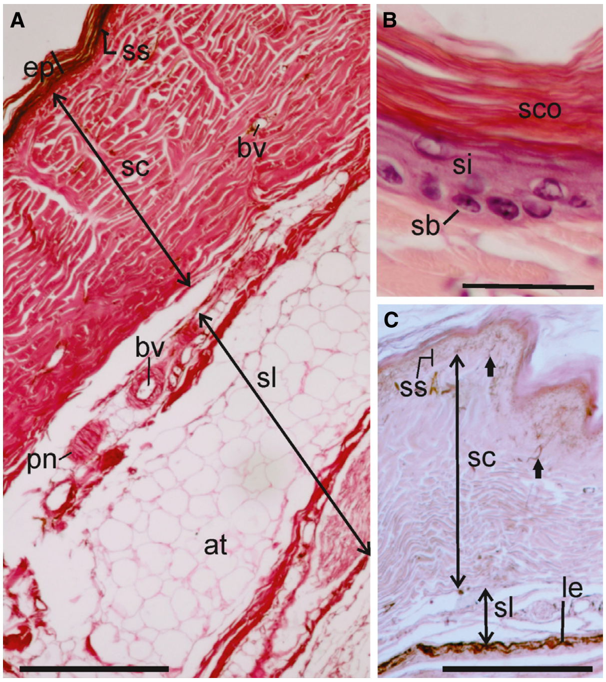

Fig. 2—General view of epidermis and dermis of an adult of greater rhea —**A.** Haematoxylin- and eosin-stained section showing the general arrangement of epidermis and dermis of an adult bird (pelvic region), at: adipose tissue, bv: blood vessel, ep: epidermis, pn: peripheral nerve, sc: stratum compactum, sl: stratum laxum and ss: stratum superficiale. Scale bar = 400 μm —**B.** Haematoxylin- and Eosin-stained section showing the epidermal layers in the pelvic region, sco: stratum corneum, si: stratum and sb: stratum basale. Scale bar = 40 μm —**C.** Orcein-stained section of the alar region showing elastic fibres (arrows) in dermis and the lamina elastica, le: lamina elastica, ss: stratum superficiale, sc: stratum compactum and sl: stratum laxum. Scale bar = 400 μm.

feathered regions (dorsal and pelvic regions) (Fig. 4A,B). The Herbst corpuscles were located adjacent to the feather follicles (Fig. 4A), but were also found in the alar region without relation to them (Fig. 4C).

The *lamina elastica* (Fig. 2C) was formed by elastic fibres interspersed with some collagen and reticular fibres. Throughout the dermis, pigment cells were observed (Fig. 3B) including the *lamina elastica* and around blood vessels.

The skin of young birds

In the epidermis, the *stratum corneum* and the cellular layers were similar to the adult structure (Fig. 5A,B). In the dermis, the *stratum compactum* of the 7-day-old chicks showed fibroblast (Fig. 5C) and the collagen fibres showed an irregular arrangement (Fig. 5C) compared to adults. Birds of 7 days old and 5 months old showed a *stratum laxum* scarce in adipose tissue. Dermal papillae were not observed in any region or any age.

Metrical features

Epidermis. The *stratum corneum* was the thickest layer at all ages and in all regions, ranging between 55% and 80%, whereas the cellular layers occupied a lower percentage (from 19% to 44%, Fig. 6A–C). Significative differences through ages and between regions were observed in the *stratum corneum* and the cellular layers (Table 1). In general, older birds had thicker epidermal layers than those younger ones. Regional differences were observed in 7-day-old birds and adults, where the *stratum corneum* of the alar region was thicker than that of the dorsal region (Table 1). Also in 5-month-old birds, the cellular layer of the alar region was thicker than that of the pelvic region (Table 1).

