Morphological Features of Herbst Corpuscles in the Oropharynx of the Ostrich (*Struthio camelus*) and Emu (*Dromaius novaehollandiae*)

MARTINA R. CROLE,[1*] LIZETTE DU PLESSIS,[2] AND JOHN T. SOLEY[1]

[1]Anatomy section, Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, South Africa

[2]Electron Microscopy Unit, Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, South Africa

The final publication is available at http://onlinelibrary.wiley.com/doi/10.1002/ar.23088/abstract

ABSTRACT

The distribution of Herbst corpuscles in the oropharynx of the ostrich and emu has recently been documented. However, although the morphology of these mechanoreceptors is well known in neognathous birds, little structural information is available on the Herbst corpuscles of ratites. Tissue sections from those regions of the oropharynx known to possess a high concentration of Herbst corpuscles were sampled from ostrich and emu heads collected after slaughter and prepared for light and transmission electron microscopy. Intra-oral Herbst corpuscles in the ostrich and emu displayed the same basic components (capsule, outer zone, inner core and axon) described in neognathous birds. However, some important differences were observed, notably, the presence of myofibroblasts in the capsule, sensory cilia in cells of the outer layers, a relatively larger, less organised outer zone and narrower inner core, and variations in the shape of the axon. The previously unreported presence of myofibroblasts in the capsule possibly indicates its ability to contract, thus altering the tension of the capsule, which in turn has implications for the conduction of vibrational stimuli. The sensory cilia in the myofibroblasts of the capsule bordering the outer zone, and in the fibroblasts of the outer zone itself, may play a regulatory role in controlling the contraction of the capsule. Such a function has not previously been reported for Herbst corpuscles in any species of bird.

Keywords: Ratite; Herbst corpuscle; Ultrastructure; Myofibroblast; Sensory cilium

Descriptions of the avian Herbst corpuscle, as well as its developmental origin (Saxod, 1973; Malinovský and Páč, 1985) and function (Schildmacher, 1931), have been almost exclusively limited to neognathous birds (Berkhoudt, 1980). Based on these studies the Herbst corpuscles of neognaths demonstrate the following general structural features: a central axon surrounded by an inner core formed by specialised Schwann cells, an outer zone which contains collagen fibres and surrounds the lamellated inner core, and an outer capsule derived from the perineurium (Gottschaldt, 1985; Malinovský and Páč, 1985). The basic structure of Herbst corpuscles in the oropharynx of ratites has been briefly described by light microscopy in the ostrich (*Struthio camelus*) (Palmieri et al., 2002; Tivane et al., 2006; Guimarães et al., 2007; Tivane, 2008), emu (*Dromaius novaehollandiae*) (Crole, 2009; Crole and Soley, 2009) and greater rhea (*Rhea americana*) (Feder, 1972) as well as in the bill of the kiwi (*Apteryx* spp.) (Cunningham et al., 2007). It appears from these studies that the structural features of Herbst corpuscles in ratites are similar to those of other birds. However, recent findings from an immunohistochemical investigation into Herbst corpuscles of the emu skin, suggests that the neurotransmitters of Herbst corpuscles may differ between flightless and volant species of birds (Weir and Lunam, 2006).

The complex ultrastructure of Herbst corpuscles has been described in numerous birds including the chicken (Andersen and Nafstad, 1968; Nafstad and Andersen, 1970), Japanese quail (Halata and Grim, 1993), duck (Saxod, 1968; Chouchkov,

1973; Watanabe et al., 1985), pigeon (Halata and Munger, 1980; Malinovský and Páč, 1980), some aquatic birds (Quilliam and Armstrong, 1963; Halata, 1971) and the goose (Gottschaldt et al., 1982). In contrast, the only documented ultrastructure of Herbst corpuscles in ratites is a preliminary comparative study of these structures in the oropharynx of the ostrich and emu (Crole et al., 2009).

The brief reports on the structure (Palmieri et al., 2002; Tivane et al., 2006; Guimarães et al., 2007; Tivane, 2008; Crole, 2009; Crole and Soley, 2009; Crole et al., 2009) of Herbst corpuscles in the oropharynx of the ostrich and emu require further examination to determine whether they share morphological features between the two species and with neognathous birds. This information becomes particularly relevant in view of a recent study demonstrating differences between these two ratites in respect of the comparative distribution of Herbst corpuscles in the oropharynx and the significance of their location (Crole and Soley, 2014).

MATERIALS AND METHODS

A total of fifteen adult ostrich and fifteen adult emu heads, from birds of either sex, were collected after slaughter from the Oryx Abattoir (Krugersdorp, Gauteng Province, South Africa) and Emu Ranch (Rustenburg, North-West Province, South Africa), respectively. All the heads were thoroughly rinsed with either distilled water, phosphate buffer or running tap water to remove mucus, blood and regurgitated food before further processing.

Light microscopy

Five ostrich and five emu heads were immersion-fixed in 10% neutral-buffered formalin. Formalin contact with all parts of the oropharynx was ensured by wedging the bill open with a small block of wood before immersing the heads in fixative. The right half of the entire oropharynx from the bill tip to the pharyngeal folds and larynx was divided into 20 regions from each of which approximately 5 tissue samples of the mucosa and underlying connective tissue were removed (Crole and Soley, 2014). The samples were trimmed, dehydrated through a graded ethanol series (70%, 80%, 96%, and 2X 100% ethanol) and further processed through 50:50 ethanol: xylol, 2X xylol and 2X paraffin wax (60-120 minutes per step) using a Shandon model 2LE Automatic Tissue Processor (Shandon, Pittsburgh, PA, USA). The samples were then imbedded manually into paraffin wax in plastic moulds. Sections were cut at 4-6 μm and stained with H&E (Bancroft and Gamble, 2002). Histological sections were viewed and features of interest digitally recorded using an Olympus BX63 light microscope (Olympus Corporation, Tokyo, Japan) equipped with a DP72 camera and Olympus cellSens imaging software (Olympus Corporation, Tokyo, Japan).

Immunohistochemistry

Sections for immunostaining of neurofilament (sampled from the glandular oropharyngeal region of five ostrich and five emu heads) were mounted on positively-charged microscope slides (SuperFrost® Plus, Menzel-Glasser®) and dried overnight in a 38-40ºC oven. Routine dewaxing took place in xylene for 10 minutes, followed by rehydration through a graded ethanol and distilled water series (100%, 96% and 70%). Sections were subsequently rinsed in distilled water, incubated with 3% hydrogen peroxide in methanol for 10 minutes at room temperature and then rinsed in distilled water. Heat-induced epitope retrieval (HIER) was performed via microwave heating (96ºC) in a plastic container in Tris-EDTA Buffer (pH 9.0) for 21 minutes. Thereafter the test sections (still in buffer) were allowed to cool for 15 minutes on the bench before rinsing three times in distilled water and then in phosphate buffered saline/bovine serum albumin (PBS/BSA) buffer (pH 7.6) for 5 minutes. Sections were incubated with the primary monoclonal mouse anti-human Neurofilament protein antibody (catalogue number M0762, Dako, Denmark) at a dilution of 1:50 for 30 minutes in a humidified chamber at room temperature. Sections were rinsed in distilled water and kept in buffer for 10 minutes. The LSAB+ System was applied according to the manufacturer's instructions (catalogue number K0679, Dako, Denmark). Sections were incubated for approximately 30 seconds in 3,3' diaminobenzidine (DAB) chromogen. They were then rinsed in distilled water, counterstained with Mayer's haematoxylin for 1 minute and rinsed under running tap water for 10 minutes to remove excess substrate. Sections were routinely dehydrated through increasing ethanol concentrations and xylol, and mounted with cover slips. Large peripheral nerves present in the sections served as a positive internal control.

Transmission Electron Microscopy

For a description of the ultrastructural features, the remaining five ostrich and five emu heads were sampled on-site immediately after slaughter. These samples were immersion-fixed in 2.5% glutaraldehyde in 0.13 M Millonig's phosphate buffer (pH 7.4) at room temperature for a minimum period of 24 hours. Sampling was restricted in both species to the rostral mandibular *Rhamphotheca* and rostral non-glandular region of the roof of the oropharynx known to contain a high density of Herbst corpuscles (see Crole and Soley, 2014), as well as the median palatine ridge in the ostrich. The samples were subsequently rinsed in Millonig's phosphate buffer for 10 minutes, post-fixed for 1 hour in similarly buffered 1% OsO4 with two final buffer washes of 10 minutes each. Samples were then rinsed in distilled water for 20 minutes and dehydrated through a graded series of ethanols (50%, 70%, 80%, 96% and 2 X 100%) for 30 minutes per step. Samples were placed in 2X propylene oxide (PO) for 20 minutes each followed by infiltration in PO: Epoxy resin (2:1) for two hours and PO: Epoxy resin (1:2) overnight. The tissue blocks were then embedded in 100% Epoxy resin and cured at 65°C overnight. Semi-thin sections were cut at 0.3 μm, mounted and stained with 1% toluidine blue and examined with the light microscope to identify areas of interest. Ultra-thin sections (50-90 nm) were stained with uranyl acetate and lead citrate, viewed and images digitally recorded using a Philips CM10 transmission electron microscope (FEI, Eindhoven, The Netherlands) equipped with a Mega View III Soft Imaging System camera and iTEM Soft Imaging System software.

