

Bony Pits in the Ostrich (*Struthio camelus*) and Emu (*Dromaius novaehollandiae*) Bill Tip

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Abstract

A specialized region of the bill tip characterized by a complex arrangement of mechanoreceptors and referred to as a bill tip organ, has been identified in numerous avians. A bill tip organ was initially inferred in kiwi species by the presence of numerous, bony pits in the rostrum of the bill, and later confirmed histologically. This study enumerates and compares the number and distribution of pits present in the bill tip in the ostrich and emu. The heads from 10 ostrich and 5 emu were prepared for osteological examination. The pattern and total number of pits was similar between the two species. However, the ostrich had significantly more pits in the regions underlying the *Culmen* and *Gonys*, whereas the emu displayed significantly more pits in the dorsal part of the mandibular rostrum. The relatively even distribution of pits in the inner and outer surfaces of both the mandibular and maxillary rostra suggest that the bill tip of the ostrich and emu are equally sensitive externally and intra-orally, as opposed to probing birds, where the major concentration of pits is located on the outer surfaces of the bill tips. The presence of pits in the bill tips of extant palaeognaths may be of relevance in interpreting the pits in the rostra of extinct theropod dinosaurs. The presence of bony pits in a region which is also well supplied with sensory nerves is highly suggestive of a bill tip organ in the ostrich and emu and which needs to be confirmed histologically.

Keywords: Premaxilla; rostrum; pits; bill tip; ratite

INTRODUCTION

In the ostrich and emu, Herbst corpuscles are reportedly present in bony pits on the intra-oral surfaces of the bill tip (Crole and Soley, 2014) as well as in pits occupying the most rostral extremity of the bill tip (Crole and Soley, 2016). Based on these studies it would appear that the bill tip of the ostrich and emu bears bony pits, yet despite numerous osteological studies in various ratite species (see below) these pits have not been described nor their significance determined. Earlier studies of the ratite skull and mandible have largely been restricted to their embryological development with the result that the equivalent structures in adult birds are not well documented. Parker (1866) described the early development of the skull and depicted pits in the bill tip in the ostrich (*Struthio camelus*), greater rhea (*Rhea americana*), emu (*Dromaeus Novae-Hollandiae* and *Dromaeus irroratus*) and dwarf cassowary (*Casuarius bennettii*) while the development of the cranium of the greater rhea was studied in detail by Müller (1963). More recent research on the mandible (Rayfield, 2011) and cranium (Cuff et al., 2015) of the ostrich

was aimed at validating specimen-specific finite elemental models and the basic descriptive anatomy or the presence of pits was not addressed. Although the structure of the bony palate of ratite species has been widely studied (Müller, 1963; Webb, 1957; Bock, 1963; Dzerzhinsky, 1999; Gussekloo and Bout, 2002, 2005; Maxwell, 2009; Johnston, 2011), the morphology of the maxillary and mandibular rostra (bill tip) has been largely neglected. These studies have generally been aimed at determining the phylogeny of this superorder (Palaeognathae) and have not focused on descriptions of anatomical structures per se. The embryological development of the bony part of the kiwi (*Apteryx oweni*, *A. bulleri* (presumably *A. mantelli*) and *A. haastii*) bill, including a depiction of the pits, has been described (Parker, 1891) and the rostral portion, including pits, investigated in relation to the bill tip organ in various kiwi species (*A. australis*, *A. owenii*, *A. haastii*, *A. rowi* and *A. mantelli*) (Cunningham et al., 2007). A more recent, detailed description of the rostral bill tip has been presented for *A. mantelli* using micro-computed tomography (Cunningham et al., 2013). Whereas the distal maxillary tip of the hatchling ostrich has been briefly

studied by scanning electron microscopy (Richardson et al., 1998), no mention of bill tip pits was made in this species.

A specialized region of the bill tip characterized by a complex arrangement of mechanoreceptors and referred to as a bill tip organ, has been identified in numerous birds including the woodcock (*Scolopax*) and snipe (*Gallinago*) (Goglia, 1964), sandpipers (*Limicolae*) (Bolze, 1968), geese (*Anser anser* and *Anser albifrons*) (Gottschaldt and Lausmann, 1974), finches (*Fringillidae*) (Krusis, 1978), ducks (*Anas platyrhynchos*, *Aythya fuligula* and *Anas clypeata*) (Berkhoudt, 1976; 1980), chicken (*Gallus domesticus*) (Gentle and Breward, 1986), Japanese quail (*Coturnix japonica*) (Halata and Grim, 1993) and ibises (*Threskiornithidae*) (Cunningham et al., 2010a, b). A bill tip organ was inferred (and confirmed histologically) in kiwi species by the presence of numerous, obvious bony pits in the rostrum of the bill (Cunningham et al., 2007) termed *Foveae corpusculorum nervosorum* (Baumel and Witmer, 1993). The sketches of Parker (1866) and digital images available on the internet (Dodds, 2013; Franzosa, 2013) of the ostrich and emu skull similarly reveal numerous small, bony pits in the bill tip although they are not specifically identified as such. Herbst corpuscle structure (Crole et al., 2015) and their distribution in the oropharynx of the ostrich and emu (Crole and Soley, 2014) has been described. Additionally, the bill tip is richly innervated (Crole and Soley, 2016). This study aims to describe a component of the hypothesized bill tip organ of the ostrich and emu by comparing the distribution, pattern and number of bill tip pits in these two species.

MATERIALS AND METHODS

A total of 10 adult ostrich and 5 adult emu heads, from birds of either sex, were collected after slaughter from the Klein Karoo Ostrich abattoir (Oudtshoorn, Western Cape, South Africa) and Oryx Abattoir (Krugersdorp, Gauteng, South Africa). All heads were thoroughly rinsed with running tap water to remove mucus, blood and regurgitated food.

Preparation of osseous elements of the upper and lower bill

The fresh ostrich (n=10) and emu (n=5) heads were prepared for osteological examination of the bony elements relevant to the structure of the mandible and premaxilla. The heads were left at room temperature for a few days to facilitate softening and subsequent removal of as much soft tissue as possible, after which they were boiled, the remaining soft tissues manually removed from the bones, and the skull and mandible de-fatted in trichloroethylene. The relevant gross anatomical features of the skull and mandible and the structure of the premaxilla and mandibular rostrum was noted and digitally recorded with a Canon EOS 5D digital camera (Canon, Oita, Japan) equipped with a Canon Macro 100mm lens. As it was not possible in the prepared specimens to determine which pits carried Herbst corpuscles (*Foveae corpusculorum nervosorum* (sensory pits) (Baumel and Witmer, 1993)), blood vessels and nerves (*Foramina [Pori] neurovascularia* (neurovascular pits) (Baumel and Witmer, 1993)) or both, all the pits in the body of the premaxilla, the mandibular rostrum and distal mandibular arms of each bird were identified by stereomicroscopy, counted and the mean values determined for the ostrich and emu.