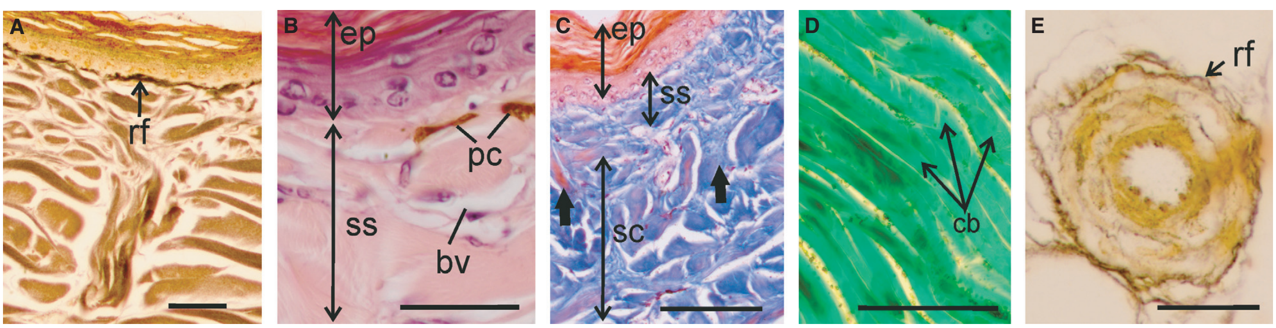

Fig. 3—Structure of the dermis in an adult bird. —**A.** Gomori-stained section showing reticular fibres (arrows) near to the basal lamina in the dorsal region, rf: reticular fibres. —**B.** Haematoxylin and Eosin technique section showing the stratum superficiale with pigmentary cells and abundant blood vessels (pelvic region), ep: epidermis, ss: stratum superficial, pc: pigmentary cells and bv: blood vessel. —**C.** Masson's trichromic stained section of the pelvic region showing the perpendicular bundles of collagen fibres (arrows) in the stratum compactum, ep: epidermis, sc: stratum compactum and ss: stratum superficiale. —**D.** Masson's trichrome (with fast green stain) of the dermis of the alar region showing the parallel bundles of collagen fibres (arrows) in the stratum compactum, cb: collagen bundles. —**E.** Gomori reticulin-stained section showing a blood vessel with reticular fibres in the dorsal region, rf: reticular fibres. Scale bar = 40 μm.

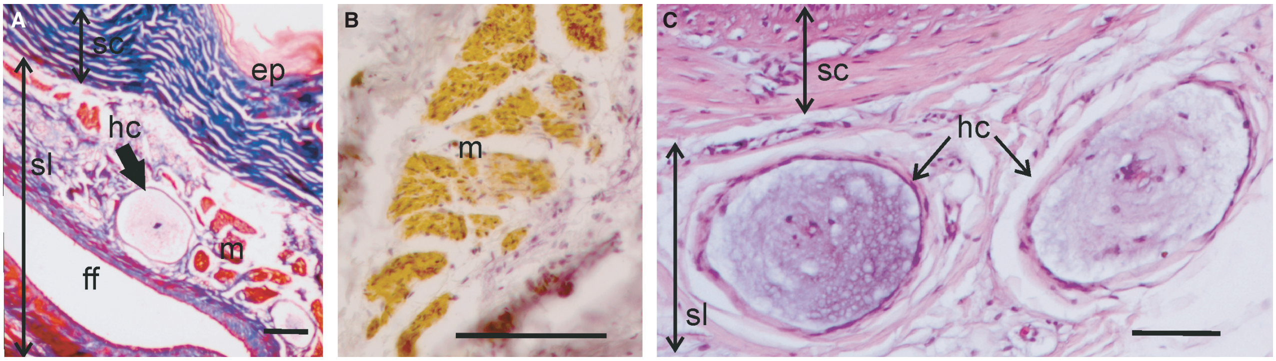

Fig. 4—Herbst corpuscles and feathers. —**A.** Masson's trichromic stained section of the dorsal region of an adult showing a Herbst corpuscle near to feather follicle, ep: epidermis, ff: feather follicle, hc: Herbst corpuscle, m: feather muscle, sc: stratum compactum and sl: stratum laxum. —**B.** Fascicles of feather muscles (desmin technique), m: feather muscle. —**C.** Herbst corpuscle not associated with feathers or muscle (alar region), haematoxylin and eosin technique, hc: Herbst corpuscle, sc: stratum compactum and sl: stratum laxum. Scale bar = 40 µm.