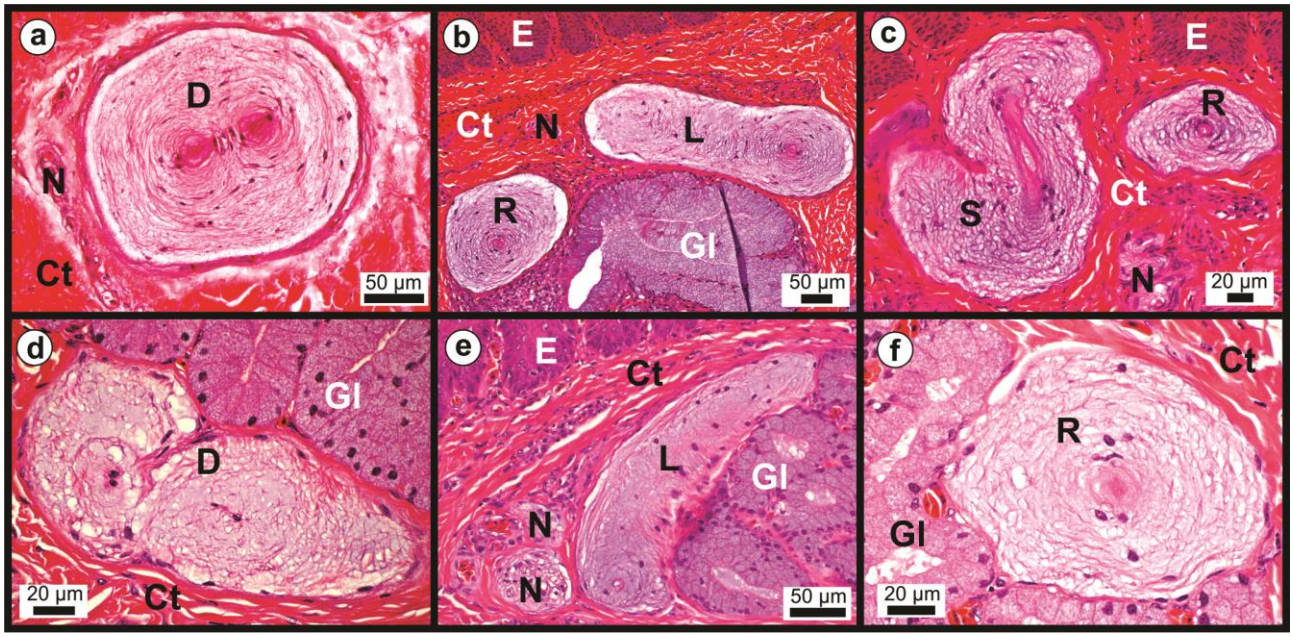

Fig. 1. Morphology of Herbst corpuscles in the ostrich (top) and emu (bottom) oropharynx. Some Herbst corpuscles display double (D) axons (**a** and **d**), long, oval profiles (L) with an eccentric axon in transverse section (**b** and **e**), oddly-shaped profiles such as an S-shape (S) (**c**) and round profiles (R) in transverse section (**a**, **b** and **f**). Connective tissue (Ct), nerve (N), gland (Gl) and stratified squamous epithelium (E).

RESULTS

Light Microscopy and Immunohistochemistry

Herbst corpuscles displayed a wide variety of shapes and sizes in the ostrich and emu throughout the oropharynx (Fig. 1) but were basically ovoid structures. The shape, depending on the plane of sectioning, appeared either round (transverse section) (Fig. 1a, b, c and f), oval (Fig. 1b and e), s-shaped (Fig. 1c) or irregular. Those occurring in the compressed connective tissue layer underlying the keratinised epithelium of the mandible and rostral oropharyngeal roof were more consistent in form and dimensions than those associated with the glands. Each corpuscle was composed of an outer capsule, outer zone, inner core and axon which were clearly demonstrated with H&E staining (Fig. 1), immunohistochemical labelling for neurofilament protein (Fig. 2) and toluidine blue staining (Fig. 3a). Positive IHC labelling clearly demonstrated the central axon as well as nerve fibres running directly outside the capsule (Fig. 2). The outer zone was composed of rings of collagen fibres and intervening fibroblasts (generally identified only by their nuclei) arranged concentrically around the inner core. Some corpuscles displayed a double (Fig. 1a and d) or branched axon, each surrounded by an inner core and outer zone but contained within a common capsule (Fig. 1a and d).

Transmission Electron Microscopy

Outer connective tissue capsule. The capsule consisted of concentrically arranged, alternating layers of cellular and acellular lamellae (Fig. 4, 5a) and was continuous with the perineurium of the accompanying nerve fibre (Fig. 3a). The capsule in both species shared similar basic features although in the emu it was more compact in nature. In the emu some Herbst corpuscles displayed an ill-defined capsule which was composed of loosely arranged acellular lamellae only.

Based on morphological characteristics the capsule could be divided into two parts. The outermost part typically displayed a series of relatively wide acellular lamellae (containing collagen microfibrils) separated by equally wide alternating cellular lamellae (Fig. 4). This region formed approximately three quarters of the width of the capsule in the ostrich but only half the width in the emu. The outer part of the capsule was vascularised by a system of small arterioles located at the periphery of the capsule and by a bed of deeper lying capillaries sandwiched between the cellular lamellae. The wide acellular lamellae were packed with similarly sized collagen microfibrils that were generally oriented either horizontally or longitudinally within a particular lamellum, although variable microfibril orientation within a given lamellum was occasionally observed (Fig. 4). In some areas the orientation of the microfibrils alternated between neighbouring acellular lamellae although most of the microfibrils lay parallel to the long axis of the corpuscle. Giant collagen microfibrils (up to 10 times larger in diameter) were occasionally present between the regular sized microfibrils. The cellular lamellae were formed by the cytoplasmic extensions of numerous myofibroblasts (Fig. 4, 5a). The long, cigar-shaped nuclei of these cells were vesicular in appearance and displayed a layer of dense marginal chromatin (Fig. 5). The cytoplasm was composed of a fine, homogenous matrix containing numerous micro-pinocytotic vesicles, mitochondria, swollen profiles of RER, microtubules and bundles of myofilaments (Fig. 4, 5). Accumulations of small

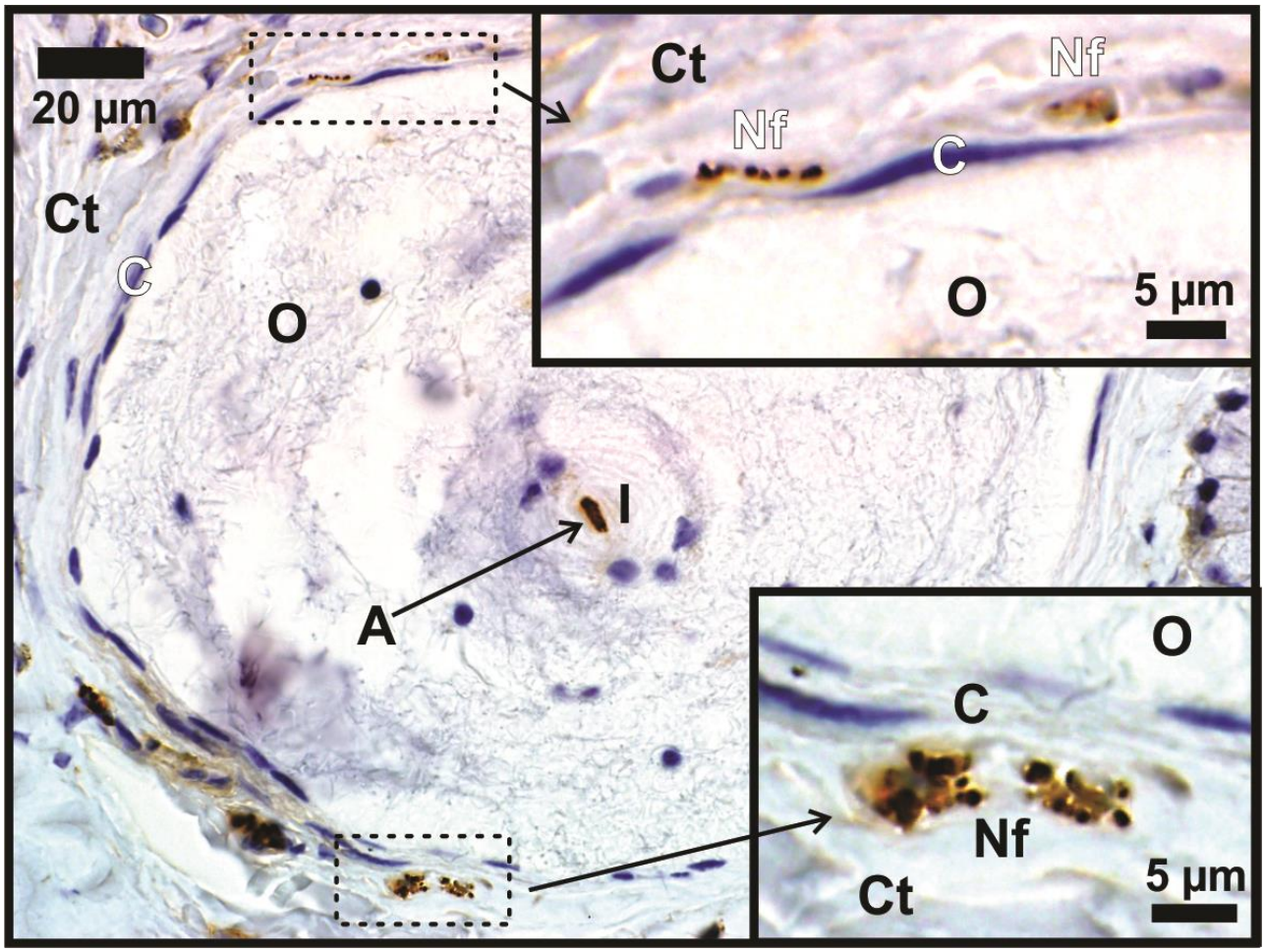

Fig. 2. Positive IHC labelling for neuro-filament (Nf) in a Herbst corpuscle in the emu. The close relationship between nerve fibres and the capsule (C) is clearly demonstrated on light microscopy with IHC labelling. Connective tissue (Ct), outer zone (O), inner core (I) and axon (A).

electron dense granules of similar dimensions to glycogen granules were also commonly observed (Fig. 5b). Isolated structures resembling lysosomes were also present (Fig. 4). The cell membrane displayed numerous marginal densities and in places was bordered by an incomplete layer of basal lamina-like material (Fig. 4). Adjoining cytoplasmic processes of the myofibroblasts either overlapped or interdigitated and were connected by small adhering junctions.

The inner part of the capsule was more compact in nature with the acellular component forming narrow bands between the wider, predominant cellular lamellae (Fig. 5a). However, the ultrastructural features of both types of lamellae were similar to those observed in the outer part of the capsule (Fig. 4). The nuclei of the myofibroblasts bordering the inner margin of the capsule and the outer zone were more rounded in appearance, often bulging into the substance of the outer zone. In some of the marginal myofibroblasts a single sensory cilium was observed in the vicinity of the nucleus and partially embedded within the cell (Fig. 5). The core of the cilium displayed a 9 X 2 + 0 arrangement of microtubules and was surrounded by an intracellular canal (Fig. 5b). A thin layer of electron dense material reinforced the cytoplasmic face of the canal (Fig. 5b).