Stereomicroscopy was performed using an Olympus SZX16 stereo microscope (Olympus Corporation, Tokyo, Japan) equipped with a DP72 camera and Olympus cellSens imaging software (Olympus Corporation, Tokyo, Japan).

Statistical Analysis

The null hypothesis, that the ostrich and emu were similar (in respect of the aspects studied), was tested by a Student's t test (a 2 sample assuming unequal variances) or the Mann-Whitney Rank Sum Test (where the normality test (Shapiro-Wilk) and/or equal variance test has failed (<0.05)). Values expressed were calculated using SigmaPlot, version 12.0 (Systat Software, San Jose, CA, USA) and comprised the mean, median, standard deviation, standard error of the mean, Mann-Whitney U statistic, significance and power of the test performed with alpha. Significance was set at p=0.05. A value of p<0.05 rejected the null hypothesis and a value of p>0.05 supported the null hypothesis.

RESULTS

Gross morphology

Left and right mandibular arms (*Ramus mandibulae*), which united rostrally at the mandibular symphysis, formed the mandibular rostrum in both the ostrich and emu (Fig. 1). The dentary bone (*Os dentale*) was the most rostral component and formed the mandibular rostrum (*Rostrum mandibulae*) and the distal portion of the mandibular arm (Fig. 1). Three parts of the dentary bone could be identified, the dorsal part (*Pars dorsalis*), the ventral part (*Pars ventralis*) and the symphyseal part (*Pars symphysealis*) (Fig. 1). On the ventral aspect a raised rectangular bony prominence was present overlying the region of the mandibular symphysis. This prominence was considerably wider in the ostrich than in the emu and supported the overlying, raised thickening of rhamphotheca, the *Gonyx* ("the midventral ridge of rhamphotheca lying superficial to the *Rostrum [Symphysis] mandibulae*" (Clark, 1993)) (Fig. 2e,f, 3a,b).

The rostral portion of the upper bill was supported by the premaxillary bone (Fig. 4) which represented the most rostral portion of the skull in the ostrich and emu. The relationship of the premaxilla to the surrounding bones (maxilla, palatal bone, vomer, rostrum (ostrich only), as depicted by Webb (1957) and confirmed in this study, of the ventral aspect of the skull is shown in Figure 4a and b. The premaxilla was composed of a rostral body (*Corpus ossis premaxillare*) which formed the maxillary rostrum (Fig. 4a,b) and three (in the ostrich) (Fig. 4c) or five (in the emu) (Fig. 4d) caudally directed processes. These comprised the maxillary processes, frontal process and, in the emu, the palatal processes. The median frontal process (*Processus frontalis*) was 3 to 4 times wider in the ostrich than in the emu (Fig. 4c,d). In both species the frontal process merged with the body of the premaxilla forming a raised area which, together with the frontal process, constituted the underlying bony support of the Culmen ("the middorsal ridge of the *Rostrum maxillare* extending from the tip of the bill to the base of the feathers on the forehead at or near the craniofacial angle" (Clark, 1993)) (Fig. 3a-d, 4c-d).

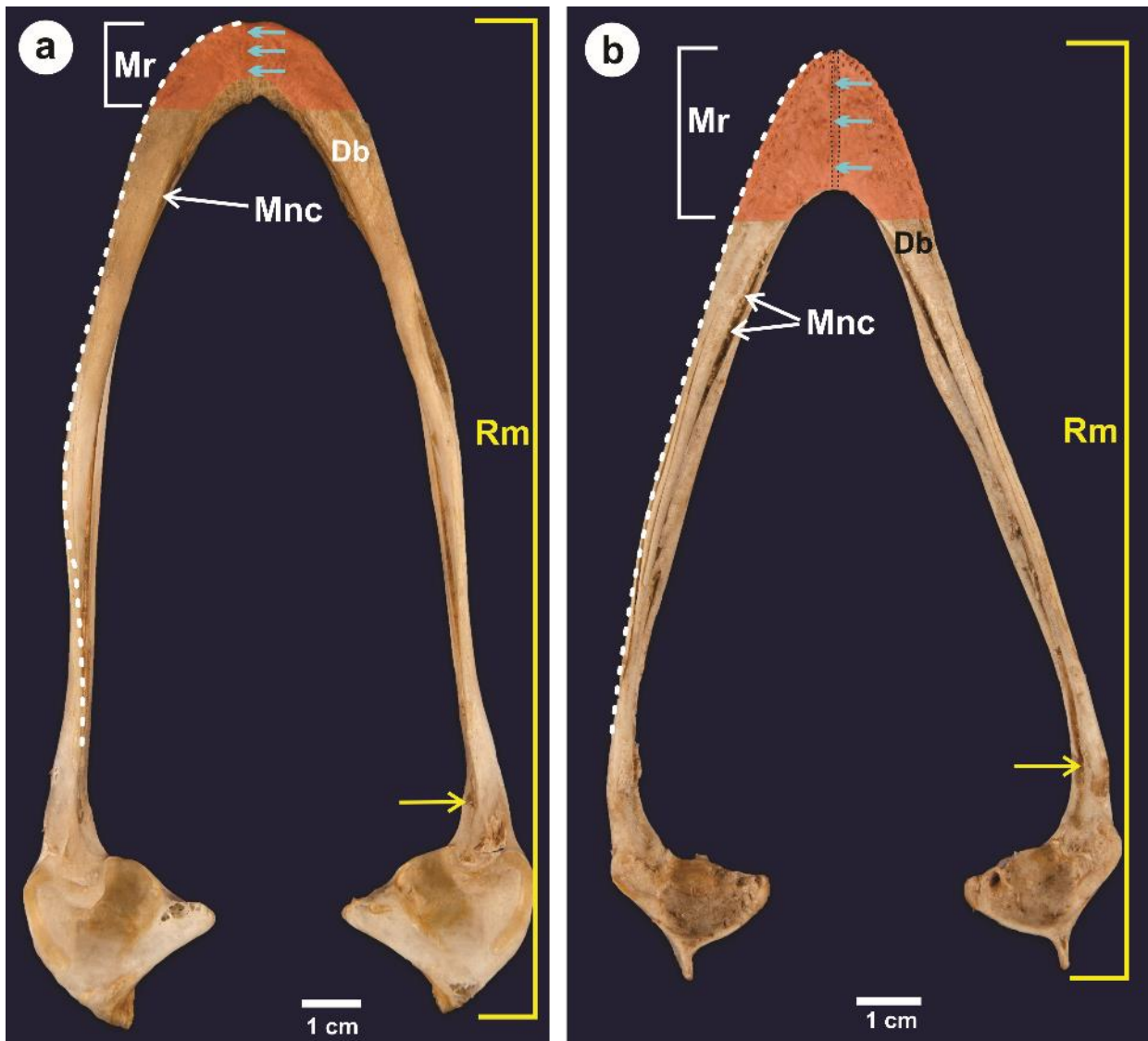


Fig. 1. Dorsal view of the ostrich (a) and emu (b) mandible showing the *Ramus mandibulae* (Rm) and mandibular rostrum (Mr). Two components of the dentary bone (Db) are visible, namely, the *Pars dorsalis* (shaded red) and the *Pars symphyialis* (turquoise arrows). The tomial crest (dotted white line) is located dorso-laterally on the mandibular rostrum and *Ramus mandibulae*. The point of entry of the intramandibular nerve into the mandibular neurovascular canal (Mnc) is indicated by yellow arrows. Black dotted line in Fig. 1b indicates the shallow groove of the mandibular symphysis.