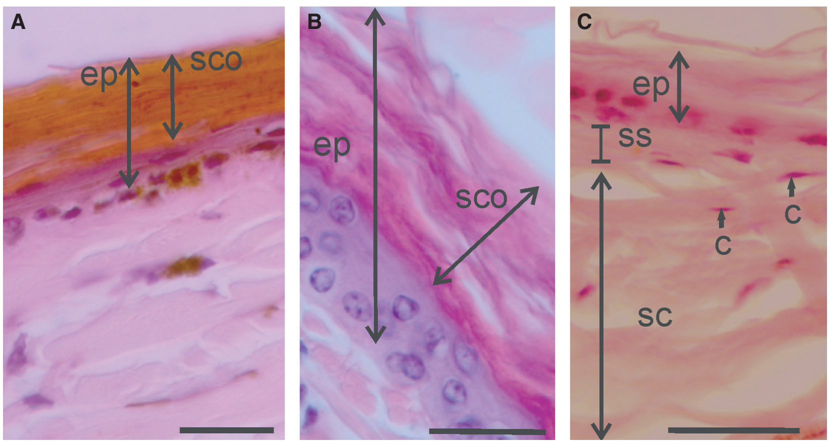

Fig. 5—Main ontogenetic changes of the skin. —**A.** and **B.** Skin of an adult and a 5-month-old bird, respectively, showing the stratum corneum of the dorsal region, ep: epidermis, sco: stratum corneum and haematoxylin and eosin technique. —**C.** Skin of 7-day-old chicks showing, respectively, the presence of cells in the dermis. Note the irregular arrangement of collagen fibres (alar region), haematoxylin and eosin technique, c: cells, ep: epidermis, ss: stratum superficiale and sc: stratum compactum. Scale bar = 40 µm.

Dermis. Of the four layers of the dermis, both the *stratum laxum* and the *stratum compactum* presented greater percentage (Fig. 7A–C), ranging between 29% and 75% and between 19% and 50%, respectively. In general, in all ages and regions, the *stratum laxum* occupied greater percentage than the *stratum compactum* (Fig. 7A–C), except the alar region of the 7-day-old birds (Fig. 7A) where the *stratum compactum* obtained a higher percentage. In all ages and regions, the *lamina elástica* and the *stratum superficiale* represented only between 1% and 10% (Fig. 7A–C). Through different ages, it was observed that the dermal layers were significantly thicker in older birds with respect to younger ones, especially the *stratum compactum*, *stratum laxum* and the *lamina elastica* (Table 2). Also, differences were observed between regions; for example in 5-month-old chicks, the *stratum compactum* of the alar region was thicker than that of the pelvic region (Table 2). The same occurred in the *stratum superficiale* of alar and pelvic regions of adults and 7-day-old birds (Table 2). Finally, it was observed that the *stratum superficiale* showed no significant difference in thickness between regions. Only the pelvic region showed differences among ages, being this stratum thicker in the pelvic region of adult birds than in 7-day-old birds (Table 2).

Discussion

The skin histology of greater rhea was similar to that described in Palaeognathae and Neognathae birds (Ahmed *et al.* 1968; Lucas and Stettenheim 1972; Hodges 1974; Weir and Lunam 2004, 2011), although some peculiarities can be highlighted. In the adult skin, the dermis was characterized by general scarcity of elastic fibres (except the *lamina elastica*), a shared feature with ostriches and emus (Weir and Lunam 2004, 2011). The thin *stratum superficiale* of greater rhea was similar to emu and ostrich as thin collagen fibres and abundant blood vessels were found in their limit with the *stratum compactum* (Weir and Lunam 2004, 2011). Collagen fibres of this stratum showed irregular arrangement in greater rhea, whereas in emu and ostrich, fibres ran parallel to the surface of the skin (Weir and Lunam 2004, 2011). As pointed out by Weir and Lunam (2011), the remarkable vascularization of the *stratum*