Outer zone (subcapsular space). The bulk of the corpuscle was formed by the outer zone which was composed of scattered stellate fibroblasts and numerous randomly scattered collagen microfibrils (Fig. 3, 6, 7, 8). Although widely dispersed, the elements of the outer zone, at low magnification, were observed to adopt a concentric arrangement (Fig. 3, 8), forming ill-defined cellular (fibroblasts) and acellular (matrix and collagen microfibrils) lamellae. The acellular component formed the bulk of the outer zone. The collagen microfibrils were mostly oriented along the long axis of the corpuscle and displayed two sizes, those of similar dimension to the microfibrils present in the capsule (Fig. 5), and giant microfibrils that were up to 10 times larger in diameter (Fig. 6a). The microfibrils were embedded in a matrix of interconnected flocculent material and variably sized, intervening clear spaces (Fig. 6a). Fibroblasts were sparse, although they increased in number (as did the density of the collagen microfibrils) towards the inner core. These cells were

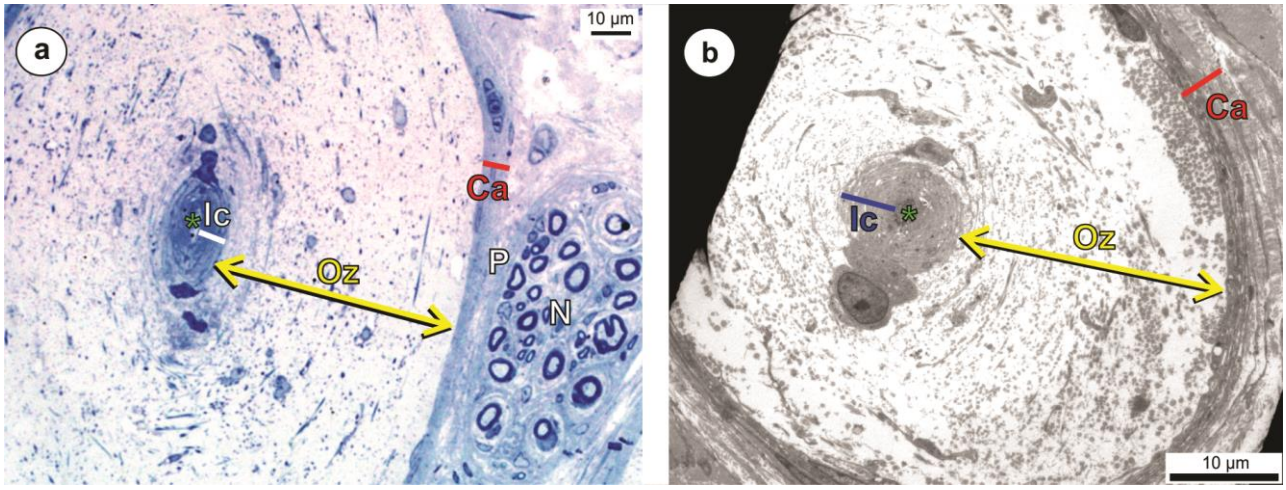

Fig. 3. Low magnification transverse sections of Herbst corpuscles from the oropharynx of the ostrich (a) (LM - toluidine blue stain) and emu (b) (TEM). A nerve (N) is closely associated with the capsule (Ca) in the ostrich and shows a degree of continuity between the perineurium (P) and the outer face of the capsule. The four basic components of the Herbst corpuscle are, from without inwards, the capsule, outer zone (Oz), inner core (Ic) and axon (*). Note the concentric arrangement of the elements of the wide outer zone.

typically mesenchymal in appearance and the nuclei were more rounded than those of the myofibroblasts in the capsule. The cytoplasm contained conspicuous collections of granular endoplasmic reticulum, scattered mitochondria, numerous small vesicles, Golgi fields, centrioles (Fig. 8) and an occasional sensory cilium (Fig. 6b). When present, the cilium, with its 9 X 2 + 0 microtubular arrangement, emerged from a typical basal body and was partly enclosed within an intracellular canal (Fig. 6b). The patchy basal lamina-like material and marginal cytoplasmic densities typical of the capsular myofibroblasts were not observed in the fibroblasts of the outer zone. The attenuated cytoplasmic extensions of the fibroblasts were sparsely scattered throughout the matrix. The morphological characteristics outlined above were similar in both the ostrich and emu.

Inner core. The inner core, which shared similar features in the two species, occupied the centre of the Herbst corpuscle (Fig. 3) and was formed by two sets of closely opposed, interdigitating, semi-circular cytoplasmic lamellae (Fig. 7, 9) emanating from terminal Schwann cells situated on either side (opposite poles) of the afferent nerve fibre (receptor axon) (Fig. 7). Oblique sections of Herbst corpuscles revealed a row of Schwann cell bodies indicating that individual Schwann cells only formed a short segment of the inner core along the length of the corpuscle. A number of relatively large fibroblasts were sometimes observed in close proximity to the inner core. The location of the Schwann cell bodies, the particular radiation of the cytoplasmic processes (lamellae) and the central positioning of the axon resulted in the inner core being subdivided into two semi-circular units by intervening clefts devoid of cytoplasmic material (Fig. 7). The outer lamellae were more loosely arranged than those closer to the axon (Fig. 7, 9). Scattered collagen microfibrils were randomly situated in the inter-lamellar spaces (Fig. 9) and were concentrated towards the poles of the inner core in the vicinity of the Schwann cell bodies. The collagen microfibrils were oriented along the long axis of the corpuscle. The more compactly arranged inner lamellae lay closely apposed with only a thin intervening layer of extracellular matrix between them (Fig. 7, 9, 10).

The large round nucleus of the Schwann cells displayed a prominent nuclear cleft and contained aggregations of mostly peripherally situated heterochromatin (Fig. 7). A prominent nucleolus was also evident (Fig. 7). The cytoplasm of the Schwann cell body, compared to the adjacent fibroblasts, was relatively electron-dense and surrounded by a discontinuous basal lamina (Fig. 7). The most conspicuous organelles were mitochondria, micro-pinocytotic vesicles and lysosome-like structures (dense bodies). Also present were collections of rough endoplasmic reticulum (normal and swollen profiles), scattered free and polyribosomes, elements of the Golgi apparatus and numerous microtubules. The cytoplasmic lamellae were composed of a featureless, moderately electron-dense matrix containing microtubules and micro-pinocytotic vesicles (Fig. 9) (many of which were coated). Mitochondria were also occasionally observed (Fig. 9) and were frequently situated at the termination of the lamellum giving it a club-shaped appearance. The presence of mitochondria was generally indicated by a localized thickening of the lamellum (Fig. 9). Basal lamina-like material appeared sporadically on the surface of the loosely arranged outer lamellae but was not observed on the inner, tightly-packed lamellae. Prominent hemi-desmosomes were present between the inner lamellae and axolemma (Fig. 10, 11) and were particularly obvious at the tips of the lamellae that curved inwards to contact the axolemma (Fig. 10). Occasional desmosome-like adhering junctions connected adjacent lamellae.

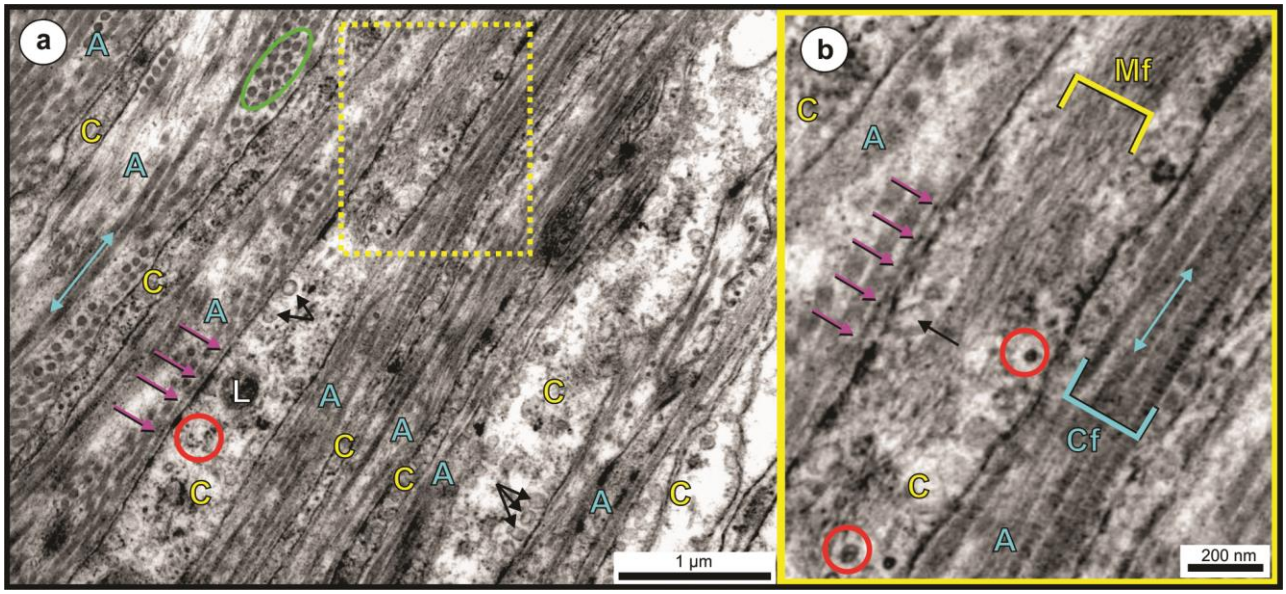

Fig. 4. (a). Outer region of the capsule in the ostrich with the yellow square enlarged in (b). The acellular lamellae (A) with collagen fibrils (Cf) oriented in both longitudinal (double-headed blue arrow) and transverse (green circle) profile, and the cytoplasmic lamellae (C) formed by the myofibroblasts alternate with one another. The broad cellular lamellae display numerous micro-pinocytotic vesicles (black arrows), microtubules (red circles), isolated lysosome-like structures (L) and occasional bundles of myofilaments (Mf). In places the plasmalemma rests on a discontinuous layer of basal lamina-like material (pink arrows).

