Mechanosensory structures in the beaks of probe-foraging birds in relation to their foraging ecology

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Summary

Some taxa of probe-foraging birds (ibises, kiwi and scolopacid shorebirds) possess the sensory of capability of “remote-touch”, allowing them to detect mechanical vibrations in their foraging substrates using a specialised bill-tip organ in their beaks. This enables them to remotely detect the location of prey submerged in opaque substrates in the absence of all other sensory cues. The bill-tip organ that facilitates remote-touch is made up of mechanoreceptors housed in dense clusters of foramina in the distal portions of the beak bones (each unit of foramen and associated receptors is referred to as a “sensory pit”). Previous research showed that in ibises (Family: Threskiornithidae), species which live in more aquatic habitats tend to have more extensively pitted beak bones (i.e., the relative size of the bill-tip organ increases with increasing aquatic habitat usage of the species) than species living in drier habitats.

The first three data chapters of this thesis investigate this trend, using three species of southern African ibises. These three species represent a spectrum of habitat usage, ranging from mainly terrestrial (Hadeda Ibises) to mainly aquatic (Glossy Ibises), with African Sacred Ibises a generalist species. My main hypothesis is that the interspecific differences in bill-tip organ morphology are related to differences in the moisture content of the birds’ foraging substrates, as this affects how well these substrates transmit vibrations that the birds are sensing using remote-touch.

The morphology of the bill-tip organs of the three species (Chapter 2) and their foraging behaviour in the wild (Chapter 3) indicate that species which forage in less saturated substrates have higher densities of mechanoreceptors in their bill-tip organs, suggesting that they are more sensitive to vibratory cues. This follows logically from the fact that drier substrates transmit vibrations more poorly than wetter ones, thus I hypothesize that species which forage frequently in dry substrates may have faced evolutionary pressure selecting for more sensitive bill-tip organs. My data on foraging behaviour of all three species of ibis in the wild suggests that bill-tip organ pitting extent on the beak bones is linked to depth of probing, which is in turn related to the penetrability of their probing substrates. As substrate penetrability is strongly affected by moisture content, the extent of pitting on the bill-tip organ is a good osteological correlate for the water content of the foraging substrate in the absence of soft tissue histology in ibises.

Experiments using captive Hadeda Ibises (Chapter 4) provide further support for the hypothesis that species foraging in drier substrates require more sensitive bill-tip organs as their success rate using remote-touch was positively affected by substrate moisture content. Additionally, as this species' recent range expansion across southern Africa has been closely tied to increased soil irrigation in urban and agricultural habitats, I suggest that this in part due to Hadeda Ibises being better able to detect prey in more saturated substrates.

The final data chapter of this thesis concerns the evolution of the remote-touch bill-tip organ in modern birds: the three families which possess remote-touch capability are widely phylogenetically separated, indicating that it evolved convergently. Kiwi (order: Apterygiformes) present an interesting case, as they are part of the palaeognath clade of Neornithes and are the only members of this clade which use remote-touch probe-foraging. However, various other palaeognathous birds (ostriches & emu) possess a bill-tip organ, though its function in these taxa is unknown. I show that all species of modern palaeognathous birds (including the extinct moa and elephant birds) have the same beak morphology (bony pits containing numerous mechanoreceptors). This is at odds with the fact that none use the organ or possess the neuroanatomical correlates that would allow them to do so, indicating that the organ is vestigial in most palaeognaths.

I thus hypothesized that the trait is plesiomorphic in palaeognathous birds, inherited from a common ancestor that used remote-touch probe-foraging. As the bill-tip organ is characterized by pitting in the beak bones, I was able to study the fossilized beaks of the oldest known palaeognaths, the lithornithids (which evolved during the Cretaceous period). By comparing them to an extensive sample of extant birds' beak bones, I showed that these ancient palaeognaths had bill-tip organs which were probably capable of remote-touch. Aside from supporting the hypothesis that the remote-touch bill-tip organ in palaeognaths is plesiomorphic, this indicates that remote-touch is one of the oldest documented foraging specialisations in modern birds.

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Pictured: Study individual “Solas” (Hadeda Ibis, serial worm thief and leg biter) from behavioural assay experiments and myself; and me (~5 years old) following a Sacred Ibis in an aviary

Funding and Permits

Experiments with captive Hadeda Ibises in Chapter 4 of my thesis were permitted under an animal ethics clearance (number 2019/V19/SC) from the University of Cape Town's Science Faculty Animal Ethics Committee. Import of animal tissues (bird beaks) preserved in ethanol (used in Chapter 5 of this thesis) to South Africa from the Smithsonian Museum of Natural History's Fluid Collection (USA) was carried out under a Veterinary Import Permit for Preserved Animal Material (Permit no: 13/1/1/30/2/0-201809004887) from the South African Department of Agriculture, Forestry and Fisheries. No birds were harmed or killed during the work in this thesis, and any tissue samples or bones analysed were from birds which died for reasons not related to this study.

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Publication Arising from Thesis

Published prior to submission for examination:

du Toit, C.J., Chinsamy, A. & Cunningham, S.J. 2020. Cretaceous origins of the vibrotactile bill-tip organ in birds. *Proceedings of the Royal Society B.* 287(1940):20202322.

The data published in the above paper are presented in Chapter 5 of this thesis. The contents of the paper have been reformatted from the published version to fit the style of the rest of the thesis (reference style, order of figures and tables, etc.). The supplementary methods and figures included online with the paper have been included in the main text of Chapter 5. Some of the supplementary methods of the paper have been moved to Chapter 2 of this thesis (as it appears before Chapter 5, and the same methodology was used to prepare the histological material – methodology which I developed for the work on both chapters) to avoid repetition within the thesis. The three supplementary tables from the paper are included in the appendix of this thesis. I am the lead author of the paper, I performed all data collection and analyses, and wrote the full draft of the paper. My co-authors are my PhD supervisors, and their contributions to the paper were as advisors to my work and assisting in the final proof editing of the manuscript drafts.

Declarations

I, Carla Jacquelyn du Toit, hereby declare that the work on which this thesis is based is my original work, and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

I confirm that I have been granted permission by the University of Cape Town's Doctoral Degrees Board to include the following publication in my PhD thesis (see previous page for details), and my co-authors (who are both my doctoral supervisors) have agreed that I may include the publication in this thesis (see letter on following page):

> du Toit, C.J., Chinsamy, A. & Cunningham, S.J. 2020. Cretaceous origins of the vibrotactile bill-tip organ in birds. *Proceedings of the Royal Society B.* 287(1940):20202322.

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30 September 2021

Doctoral Degrees Board

RE: Consent to include a publication as a chapter in Carla Du Toit's Doctoral thesis

We wish to confirm that as Carla du Toit's PhD supervisors and co-authors of the following paper, we provided guidance and supervision of the research, which formed an integral part of her PhD dissertation:

CJ Du Toit, A Chinsamy & SJ Cunningham (2020) Cretaceous origins of the vibrotactile bill-tip organ in birds. *Proceedings of the Royal Society B: Biological Sciences.* **287**: 20202322. https://doi.org/10.1098/rspb.2020.2322

We fully support the inclusion of the publication as a chapter of her doctoral dissertation.

If you have any queries, please do not hesitate to contact us.

Yours sincerely,

Dr Susan J Cunningham	Prof Anusuya Chinsamy-Turan
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I support the inclusion of the above chapter (article) in Carla Du Toit's thesis.

Signatures Removed

Dr Jacqueline Bishop

Acting Head of Department of Biological Sciences

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CHAPTER 1:

General Introduction

Glossy Ibis (photograph by CJ du Toit)

Introduction:

I present here a brief overview of previous research and data on the sensory capability of remote-touch and its associated bill-tip organ in Neornithes. Since the first three data chapters of my thesis focus on the ibises as a model study system, I discuss the ecomorphology of the remote-touch bill-tip organ in these birds. Thereafter, I provide some background about the little that is known about the evolution of the remote-touch bill-tip organ, which is the topic of the fourth data chapter of this thesis. Finally, I outline the aims and objectives of this thesis.

1.1: Remote-touch and probe-foraging in wading birds

Standing still in water or soft mud and searching for prey is ubiquitous in wading birds (Kushlan, 1978). Many waders will also walk slowly in the mud/water to widen their search zone, or to stalk faster moving prey. This can involve both visual and non-visual search components. Non-visual methods usually involve some type of active foraging, to detect prey using another sense, such as olfaction or tactile cues (Kushlan, 1978).

Probe-foraging is a foraging method used by some wading birds, whereby their beaks are probed in and out of the water/mud. This tactic of feeding is often associated with specialized morphologies, particularly in the beak structure, with most probing birds having long, slender, and often down-curved bills (Remsen Jr & Robinson, 1990). Probing exposes the beak area to the substrate (water or mud) that the birds are foraging in, allowing them to detect tactile cues from their prey that would otherwise be hidden from sight and even hearing.

Probe-foraging birds can detect their prey hidden in the soft substrates they feed in using two tactile methods: direct and remote-touch (Gerritsen & Meiboom, 1985; Piersma *et al.*, 1995). Direct touch, whereby prey is sensed when it comes into physical contact with the probing beak, was long believed to be the main sensory method used by probing birds, sometimes combined with other sensory cues (visual, auditory, chemical) (Hamilton, 1975; Franklin, Clark & Clark, 1979; Hulscher, 1982; Pierce, 1986; Zembal & Fancher, 1988; Cullen, 1994). However, this method involves a degree of chance and does not allow the birds to gather much information about the abundance of prey in the surrounding substrate (Gerritsen & Meiboom, 1985). Empirical evidence exists that direct touch is used as a method for locating prey by some probing birds, such as oystercatchers, and relies on fairly high prey densities to be energetically efficient (Hulscher, 1982). Remote-touch refers to the bird's ability to detect prey in the substrate via tactile cues at a distance from their beak before coming into direct contact with the prey item (Gerritsen & Meiboom, 1985; Piersma *et al.*, 1995). This was first shown experimentally in scolopacid shorebirds (Gerritsen & Meiboom, 1985; Piersma *et al.*, 1998), and has subsequently been demonstrated experimentally using sensory assays in ibises (Cunningham, Castro *et al.*, 2010) and kiwi (Cunningham, Castro & Potter, 2009), as well as via anatomical and neurological studies (see section 1.2).