Numerous openings, presumably both neurovascular foramina (*Foramina [Pori] neurovascularia*) as well as sensory pits (*Fovea corpusculorum nervosorum*), were present on the dorsal and ventral surfaces of the mandibular rostrum (Fig. 2, 3a,b) and premaxillary body (Fig. 3c-f, 5) where they were arranged in specific patterns in both the ostrich and emu (see below).

The distribution, pattern and number of pits in the bill tip

Distribution and pattern of pits. Numerous neurovascular openings and sensory pits were present on the dorsal and ventral surfaces of the mandibular and maxillary rostra and extended a short distance onto the distal region of the intermediate part of the mandibular arms (Fig. 2, 3). The pits were round to oval in shape and slightly larger in the ostrich than in the emu. The majority of the pits opened vertically onto the surface; however, some of the pits emerged at a slight angle which corresponded to the location of these structures. The more rostrally positioned pits tended to slant rostrally, laterally positioned pits slanted laterally and those located more caudally slanted caudo-laterally.

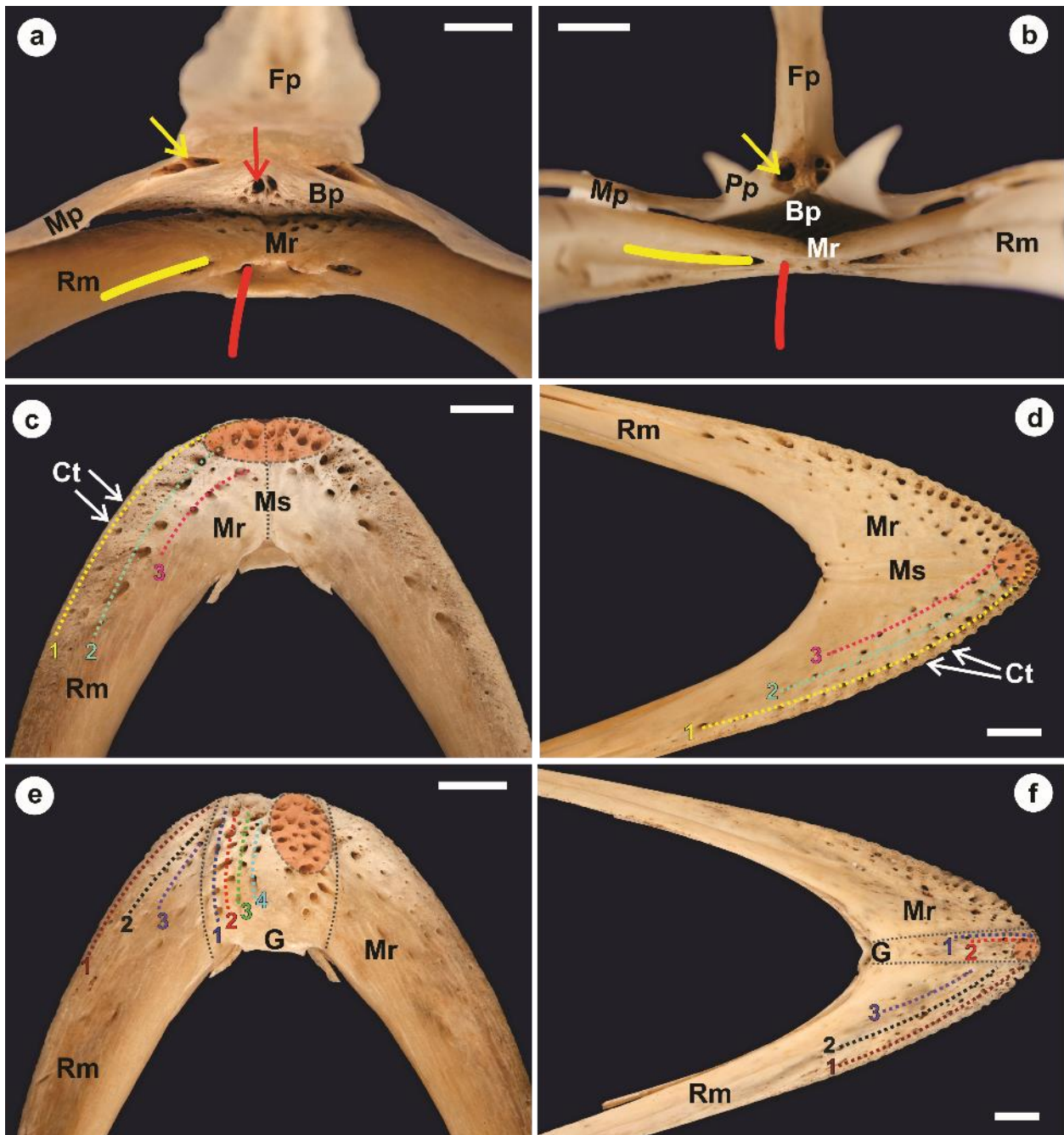


Fig. 2. Distribution of pits in the bill tip of the ostrich (a, c, e) and emu (b, d, f). (a and b). Caudal (intra-oral) view of the bill tip. Note the body (Bp), frontal process (Fp), maxillary processes (Mp) and palatal processes (Pp) (emu only) of the premaxilla, and the mandibular rostrum (Mr) and *Ramus mandibulae* (Rm) of the lower bill. *Canalis neurovascularis maxillae* (yellow arrow), *N. intramandibularis* (yellow line), branch of the sublingual artery (red line) and foramen for the median palatine artery (red arrow, ostrich only). (c and d). Dorsal (intra-oral) view of the mandibular rostrum (Mr). Three main rows of pits (numbers 1-3 and dotted lines) are present in both species. Concentration of pits at the rostral tip (orange-shaded region), mandibular symphysis (Ms), *Ramus mandibulae* (Rm) and *Crista tomialis* (Ct). (e and f). Ventral (external) view of the mandibular rostrum (Mr). The raised portion of the rostrum which corresponds to the external *Gonys* (G) is outlined in grey for clarity and which in the ostrich displays 4 rows, and in the emu, 2 rows of pits (numbers and dotted lines) on either side of the midline. In both species 3 rows of pits (numbers 1-3 and dotted lines) extend a short distance onto the *Ramus mandibulae* (Rm). Concentration of pits at the rostral tip (orange-shaded region). Scale bars = 5 mm.