Receptor axon. The receptor axon of the Herbst corpuscles displayed similar features in both species and typically contained many mitochondria, microtubules and neurofilament (Fig. 10, 11). The axon could be morphologically divided into two components, namely the myelinated part (Fig. 8) and the non-myelinated part (Fig. 3, 7, 9, 11). The non-myelinated part represented the greatest length of the axon within the corpuscle. In both species the profile varied from round to oval; however, the myelinated portion always displayed a round profile.

The axon, as it entered the Herbst corpuscle, was surrounded by a myelin sheath (Fig. 8) supported by fibroblasts and their processes which was soon replaced by the cytoplasmic lamellae of the inner core (see above) (Fig. 7, 9, 11). The myelinated axon displayed the typical features of peripheral myelinated nerves, namely, a Schwann cell with a basal lamina displaying an outer collar of cytoplasm, an outer mesaxon, a myelin sheath (Fig. 8), an inner mesaxon and an inner collar of cytoplasm surrounding the axon. The non-myelinated receptor axon was held firmly in position by the numerous hemi-desmosomes of the inner core lamellae which attached them to the axolemma (Fig. 9, 10, 11). Transducer sites (Fig. 11) were marked by slender axon processes projecting into the cellular lamellae of the inner core (Fig. 11). The axoplasm at the base of the processes was devoid of mitochondria, displaying only clear vesicles and neurofilaments embedded in the cytoplasmic matrix (Fig. 11).

DISCUSSION

Light Microscopy

The intra-oral Herbst corpuscles assumed a variety of shapes in both the ostrich and emu, although the round to oval form was the most prominent. Unusually shaped Herbst corpuscles have previously been reported (Malinovský, 1996) including their occurrence in other regions of the ostrich such as the copulatory organ (Palmieri et al., 2006). Double axons have been reported in the ostrich and were confirmed in the present study, as well as in the emu. This could either be a result of axonal branching or a 180 degree bending of the axon, both of which have been demonstrated in the ostrich (Palmieri et al., 2002, 2006). Double or multiple axons are not common but have been reported in other birds (Winkelmann and Myers, 1961; Nafstad and Andersen, 1970; Wight et al., 1970; Ziswiler and Trnka, 1972; Halata and Munger, 1980; Gottschaldt, 1985). The significance of multiple axons is not known.

Although both Herbst and Grandry corpuscles have been identified in the bill skin of geese (Gottschaldt and Lausmann, 1974) and ducks (Saxod, 1968; Berkhoudt, 1980; Watanabe et al., 1985), only structures resembling Herbst corpuscles were observed in the bill skin (Rhamphotheca) and oropharynx of the ostrich and emu, thus leading to the conclusion that Grandry corpuscles do not occur in this specific location in the ratites studied. Although Grandry corpuscle-like structures were identified in the kiwi, termed terminal cell receptors (Cunningham et al., 2007), these structures appear similar to the myelinated afferent nerve fibres observed in the ostrich and emu and which were associated with clusters of Herbst corpuscles (Crole and Soley, 2014).

Ultrastructure

Herbst corpuscles in the ostrich and emu display the same basic components to those described for birds in general (the capsule, outer zone, inner core and axon (Berkhoudt, 1980); however, there appear to be some differences in the organisation of these components. The comparisons made in the following sections are broadly based on comparable regions in other birds.

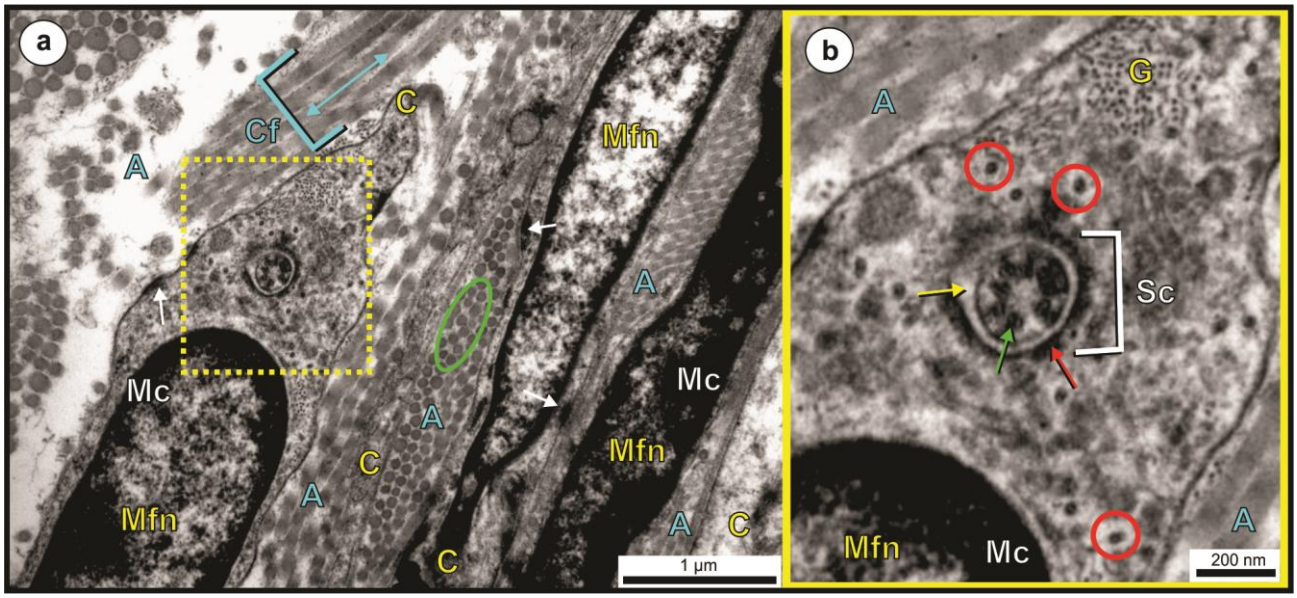

Fig. 5. (a). Innermost region of the capsule in the ostrich. Note the narrow acellular lamellae (A) with collagen microfibrils (Cf) orientated in longitudinal (blue double-headed arrow) and transverse (green circle) profile alternating with cellular lamellae (C). The cigar-shaped myofibroblast nuclei (Mfn) display a layer of dense marginal chromatin (Mc). Marginal densities (white arrows) and a sensory cilium (yellow square – enlarged in b) are also apparent. (b). The sensory cilium (Sc) displays a 9 x 2 + 0 arrangement of microtubules (green arrow) and is surrounded by an intracellular canal (yellow arrow) supported by a thin layer of electron-dense material (red arrow). Microtubules (red circles) and presumptive glycogen granules (G) are also present. Acellular lamellae (A).

Capsule. Herbst corpuscles are less bulky than Pacinian corpuscles, although the capsule is better developed, being two to three times thicker and more rigid than that of Pacinian corpuscles (Quilliam and Armstrong, 1963). The structure of the avian Herbst corpuscle capsule has received little attention and the most in-depth description is that of Halata (1971) in the Canada goose (*Branta canadensis*) and mute swan (*Cygnus olor*). The capsule of Herbst corpuscles is composed of alternating cytoplasmic cellular lamellae and acellular lamellae (collagen fibrils) (Quilliam and Armstrong, 1963). The collagen fibrils mechanically strengthen the capsule and form a network between which the slender cellular lamellae are found (Halata, 1971). This arrangement was noted in the Canada goose and mute swan (Halata, 1971) as well as in the ostrich and emu (present study) and appears to be typical for birds.

The cytoplasmic features of the cellular lamellae in both ratites, as well as their basic structure, was similar to that described in the Canada goose, mute swan (Halata, 1971), duck (Watanabe et al., 1985) and Japanese quail (Halata and Grim, 1993). In the goose and swan fibroblasts formed the cellular lamellae and the presence of many micro-pinocytotic vesicles was taken as an indication of the cell's high level of activity (Halata, 1971). Additionally, in the ostrich and emu, myofilaments, a discontinuous basal lamina, marginal densities and presumptive glycogen granules in the cytoplasm of the cellular lamellae were indicative of myoid properties, thus making these cells myofibroblasts. This is in sharp contrast to the fibroblasts or fibroblast-like cells reported in the capsule of the chicken (Wight et al., 1970) and other birds (Halata, 1971; Watanabe et al., 1985; Halata and Grim, 1993). However, the observation that filaments (Watanabe et al., 1985) and basal lamina-like material (Halata, 1971; Halata and Grim, 1993) are associated with the cellular component of the capsule of some birds may suggest the more widespread occurrence of contractile elements in the capsule of avian Herbst corpuscles.

Based on the thickness of the Herbst corpuscle capsule (in comparison to the Pacinian corpuscle) and its possible contractile properties, it is plausible that the capsule of the Herbst corpuscle in the ostrich and emu may be able to alter the frequency to which the corpuscle responds by tightening (response to higher frequencies) or relaxing (response to lower frequencies) the capsule. This proposed property of the ratite Herbst corpuscle capsule may compensate for the less organised outer zone reportedly present in the ostrich and emu compared to other birds (see below), and which itself is less organised than in the Pacinian corpuscle. The manner in which the myofibroblasts in the capsule are signalled to vary the tension of the capsule may be related to the presence of sensory cilia in the myofibroblasts in the capsule bordering the outer zone and the fibroblasts located in the outer zone (see below) of the Herbst corpuscle. Sensory cilia in the mammalian kidney have been shown to function in sensory transduction, where the cilium may be bent by the flow of fluid (see Pazour and Witman, 2003). It has also been proposed that sensory cilia possess chemical receptors (Pazour and Witman, 2003); however, in a specialised mechanoreceptor such as the Herbst corpuscle, sensory cilia would most likely react to mechanical signals rather than to chemical signals. If the sensory cilia in the ratite Herbst corpuscle do react to mechanical displacements, they may regulate and coordinate the contraction of the myofibroblasts, thus controlling the tensioning of the capsule for optimum signal enhancement and transduction. Whether the apparent absence of sensory cilia in the emu samples studied indicated inter-species variation, or simply reflected the