There is little evidence that most probing birds use taste or olfaction to detect hidden prey buried in the substrate, as these senses do not provide much directional information over larger spatial scales without significant air or water flow (Dusenbery, 1992). There are exceptions, however. Most notable are the kiwi of New Zealand, which have been shown to use olfaction to detect the presence of prey buried under dry leaves or in the soil (Benham, 1906; Bang, 1971; Wenzel, 1971). However, studies have shown that kiwi can make use of remote-touch when olfactory cues are absent (Cunningham, Castro & Potter, 2009). Additionally, some sandpipers (genus *Calidris*) may use taste to select where to forage and in prey quality assessment (once caught), but whether they can use taste to accurately locate buried prey has not been established (Gerritsen, Van Heezik & Swennen, 1982).

Birds can use remote-touch to detect submerged or buried prey in two ways: i) through detection of high frequency vibratory/“seismic” cues made by invertebrate prey moving around in the substrate (Gerritsen & Meiboom, 1985; Cunningham, Castro & Potter, 2009; Cunningham, Castro *et al.*, 2010); or ii) by detecting changes in interstitial water pressure gradients around hard-shelled sessile prey (e.g., molluscs), which are caused by the birds probing rapidly and repeatedly in the mud and deformed by the presence of hard-shelled prey [shown in Red Knots by Piersma *et al.* (1998)].

1.2: Morphology of the remote-touch bill-tip organ

Remote-touch is facilitated in birds by a specialized bill-tip organ, found in the distal portions of their beaks (Bolze, 1968; Gottschaldt, 1985; Piersma *et al.*, 1998; Nebel, Jackson & Elner, 2005; Cunningham, Castro & Alley, 2007; Cunningham, Alley *et al.*, 2010). It was first described over a century ago in shorebirds (Leydig, 1868; Clara, 1925), but it was not until the late 20th century that its function was first ascribed (Bolze, 1968). The remote-touch bill-tip organ is made up of mechanoreceptors [predominantly Herbst corpuscles (Gottschaldt, 1985), see below] which are grouped together into distinct units inside foramina in the bones of the beak. The foramina are numerous, with hundreds or even thousands of them perforating the distal beak bones, and can be clustered closely together to form a honeycomb-like pattern on the surface of the bone (Bolze, 1968; Cunningham, 2010), as can be seen in Figure 1.1. The foramina, with their accompanying soft tissues and receptors are referred to as "sensory pits", as the openings of the foramina on the surfaces of the beak bones resemble small holes or "pits" (Cunningham, Castro & Alley, 2007; Cunningham, Alley *et al.*, 2010). A nerve fiber runs down the central axis of each sensory pit and connects to the central nerve canals of the beak bones, forming branches of the trigeminal nerve [which relays sensory information from the beak to the brain (Barnikol, 1954)]. The Herbst corpuscles are clustered around and attached to the nerve fiber of the sensory pit containing them (Bolze, 1968). The bill-tip organ refers to the entire structure of multiple sensory pits and associated neurovascular tissues in the distal regions of the beak.

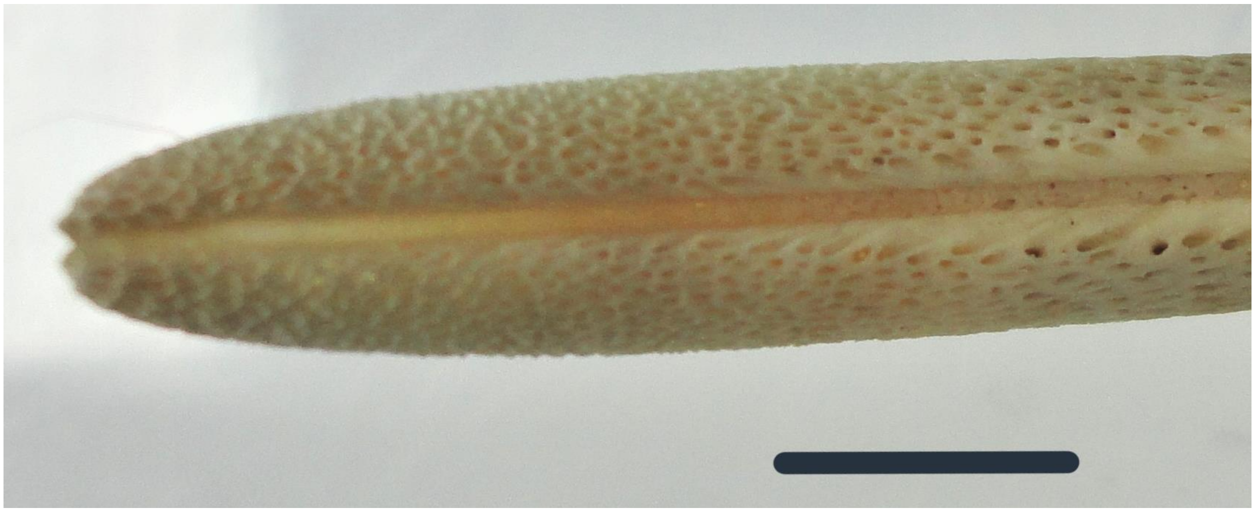

Figure 1.1: Photograph of the distal portion (ventral view) of a Sacred Ibis's (*Threskiornis aethiopicus*) mandible, showing the "honeycomb" pattern of foramina (which form the "sensory pits") in the bone, characteristic of the remote-touch bill-tip organ. Scale bar: 1cm. (photograph by CJ du Toit)

There are two other types of bill-tip organs described in modern birds. The first is found in ducks and geese (order: Anseriformes), and comprises mechanoreceptors organized into specialized cornified structures called touch papillae, which extend through the bone and rhamphotheca at the beak tip, and can be seen on the surface of the beak as small nodules (Gottschaldt & Lausmann, 1974; Gottschaldt, 1974; Berkhoudt, H., 1979; Soliman & Madkour, 2017; Avilova, Fedorenko & Lebedeva, 2018; Avilova, 2018; Schneider *et al.*, 2019). This type of bill-tip organ is used for dabbling, whereby small food particles are differentiated and extracted from the water. The second is found in parrots (order: Psittaciformes) and comprises sensory units (again clusters of mechanoreceptors) which are restricted to the keratinized rhamphotheca surrounding the beak (Goujon, 1869; Demery, Chappell & Martin, 2011; Martin & Martin, 2021). This type of bill-tip organ is thought to be used for fine scale manipulation of food objects by the beak. None of the three types of bill-tip organs (remote-touch, Anseriform or Psittaciform) contain any types of receptors aside from mechanoreceptors (i.e., no chemoreceptors or thermoreceptors).

In the remote-touch bill-tip organ, Herbst corpuscles are the mechanoreceptors believed to facilitate the detection of remotely-sourced vibratory cues (Zweers & Gerritsen, 1997; Piersma *et al.*, 1998; Nebel, Jackson & Elner, 2005; Cunningham, 2010). Herbst corpuscles are mechanoreceptors common to most (if not all) birds, and they detect changes in acceleration of high frequency (50-2000 Hz) vibratory stimuli which originate from sources that are not in direct contact with the structure they are a part of (Gottschaldt, 1985). Each Herbst corpuscle has a specific range of frequencies to which it responds, therefore possession of groups of Herbst corpuscles with different response thresholds in the bill-tip organ likely allows birds to differentiate fine-scale information about direction and distance of the source of vibrations (Zweers & Gerritsen, 1997).

In biology, “sensitivity” refers to how strong a stimulus must be before a sensory system (made up of multiple receptors) reacts to it – i.e., systems with higher sensitivities can detect weaker stimuli than those with lower sensitivities. In this case, the “stimulus” or “signal” is the vibratory tactile cues in the substrates the birds probe in; the “system” is the remote-touch bill-tip organ; and the “receptors” making it up are the Herbst corpuscles and other mechanoreceptors. Thus, more sensitive bill-tip organs will be able to detect weaker vibratory signals than less sensitive ones. Anatomically speaking, Zweers & Gerritsen (1997) showed mathematically that more sensitive bill-tip organs would require higher numbers and densities of Herbst corpuscles. As to how this translates to the birds’ behavior, species with more sensitive bill-tip organs would a) be able to detect weaker signals (see below) than species with less sensitive organs; and b) if the same signals are available, be more successful at using remote-touch to locate prey (i.e., find prey items faster) than the less sensitive species.

The strength of a signal (the pressure waves travelling through the substrate) when it reaches a receptor is affected by multiple factors, including its strength at the source (i.e., the object it originates from), the physical properties of the medium it travels through (in this case, soil or water the birds are probing in), the distance between the source of the signal and the receptor (the distance between the surface of the bird’s beak and the prey item) and the amount of background “noise” in the medium coming from other sources of mechanical vibrations in the substrate (Knight, Jones & Field, 2019). Background noise in the substrate and distance of the prey object from the bird’s beak both negatively affect the strength of vibratory signals when they reach the bill-tip organ. The source of the signal is either the moving prey item (Gerritsen & Meiboom, 1985) or the bird's beak itself [when considering the example of the Red knots studied by Piersma *et al.* (1998)], though the signal that reaches the bill-tip organ in the latter case is the deformed pressure waves that have been distorted in the medium by the presence of a stationary, hard-shelled prey item (see section 1.1). In both cases, the strength of the signal at its source will be affected by the movement speed and magnitude of displacement of the source, and the physical properties (size, density and shape) of the source (Zweers & Gerritsen, 1997). The physical properties of the substrates that affect the transmission of the vibratory cues (and their strength when they reach the receptors) include water content (positive effect on transmission), soil particle size (positive effect) and porosity (negative effect) (Biot, 1956 & 1962).

Herbst corpuscles comprise a central nerve axon, surrounded by a myelinated sheath of Schwann cells (see Figure 1.2). The nerve axon connects to the central nerve fiber of the sensory pit and terminates distally as a round (in most cases) or disc shaped protrusion [the latter shape described from kiwi's bill-tip organs (Cunningham, Castro & Alley, 2007)]. The axon is enclosed by an outer membrane of collagen, and between them lies an interconnected matrix of lamellae, which in turn are connected to the central nerve axon.