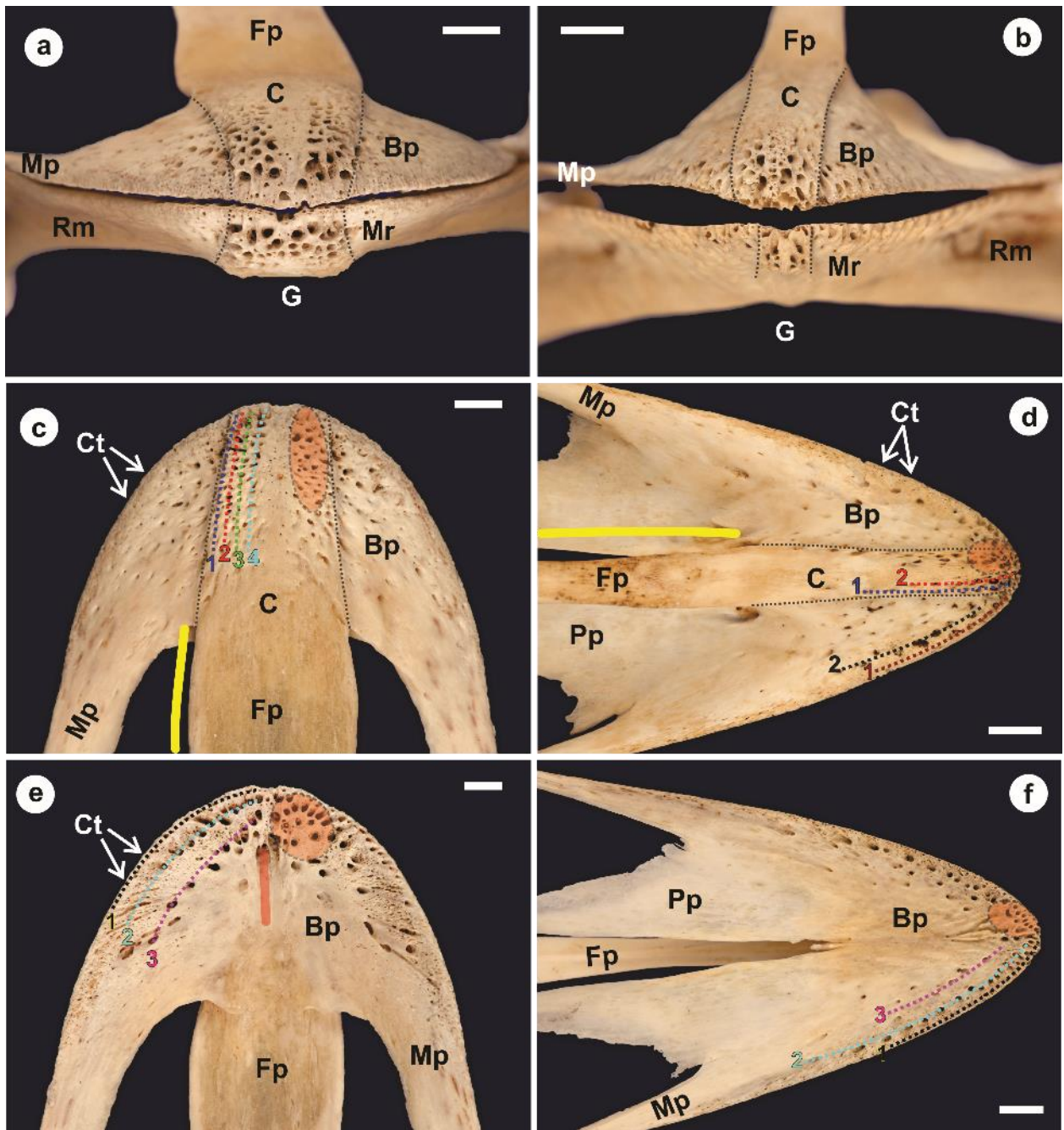


Fig. 3. Distribution of pits in the bill tip of the ostrich (a, c, e) and emu (b, d, f). (a and b). Rostral (external) view of the bill tip. Note the body (Bp), frontal process (Fp) and maxillary processes (Mp) of the premaxilla, and the mandibular rostrum (Mr) and *Ramus mandibulae* (Rm) of the lower bill. The part of the premaxilla (C) and mandibular rostrum (G) supporting the overlying *Culmen* and *Gonys*, respectively, are outlined in grey. Note the honey-comb appearance of the rostral bill tip due to the concentration of pits in this region. (c and d). Dorsal view of the premaxilla. Rows of pits (numbers and dotted lines) occur on the bony region underlying the *Culmen* (C). Pits on the lateral parts of the body of the premaxilla (Bp) form rows (numbers and dotted lines) in the emu but are randomly dispersed in the ostrich. Concentration of pits at the rostral tip (orange-shaded region). *Crista tomialis* (Ct), *N. ophthalmicus* *R. medialis* (yellow line) and palatal process (Pp) (emu only) of the premaxilla. (e and f). Ventral (intra-oral) view of the premaxilla. Three to four rows of pits (numbers and dotted lines) are present in both species. Median palatine artery (red line). Concentration of pits at the rostral tip of the rostrum (orange-shaded region). Scale bars = 5 mm.