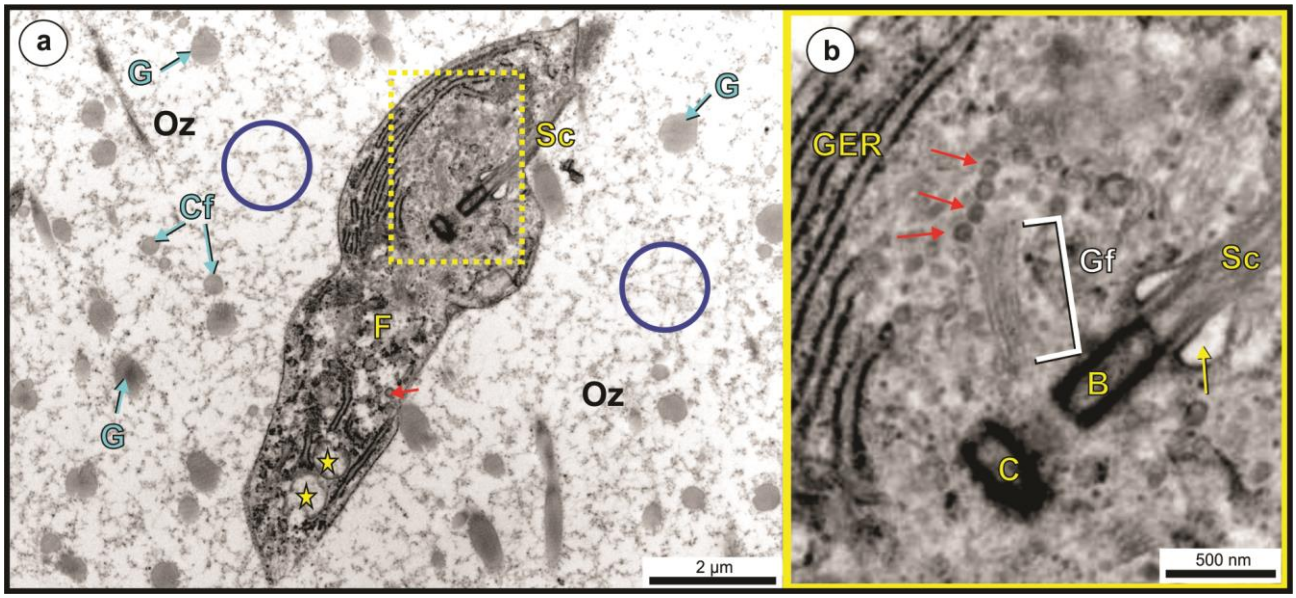

Fig. 6. A fibroblast (F) located in the outer zone (Oz) of an ostrich Herbst corpuscle (a) with the yellow square enlarged in (b). The outer zone demonstrates a matrix of flocculent material (blue circles), and normal (Cf) and giant sized (G) collagen fibrils. The fibroblast cytoplasm displays strands of granular endoplasmic reticulum (GER), Golgi fields (Gf), mitochondria (stars), small vesicles (red arrows) and a sensory cilium (Sc). The basal body (B) and an underlying centriole (C) appear to form a typical diplosome. Intracellular canal (yellow arrow).

randomness inherent in the sectioning of material for TEM, could not be determined.

Outer zone. The outer zone is an enlargement of the endoneural space (Halata, 1971; Gottschaldt, 1985) and characterised by collagen microfibrils and sparse fibrocytes, a feature also apparent in the ostrich and emu. Halata (1971) refers to the outer zone as the subcapsular space and states that it is functionally part of the capsule. The collagen microfibril system forms incomplete lamellae (Quilliam and Armstrong, 1963; Halata, 1971; Berkhoudt, 1980) which exist in the form of a two-dimensional coiled network of fibres emanating from flattened fibrocytes (Gottschaldt, 1985). This particular arrangement was not confirmed in the present study, and the elements of this zone were not as regular and concentrically arranged as in domestic poultry (Saxod, 1968; Cobb and Bennett, 1970; Wight et al., 1970; Halata and Munger, 1980; Gottschaldt et al.,1982; Gottschaldt, 1985; Watanabe et al., 1985; Zelená et al., 1997) and other birds (Schildmacher, 1931; Halata, 1971; Ziswiler and Trnka, 1972; Halata and Grim, 1993; Genbrugge et al., 2012). In general, the outer zone of the Herbst corpuscle is less highly organised than that of the Pacinian corpuscle (Quilliam and Armstrong, 1963; Halata, 1971; Malinovský, 1996). In the ostrich and emu the outer zone was much larger in proportion to the inner core and axon in the majority of the Herbst corpuscles. This differs from the Herbst corpuscles depicted in the bill tip organ in the kiwi (Cunningham et al., 2007) and in other birds (Bolze, 1968; Cobb and Bennett, 1970; Watanabe et al., 1985; Toyoshima et al., 1992; Halata and Grim, 1993) where the outer zone is relatively narrow and the inner core forms a considerable component of the corpuscle. This feature of a larger outer zone and smaller inner core was briefly noted by Guimarães et al. (2007) when comparing the Herbst corpuscles in the ostrich palate to those in the chicken. As in the capsule of the ostrich, a fibroblast displaying a sensory cilium was identified in the outer zone, a phenomenon not previously reported in avian Herbst corpuscles. Although similar structures have not been noted in fibroblasts of the emu corpuscle, their presence cannot be excluded. The relative scarcity of cells displaying single cilia in the ostrich can be ascribed to the limited chance of sectioning the region of the cell from where the cilium emerges. The presence of cilia in both the outer zone and capsule of the ostrich may be a unique feature of this species, and possibly of ratites in general.

Inner core. The inner core of the avian Herbst corpuscle is formed by 8-30 pairs of Schwann cells (Gottschaldt, 1985). In the greater rhea 30-40 pairs of cells form this part of the corpuscle (Feder, 1972), but were not counted in the present study due to the scarcity of appropriate longitudinal sections. The presence of bilateral, symmetrical rows of Schwann cell nuclei are another feature which distinguish Herbst corpuscles from Pacinian corpuscles (Quilliam and Armstrong, 1963). There is reportedly great variation between Herbst corpuscles of different species and from different regions regarding the number, thickness and distance between the lamellae of the inner core (Gottschaldt, 1985). However, the structure of the inner core in the ostrich and emu was generally similar to that collectively described in other birds (Andersen and Nafstad, 1968; Halata, 1971; Gottschaldt, 1985; Watanabe et al., 1985; Halata and Grim, 1993). The non-myelinated nerve fibres demonstrated in the outer fibrous wall (capsule) (Anderson and Nafstad, 1968) and outer zone and inner core of the chicken

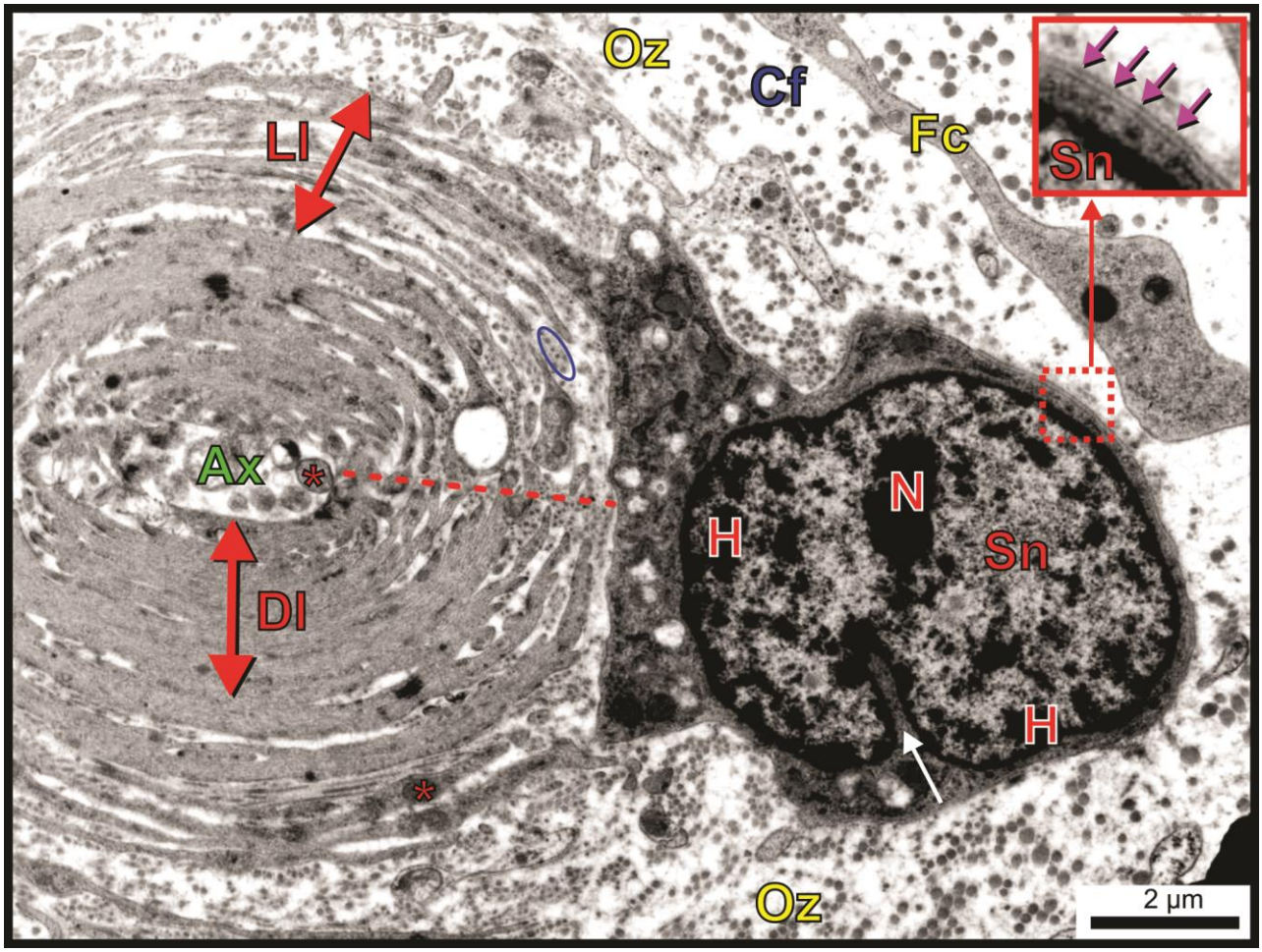

Fig. 7. Inner core of a Herbst corpuscle in the ostrich. Note the large, indented (white arrow) Schwann cell nucleus (Sn) with a prominent nucleolus (N) and clumps of heterochromatin (H). The outer layers of cytoplasmic lamellae are loosely arranged (Ll) whereas the inner lamellae are more densely packed (Dl). The outer lamellae are interspersed with collagen fibrils (blue circle). Fibroblast cell processes (Fc), collagen fibrils (Cf), outer zone (Oz), mitochondria (red *) and axon (Ax). The position of the cleft dividing the inner core into semi-circular units is indicated by a red dotted line. The enlargement shows a short, section continuous of Schwann cell basal lamina (pink arrows).