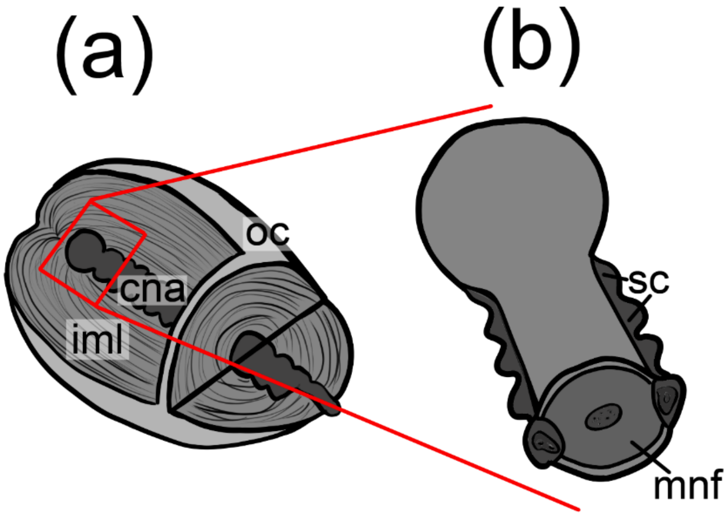

Figure 1.2: Rough diagram of cross sections of a Herbst corpuscle, showing a 3D view of (a) the entire corpuscle, dissected to show inner structures and (b) the head of the central nerve axon. cna = central nerve axon; iml = interconnected matrix of lamellae; mnf = myelinated nerve fibre; oc = outer capsule; sc = Schwann cell nucleus. [Figure adapted from Gottschaldt (1985)]

The regions of the brain which are responsible for processing tactile information from the beak are the principal trigeminal nucleus and the nucleus basorostralis (Berkhoudt, Herman, Dubbeldam & Zeilstra, 1981). Both regions show enlargement/hypertrophy in species of birds which process tactile information from their beaks (remote-touch probing birds, parrots and waterfowl), compared to other birds which do not use their beaks for specialized tactile foraging (Pettigrew & Frost, 1985; Martin *et al.*, 2007; Gutierrez-Ibanez, Iwaniuk & Wylie, 2009; Cunningham, Corfield, *et al.*, 2013).

1.3: Remote-touch in ibises, and ecomorphological trends between bill-tip organ structure and foraging ecology

Cunningham, Alley *et al.* (2010) and Cunningham (2010) showed that though the overall morphology of the remote-touch bill-tip organ is conserved across the taxa that possess it, in kiwi and ibises there are some interspecific differences in bill-tip organ morphology that correlate with differences in the birds' habitats. Specifically, there is a trend whereby the extent of pitting on the beak bones is larger in species living in more aquatic habitats compared to those in more terrestrial, drier habitats. The authors hypothesized that the increased relative size of the bill-tip organ is linked to species probe-foraging in substrates with different water contents, as the latter can affect how well vibrations are transmitted through the substrate and how the prey items move through the substrate. Cunningham (2010) tentatively suggested that bill-tip organ size (represented by the extent of pitting and number of sensory pits) could be an indicator of bill-tip organ sensitivity. However, she could not comment further, as information on the numbers and arrangement of Herbst corpuscles within the bill-tip organ is the only anatomical way to assess the sensitivity of the bill-tip organ (Zweers & Gerritsen, 1997). Only one species of ibis (Australian White Ibis) had been sectioned to describe the soft tissue histology of their bill-tip organs (Cunningham, Alley *et al.*, 2010), and multiple species from different habitats were needed to investigate this further.

Among the Pelecaniformes, ibises and spoonbills form the family Threskiornithidae, with herons (family: Ardeidae) being their closest living relatives, based on recent genetic studies (Jarvis *et al.*, 2014; Prum *et al.*, 2015). Originally, based on their morphology, ibises and spoonbills were considered to form two distinct sister subfamilies (Matheu & del Hoyo, 1992). However, genetic evidence indicates that spoonbills are rather nested within the Old World ibises, with their closest relatives belonging to the genus *Threskiornis* (Chesser *et al.*, 2010; De Pietri, 2013; Ramirez, Miyaki & Del Lama, 2013).

All members of Threskiornithidae have long, thin, and generally downcurved bills, which they probe into water, mud and/or soil to extract buried prey items. The exception is the spoonbills, which have long, thin bills that are relatively straight (viewed laterally). The distal portion of their bills form an expanded flattened “spoon” shape (from dorsal/ventral view), which they swing side-to-side through the water to catch small fish and other aquatic prey. While spoonbills forage exclusively in open water (sometimes at depths up to their bellies) (Lowe, 1982; Matheu & del Hoyo, 1992; Hockey, Dean & Ryan, 2005; Swennen, Cornelis Kees & Yu, 2005; Sullender, Barzen & Silbernagel, 2016), the ibises are more varied in their foraging habitats (Cramp *et al.*, 1983; Ali & Ripley, 1983; Matheu & del Hoyo, 1992; Hockey, Dean & Ryan, 2005). There are a few ibis species that forage in dry habitats, sometimes many kilometers away from the nearest water body [e.g., both *Geronticus* (bald ibis) species and the Red-Naped Ibis, *Pseudibis papillosa* (Manry, 1982; Cramp *et al.*, 1983; Hockey, Dean & Ryan, 2005)]. These species often make use of foraging techniques other than probing – e.g.,visual hunting and pursuit of prey (Manry, 1982; Ali & Ripley, 1983; Cramp *et al.*, 1983; Matheu & del Hoyo, 1992; Hockey, Dean & Ryan, 2005), and sometimes even feed on carrion (Khan, 2015). Most ibis species do however tend to forage in wetlands, either along the soft mud at the edge of the water or wading in the shallows (Kushlan, 1977; Kushlan, 1978; Cramp *et al.*, 1983; Ali & Ripley, 1983; Matheu & del Hoyo, 1992; Hockey, Dean & Ryan, 2005).

There are three species of ibis which occur commonly in southern Africa: Hadeda Ibis (*Bostrychia hagedash*), African Sacred Ibis (*Threskiornis aethiopicus*) and Glossy Ibis (*Plegadis falcinellus*) (Figure 1.3). They present an interesting case to further study the interspecific differences in the bill-tip organs of ibises, as they utilize a spectrum of different habitats (Hockey, Dean & Ryan, 2005). Hadeda Ibises are mainly terrestrial, historically living in forests (Macdonald, Richardson & Powrie, 1986), though in the past century they have experienced a significant range expansion across southern Africa, and now occupy various human-modified environments (agricultural land and gardens) (Macdonald, Richardson & Powrie, 1986; Duckworth, Altwegg & Harebottle, 2012; Duckworth & Altwegg, 2014). Though they have been recorded foraging in wetlands and shallow water, they spend most of their time on relatively dry land (Skead, 1951). They do not forage in very arid areas, and their expansion into urbanized habitats seems to have been facilitated by artificial irrigation by humans (Duckworth, Altwegg & Guo, 2010; Duckworth & Altwegg, 2014). Glossy Ibises are almost exclusively aquatic foragers, living and feeding in and around bodies of fresh water or wetlands (Matheu & del Hoyo, 1992; Frederick & Bildstein, 1992; Hockey, Dean & Ryan, 2005; Taylor & Taylor, 2015). Sacred Ibises represent an intermediate of the three species, being generalist in both foraging habitat and behaviour (Hockey, Dean & Ryan, 2005; Clergeau & Yésou, 2006; Yésou *et al.*, 2017). Though they regularly make use of probe-foraging, they will often use visual hunting to capture large vertebrate prey (Williams & Ward, 2006), or sometimes even feed on carrion (Chane & Balakrishnan, 2016). Many populations of Sacred Ibis have become urban exploiters, feeding on human refuse (Clark, 1979; Clergeau & Yésou, 2006; Calle & Gawlik, 2011; Yésou *et al.*, 2017).

Figure 1.3: Three study species of southern African Ibis and their general habitats. From left to right: Hadeda Ibis (*Bostrychia hagedash*) – mainly terrestrial; African Sacred Ibis (*Threskiornis aethiopicus*) – generalist; and Glossy Ibis (*Plegadis falcinellus*) – mainly aquatic. (Image is an artistic rendering from photographs of the three species to visually represent their habitat preferences, made by CJ du Toit)

Aside from the fact that all three species fall within the “Old World” clade of ibises (defined as species not endemic to the Americas), and that the genus *Threskiornis* is sister to *Platalea* (the spoonbills), there is unfortunately no higher resolution of the relationships between the three species based on genetic data (Ramirez, Miyaki & Del Lama, 2013).

Data on stomach contents [Table 1.1, data from Kushlan (1978)] shows that Sacred Ibises have the most varied diet, with a large range of prey from both aquatic and terrestrial habitats (Kushlan, 1978). Hadeda Ibises feed mostly on terrestrial prey, and Glossy Ibises feed mainly on aquatic species. This could be used as a rudimentary indication of the feeding habitat of these ibises, but is limited in that it does not provide any indication of the ratios in which the different prey items were consumed by the different species.

Table 1.1: Prey consumed by three ibis species [data obtained from Kushlan (1978)]. *Asterisks indicate prey found mainly in aquatic habitats

	Glossy Ibis	Hadeda Ibis	Sacred Ibis
*Leeches	X		
Earthworms	X	X	X
*Aquatic insects	X	X	X
Land insects	X	X	X
*Crabs	X		
*Prawns/crayfish	X	X	
*Other crustacea		X	X
Spiders		X	X
Other arthropods		X	X
Snails		X	X
*Freshwater fish			X
*Frogs/toads	X		X
*Tadpoles/newts	X		X
Snakes/lizards	X	X	
Eggs			X
Carrion			X

1.4: Evolution of remote-touch in modern birds

Remote-touch and the associated bill-tip organ are found in three phylogenetically widely separated families of modern birds, occurring in both palaeognathous (in kiwi) and neognathous (in ibises and scolopacid shorebirds) birds (see Figure 1.4). Therefore, the organ and associated sensory capability appear to have evolved convergently in the three groups, between palaeognathous and neognathous birds (Cunningham, Castro & Alley, 2007; Cunningham, Castro *et al.*, 2010).