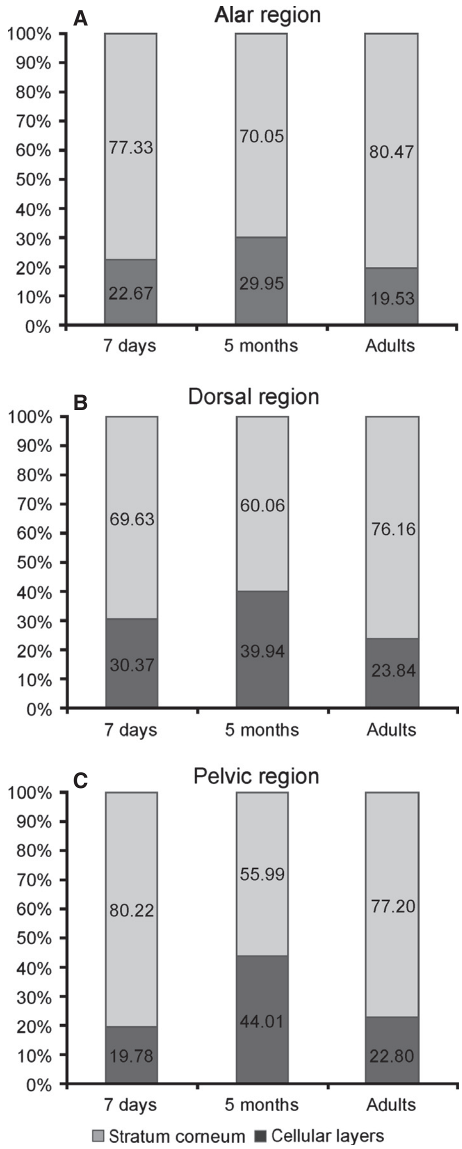

Fig. 6—Percentages of epidermal layers. —**A.** alar region. —**B.** dorsal region. —**C.** pelvic region. CL: cellular layers (*stratum intermedium + stratum basale*), SCO: *stratum corneum.*

superficiale can be related to the control of thermoregulation (Lucas and Stettenheim 1972; Phillips and Sanborn 1994), although the role of the skin in thermoregulation in the greater rhea has not been studied yet.

The *stratum compactum* of greater rhea showed a network of collagen fibres running parallel to the skin surface as in emu and ostrich (Weir and Lunam 2004, 2011). But unlike them, in greater rhea, the pelvic region presented collagen bundles that ran also perpendicularly to the skin surface; instead, in emu and ostrich, the presence of this type of collagen bundles was sporadic (Weir and Lunam 2004, 2011). The *stratum laxum* of greater rhea was an extensive layer, as in the emu (Weir and Lunam 2004, 2011), but in the case of 7-day-old chicks, the *stratum compactum* was thicker than the *stratum laxum* (see Fig. 7A). This last stratum presented adipose tissue, collagen and elastic fibres, as well as in the feathered region, the feather follicles and their muscles. The *lamina elastica* in *R. americana* is distinguished by the presence of reticular and collagen fibres accompanying the elastics fibres. It is worth mentioning that the *stratum laxum* and the *lamina elastica* of the ostrich have not been described to date yet (Weir and Lunam 2011).

Table 1 Total thickness of epidermis (TT) (μm), mean thickness (μm) and standard deviation (SD) of each layers of epidermis