Herbst corpuscle (Nafstad and Anderson, 1970) were not confirmed in the present study with either IHC labelling for neurofilament protein or by TEM. Intra-corpuscular non-myelinated nerve fibres are also not present in the goose (Gottschaldt et al., 1982). Why these fibres would appear in one species and not in others remains unexplained.

Receptor axon. The axon of the Herbst corpuscle displays three parts, the initial myelinated part (not surrounded by an inner core), the non-myelinated part, and the dilated, terminal end-bulb (also non-myelinated) (the latter two surrounded by the inner core) (Andersen and Nafstad, 1968; Saxod, 1968; Halata, 1971; Halata and Munger, 1980; Gottschaldt, 1985; Toyoshima et al., 1992; Halata and Grim, 1993). This was the typical pattern observed in both the ostrich and emu. The myelinated part displayed features of a typical myelinated peripheral nerve described in mammals and birds (Bubień-Waluszewska, 1985; Pavelka and Roth, 2010) and represented that portion of the nerve up until it reaches the inner core (Gottschaldt, 1985). The non-myelinated axons in the present study displayed the same basic ultrastructural features and attachments to the inner lamellae of the inner core as noted in other birds (Halata, 1971; Chouchkov, 1973; Halata and Munger, 1980; Gottschaldt, 1985; Watanabe et al., 1985; Halata and Grim, 1993). The terminal end-bulb of the axon was not demonstrated in the present study in the ostrich and emu. The axon in the ostrich and emu displayed attachment to the inner core lamellae via hemi-desmosomes, but no synapses as described by Saxod (1968) and Halata (1971) were found. This may simply reflect differences in the preparation of material for electron microscopy.

Morpho-Functional Properties

Although the axon in the ostrich and emu sometimes appeared more round in transverse section (also described in the small Herbst corpuscles in the beak of the Japanese quail

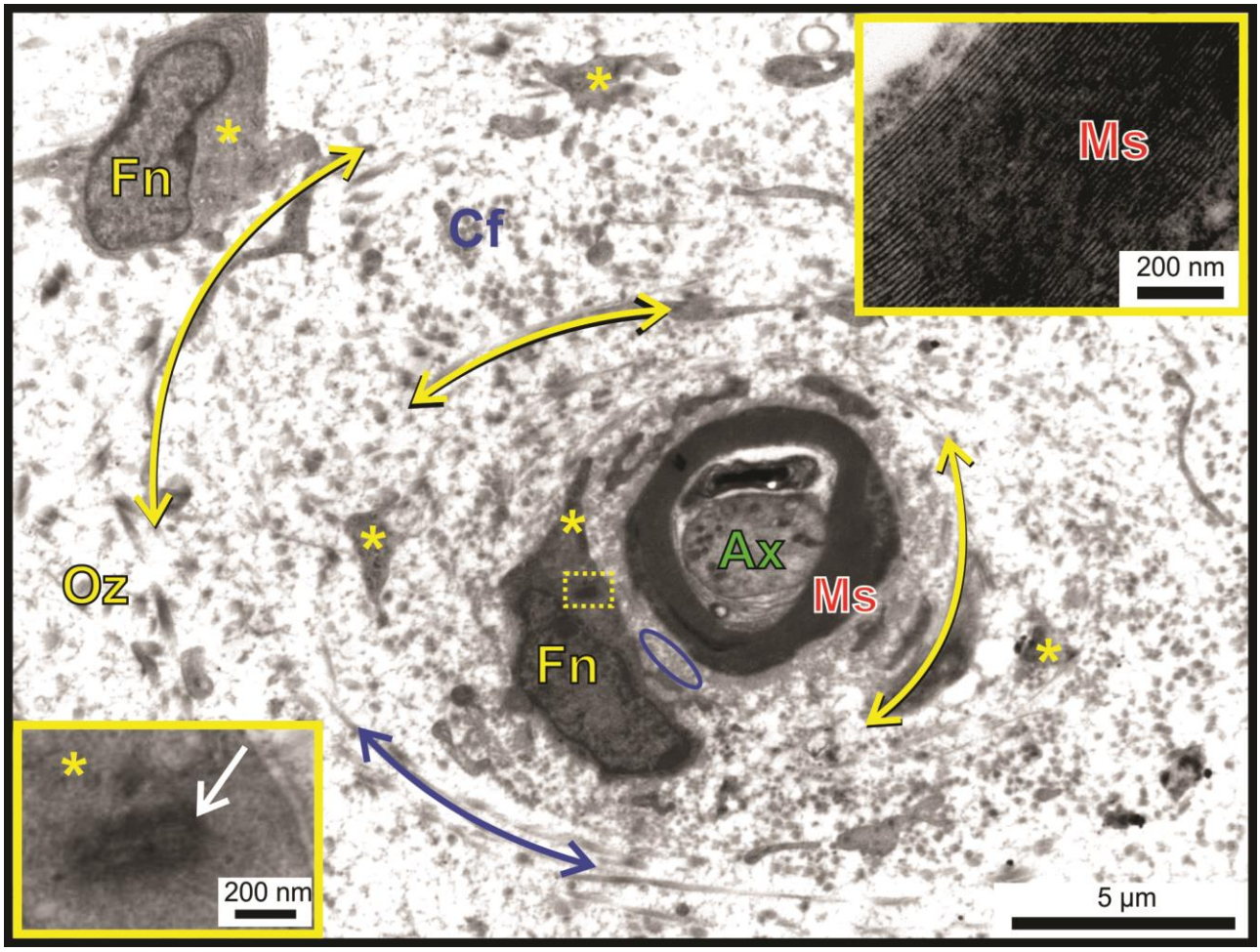

Fig. 8. Myelinated axon (Ax) in the emu. The cytoplasmic lamellae (curved yellow arrows) and the collagen fibrils (Cf and blue curved arrow) of the outer zone (Oz) are concentrically arranged around the myelinated axon. A distinct endoneurium of tightly packed collagen fibrils (blue circle) is present. Fibroblast nucleus (Fn), fibroblast cytoplasm (*), centriole (white arrow) in the fibroblast cytoplasm (dotted yellow square, enlarged in the bottom inset). Top inset: High magnification of the myelin sheath (Ms) illustrating the characteristic lamellar structure.

(Halata and Grim, 1993) oval shaped axons were also observed as described in other birds (Andersen and Nafstad, 1968; Halata, 1971; Gottschaldt et al., 1982; Gottschaldt, 1985, Halata and Grim, 1993). In Pacinian corpuscles the oval shape of the axon reflects the ability of the corpuscle to detect direction (Ilyinsky et al., 1976). Depolarization occurs with an increase in surface membrane area (deformed along the shorter axis) and hyperpolarization occurs with a decrease in surface membrane area (deformed along the longer axis) (Ilyinsky et al., 1976). Thus the Herbst corpuscles in the ostrich and emu with round axons are most likely not direction sensitive. This seems plausible in light of the fact that the majority of the Herbst corpuscles in the oropharynx are situated in a very thin layer of dense, irregular connective tissue (Crole and Soley, 2014) and can only be stimulated from a dorso-ventral plane (between the upper and lower bills), negating the need for a directional sensitivity. However, Hersbt corpuscles present in the median palatine and ventral ridges, which project into the oropharynx of the ostrich (Crole and Soley, 2014), would be able to detect vibrations from a variety of directions.

The specific structure of Herbst corpuscles determines the response properties displayed by these receptors and the discussion that follows is summarised from Gottschaldt (1985). The outer zone and inner core (auxiliary structures) attenuate the velocity and amplitude of mechanical stimuli. Higher frequencies are transmitted more easily through the auxiliary structures. The mucous fluid in the outer zone and between the inner core lamellae may play a role in the particular mechanical filtering characteristics of the auxiliary structures. A receptor potential is generated when the axon processes become deflected by displacements of the inner core lamellae. The deflection of the axon process is facilitated by fixing of the base, by desmosome-like attachments, to the inner core lamellae, while the inherent elasticity of the microfilaments return the axon process to its original position when the deformation of the inner core lamellae ceases. The structure of the Herbst corpuscles in the ostrich and emu supports the