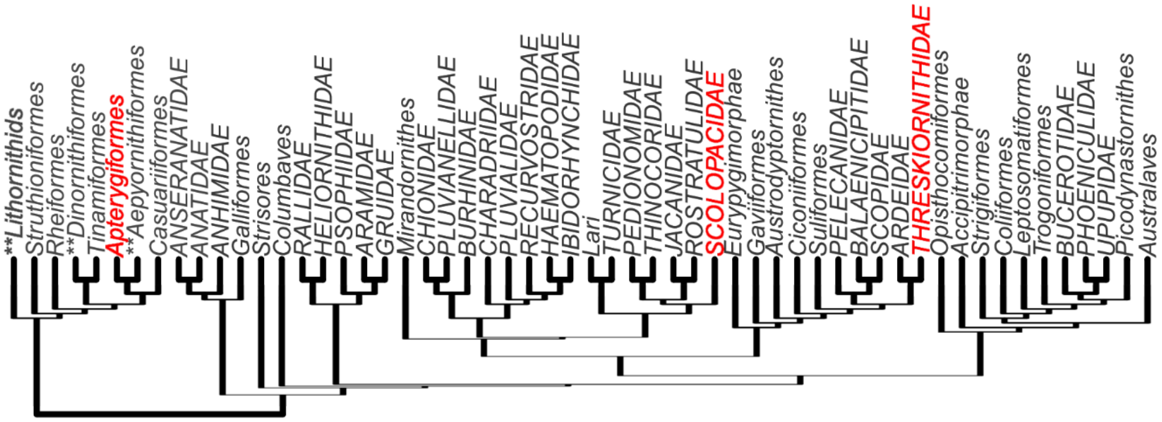

Figure 1.4: Phylogeny showing the occurrence of the remote-touch bill-tip organ across Neornithes. Large clades in which all orders have the same beak morphololgy have been collapsed for simplicity (e.g., Australaves), and some orders have been expanded to show the constituent families in capital letters. The three groups which use remote-touch probe-foraging are shown in red. (Figure simplified from Chapter 5, Figure 5.7C)

As yet, there is no study into the genetic markers associated with this or any other type of tactile bill-tip organ in an evolutionary or phylogenetic context. Furthermore, until now there has been little published data on its evolution in the fossil record. Evidence of sensory foramina (or the absence thereof) on the beak bones has only been commented on in a few publications. In ibises, a specimen of a maxilla from a Late Eocene threskiornithid has been described as lacking sensory foramina (Jadwiszczak, GaŹdzicki & Tatur, 2008). Jadwiszczak, GaŹdzicki & Tatur (2008) suggested that this could mean either that the specimen belongs to another undescribed group of birds, or that these earlier ibises did not use remote-touch foraging. The specimen is much larger than extant ibises and does differ in other characteristics as well. Houde (1988) noted the numerous foramina on the beaks of the palaeognathous lithornithids (order: Lithornithiformes) and suggested they may represent a sensory organ similar to that found in Scolopacid shorebirds (the only taxa known at that stage to possess this type of bill-tip organ). However, no further investigation was made to differentiate these foramina from those found on most birds' beaks, or to conclusively assess whether they represented a tactile bill-tip organ in these fossil birds. Olson (1975) and Cunningham (2010) noted that the beaks of the extinct Snipe-rail from New Zealand (*Capellirallus karamu*) appeared to show characteristics of a remote-touch bill-tip organ, possibly indicating that a fourth family of modern birds (Rallidae) have evolved this sensory modality.

The palaeognathous birds present an interesting conundrum: only kiwi use remote-touch probe-foraging (Cunningham, Castro & Potter, 2009), but ostriches and emu (order: Struthioniformes) possess a similar morphology in their beaks (i.e., numerous Herbst corpuscles housed within foramina in the bones of their beaks) (Crole & Soley, 2014; Crole & Soley, 2017), which was previously believed to be unique to remote-touch probing birds. This is at odds with the fact that ostriches and emu do not use any specialized tactile foraging methods (Tomlinson, 2000; Gussekloo & Bout, 2005), and they lack the hypertrophy of their brain regions found in tactile foraging birds (Cunningham, Corfield, *et al.*, 2013). This suggests that they are not using the organ for tactile foraging or any other specialized tactile purpose. As there are no other types of receptors aside from mechanoreceptors found in their beaks, they are unlikely to be used for any other function. Furthermore, Schneider *et al.* (2019) showed that in ducks (which possess a different type of tactile bill-tip organ, see section 1.2), the expansion of mechanoreceptive neurons in the trigeminal ganglia come at the expense of temperature and pain receptors, indicating that tactile specializations in the beak tend to preclude other sensory information in the region [a trend common in multiple vertebrates (Lallemend & Ernfors, 2012)]. Therefore, the high density of mechanoreceptors in the beaks of Struthioniformes likely limits any other sensory specialisations from the distal portions of the beak.

Thus, it appears that Struthioniformes possess a bill-tip organ that they do not use and which is likely vestigial, or possibly used for an as yet undescribed function. As Struthioniformes are not a sister group to Apterygiformes (kiwi) (Mitchell *et al.*, 2014), it is unlikely the bill-tip organ represents an ancestral state to only these two orders of palaeognathous birds. Furthermore, other modern palaeognathous birds may also possess a similar “vestigial” organ, based on preliminary examinations of their beak bone morphology (Crole & Soley, 2014). This suggests that the bill-tip organ may be plesiomorphic in all modern palaeognathous birds, inherited from a previous common ancestor which used remote-touch probe-foraging.

As exquisitely preserved specimens of lithornithid beaks exist in the fossil record, and numerous foramina in these beaks had been previously noted by Houde (1988), they are good candidates to study the ancestral state of palaeognaths. Having evolved during the Cretaceous period and surviving the K-Pg extinction event, they are also the oldest known palaeognathous birds, and are considered as a sister taxon to all modern palaeognathous birds (Houde & Olson, 1981; Clarke *et al.*, 2005; Nesbitt & Clarke, 2016; Yonezawa *et al.*, 2017). Houde’s hypothesis that they were probe-foragers is supported by the fact that their overall beak and leg morphology suggests that they were wading birds (Houde, 1988; Grande, 2013; Mayr, 2016). However, as several of the non-probing extant palaeognaths show high numbers of foramina on their beak bones (Crole & Soley, 2014), the presence of these alone as noted by Houde (1988) are not enough to infer the presence of a remote-touch capable bill-tip organ.

1.5: Aims and Objectives:

In this thesis, I investigate three broad questions regarding remote-touch in Neornithes:

1) What drives the trend in increased bill-tip organ size in remote-touch foraging species which live in more aquatic habitats compared to more terrestrial species?
2) Can we use the morphology of the bill-tip organ to infer information about the foraging habits and sensory ecology of fossil birds?
3) How and when did remote-touch first evolve in modern birds?

Question 1 is investigated comprehensively in Chapters 2-4, using ibises as a model family of remote-touch foraging birds. Question 2 is covered in Chapters 2, 3 and 5, by studying ibises and palaeognathous birds. Question 3 is the focus of Chapter 5, where I study palaeognathous birds, and compare the fossil lithornithids to an extensive sample of extant birds' beaks covering all orders and most families of living birds.

In this thesis, each of the next four chapters stand alone in terms of methodology and their specific aims and objectives, and they are intended to be published as separate papers. Together, they synthesize to answer the three questions detailed above, which is discussed in the final chapter of this thesis. Below I outline the specific aims of each chapter.

Chapter 2:

This chapter covers the interspecific differences in the morphology and soft tissue histology of the remote-touch bill-tip organs of ibises, using three species of southern African ibises as a study model.

Aims: To investigate whether the extent and number of sensory pits on the beak bones of ibises is:

A) Correlated with aquatic habitat usage in the three study species of southern African ibises
B) Correlated with differences in the density of mechanoreceptors in their bill-tip organs, which is the best anatomical measure of bill-tip organ sensitivity

Objective (B) has not been previously investigated and will provide valuable information as to the interspecific differences in the underlying soft tissues of the bill-tip organ. This chapter provides the morphological “backbone” to the subsequent two chapters, which investigate whether the interspecific differences in bill-tip organ morphology correlate with differences in the birds’ behavioural and sensory ecology.

Chapter 3:

This chapter focuses on investigating links between ibises' foraging behaviour, foraging substrate use and the morphology of their bill-tip organs.

Aims: The main aim of this chapter is to investigate whether the interspecific differences in bill-tip organ morphology described in Chapter 2 correlate to the birds' foraging behaviour in the wild, with specific reference to their probing behaviour, tactile foraging success (particularly while probe-foraging) and use of different foraging substrates.

This chapter also aims to investigate the hypothesis that the physical properties of the birds' foraging substrate affect the tactile foraging success of the three species. I specifically examined whether:

A) the extent of pitting on the bill-tip organ is related to depth of probing and the penetrability of the probing substrate
B) the extent of pitting on the *inside* surfaces of the bill-tip organ is related to the use of probing with the beak held open and the use of more saturated substrates
C) the moisture content of the substrate affects the birds' tactile foraging success
D) species adapted for drier substrates are better at using tactile foraging techniques than those adapted for wetter substrates

Chapter 4:

This chapter comprises a set of sensory assay experiments to test the effects of foraging substrate water content on remote-touch foraging success rate in birds, using Hadeda Ibises as a model species.

Aims: The main aims of this chapter are to:

A) Confirm whether Hadeda Ibises can use remote-touch to locate prey (as has been shown previously in other species of ibis)
B) (If A is true) Investigate whether their ability to use remote-touch to locate prey is affected by substrate water content

In this study I test (under controlled conditions with captive birds) the hypothesis that substrate moisture content positively affects the remote-touch capability of birds through improving transmission of vibratory cues in the substrate.

Chapter 5:

This chapter focuses on the evolution of the remote-touch bill-tip organ in Neornithes, with specific reference to its presence in palaeognathous birds

Aims: The aims of this chapter are to investigate:

A) Whether the complex morphology of a bill-tip organ is common to all modern palaeognathous birds (thus indicating it is plesiomorphic to the clade)

B) Whether the sensory pits on the beak bones of birds (both palaeognathous and neognathous) are a good osteological correlate for the underlying soft tissue organisation of a tactile bill-tip organ

C) Whether the foramina on the bones of extant birds can be used in isolation to determine the presence and probable function of a bill-tip organ [most significantly, following (A), whether it is possible to differentiate the remote-touch capable bill-tip organ from the vestigial plesiomorphic organ common to all palaeognathous birds]

D) Whether lithornithids possessed bill-tip organs that were capable of remote-touch

Chapter 6:

The final chapter is a general synthesis of the results and outcomes of the four data chapters (Chapters 2-5). I also provide some personal views regarding possible future research into tactile sensory specialisations in the beaks of modern birds, and summarize the conclusions reached in my thesis as a whole.

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CHAPTER 2:

Interspecific differences in the morphology and soft tissue histology of the remote-touch bill-tip organs of ibises in relation to their sensory ecology.