A	R	TT	CL Mean ± SD	SCO Mean ± SD
7 days old	Alar	26.76	6.07 ± 1.82^{a}	$20.69 \pm 2.93^{g,h}$
	Dorsal	20.59	6.25 ± 1.02	$14.34 \pm 2.95^{g,i}$
	Pelvic	25.18	$4.98 \pm 0.02^{c,d}$	20.20 ± 1.92^{j}
5 months old	Alar	31.75	$9.51 \pm 0{,}23^{a,e}$	22.24 ± 2.22^{n}
	Dorsal	31.47	12.57 ± 2.09^{b}	18.90 ± 1.45^{k}
	Pelvic	30.35	$13.36 \pm 1.98^{c,e,f}$	16.99 ± 2.54^{l}
Adults	Ala	41.24	8.06 ± 1.02	$33.19 \pm 2.4^{h,n,m}$
	Dorsal	38.81	9.25 ± 0.9^{b}	$29.56 \pm 2.10^{i,k,m}$
	Pelvic	41.30	$9.42 \pm 1.3^{d,f}$	$31.88 \pm 1.00^{j,l}$

Results of ANOVA are indicated by superscript letters (identical letters indicate significant differences at $P < 0.05$).

A, ages; R, regions; CL, cellular layers (*stratum intermedium + stratum basale*); SCO, *stratum corneum.*

In greater rhea (both adults and chicks), Herbst corpuscles were found to be associated with the feather follicles as well as not associated with them. These mechanoreceptors are one of the most widely distributed in the body of birds (Gottschaldt 1985), as they have been found in different regions (e.g. bill, wings, legs Hörster *et al.* 1983 and literature cited therein). Also, these mechanoreceptors show variations in their physiological properties according to their location (Hörster *et al.* 1983). In general, Herbst corpuscles located close to feather follicle detect the position of the feather and the forces pressing on it (Dorward 1970; Hörster *et al.* 1983). Their presence in unfeathered regions would be an interesting topic to study possible differences in the physiological properties of these mechanoreceptors between feathered and unfeathered regions of greater rhea skin. In comparison with other Ratitae, the distribution of Herbst corpuscles of emu is similar to greater rhea, whereas in ostrich, these sensorial organs are always related to the attachment of the muscles of the feather follicle (Weir and Lunam 2004, 2011).

The ontogenetic changes observed in greater rhea skin were similar to those described in domestic birds (*Gallus gallus* or *Meleagris gallopavo*) (Lucas and Stettenheim 1972). Changes in the epidermis were associated mainly with their thickness, while in the dermis, both qualitative and quantitative changes were observed. Qualitative changes of the dermis

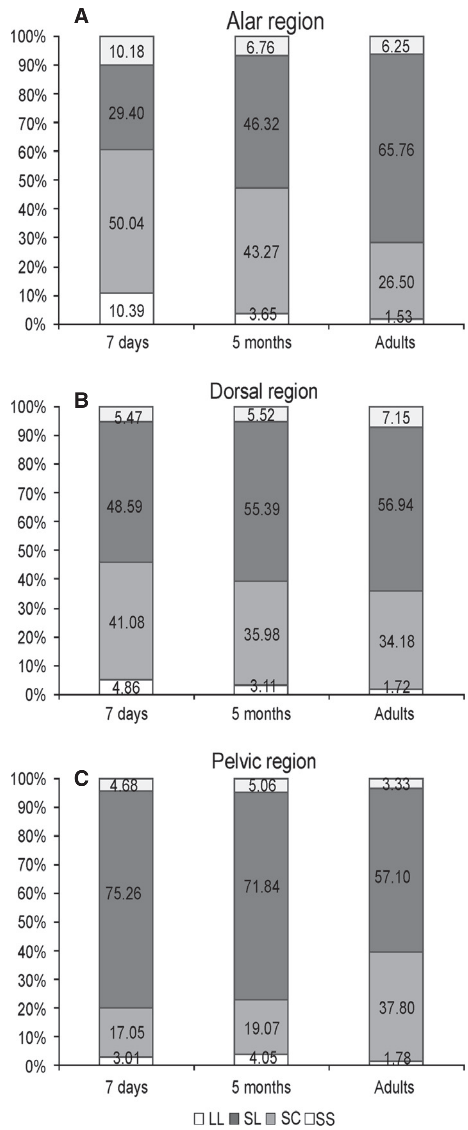

Fig. 7—Percentages of dermal layers. —**A.** alar region. —**B.** dorsal region. —**C.** pelvic region. SS: *stratum superficiale*, SC: *stratum compactum*, SL: *stratum laxum*, LE: *lamina elastica*.

included the progressive increment of blood vessels in the *stratum superficiale* and the increased connective tissue organization of the s*tratum compactum,* where collagen fibres were arranged irregularly in chicks while in adults these were ordered forming bundles with parallel and perpendicular directions.