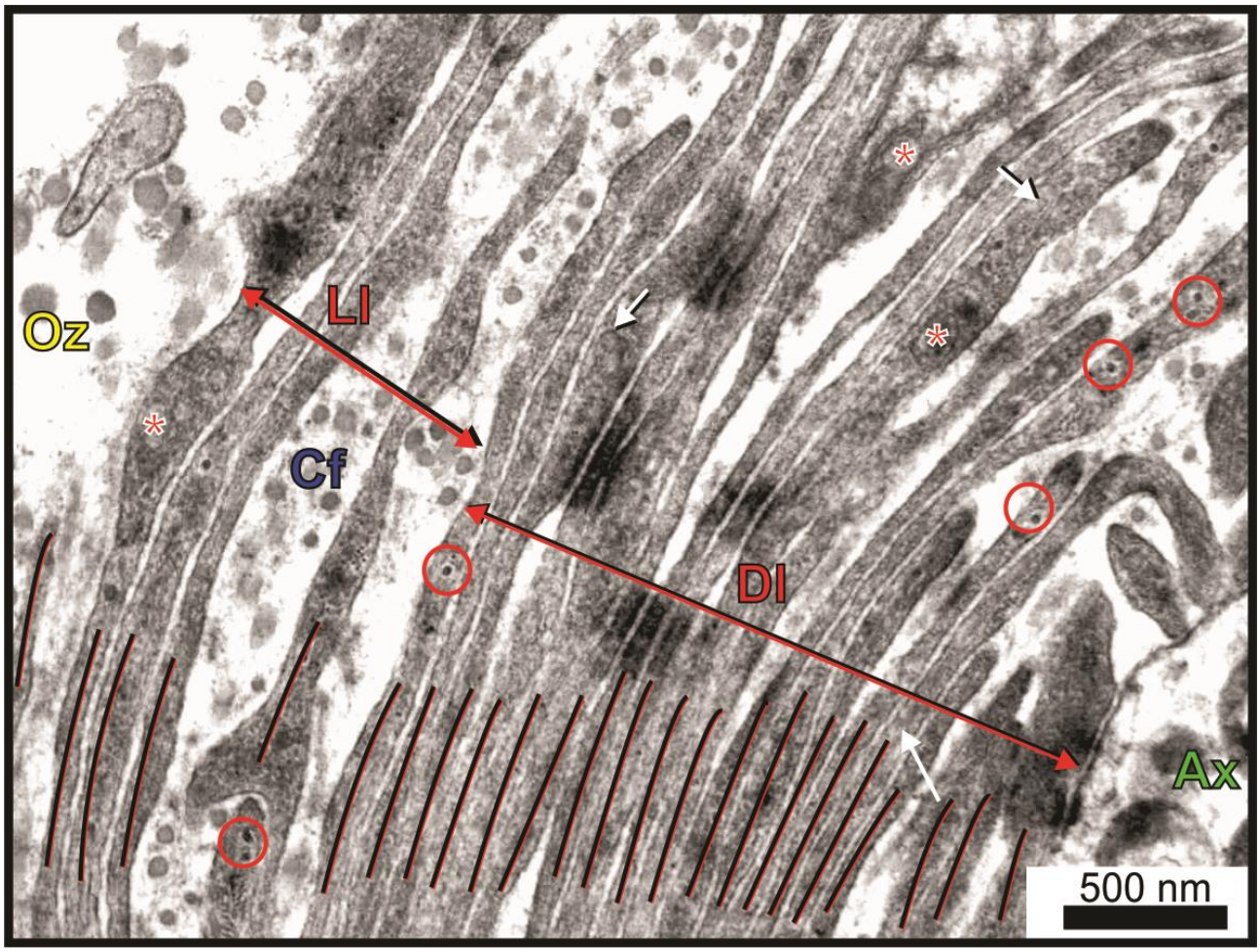

Fig. 9. Cytoplasmic lamellae of the inner core in the ostrich. A total of 24 lamellae (red lines) form loosely arranged (Ll) and densely packed lamellae (Dl) closer to the axon (Ax). Mitochondria (red *), microtubules (red circles) and micro-pinocytotic vesicles (white arrows). Collagen microfibrils (Cf) mainly oriented with their long axis parallel to the long axis of the Herbst corpuscle occur between the looser lamellae. Outer zone (Oz).

functioning of the corpuscles as described above particularly in respect of the hemi-desmosomes attaching the tips of the inner lamellae of the inner core to the axon. However, the physical effect of a contractile capsule on the response properties of Herbst corpuscles in these two species, as well as the presence of sensory cilia, adds an interesting dimension to this hypothesis, and which remains to be determined.

AUTHOR CONTRIBUTIONS

The research design was the concept of MRC. MRC and JTS collected the samples. LdP prepared the samples for TEM and digitally recorded the images. MRC compiled the manuscript and JTS acted in a supervisory role on all aspects of the work.

ACKNOWLEDGEMENTS

We thank Mrs. Petra Rough for provision of the emu heads and Tanya Claassen (Oryx Abattoir) for the ostrich and emu heads, Dr Sarah Clift for assistance with the immunohistochemistry, and the support staff of the Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria for their assistance. This work was funded by the University of Pretoria.

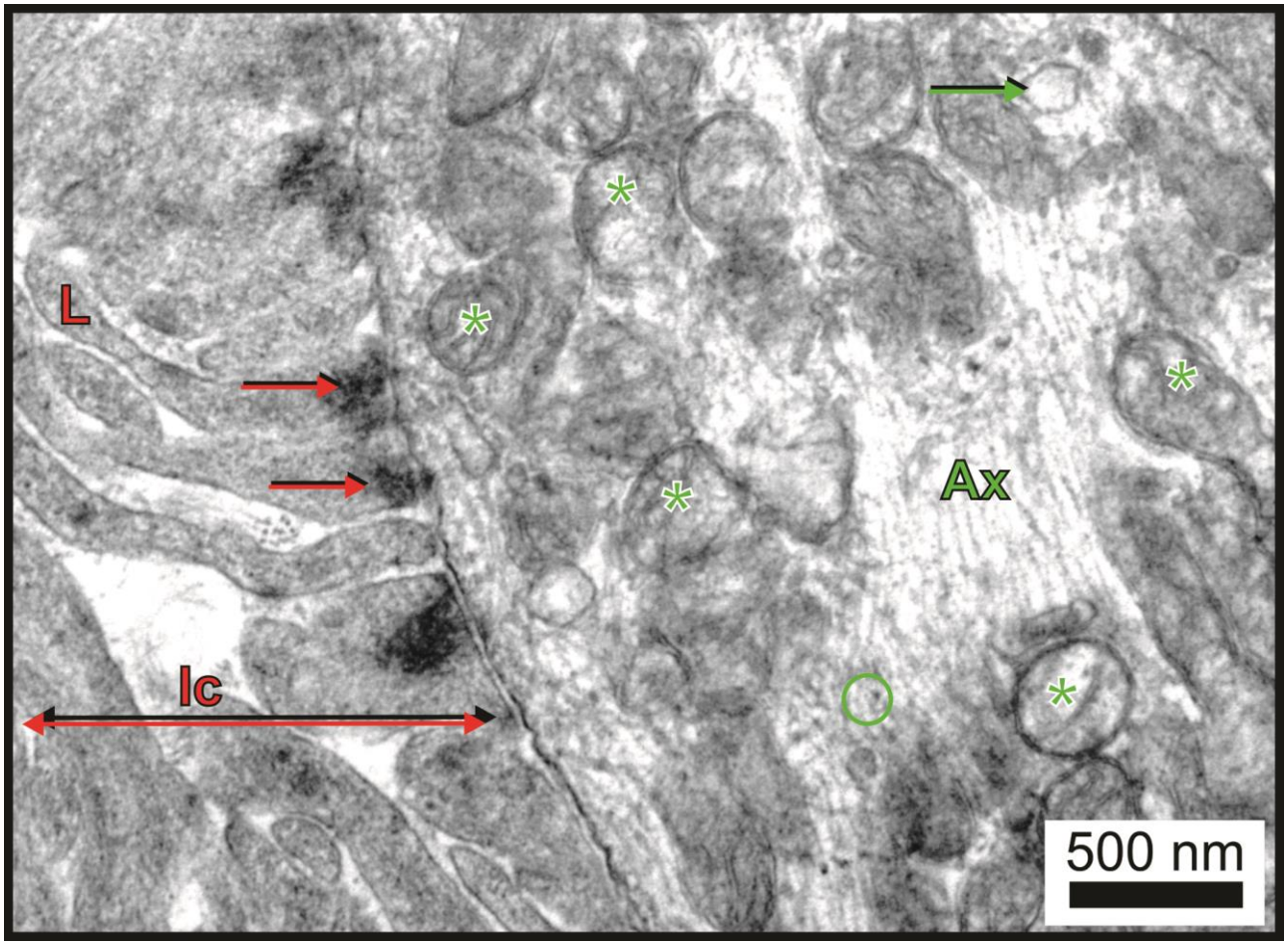

Fig. 10. Cytoplasmic lamellae (L) of the inner core (Ic) in the emu. Note how the lamellae curve towards and attach to the axolemma of the axon (Ax) via hemi-desmosomes (red arrows). Mitochondria (*), microtubule and neurofilament (green circle), vesicle (green arrow).

LITERATURE CITED

Andersen AE, Nafstad PHJ. 1968. An electron microscopic investigation of the sensory organs in the hard palate region of the hen (*Gallus domesticus*). Z Zellforsch Mikrosk Anat 91:391-401.

Bancroft JD, Gamble M. 2002. Theory and Practice of Histological Techniques. 5th ed. China: Churchill Livingston Elsevier.

Berkhoudt H. 1980. The morphology and distribution of cutaneous mechanoreceptors (Herbst and Grandry corpuscles) in bill and tongue of the mallard (*Anas platyrhynchos* L.). Neth J Zool 30:1-34.

Bolze G. 1968. Anordnung und Bau der Herbstchen Körperchen in Limicolenschnäbeln im Zusammenhang mit der Nahrungsfindung. Zool Anz 181:313-355.

Bubień-Waluszewska A. 1985. Somatic Peripheral Nerves. In: King AS, McLelland J, editors. Form and function in birds. Volume 3. London: Academic Press. p. 149-193.

Chouchkov C. 1973. On the ultrastructure of the Herbst corpuscles in the bill skin of the Pekin duck. Cr Avad Bulg Sci 26:1705–1708.

Cobb JLS, Bennett T. 1970. Herbst corpuscles in the smooth muscles in the wings of chicks. Experientia 26:768–769.

Crole MR. 2009. A gross anatomical and histological study of the oropharynx and proximal oesophagus of the emu (*Dromaius novaehollandiae*) [dissertation]. Gauteng: University of Pretoria. 175 p. Available from: http://upetd.up.ac.za/thesis/available/etd-05132009-171429/

Crole MR, Soley JT. 2009. Morphology of the tongue of the emu (*Dromaius novaehollandiae*). II. Histological features. Onderstepoort J Vet Res 76:347-361.

Crole MR, Soley JT. 2014. Comparative distribution and arrangement of Herbst corpuscles in the oropharynx of the ostrich (*Struthio camelus*) and emu (*Dromaius novaehollandiae*). Anat Rec 297:1338-1348.

Crole MR, Soley JT, du Plessis L. 2009. The ultrastructure of Herbst corpuscles in the oropharynx of the emu *(Dromaius novaehollandiae*) and ostrich (*Struthio camelus*). Proc Microsc Soc South Afr 39:27.