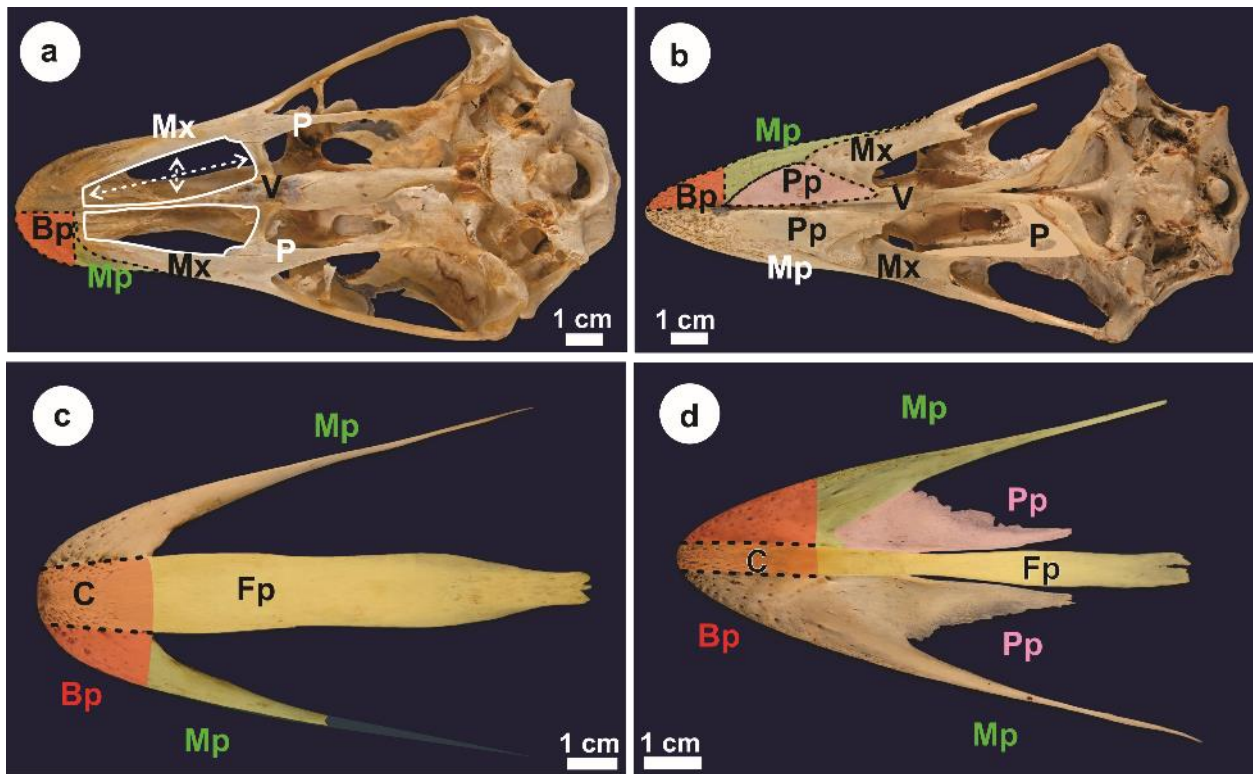


Fig. 4. Ventral view of the ostrich (a) and emu (b) skull and dorsal view of the ostrich (c) and emu (d) premaxilla. (a and b).The premaxilla is shown in relation to the skull. Dried rhamphotheca covers the premaxilla in the ostrich. Note the large gaps (white outline and dotted arrows) enclosed by the body of the premaxilla (Bp), maxillary process (Mp), maxilla (Mx) and vomer (V) in the ostrich. The corresponding region in the emu is filled by the palatal process (Pp). The palatal bone (P) is not shown in the emu skull as it was lost during boiling, but has been schematically drawn in on the right side. (c and d). Note the wider region of the premaxilla underlying the *Culmen* (C) in the ostrich compared to the emu. The body of the premaxilla (Bp) is U-shaped in the ostrich and V-shaped in the emu. Frontal process (Fp), maxillary process (Mp) and palatal process (Pp) (emu only).

The most rostral extremity of the bill tip in the ostrich and emu was densely packed with pits giving this region a honeycomb appearance (Fig. 3a,b). Viewed rostrally, this concentration of pits was generally confined to the bony regions underlying the *Culmen* and *Gonys* in both species (Fig. 3a,b), although in the emu the immediate surrounding bone was also heavily pitted (Fig. 3b). The large openings visible in caudal view (Fig. 2a,b) were not considered to represent bill tip pits. These cavities allowed the entry of large nerves such as the *N. ophthalmicus* *R. medialis* and the *N. intramandibularis* (Crole and Soley, 2016) and blood vessels such as the median palatine artery and a branch of the sublingual artery (Crole and Soley, 2014) into the premaxilla and mandibular rostrum respectively (Fig. 2a,b, 3c-e).

The pits displayed a discernible pattern of distribution which was basically similar in both species (Fig. 2, 3, 5). Three main rows of pits were present on the dorsal surface of the mandibular rostrum (Fig. 2c,d). Row 1 lay adjacent to the *Crista tomialis* and rows 2-3 were situated medial and parallel to row 1 (Fig. 2c,d). In the ostrich the pits in row 1 were smaller than those in rows 2-3, which were similarly sized (Fig. 2c), whereas in the emu all the pits in rows 1-3 were similar in size (Fig. 2d). The more peripheral 1st and 2nd rows extended further onto the mandibular arms than the inner 3rd

row (Fig. 2c,d). The pits were positioned closer to each other towards the tip of the rostrum resulting in a higher concentration in this region (Fig. 2c,d). The distribution of pits differed slightly between the ostrich and emu on the ventral surface of the mandibular rostrum. On the raised bony region underlying the *Gonys* in the ostrich were 4 rows of pits, on either side of the midline (Fig. 2e), and only 2 rows of pits in the emu (Fig. 2f). This phenomenon reflected the broader region underlying the *Gonys* in the ostrich. In both species, 3 rows of pits were present on the remainder of the rostrum and the *Ramus mandibulae* and distal aspect of the mandibular arms (Fig. 2e,f). However, the rows did not extend as far onto the mandibular arms as on the dorsal surface (compare Fig 2c,d to Fig 2e,f). As observed on the dorsal surface, in the ostrich the pits in row 1 were smaller than those in rows 2-3 and not as well-defined (Fig. 2e), whereas in the emu the pits in rows 1-3 were similarly sized (Fig. 2f). Row 3 in both species was ill-defined (Fig. 2e,f). The pits on the ventral surface of the mandibular rostrum were also concentrated at the rostral tip (Fig. 2e,f).

In the premaxilla the bony region underlying the *Culmen* was wider in the ostrich than in the emu (Fig. 3a-d). In both species the midline of this region was free of pits whereas on the lateral edges were 4 rows of pits in the ostrich (Fig. 3c) and

2 rows in the emu (Fig. 3d). As in the lower bill, the pits were most heavily concentrated at the tip of the rostrum, both dorsally and ventrally (Fig. 3c-f). In the ostrich, the lateral parts of the dorsal premaxillary rostrum did not display a discernible pattern of pits (Fig. 3c), whereas in the emu, 2 ill-defined rows of pits were present (Fig. 3d). Three to four rows of pits were present on the ventral surface of the premaxillary rostrum in both species (Fig. 3e,f). Row 1, adjacent to the tomial crest, was formed by small pits, and rows 2-3 composed of larger pits were ill-defined in both species (Fig. 3e,f). The pits on the ventral surface in the ostrich were markedly larger than those in the emu (Fig. 3e,f).