African Sacred Ibis (photograph by CJ du Toit)

Abstract

Ibises (Order: Pelecaniformes, Family: Threskiornithidae) are probe-foraging birds that use “remote-touch” to locate prey items hidden in opaque substrates. This sensory capability allows them to locate their prey using high frequency substrate vibrations, in the absence of other sensory cues. Remote-touch is facilitated by a specialised bill-tip organ, comprising high densities of mechanoreceptors (Herbst corpuscles) embedded in numerous foramina in the beak bones. Each foramen and its associated Herbst corpuscles make up a sensory unit, called a “sensory pit”, which are densely clustered in the distal portion of the beak. This chapter investigates the interspecific differences in the bone and soft tissue histology of the bill-tip organs of three species of southern African ibises from different habitats (mainly terrestrial to mainly aquatic). I analysed the external pitting pattern on the bones, as well as internal structure of the beak using micro-CT scans and soft tissue histological sections of each species’ bill-tip organ. Clear interspecific differences are evident between the species’ bill-tip organs, both in terms of bone morphology and soft tissue histology. Glossy Ibises, which forage exclusively in wetter substrates, have a greater extent of pitting but lower numbers of Herbst corpuscles in each pit, while species foraging in drier substrates (Hadeda and Sacred Ibises) have more robust beaks, fewer pits and higher densities of Herbst corpuscles per sensory pit. I hypothesize that these differences are related to each species’ foraging behaviour and substrate usage, which is further tested in the next two chapters.

2.1: Introduction

Probe-foraging birds feed on prey that are either submerged in water or buried in mud/soil, and thus often face the challenge of detecting “invisible” prey concealed within opaque substrates. Some species (such as oystercatchers, stilts, rails, and ibisbills) use a method of “random” probing reliant on direct tactile contact with prey, combined with other sensory cues (visual, auditory, chemical and/or olfactory) to locate their prey (Hamilton, 1975; Franklin, Clark & Clark, 1979; Hulscher, 1982; Pierce, 1986; Zembal & Fancher, 1988; Cullen, 1994). Such direct-contact probing requires prey density to be relatively high to be energetically cost effective (Hulscher, 1982). Other species possess a specialised sensory modality known as “remote-touch”. Remote-touch refers to the detection of buried prey at a distance from the beak through perception of high frequency acceleration components of mechanical vibrations in the substrate (Gottschaldt, 1985). These stimuli result from either the movement of the prey within the substrate, or from changes in pressure gradients in wet granular substrate caused by the birds’ repeated probing action around hard-shelled prey items (Gottschaldt, 1985; Gerritsen & Meiboom, 1985; Piersma *et al.*, 1998). Birds that use remote-touch to locate submerged prey can do so in the absence of all other sensory cues, though they generally do so in combination with their other senses (Van Heezik, Gerritsen & Swennen, 1983; Gerritsen & Meiboom, 1985; Cunningham, Castro & Potter, 2009).

Remote-touch probing appears to have evolved independently in three families of modern birds: ibises (Threskiornithidae), shorebirds (Scolopacidae) and kiwi (Apterygidae) (Gerritsen & Meiboom, 1985; Piersma *et al.*, 1998; Nebel, Jackson & Elner, 2005; Cunningham, Castro & Alley, 2007; Cunningham, Castro *et al.*, 2010).

The ability to use remote-touch as a tactile sensibility for foraging in these families has been confirmed in various behavioural assays: *Calidris alba* (Sanderlings, Family: Scolopacidae) were shown to be able to detect prey using the vibrations in the substrate caused by their prey's movement (Gerritsen & Meiboom, 1985). Piersma *et al.* (1998) showed that *C. canutus* (Red knots) detect stationary hard-bodied prey using changes in pressure gradients in the substrate. The ability to successfully locate moving prey in the absence of all other sensory cues has been shown in Madagascar Crested Ibises (*Lophotibis cristata*) (Cunningham, Castro *et al.*, 2010) and in kiwi (Cunningham, Castro & Alley, 2007).

Anatomical studies of the sensory regions of the brains of birds that use remote-touch provide further evidence to support its importance. Both scolopacid shorebirds and kiwi species possess hypertrophy in the brain regions responsible for the processing of tactile information from the beak (predominantly the principal sensory trigeminal nucleus) compared to other bird species which do not use remote-touch (Cunningham, Corfield, *et al.*, 2013). The same is true for other taxa of birds which use their beaks for different types of specialized tactile foraging or food manipulation, including waterfowl (ducks and geese) and parrots (Cunningham, Corfield, *et al.*, 2013; Martin & Martin, 2021).

Remote-touch is facilitated by a specialised tactile bill-tip organ. This comprises groups of mechanoreceptors (Herbst corpuscles) embedded within densely clustered foramina in the distal regions of both the premaxilla and mandible (Bolze, 1968; Gottschaldt, 1985; Nebel, Jackson & Elner, 2005; Cunningham, Alley *et al.*, 2010; Cunningham, Corfield, *et al.*, 2013) (see Chapter 5 for more details). Herbst corpuscles are avian mechanoreceptors which detect changes in acceleration components of mechanical vibrations and detect vibrations that originate from sources that are not in direct contact with the structure they are part of (Gottschaldt, 1985). The clusters of Herbst corpuscles are centred around nerve fibres that run through the foramina into the central nerve canals of the beak bones, forming branches of the trigeminal nerve [responsible for relaying sensory information from the beak (Barnikol, 1954; Dubbeldam & Karten, 1978)]. The foramina in the bone and the soft tissues within them are together referred to as “sensory pits”, as the external openings of the foramina on the surface of the beak bones resemble small pits/holes. The bill-tip organ refers to the entire structure of the multiple sensory pits and associated neurovascular tissues in the distal portion of the beak. In the absence of soft tissues, the presence of a tactile bill-tip organ can be inferred from the number and proximity of the sensory pits on the external surfaces of the beak bones, and when combined with the shape of the beak, the function of the organ can also be determined (Chapter 5, section 5.4.1).

A potential link between habitat usage and interspecific differences in bill-tip organ morphology was proposed by Cunningham, Alley *et al.* (2010), based on differences in the number of pits in the beak bones and the extent (percentage of beak length) of pitting between species of ibises. These differences in bill-tip organ morphology are correlated with the birds’ habitat usage: birds living in more aquatic habitats have larger bill-tip organs (relative to beak size) and higher numbers of sensory pits on their beak bones. This pattern holds when including data from kiwi species, which live in drier habitats and have fewer pits in their bill-tip organs compared to ibises. Cunningham (2010) proposed that these differences in the bill-tip organ morphology may be linked to different sensitivities required to detect the vibrations in substrates with different water contents.

If more extensive pitting does correlate with higher sensitivity [as was tentatively suggested by Cunningham (2010)], the finding that species living in more aquatic habitats have more extensively pitted bill-tip organs than species from terrestrial habitats is at odds with the fact that substrates with higher water content transmit vibrations more easily (Biot, 1956 & 1962). Cunningham (2010) attempted to address this potential conundrum by hypothesizing that there will be more “noise” in wetter substrates from other sources that needs to be filtered out to detect useful signals coming from prey. However, there is as yet no behavioural evidence that higher extent of pitting correlates with higher sensitivity of the bill-tip organ to vibratory stimuli, and a better anatomical measure of sensitivity would be a comparison of the mechanoreceptor arrangements (Zweers & Gerritsen, 1997).

Ibises and spoonbills form the family Threskiornithidae, which falls within the Pelecaniformes order (Jarvis *et al.*, 2014; Prum *et al.*, 2015). Though originally believed to be split into two sister subfamilies [ibises and spoonbills, based on morphology (Matheu & del Hoyo, 1992)], recent genetic and morphological studies show that the spoonbills are nested within the ibises, with the genus *Threskiornis* being their closest relatives (Chesser *et al.*, 2010; De Pietri, 2013; Ramirez, Miyaki & Del Lama, 2013).

Ibises have long, thin, downcurved bills, which they probe into water, mud and/or soil to extract buried prey items. Ibises occupy various foraging habitats (Cramp *et al.*, 1983; Ali & Ripley, 1983; Matheu & del Hoyo, 1992; Hockey, Dean & Ryan, 2005), though most ibis species do tend to forage in wetlands (Kushlan, 1977; Kushlan, 1978; Cramp *et al.*, 1983; Ali & Ripley, 1983; Matheu & del Hoyo, 1992; Hockey, Dean & Ryan, 2005). Remote-touch has been experimentally confirmed in ibises (Cunningham, Castro *et al.*, 2010; see also Chapter 4), and all studies of ibis beak morphology indicate the presence of remote-touch capable bill-tip organs (Swennen & Yu, 2004; Cunningham, Alley *et al.*, 2010) (see also Chapter 5).

In this study, I investigate whether ibis species foraging in wetter habitats have more sensitive bill-tip organs than those adapted for terrestrial habitats, by assessing whether there is a positive correlation between sensory pitting extent and density of Herbst corpuscles. I compare the soft tissue histology and bone morphology of the bill-tip organs of three southern African ibis species from different habitats: Hadeda Ibis (*Bostrychia hagedash*), African Sacred Ibis (*Threskiornis aethiopicus*) and Glossy Ibis (*Plegadis falcinellus*). I describe the interspecific differences in soft tissue histology of the bill-tip organs with specific reference to the Herbst corpuscle arrangement, as the receptors themselves (not just the foramina in the bones) are likely to be driving the sensitivity of the bill-tip organs (Zweers & Gerritsen, 1997). I discuss the implications of these findings for understanding the foraging ecology of ibises and other remote-touch capable birds. This chapter provides the morphological context for the subsequent chapters of my thesis, which investigate the ibises' foraging behaviour and sensory ecology in relation to the interspecific differences in their bill-tip organs described here.

2.2: Materials and Methods

2.2.1: Ecology of study species

All three species of ibises studied in this chapter (Hadeda, Sacred and Glossy Ibises) use probe-foraging as their primary foraging technique (see Chapter 3, section 3.4.3 and Figure 3.3), but differ in their foraging habitats: Hadeda Ibises are mostly terrestrial, though they will also use wetlands on occasion (Skead, 1951, Hockey, Dean & Ryan, 2005, Chapter 3, section 3.3.1). In the past century they have experienced a range expansion in South Africa, now using urban gardens and agricultural land alongside their historical forested habitats (Macdonald, Richardson & Powrie, 1986), apparently facilitated by artificial irrigation and growing of lawns and pastures (Duckworth, Altwegg & Guo, 2010). Glossy Ibises represent the other end of the habitat spectrum, in that they forage almost exclusively in or around bodies of fresh water (Matheu & del Hoyo, 1992; Frederick & Bildstein, 1992; Hockey, Dean & Ryan, 2005; Taylor & Taylor, 2015) (see also Chapter 3, section 3.3.1). Sacred Ibises are generalist in both their foraging habitat and behaviour: they forage in terrestrial and aquatic habitats (Hockey, Dean & Ryan, 2005; Clergeau & Yésou, 2006; Yésou *et al.*, 2017), sometimes foregoing probing to capture large vertebrate prey [e.g., eggs and chicks of other birds (Williams & Ward, 2006)], and will also feed on carrion (Chane & Balakrishnan, 2016). In recent years, many Sacred Ibises have become urban exploiters, feeding on human refuse (Clark, 1979; Clergeau & Yésou, 2006; Calle & Gawlik, 2011; Yésou *et al.*, 2017) (see also Chapter 3, section 3.3.4).