Quantitative changes, as differences in thickness, were observed through regions and ages. Regarding regional differences, the *stratum corneum* of the alar region (unfeathered) was thicker than that of the dorsal region at least in adults and 7-day-old birds. Comparing with emu, Weir and Lunam (2011) found that this region was thinner with respect to other corporal regions. Possibly, the greatest thickness in this region in greater rhea is due to the fact that this is a zone of friction and wear against the medial surface of the proximal segment of the wing (see Fig. 1). In other vertebrates like mammals, the presence of a thicker *stratum corneum* is also verified in bare areas subject to friction (Sokolov 1982).

In greater rhea, the thickness of each layer of epidermis increased with age, but Weir and Lunam (2011) found that in the emu, cellular layers and the *stratum corneum* were thicker in 7-day-olds than in older birds. Further studies focused on postnatal ontogeny will be necessary to understand the possible factors that generate differences in skin structure between ratites species. Finally, Weir and Lunam (2004) found that both layers of epidermis were thicker in males, but this could not be confirmed in the greater rhea, so further studies with more specimens are needed. The epidermis of ratites studied to date showed differences between species related to age, sex and body regions, but it must be taken into account that the number of specimens used in this and previous works (Weir and Lunam 2004, 2011) was low. Consequently, more studies with a larger number of specimens are needed to elucidate and establish more effectively the presence of variation in skin layers.

In the dermis, the *stratum superficiale* showed little variation in thickness, this could be associated to their functions of maintenance and nutrition, which remain constant throughout life. However, in the remaining layers, significant differences in the thickness occurred among older birds with respect to younger ages. These differences can be associated with the normal pattern of growth and postnatal maturation of the tissues, although more research is required to interpret more accurately the changes and differences in dermal layers.

Some functional implications of the characteristics of the skin of *R. americana* can be considered, especially concerning the dermis, because it is the main component of the leather (Engelbrecht *et al.* 2009), and also, their collagen network is responsible for the mechanical properties of the skin (Jor *et al.* 2011). The general cross-weave arrangement of the collagen fibres found in adults of greater rhea (as well as in other ratites – emu and ostrich – Weir and Lunam 2004, 2011) along with the scarcity of elastic fibres provides strength and flexibility to the leather of ratites (Mellett *et al.* 1996 in Cooper 2001; Weir and Lunam 2011). This regular arrangement of collagen fibres at adult age suggests that this is the most favourable age to obtain optimal leather quality, as it is also suggested to ostrich (Swart 1981 in Sales 1999). Another important feature is the high vascularity of the *stratum superficiale* that in ostrich skin was related to the susceptibility to lamination and bruising which also influence the skin quality (Meyer 2003 in Engelbrecht *et al.* 2009). In greater rhea, this aspect has not been analysed yet and would be interesting to study whether the irregular arrangement of collagen fibres observed here produce any influence on leather quality.

This study provides for the first time a histological description of the skin of greater rhea, and this contribution constitutes a robust basis for future comparative studies.

Table 2 Total thickness of dermis (TT), mean thickness (μm) and standard deviation (SD) of each layers of dermis