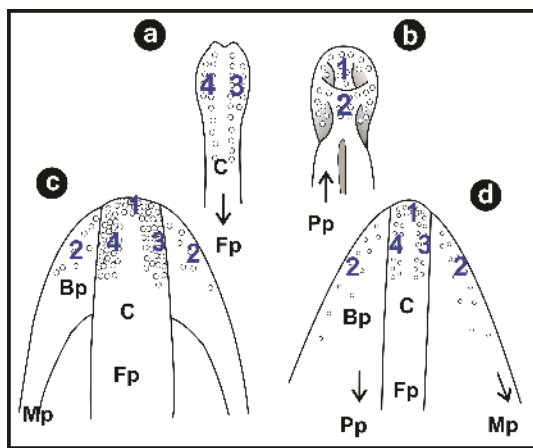


Fig. 5. Schematic representation of the pattern of pits on the premaxilla in the kiwi chick (a and b) and adult ostrich (c) and emu (d). (a). Dorsal premaxilla. Dorsal zones (3 and 4), bony region underlying the Culmen (C) which caudally forms the frontal process (Fp). (b). Ventral premaxilla. Zone 1 represents the tactile disc and zone 2 the pre-oral zone. Palatal process (Pp). (c and d). Dorsal views. Bony area underlying the Culmen (C), body of the premaxilla (Bp), maxillary (Mp), frontal (Fp) and palatal (emu) (Pp) processes. The sketches are not drawn to scale. (Kiwi data adapted from original sketch of the bill by Parker (1891) and numbered zones and pits depicted by Cunningham et al. (2007)).

Number of pits. In the following section reference to the mandibular rostrum implies the rostrum and distal extremity of the mandibular arms, and reference to the premaxilla implies the body of the premaxilla. The number of pits present on the dorsal and ventral surfaces of the mandibular rostrum and premaxilla of the ostrich and emu is reflected in Tables 1 and 2. Relevant data for the individual birds is presented in supplementary tables 1 and 2.

The emu displayed more pits on the mandibular rostrum than the ostrich due to the greater number of pits on the dorsal (intra-oral) surface (Table 1). Although the number of pits on the ventral surface of the mandibular rostrum was similar between the two species, the bony region underlying the *Gonys* in the ostrich contained twice as many pits as the comparable region in the emu (Table 1). Thus the ostrich displayed a regional concentration of pits on the ventral mandibular rostrum, whereas in the emu the pits were more evenly distributed over the entire ventral surface.

The ostrich demonstrated a greater number of pits on the dorsal surface of the premaxilla than in the emu as well as on the premaxilla as a whole (Table 2). This difference was due to the bony region underlying the *Culmen* containing twice as many pits as the comparable region in the emu (Table 2). The remaining regions of the premaxilla reflected a similar number of pits between the two species (Table 2).

As a unit, the bill tip of the ostrich displayed more pits on the outer surfaces (ventral mandibular rostrum (Table 1) and dorsal premaxilla (Table 2)) than on the inner (intra-oral) surfaces. The regional concentration of pits in the raised bony areas underlying the *Gonys* (Table 1) and *Culmen* (Table 2) appeared to be responsible for this effect in the ostrich. In both species the bony region underlying the *Culmen* (Table 2) contained twice as many pits as the corresponding *Gonys* (Table 1), whereas in total the ostrich displayed twice as many pits to that in the emu for these regions (Tables 1 and 2). The only region in which the emu displayed more pits than the ostrich was on the dorsal surface of the mandibular rostrum (Table 1). In the emu, the premaxilla and mandibular rostrum contained a similar number of pits, whereas in the ostrich more pits occurred on the premaxilla. If the data in table 1 and 2 are combined, no statistically significant difference ($p = 0.3$) in the total number of pits in the bill tip (mandibular rostrum and premaxilla) of the ostrich (350 ± 35.06) and emu (329.6 ± 32.62) is apparent.

DISCUSSION

Number and distribution of pits in the bill tip

There was no statistically significant difference ($p=0.3$) between the total number of pits in the bill tip in the ostrich and emu. However, their distribution differed significantly between the two birds with pits being more concentrated in the regions underlying the *Culmen* and *Gonys* in the ostrich whereas in the emu more pits were present on the dorsal surface of the mandible. These two phenomena could be related to the physical size of these regions, with the raised bony regions underlying the *Culmen* and *Gonys* being much wider in the ostrich than the equivalent regions in the emu, and the mandibular rostrum in the emu displaying a larger surface area than in the ostrich. It has been noted that in different sized species of ducks, specialized dermal papillae in the bill (originating from the deep dermis and containing Herbst corpuscles in a highly organized fashion), although of similar size, varied in number depending on the size of the duck (Berkhoudt, 1976). Thus the statistical significance in the number of pits present in the region of the *Gonys* and *Culmen* (ostrich>emu) may be directly related to the space available for the pits. Similarly, more pits were present in the dorsal part of the mandibular rostrum of the emu (which compared to the ostrich is larger in the emu).

The only other ratite family which has been studied with regards to the number and distribution of pits in the bill tip is the Apterygidae. The sensory pits were counted in 5 different kiwi species (*A. australis*, *A. haastii*, *A. mantelli*, *A. owenii*, *A. rowi*) ($n=22$) and the data on the five individual species as well as the average of the 22 specimens examined (Cunningham et al., 2007) was presented. A further study which counted the pits in a limited region of the bill tip in *A. mantelli* ($n=3$)

TABLE 1. Comparative data on the number of pits present on the mandibular rostrum of the ostrich (n=10) and emu (n=5).

Ostrich	Ventral (external) MR			Ventral MR Total	Dorsal (intra-oral) MR		Dorsal MR Total	MR Total
	Left	<i>Gonys</i>	Right		Left	Right		
Mean	22.50	40.50*	25.60	88.60	25.20	25.00	50.20	138.80
Median	21	40.5	25	88	25.50	26	52	142
SD	7.17	5.68	8.34	16.61	3.16	4.50	6.61	18.79
SEM	2.27	1.80	2.64	5.25	-	1.42	2.09	5.94
Emu								
Mean	30.40	20.00	31.8	82.20	40.40	44.00*	84.40*	166.60*
Median	29	21	32	82	41*	45	81	160
SD	8.82	2.92	4.76	14.53	-	4.18	10.31	22.81
SEM	3.95	1.30	2.12	6.50	-	1.87	4.61	10.20
NT	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
EVT	Pass	Pass	Pass	Pass	Fail	Pass	Pass	Pass
MUS	-	-	-	-	0	-	-	-
t	-1.87	7.49	-1.52	0.73	-	-7.88	-7.87	-2.52
df	13	13	13	13	-	13	13	13
p value	0.08	<0.001*	0.15	0.48	0.002*	<0.001*	<0.001*	0.03*
Pa	0.30	1	0.18	0.05	-	1	1	0.57

The ventral surface of the rostrum is divided into the *Gonys* and left and right regions on either side of it including the distal parts of the mandibular arms. The dorsal surface of the rostrum is divided into left and right halves. Mandibular rostrum (MR), Standard Deviation (SD), Standard Error of the Mean (SEM), Normality Test (Shapiro-Wilk) (NT), Equal Variance Test (EVT), Mann-Whitney U Statistic (MUS), t value (t), degrees of freedom (df), significance (p value), power of the test performed with alpha (Pa). Asterisks (*) indicate a significant difference.