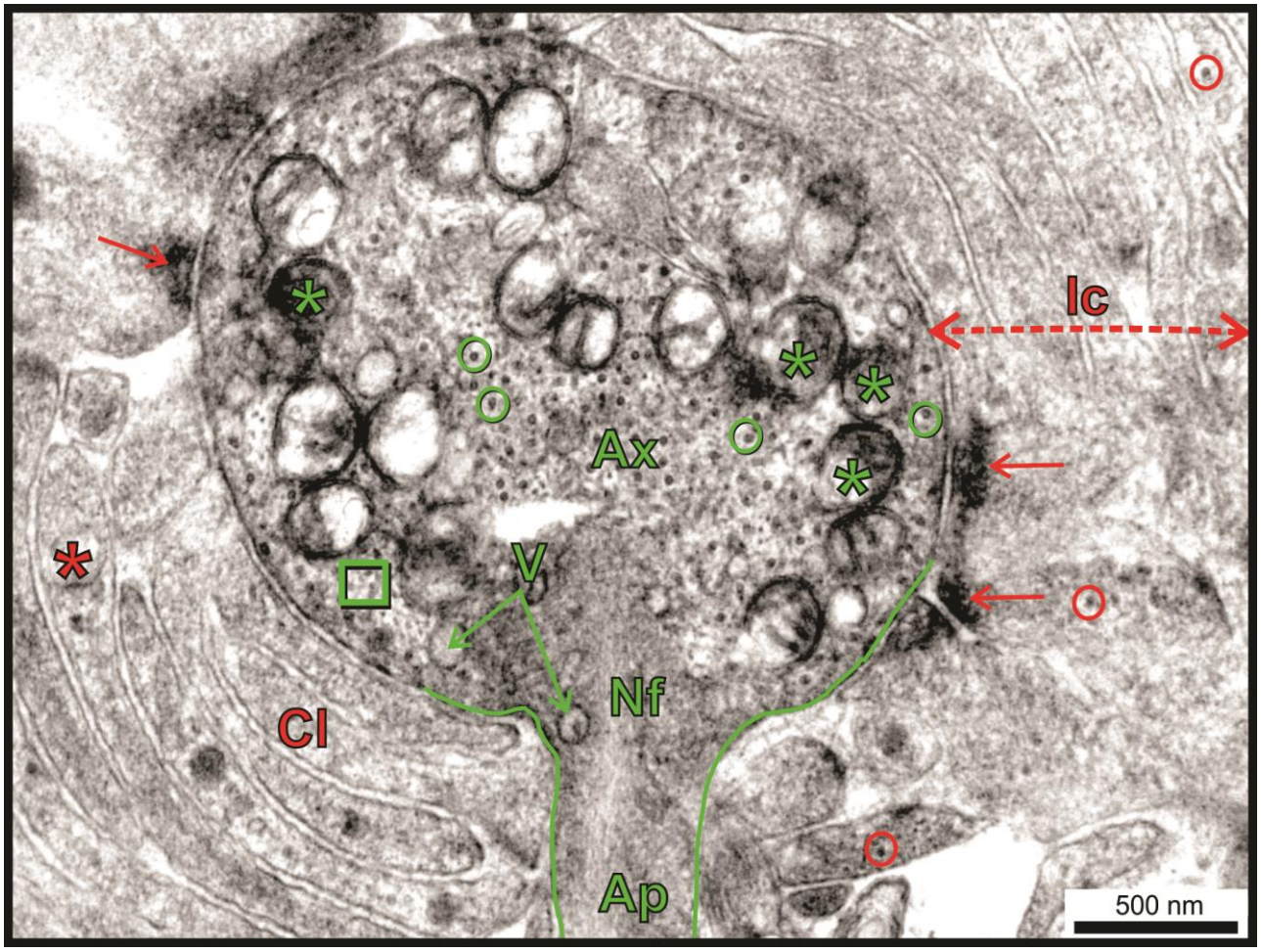

Fig. 11. Transducer site of the receptor axon (Ax) in the emu. Note the finger-like axon process (Ap) emanating from the axon and the lack of mitochondria but presence of clear vesicles (V) and neurofilament (Nf) at the point of origin. Both the axon and the process are surrounded by cytoplasmic lamellae (Cl) of the inner core (Ic) which display hemi-desmosomes (red arrows). Mitochondria (green *), axolemma (green lines) microtubules (green circles) and microfilaments (green square) are the main features of the axon. Mitochondria (red star) and microtubules (red circles) in the cytoplasmic lamellae. The swollen profiles of the mitochondria in the axon indicate inadequate ultrastructural preservation of the material.

Cunningham S, Castro I, Alley M. 2007. A new prey-detection mechanism for kiwi (*Apteryx* spp.) suggests convergent evolution between paleognathous and neognathous birds. J Anat 211:493-502.

Feder F-H. 1972. Zur mikroskopischen Anatomie des Verdauungsapparates beim Nandu (*Rhea americana*). Anat Anz 132:250-265.

Genbrugge A, Adriaens D, De Kegel B, Brabant L, Van Hoorebeke L, Podos J, Dirckx J, Aerts P, Herrel A. 2012. Structural tissue organization in the beak of Java and Darwin's finches. J Anat 221:383-393.

Gottschaldt K-M. 1985. Structure and Function of Avian Somatosensory Receptors. In: King AS, McLelland J, editors. Form and function in birds. Volume 3. London: Academic Press. p 375-462.

Gottschaldt K-M, Fruhstorfer H, Schmidt W, Kräft I. 1982. Thermosensitivity and its possible fine-structural basis in mechanoreceptors in the beak skin of geese. J Comp Neurol 205:219-245.

Gottschaldt K-M, Lausmann S. 1974. The peripheral morphological basis of tactile sensitivity in the beak of geese. Cell Tiss Res 153:477-496.

Guimarães JP, Mari RB, Miglino MA, Hernandez-Blasquez FJ, Watanabe I. 2007. Mecanoreceptores da mucosa palatine de avestruz (*Struthio camelus*): setudo ao microscópio luz. Pesquisa Vet Brasil 27:491-494.

Halata Z. 1971. Die Ultrastruktur der Lamellenkörperchen bei Wasservögeln (Herbstche Endigungen). Acta Anat 80:362-376.

Halata Z, Grim M. 1993. Sensory nerve endings in the beak skin of Japanese quail. Anat Embryol 187:131-138.

Halata Z, Munger BL. 1980. The ultrastructure of Ruffini and Herbst corpuscles in the articular capsule of domestic pigeon. Anat Rec 198:681-692.

Ilyinsky OB, Volkova NK, Cherepnov VL, Krylov BV. 1976. Morphofunctional properties of Pacinian corpuscles. Prog Brain Res 43:173-186.

Malinovský L. 1996. Sensory nerve formations in the skin and their classification. Microsc Tes Techniq 34:283-301.

Malinovský L, Páč L. 1978. Ultrastructure of simple sensory corpuscles in the domestic pigeon. Folia Morph, Prague 26:170-171.

Malinovský L, Páč L. 1980. Ultrastructure of Herbst corpuscle from beak skin of pigeon. Z Mikrosk Anat Forsc 94:292-304.

Malinovský L, Páč L. 1985. Ultrastructural development of the Herbst corpuscles in the skin of the beak of the domestic duck (*Anas platyrhynchos, f. domestica*). Folia Morphol 33:150-155.

Nafstad PHJ, Andersen AE. 1970. Ultrastructural investigation on the innervation of the Herbst corpuscle. Z Zellforsch Mik Ana 103:109-114.

Palmieri G, Acone F, Sanna M, Bo Minelli L, Botti M, Maxia M, Corriero A, De Metrio G. 2002. On the sensitive and vegetative innervation of the ostrich's palate. It J Anat Embryol 107:5-18.

Palmieri G, Cappai MG, Costa G, Bo Minelli L, Botti M, Desantis S, Corriero A, Acone F. 2006. Sensory innervation of the copulatory organ in Struthio camelus: comparison to the corresponding district in female proctodeum. It J Anat Embryol 111:31-44.

Pavelka M and Roth J. 2010. Functional Ultrastructure. Atlas of Tissue Biology and Pathology. 2nd ed. Austria: Springer-Verlag.

Pazour GJ, Witman GB. 2003. The vertebrate primary cilium is a sensory organelle. Curr Opin Cell Bio 15:105-110.

Quilliam TA, Armstrong J. 1963. Mechanoreceptors. Endeavour 22:55-60.

Saxod R. 1973. Developmental origin of the Herbst cutaneous sensory corpuscle. Experimental analysis using cellular markers. Dev Biol 32:167-178.

Saxod R. 1968. Ultrastructure des corpuscules sensoriels cutanés de Herbst et Grandry chez le canard. Arch Anat Microsc Mo 57:379-400.

Schildmacher H. 1931. Untersuchungen über die Funktion der Herbstschen Körperchen. J Ornithol 79:374-415.

Tivane C. 2008. A morphological study of the oropharynx and oesophagus of the ostrich (*Struthio camelus*) [dissertation]. Gauteng: University of Pretoria. 99 p. Available from: http://upetd.up.ac.za/thesis/available/etd-12152008-154740/

Tivane C, Soley JT, Groenewald HB. 2006. Distribution and structure of Pacinian (Herbst) corpuscles in the non-glandular region of the palate of the ostrich (*Struthio camelus*). Proc Microsc Soc South Afr 36:64.

Toyoshima K, Seta Y, Shimamura A. 1992. Fine structure of the Herbst corpuscules in the lingual mucosa of the Finch, *Lonchura striata*. Arch Histol Cytol 55:321–331.

Watanabe I, Ususkura J, Yamada E. 1985. Electron microscope study of the Grandry and Herbst corpuscles in the palatine mucosa, gingival mucosa and beak skin of the duck. Arch Histol Japon 48:89-108.

Weir KA, Lunam CA. 2006. Immunohistochemical study of cutaneous nerves in the emu. Cell Tissue Res 326:697–705.

Wight PAL, Siller WG, Mackenzie GM. 1970. The distribution of Herbst corpuscles in the beak of the domestic fowl. Brit Poultry Sci 11:165-170.

Winkelmann RK, Myers TT. 1961. The histochemistry and morphology of the cutaneous sensory end-organs of the chicken. J Comp Neurol 117:27-31.

Zelená J, Halata Z, Szeder V, Grim M. 1997. Crural Herbst corpuscles in chicken and quail: numbers and structure. Anat Embryol 196:323–333.

Ziswiler V, Trnka V. 1972. Tastkörperchen im Schlundbereich der Vögel. Revue Suisse Zool 79:307-318.