A	R	DT	SS Mean ± SD	SC Mean ± SD	SL Mean ± SD	LE Mean ± SD
7 days old	Alar	172.42	17.91 ± 5.91	$86.27 \pm 30.60^{b,c}$	$50.68 \pm 13.05^{j,k}$	17.55 ± 5.86^{q}
	Dorsal	416.65	16.72 ± 3.82	127.94 ± 52.54^{d}	245.46 ± 122.33^{p}	26.51 ± 6.99^{r}
	Pelvic	430.42	12.97 ± 5.09^{a}	73.37 ± 42.34^{e}	$323.92 \pm 31.45^{j,l}$	20.16 ± 8.35^{s}
5 months old	Alar	663.88	24.23 ± 5.90	$287.26 \pm 78.08^{b, f}$	$307.54 \pm 46.62^{k,m}$	44.85 ± 14.81^{t}
	Dorsal	716.62	21.78 ± 4.10	260.50 ± 93.27^{g}	477.59 ± 118.14^{n}	38.74 ± 12.88^{u}
	Pelvic	483.83	19.61 ± 6.71	$92.25 \pm 49.21^{f, h}$	347.56 ± 58.55^{o}	24.41 ± 5.62
Adults	Alar	1190.17	18.19 ± 6.22	$315.36 \pm 45.27^{c, i}$	782.60 ± 64.17^{m}	$74.01 \pm 16.78^{q,t}$
	Dorsal	1324.06	22.82 ± 5.39	$452.61 \pm 39.97^{d, g}$	$753.92 \pm 136.20^{n, p}$	$94.72 \pm 3.96^{r, u, v}$
	Pelvic	1560.98	27.71 ± 6.74^{a}	$589.98 \pm 158.40^{e, h, i}$	$891.32 \pm 169.13^{l, o}$	$51.97 \pm 22.42^{s, v}$

Results of ANOVA are indicated by superscript letters (identical letters indicate significant differences at $P < 0.05$).
A, ages; R, regions; DT, dermis total thickness; SS, stratum superficiale; SC, stratum compactum; SL, stratum laxum; LE, lamina elastica.

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References

Ahmed, S. S., Das, L. N. and Biswal, G. 1968. Comparative histological study of the skin of fowl and duck. *Indian Veterinary Journal* **45**: 725–732.

Alibardi, L. 2007. Cell organization of barb ridges in regenerating feathers of the quail: Implications of the elongation of barb ridges for the evolution and diversification of feathers. *Acta Zoologica* **88**: 101–117.

Baumel, J. J., King, S. A., Breazile, J. E., Evans, H. E. and Vanden Berge, J. C. 1993. Handbook of Avian Anatomy. Publication of the Nuttall Ornithological Club No. 23, Cambridge, MA.

Cloete, S. W. P., Brand, T. S., Hoffman, L., Brand, Z., Engelbrecht, A., Bonato, M., Glatz, P. C. and Malecki, I. A. 2012. The development of ratite production through continued research. *World's Poultry Science Journal* **68**: 323–334.

Cooper, R. G. 2001. Ostrich (*Struthio camelus* var. *domesticus*) skin and leather: A review focussed on southern Africa. *World's Poultry Science Journal* **57**: 157–178.

Dorward, P. K. 1970. Response patterns of cutaneous mechanoreceptors in the domestic duck. *Comparative Biochemistry and Physiology* **35**: 729–735.

Engelbrecht, A., Hoffman, L. C., Cooper, S. W. P. and Van Schalkwyk, S. J. 2009. ostrich leather quality: A review. *Animal Production Science* **49**: 549–557.

Folch, A. 1992. Order Struthioniformes. In: Del Hoyo, J., Elliot, A. and Sargatal, J. (Eds): Handbook of Birds of the World, pp. 75–110. Lynx, Barcelona.

Glatz, P. C. and Miao, Z. H. 2008. Husbandry of ratites and potential welfare issues: A review. *Australian Journal of Experimental Agriculture* **48**: 1257–1265.

Gottschaldt, K. M. 1985. Structure and function of avian somatosensory receptors. In: King, A. S. and McLelland, J. (Eds): Form and Function in Birds, pp. 375–461. Academic Press, London.

Greschik, E. 1915. Zur Histologie der Vogelhaut. Die Haut des Kernbeissers und Haussperlings. *Aquila* **22**: 69–110.

Hackett, S. J., Kimball, R. T., Reddy, S., Bowie, R., Braun, E. L., Chojnowski, J. L., *et al.* 2008. A phylogenomic study of birds reveals their evolutionary history. *Science* **320**: 1763–1768.