TABLE 2. Comparative data on the number of pits present on the premaxilla of the ostrich (n=10) and emu (n=5).

Ostrich	Dorsal (external) PM			Dorsal PM Total	Ventral (intra-oral) PM		Ventral PM Total	PM Total
	Left	<i>Culmen</i>	Right		Left	Right		
Mean	25.30	87.00*	27.20	139.50*	35.10	36.60	71.70	211.20*
Median	22.50	87	28.5	133.5	32	35	70	204
SD	7.24	10.51	6.09	19.67	4.56	6.77	10.42	22.04
SEM	-	3.23	1.93	6.22	-	-	-	6.97
Emu								
Mean	23.00	44.80	24.60	92.40	37.00	33.60	70.60	163.00
Median	23	42	25	96	34	33	67	163
SD	3.32	9.31	2.97	10.07	7.58	4.51	11.72	19.88
SEM	-	4.16	1.33	4.50	-	-	-	8.89
NT	Fail	Pass	Pass	Pass	Fail	Fail	Fail	Pass
EVT	-	Pass	Pass	Pass	-	-	-	Pass
MUS	25	-	-	-	21	18.5	22.5	-
t	-	7.59	0.89	4.97	-	-	-	4.11
df	-	13	13	13	-	-	-	13
p value	1	<0.001*	0.39	<0.001*	0.66	0.46	0.80	0.001*
Pa	-	1	0.05	1	-	-	-	0.97

The dorsal surface of the premaxilla is divided into the *Culmen* and left and right regions on either side of it. The ventral surface of the premaxilla is divided into left and right halves. Premaxilla (PM), Standard Deviation (SD), Standard Error of the Mean (SEM), Normality Test (Shapiro-Wilk) (NT), Equal Variance Test (EVT), Mann-Whitney U Statistic (MUS), t value (t), degrees of freedom (df), significance (p value), power of the test performed with alpha (Pa). Asterisks (*) indicate a significant difference.

(Cunningham et al., 2013) is not included in the following comparison. Taking into consideration that the counts in the kiwi (5 species), ostrich and emu were performed by different researchers any comparison should be interpreted with caution. Although the data cannot be submitted to statistical evaluation, they can, however, provide a basis for comparison between these three ratite genera. On average, the 5 kiwi species displayed a slightly smaller total number of pits in the

bill (288 ± 58) (Cunningham et al., 2007) compared to both the ostrich (361.6 ± 45.10) and emu (329.6 ± 32.62); however, in *A. australis* the count was similar (315 ± 23) while *A. rowi* displayed substantially more pits (407 ± 8) (Cunningham et al., 2007). Average pit numbers in the mandible of the 5 kiwi species (142 ± 32) (Cunningham et al., 2007) were similar to those of the ostrich (138.8 ± 18.79) and slightly less than in the emu (166.6 ± 22.81), except for *A. rowi* (203 ± 11)

(Cunningham et al., 2007) which was higher than both the ostrich and emu. The average number of pits in the premaxilla of the 5 kiwi species (146 ± 35) (Cunningham et al., 2007) was similar to that in the emu (163 ± 19.87) but markedly less than in the ostrich (211.2 ± 22.04), except for *A. rowi* (204 ± 3) (Cunningham et al., 2007) which displayed a similar value to that of the ostrich. It was not possible from the available data on the kiwi to determine whether there were any regional differences in distribution, as was seen in the ostrich and emu. However, Cunningham et al. (2013) do show that the pits are more concentrated towards the distal bill tips in *A. mantelli*. It is interesting to note that the two Australasian ratite genera (*Apteryx* and *Dromaius*) (excluding *A. rowi*) display affinities in respect of the number of pits present in the bill tips, despite the obvious size difference between these birds.

The arrangement of bony pits in rows in the premaxilla (see Fig. 3) and mandibular (see Fig. 2) rostrum was similar in the ostrich and emu. This pattern of pits in the premaxilla of kiwi species (Cunningham et al., 2007), despite structural modifications (see below) and an obvious difference in size, also resembles that of the ostrich and emu. The intra-oral aspect of the kiwi mandible displays 1-2 rows of pits along the edges (Parker, 1891; Cunningham et al., 2007) and rows of pits on the ventral surface, similar to that in the ostrich and emu. However, the mandible of the kiwi does differ from that of the ostrich and emu in that the lateral edges display a narrow groove which is also lined with pits (Cunningham et al., 2007; Cunningham et al., 2013). It was noted that the pattern of pits in the kiwi bill (a probe-foraging bird) was similar to that seen in Scolopacidae (also probing birds) (Cunningham et al., 2007), although the particular pattern was not mentioned.

The premaxilla of the kiwi (Parker, 1891; Martin et al., 2007; Cunningham et al., 2007, Cunningham et al., 2013) differs markedly in respect of its gross anatomy from that of the ostrich and emu, and the maxillary rostrum is reportedly highly specialized in this species. However, by analysing the pattern and distribution of the bony pits it is possible to draw an analogy between the ostrich, emu and kiwi (Fig. 5). The dorsal view of the kiwi bony premaxilla (Martin et al., 2007; Cunningham et al., 2007, Cunningham et al., 2013) (Fig. 5a) shows a similar distribution of pits to the bony region underlying the *Culmen* in the ostrich (Fig. 5c) and emu (Fig. 5d). In all three genera pits are present on the lateral aspect of this region and are separated by a midline tract of non-pitted bone (Fig. 5). In the kiwi these pits are referred to as “dorsal zones 3 and 4” (Cunningham et al., 2007) (Fig. 5a) and correspond to the pits identified on the right (zone 3) and left (zone 4) side of the bony region underlying the *Culmen* in the ostrich (Fig. 5c) and emu (Fig. 5d). In the kiwi, the overlapping ventral surface of the premaxilla (termed the sensory pad) has two zones, namely, the central circular tactile-disc (zone 1) and the semi-circular pre-oral zone (zone 2) (Cunningham et al., 2007) (Fig. 5b). The so-called pre-oral zone in the kiwi appears to be homologous to the lateral portions of the body of the premaxilla in the ostrich and emu (zone 2) (Fig. 5b-d). It would appear that these two regions of the premaxilla (the body) in the kiwi are greatly reduced and fold in toward each other meeting ventrally (Fig. 5b). The so-called tactile disc (zone 1) (Fig. 5b) in the kiwi appears to be either homologous to the rostral tip of the premaxilla in the ostrich and emu where a concentration of pits is encountered (zone 1) (Fig. 5c and d),