2.2.2: Specimens used

Skeletal samples of all three species were sourced from various natural history museums around the world (museum accession number abbreviations in brackets): Iziko South African Museum, Cape Town (SAM); Smithsonian National Museum of Natural History, Washington, DC (USNM); Natural History Museum at Tring, UK (NHM); University of Cambridge Zoology Museum, UK (UCZM); and Muséum National d'Histoire Naturelle, Paris (MNHN).

All the samples used for histological sectioning were sourced from recently deceased birds from the Western Cape Province of South Africa.These birds all died for reasons unrelated to this study (either as casualties of vehicle impacts or attacked by dogs) and were donated to the project by various wildlife organisations and members of the public. As no live birds were utilised in this study or killed for this research, under South African law I required no ethical approval for the research outlined here.

I sampled six skeletal specimens per species of Glossy and Sacred Ibises, and four Hadeda Ibises (for a list of skeletal specimens used, see Appendix 2.1). For the histological analyses, I sectioned two specimens per species of Glossy and Sacred Ibises, and three specimens of Hadeda Ibises (for a list of specimens sectioned for histology, see Appendix 2.2). Similar numbers per species have been used in various other studies of bird beaks (Gottschaldt & Lausmann, 1974; Piersma et al., 1998; Cunningham, Castro & Alley, 2007; Cunningham, Corfield *et al.*, 2013; Demery, Chappell & Martin, 2011; Crole & Soley, 2014).

2.2.3: Measures of pitting pattern from photographs

Each skeletal specimen (using specimens which had the rhamphotheca removed by their respective museums prior to my examination) was photographed using a digital camera (*Canon PowerShot SX620 HS*) and light table. Six images per specimen were analysed: lateral, ventral and dorsal views of both the mandible and maxilla.

Measurements extracted for each specimen were: total number of pits; distance of each pit from the tip of the bill; and bill-tip organ length (measured as the average maximum distance of pits from the distal tip of the bill; used to calculate extent of pitting and density of pits). The premaxilla length was measured from the distal tip to the craniofacial hinge and was used as a standard measure of beak length (see “PMx” on Figure 2.1A).

To compare the “tapering” in beak shape (the change in size between the base and the tip of the beak), I calculated the ratio of tip width to base width and tip depth to base depth of the premaxilla (see Figure 2.1 for points of measurement; “width” from dorsal view and “depth” from lateral view). The two ratios of tapering were calculated separately (i.e., one ratio for width and one for depth) by dividing the tip by the base measurements.

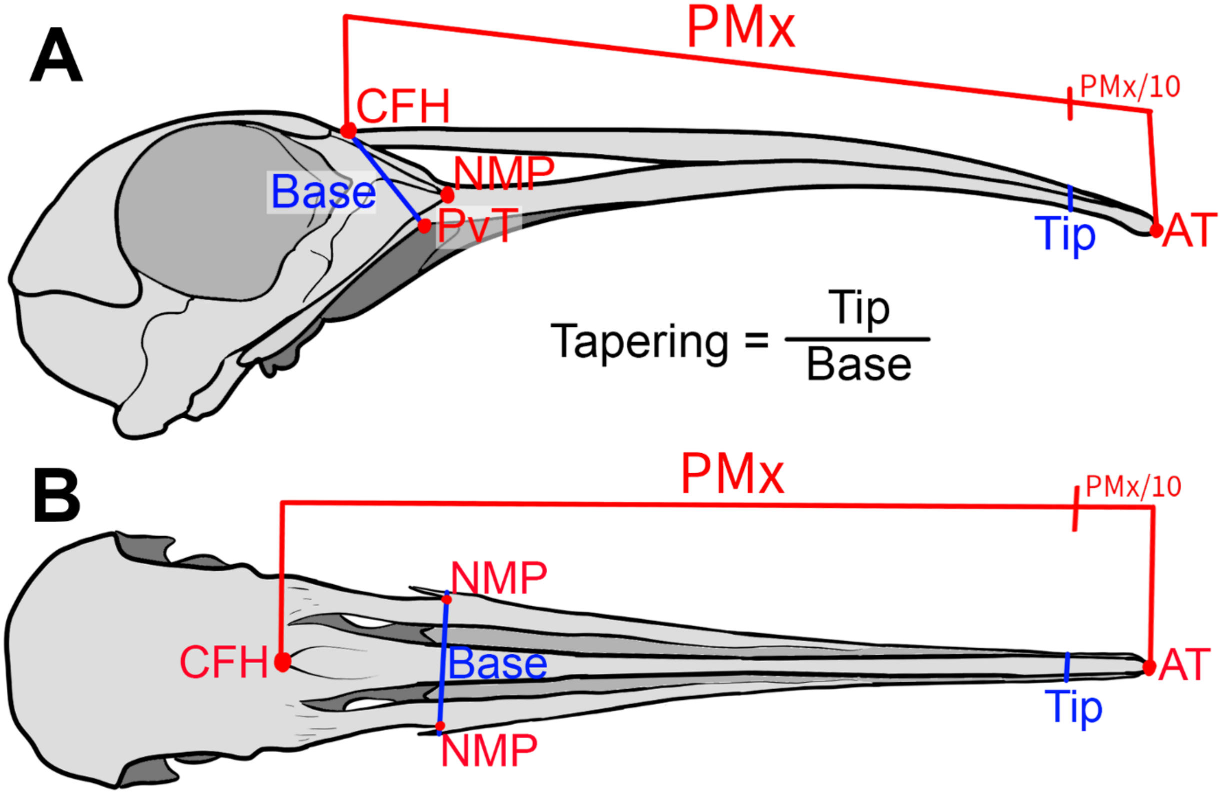

Figure 2.1: Drawing of an ibis skull, showing where measurements were taken to calculate the tapering of the beak shape. Tapering was measured as the ratio between the tip depth/width to the base depth/width (depth/width depending on lateral or dorsal view respectively). “Tip” depth/width measured at a distance (PMx/10) of 1/10th of the total premaxilla length (PMx) from the anterior tip of the premaxilla (AT). A) Lateral view showing depth measurements; “Base” depth measured as lateral distance from the craniofacial hinge (CFH) to the posteroventral tip of the premaxilla (PvT). B) Dorsal view showing width measurements; “Base” width measured across left and right symphyses of the nasal bone and maxillary processes [NMP – anatomical position indicated more clearly from lateral view in (A)].

I developed the following workflow using the software *ImageJ2* (Rueden *et al.*, 2017) to extract the various measures of pitting from the photographs: The distal tip of the beak was selected, and its position in the photograph was measured (using the *Measure* command) to give a pair of coordinates (in pixels). The origin of the photograph was then reset to these coordinates [*Image → Properties → Origin (pixels)*], so that the tip represented the 0:0 mark. The scale of the image was then set. The openings of the foramina on the beak were manually selected using the point selection tool. As there was no published information on how the "sensory pits" can be distinguished from other types of foramina on the beak in the absence of soft-tissue histology, all foramina on the surface of the beak bones were selected (see Chapter 5, section 5.4.1 for justification of using this method). Once all the pits had been selected, the *Measure* command was used to give a list of the scaled coordinates of each pit (relative to the tip of the beak). These coordinates were then extracted to *Microsoft Excel* (2013), and the distance from the tip of the beak to each pit was calculated using standard trigonometry.

2.2.4: Three-dimensional imaging of beak bones

I used X-ray micro computed-tomography (micro-CT) to image the internal bone structure of the specimens at very high resolutions, following similar methods to Cunningham, Corfield, *et al.* (2013). Scans were done by the Central Analytical Facilities Lab at Stellenbosch University (Stellenbosch, South Africa) using the *General Electric VTomex L240* (*General Electric Sensing and Inspection Technologies/Phoenix X-ray*, Germany) scanner (du Plessis, le Roux & Guelpa, 2016). Specimens were mounted vertically in the scanning chamber, and the entire beak was scanned, as well as additional scans for the distal most ~5cm portions of the bill at the higher resolutions. All scans were taken at resolutions between 7-20 µm, to visualize the pits in the bones at sufficient detail.

The program *VG Studio* (© 1997-2021*Volume Graphics*, Germany) was used to compile the voxel data from the CT scans into three dimensional models for analysis. I performed all image capture and analyses with the open-source software *MyVGL* (© 1997-2021*Volume Graphics,* Germany).

2.2.5: Histological sectioning, staining and analyses

Fresh samples were stored for a maximum of 48 hrs in a -20ºc freezer (or processed immediately where possible). The heads were removed, and were fixed and stored in a 10% neutral buffered formalin solution. The keratin of the rhamphotheca was softened by covering it in a commercially available hair removal cream (*Veet® In shower Hair Removal Cream*), as stipulated by Van Hemert *et al.* (2012), which was changed every ~6 hours for 1-3 days. Once the rhamphotheca was softened to the point where it could be easily dented by a small amount of pressure applied to the surface, the excess cream was wiped off, and the sample was rinsed under running water. The first 1cm of the beak (mandible and maxilla treated separately) was split sagittally and fixed for a minimum further 48 hrs in 10% neutral buffered formalin. Each sample was then dehydrated, cleared and embedded in paraffin blocks using a benchtop tissue processor (*Leica TP1020*, Germany). In order to decalcify the bone for sectioning, the exposed surface of the sample was submerged in Kristenson's fluid [18% formic acid buffered with formate (Wallington, 1972)] for up to 15 min at a time (longer damaged the soft tissues, see section 2.3.5). Sagittal sections of between 5-10 µm (depending on the sample) were made using a rotary microtome (*Leica RM2125RT*, Germany) and then mounted on charged glass slides.

Sections were dewaxed in xylene and then rehydrated in successive decreasing solutions of ethanol following standard procedures. The sections were stained using a Masson's trichrome staining kit (*Sigma-Aldrich*). The sections were dehydrated using increasing ethanol solutions and cleared with xylene, before final mounting in DPX Mountant (*Sigma-Aldrich*) and coverslipped.

The sections were photographed with a compound microscope (*Nikon Eclipse E200*) and attached digital camera (*Canon PowerShot D10*), and the images were captured using *NIS Elements* (*Nikon*). All measurements were taken from the images of the sections using *ImageJ2* (Rueden *et al.*, 2017).