Hodges, R. D. 1974. The Histology of the Fowl. Academic Press, London.

Homberger, D. G. and de Silva, K. N. 2000. Functional microanatomy of the feather-bearing integument: Implications for the evolution of birds and avian flight. *American Zoologist* **40**: 553–574.

Hörster, W., Shen, J. and Schwartzkopff, J. 1983. Electrophysiological studies on the vibrational sensitivity in the pigeon. *Naturwissenschaften* **70**: 151–152.

Johnston, P. 2011. New morphological evidence supports congruent phylogenies and Gondwana vicariance for palaeognathous birds. *Zoological Journal of the Linnean Society* **163**: 959–982.

Jor, J. W., Nielsen, P. M., Nash, M. P. and Hunter, P. J. 2011. Modelling collagen fibre orientation in porcine skin based upon confocal laser scanning microscopy. *Skin Research and Technology* **17**: 149–159.

Livezey, B. C. and Zusi, R. L. 2007. Higher-order phylogeny of modern birds (Theropoda, Aves: Neornithes) based on comparative anatomy. II. Analysis and discussion. *Zoological Journal of the Linnean Society* **149**: 1–95.

Lucas, A. M. and Stettenheim, P. R. 1972. Avian Anatomy – Integument (Parts I and II). United States Department of Agriculture, Washington, D.C.

Luvisutti Suárez, M. A. and Bernard, L. 2011. Cuero de ñandú, un producto con grandes oportunidades. *Visión Rural* **18**: 39–42.

Matoltsy, A. 1969. Keratinization of the avian epidermis: An ultrastructural study of the newborn chick skin. *Journal of Ultrastructure Research* **29**: 438–458.

Menon, G. K., Maderson, P. F. A., Drewes, R. C., Baptista, L. F., Price, L. F. and Elias, P. M. 1996. Ultrastructural organization of avian stratum corneum lipids as the basis for facultative cutaneous waterproofing. *Journal of Morphology* **227**: 1–13.

Nitzsch, C. L. 1867. Nitzsch's Pterylography (translated from the German). Hardwicke, London.

Ortega, H. H., Salvetti, N. R., Müller, L. A., Amable, P., Lorente, J. A., Barbeito, C. G. and Gimeno, E. J. 2007. Modulation of cytoskeletal proteins in follicular structures of cows with cystic ovarian disease. *Journal of Comparative Pathology* **136**: 222–230.

Phillips, P. K. and Sanborn, A. F. 1994. An infrared, thermographic study of the surface temperature in three ratites:

Ostrich, emu and double-wattled cassowary. *Journal of Thermal Biology* **19**: 423–430.

Picasso, M. B. J., Mosto, M. C., Tozzi, R., Degrange, F. J. and Barbeito, C. G. 2014. A peculiar association: The skin and the subcutaneous diverticula of the Southern Screamer (Chauna torquata, Anseriformes). *Vertebrate Zoology* **64**: 245–249.

Sales, J. 1999. Slaughter and products. In: Deeming, D. C. (Ed.): The Ostrich – Biology, Production and Health, pp. 191–216. CABI Publishing, Oxon, UK.

Sawyer, R. H. and Knapp, L. W. 2003. Avian skin development and the evolutionary origin of feathers. *Journal of Experimental Zoology, Part B: Molecular and Developmental Evolution* **298**: 57–72.

Sokolov, V. E. 1982. Mammalian Skin. University of California Press, Berkeley.

Stettenheim, P. R. 2000. The integumentary morphology of modern birds – an overview. *American Zoologist* **40**: 461–477.

Weir, K. A. and Lunam, C. A. 2004. A histological study of emu (*Dromaius novaehollandiae*) skin. *Journal of Zoology of London* **264**: 259–266.

Weir, K. A. and Lunam, C. A. 2011. The structure and sensory innervation of the integument of ratites. In: Glatz, P. C., Lunam, C. and Malecke, I. (Eds): The Welfare of Farmed Ratites, Animal Welfare 11, pp. 131–145. Springer-Verlag, Berlin.