which in the kiwi is folded ventrally, or to the immediate ventral surface of the rostral premaxilla in the ostrich and emu. The tactile disc is accentuated in the kiwi due to the slightly bulging nature of the ventrally folded pre-oral zones. The bill tip sensory pad is said to be unique to the kiwi. However, based on the above comparisons it would appear that it displays similar basic morphological components to those present in the ostrich and emu. The maxillary bill tip, in respect of the pattern and distribution of pits, is therefore similar in the ostrich, emu and kiwi, although the various zones display different proportions. However, this inferred homology between the above genera would have to be tested in a comparative developmental study. This comparative analysis of the distribution of bony pits and understanding how it relates to the morphology of the bill may be of relevance in similar exercises relating to extinct species, for example in ornithomimosaurian (Cuff and Rayfield, 2015) and theropod dinosaurs (Lautenschlager et al., 2013) (see below). Comparative analysis of the pattern and distribution of pits in the rostra of extinct, but related species, may similarly facilitate an understanding of the homology of structures which may appear quite dissimilar (as observed in the bill of the emu and kiwi), and provide additional data relevant for determining and assigning phylogenetic relationships.

Sensory pits have also been counted in the bills of 11 ibis species in 8 genera (*Threskiornis*, *Geronticus*, *Plegadis*, *Lophotibis*, *Eudocimus*, *Phimosus*, *Theristicus* and *Bostrychia*) (Cunningham et al., 2010a), the Eurasian woodcock (*Scolopax rusticola*) and bar-tailed godwit (*Limosa lapponica*) (Cunningham et al., 2013). The ibis species, like the kiwi and shorebirds, use their long, slender bills for probe-foraging. As such, they display a similar distribution of pits to the latter two families of birds (Cunningham et al., 2010a). The pits in the Eurasian woodcock and bar-tailed godwit are not compared here as the study did not mention on which surfaces the pits occurred (Cunningham et al., 2013). In the averaged data from the 11 ibis species, the largest number of pits was located on the outer surfaces of the upper (594.2 outside and 81.6 inside) and lower (505.5 outside and 83.3 inside) bills. Although the ostrich also displayed more pits on the outside of the upper (139.5 outside and 70.1 inside) and lower (88.6 outside and 50.2 inside) bills, the order of magnitude in respect of the difference between these surfaces was far less than that in the ibis species. In the emu the difference between the outer and inner surfaces was not as exaggerated as in the ostrich, except for the outside surface of the upper bill in the emu. The preferential placement of sensory pits on the outer surfaces of the bill in ibis species would indicate that the greatest degree of sensitivity exists on the outer surfaces of the bill; a suitable arrangement for probing. However, as the ostrich and emu do not probe it would appear, by the placement of the pits, that the inner and outer surfaces of the bill tip are equally important in these two birds.

Bony pits and the bill tip organ

The presence of bony pits in the bill tip indicates a propensity for the presence of a bill tip organ (Bolze, 1968; Cunningham et al., 2007). Cunningham et al. (2010a) speculate that the presence of this organ is favoured by a probe-foraging lifestyle as it occurs in three different families

of probe-foraging birds, namely the Apterygidae, Scolopacidae and Threskiornithidae. The presence of a bill tip organ has been confirmed in one of the five palaeognathae orders (Apterygiformes (Cunningham et al., 2007)). Pits are present in the bill tip of Struthioniformes (present study). Of the Casuariiformes, the emu (present study) displays pits in the bill tips, whereas the status of the cassowary (various species) is still undetermined. However, sketches of the bill tips of the cassowary and the remaining two orders, Rheiformes (Parker, 1866; Müller, 1963) and Tinamiformes (Parker, 1866), and images of palaeognath skulls available on the website Digital Morphology (<http://digimorph.org/index.phtml>), all display numerous pits in the rostral bill tips. If the connection between bony pits and the existence of a bill tip organ is valid, it may be reasonable to assume that a bill tip organ is synapomorphic for the Palaeognathae. Further evidence for a bill tip organ in the ostrich and emu is the presence of Herbst corpuscles within bony pits of the intraoral surfaces of the premaxilla and dentary bones (Crole and Soley, 2014). Histological evidence revealed that Herbst corpuscles were present in sheets or chains within the connective tissue between the rhamphotheca and bone and as groups within the bony pits (Crole and Soley, 2014). The bill tips of the ostrich and emu have thus been described as possessing great tactile acuity (Crole and Soley, 2014), which is further supported by the large size of the attendant nerves and their extensive branching within the ostrich and emu bill tip (Crole and Soley, 2016). Pits in the bill tips of extinct palaeognaths (Dinornithidae, Aepyornithidae and Dromornithidae) may also indicate that these birds possessed a bill tip organ. For example, bony pits in the bill tip of the extinct Australian “Demon Duck of Doom” (*Bullockornis planei*) (Murray and Megirian, 1998), are strongly suggestive that this bird possessed a bill tip organ. Although the ostrich and emu oropharynx is abundantly supplied with Herbst corpuscles (Crole and Soley, 2014), the bill tips are richly innervated (Crole and Soley, 2016), strongly pitted (present study) and Herbst corpuscles are present within the pits (Crole and Soley, 2014; 2016), it remains to be confirmed whether these two birds possess a bill tip organ. Histological studies on the bills of extant palaeognathous birds other than the kiwis will be required to confirm the common presence of a bill tip organ in this superorder of birds. Although evidence appears to indicate a bill tip organ in the ostrich and emu, its presence would be enigmatic as these two birds do not probe or use their bills in any complex oral task. Furthermore, unlike the kiwi which possesses poor eyesight (Martin et al., 2007), the ostrich (Martin and Katzir, 1995) and emu (Long, 1959) are supplied with excellent vision. Based on the presence of pits in the lateral premaxilla, maxilla and mandible of edentate ornithomimids, as well the remnants of keratinous rhamphotheca, it was determined that these animals possessed beaks (Cuff and Rayfield, 2015). Although foramina and keratinous rhamphotheca are not mutually inclusive (Cuff and Rayfield, 2015), foramina have been used as evidence for a keratinous rhamphotheca in extinct species (Kobayashi and Lü, 2003). It has been demonstrated that keratinous beaks would be important in dissipating stress during feeding as well as increasing the overall strength of the cranium, as for example, modelled in *Erlikosaurus andrewsi*, a therizinosaurid (Lautenschlager et al., 2013). Such findings may aid in interpreting the function of a possible bill tip organ

in the ostrich and emu where the simple foraging behaviour of these species would not seem to warrant the presence of such a complex sensory organ.

AUTHOR CONTRIBUTIONS

Study concept / design: MRC. Acquisition of specimens and data, data processing, interpretation / analysis: MRC, JTS. Drafting manuscript: MRC. Critical revision of the manuscript and approval of the article: MRC, JTS.

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