The general arrangement and orientation of the Herbst corpuscles in the pits in the bone are described, as well as overall descriptions of the soft tissue structure of the bill-tip organs. Quantitative measurements and counts were taken of the Herbst corpuscles in each specimen, including: average width and length of the corpuscles (measured from longitudinal sections of corpuscles where the central nerve axon could be clearly seen); and the number of Herbst corpuscles per sensory pit (counted for all pits visible in the section, then averaged per specimen).

2.2.6: Statistical analyses and generation of figures

The raw data measurements were compiled in *Microsoft Excel* (2013). All statistical analyses and plotting of data were done using *RStudio* (RStudio Team, 2016). All figures showing photographs of skulls, histological sections and images generated from the micro-CT scans were compiled and edited in *Adobe Photoshop* (2004).

All plots were generated using the *R* (R Core Team, 2018) package *ggplot2* (Wickham, 2011). Due to the small sample sizes, I used non-parametric Kruskal-Wallis H-tests to assess the statistical significance of differences in pitting numbers and density on the beak bones between the three species of ibis. Post-hoc Wilcoxon signed-rank tests were applied when statistical significance ($p<0.05$) was found in the Kruskal-Wallis H-tests. In order to plot the P-values and significance codes on the figures, I used the *R* packages *ggsignif* (Ahlmann-Eltze, 2019) and *ggpubr* (Kassambara, 2018).

No statistical tests were performed on the data from the CT scans or histological sections, as these all had small sample sizes (n=2 or 3 for each ibis species). Such small sample sizes per species are normal for these types of morphological descriptions (Gottschaldt & Lausmann, 1974; Cunningham, Castro & Alley, 2007; Cunningham, Alley *et al.*, 2010; Cunningham, Corfield, *et al.*, 2013; Avilova, Fedorenko & Lebedeva, 2018), and were sufficient for the purposes of this study. The 95% confidence intervals shown for the number of Herbst corpuscles per pit for each species were generated from the counts of Herbst corpuscles in each pit.

2.3: Results

2.3.1: Beak bone morphology

All three ibis species have similar external (Figure 2.2) and internal (Figure 2.3) beak bone structures. The premaxillae are split into three distinct processes (Figure 2.2B): the nasal process is positioned dorsally along the median of the lower two maxillary processes, which run alongside each other. All three processes are fused at the distal end of the beak, but the lateral grooves along the joints on either side between the nasal and maxillary processes create a clear distinction between them even in the distal region (see Figure 2.2B). The mandible is separated into two dentary bones, which are also fused in the distal region of the beak by a thin strip of bone (mandibular symphysis; see the coronal sections in Figure 2.3A), and then split apart proximally into two rami.

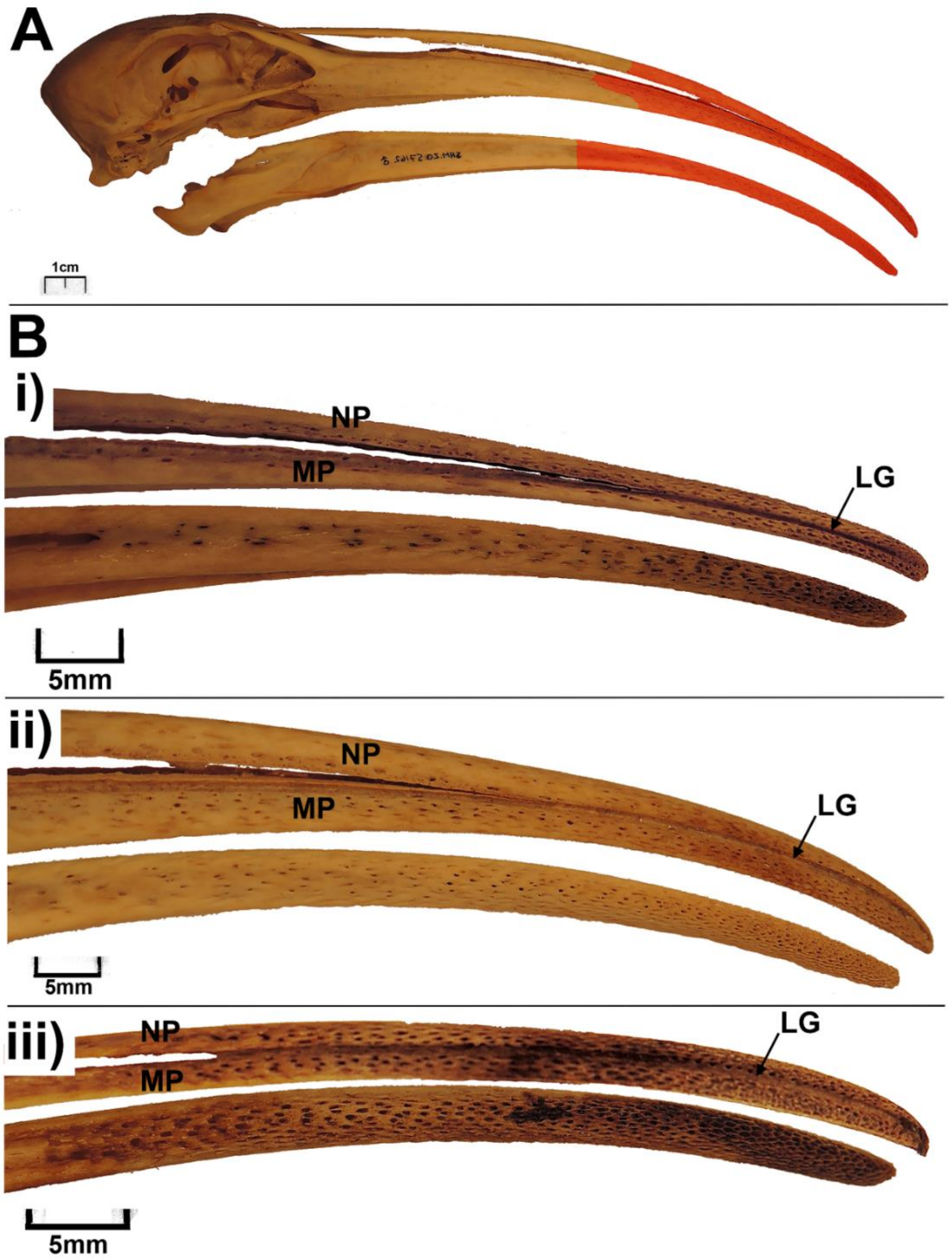

Figure 2.2: External features on the beak bones of the three species of ibis. A) Lateral view of the premaxilla and mandible of a Sacred Ibis (*Threskiornis aethiopicus,* SAM.ZO 57162). Red shaded regions indicating surface area of beak pitted. B) Distal portion of the beaks of the three ibis species, showing the external openings of the sensory foramina. i) Hadeda Ibis (*Bostrychia hagedash,* SAM.ZO 58022); ii) Sacred Ibis [same specimen as in (A)]; iii) Glossy Ibis (*Plegadis falcinellus,* SAM.ZO 58483). Note the distinction between the nasal (NP) and maxillary (MP) processes on the premaxillary bone of all three species. These are fused together on the distal potion of the rostrum, but still form distinct units separated by a lateral groove (LG) in the bone.

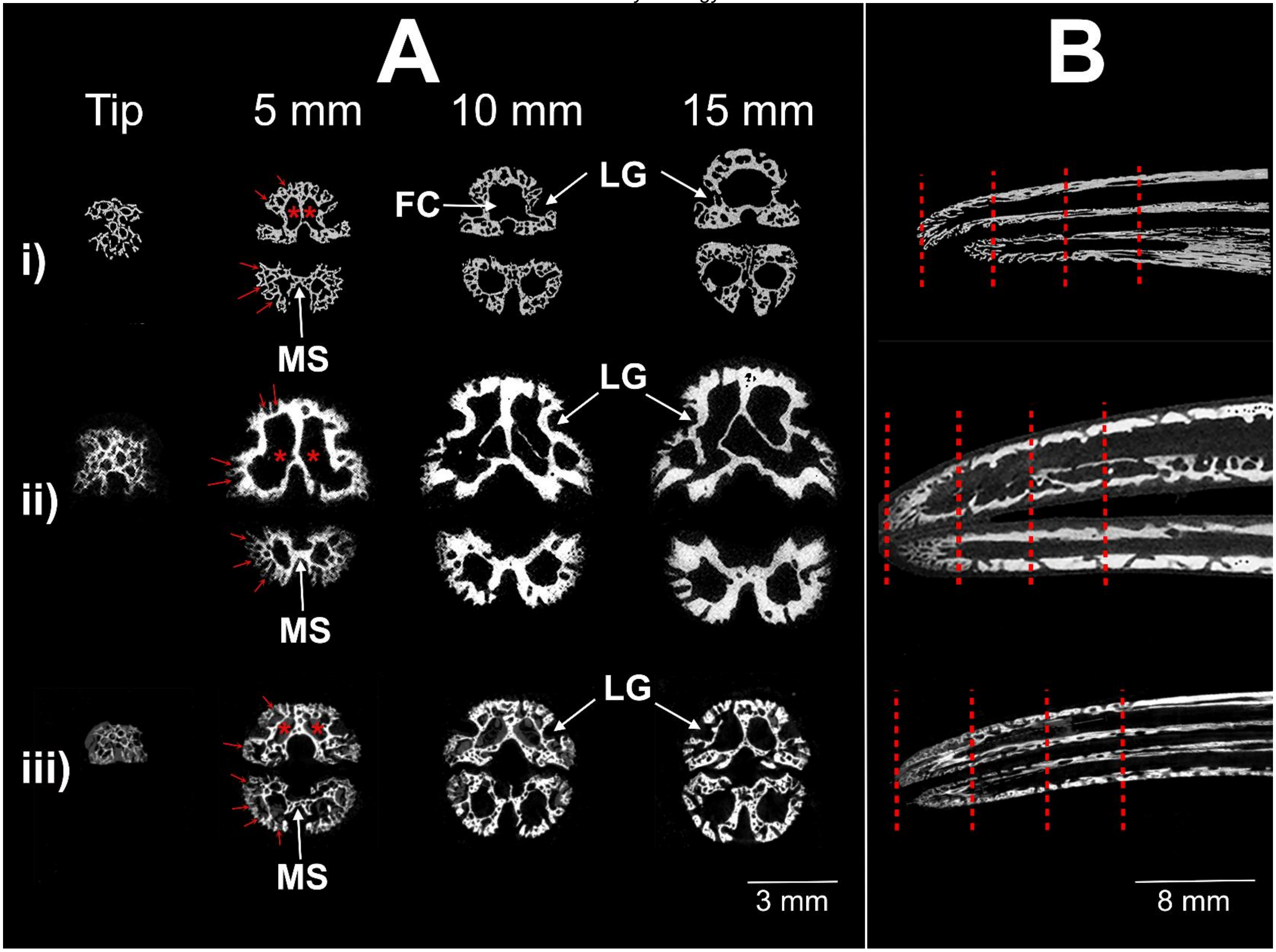

Figure 2.3: Micro-CT sections through bill-tip bones of three ibis species: i) Hadeda Ibis (*Bostrychia hagedash*); ii) Sacred Ibis (*Threskiornis aethiopicus)*; iii) Glossy Ibis (*Plegadis falcinellus*). A) Consecutive coronal sections starting from the tip of the premaxilla, with slices 5 mm apart. Red arrows showing examples of foramina (which form the ‘sensory pits’). Red asterisks indicate two branches of the neurovascular canal in distal portions of the premaxilla; these then fuse to from a single canal, which can be seen in the Hadeda Ibis (“FC”) at 10mm (fusion occurs more proximally in Sacred and Glossy Ibises). B) Sagittal sections of the same specimens. Vertical red dashed lines indicate the position of the coronal slices shown in (A). LG: lateral grooves (run along fusion lines between the nasal and maxillary processes of the premaxilla); MS: mandibular symphysis.

Two internal canals run alongside each other in the premaxilla from the distal tip, which fuse together proximally to form a single cavity. This can be seen by looking at the coronal sections of the beak bones (Figure 2.3A), and is best seen in the Hadeda Ibises [Figure 2.3A(i): at 5mm from the tip of the beak, the two canals are separate, but have fused together by 10mm (this fusion takes place more proximally in both Sacred and Glossy Ibis premaxillae, between 20-25 mm from the tip)]. The canal/s are predominantly filled by large branches of the trigeminal nerve, along with blood vessels supplying the length of the beak. The canals in the mandible remain separated in the two dentary bones.

The sensory foramina of the bill-tip organ open to the external surfaces of the beak bones (forming the “pit” structures on the surface of the bones – see Figure 2.2B) and extend through the bone to connect to the central neurovascular canals of the beak (see Figure 2.3). In cross section, the beak bones appear porous (Figure 2.3), due to the high density of foramina extending in multiple directions out of the central nerve cavity. None of the foramina cross into other processes of the beak from that in which they originate (e.g., an opening on the external surface of the left mandibular process will be connected to the neurovascular canal of the same process).

The density of foramina decreases proximally from the tip of the beak (see Figure 2.2B). The longest foramina (foramen length measured from the opening on the external surface of the bone to the joining with the central neurovascular canal) are found on the anterior surfaces of both the premaxilla and mandible (see first 5mm of the bones in Figure 2.3B). The shallowest foramina (and the lowest density of foramina) are found on the inner surfaces of the beak bones (the inside of the birds’ beaks; dorsal surface of mandible and ventral surface of premaxilla). Glossy Ibises have the shallowest foramina on average (230 ± 30 µm), followed by Sacred Ibises (400 ± 50 µm), with Hadeda Ibises having the deepest foramina on average (580 ± 110 µm).

2.3.2: Beak shape

Whether viewed laterally or dorsally, the beaks of all three species are widest at their bases and taper distally. From a dorsal view, they have a thin distal beak tip (tip width – from dorsal view; see Figure 2.1 for definitions and places of measurement), ranging from 3.7-5.9 mm on average for each species (see Table 2.1). Thereafter, the degree of tapering (the difference in size between the base and tip of the beak) is unequal between the three species (Figure 2.4). Hadeda Ibises show the greatest tapering of beak width, with the base of the beak being roughly six times wider than the tip (Figure 2.4A). From a lateral view (beak depth), all three species have a thin distal tip (Figure 2.4A). Once again, the Hadeda Ibises have the greatest degree of tapering in beak depth, with the base of their beaks measuring almost seven times thicker than the distal tip region (Figure 2.4B). Glossy Ibises have the least amount of lateral tapering, with their premaxilla bases being less than three times thicker than their tips, meaning that their beaks stay relatively thin across their entire length (Figure 2.4B). Furthermore, Glossy Ibises have the thinnest beak bases from both views [average depth of 9.5 mm, compared to 17.2 mm and 20.0 mm for Sacred and Hadeda Ibises respectively – see Table 2.1 for more details].

Table 2.1: Summary of various beak measurements for three species of ibis. Values are quoted as means. All width and depth measurements were taken from the premaxillary bone. “Width” measured from dorsal view, “Depth” from lateral view (see Figure 2.1). Species labels: “Hadeda” = *Bostrychia hagedash*; “Sacred” = *Threskiornis aethiopicus*; “Glossy” = *Plegadis falcinellus*. n = number of specimens (could not count sensory pits on all due to preservation condition of some specimens).

Species	n (total)	n (used to count pits)	Bill length (mm)	Bill-tip width (mm)[1]	Base width (mm)	Tip/base width ratio	Bill-tip depth (mm)[1]	Base depth (mm)	Tip/base depth ratio	Total number of pits	Bill-tip organ length (mm)[2]	Percent bill length pitted	Pit density (pits/mm)	Estimated total Herbst corpuscles[3]
Hadeda	4	4	141.5	3.7	21.5	0.17	3.0	20.0	0.15	2206	33.6	23.61	68.3	33400
Sacred	6	5	157.6	5.9	20.6	0.29	4.9	17.2	0.29	3155	63.5	40.54	49.4	51000
Glossy	6	4	121.7	4.6	14.1	0.33	3.3	9.5	0.35	4354	63.3	52.43	68.3	31700

[1] measured at distance 1/10th of the total premaxilla length from the distal tip of the beak (see Figure 2.1)

[2] the maximum distance of sensory pits from the distal tip of the bill (average for all surfaces of premaxilla and mandible)

[3] calculated by multiplying the total number of pits by the average number of Herbst corpuscles per pit (see Table 2.2) for each species

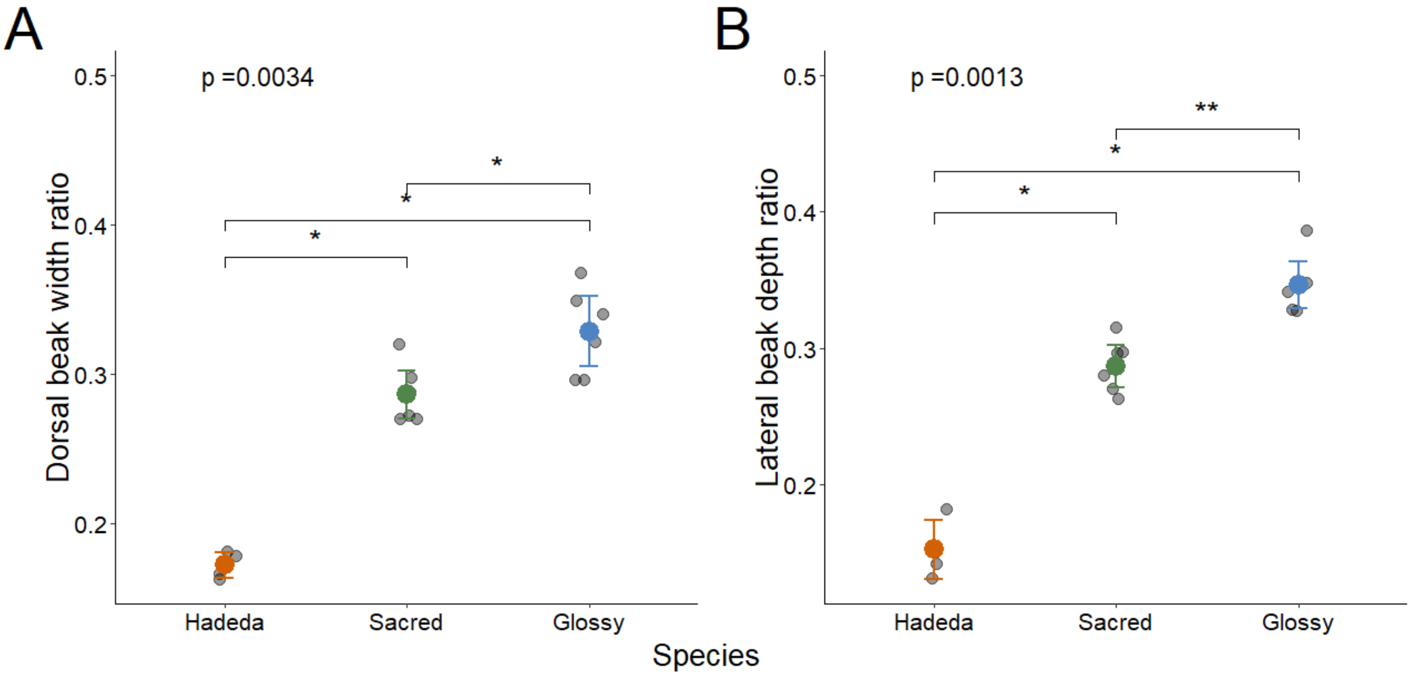

Figure 2.4: Ratio of premaxillary bone tip-to-base size in three species of ibis, showing distal tapering of beak shape, from A) dorsal (width) and B) lateral (depth) views. See Figure 2.1 for location of measurements. Small grey circles represent individual specimens (see Table 2.1). Large coloured circles show mean value for species; coloured brackets showing 95% confidence intervals. Quoted p-values from non-parametric Kruskal-Wallis tests. Significance codes between species from post-hoc Wilcoxon tests: * = $p<0.05$; ** = $p<0.01$. Species labels: “Hadeda” = *Bostrychia hagedash* (n=4); “Sacred” = *Threskiornis aethiopicus* (n=6); “Glossy” = *Plegadis falcinellus* (n=6).

2.3.3: Soft tissue histology

General features of soft tissue histology and arrangement of the Herbst corpuscles (mechanoreceptors responsible for facilitating remote-touch) are consistent across all three species of ibis.

All three species have sensory pits extending outwards in all directions from the central nerve cavities of their beaks (Figures 2.5-7), all of which contain two or more Herbst corpuscles visible per section. The majority of these pits extend outwards in an anterior direction from the central nerve canals to their external openings on the surfaces of the beak bones. All of the foramina sectioned in the distal-most ~1 cm of the beak comprised sensory pits (identifiable by the central nerve fibre surrounded by Herbst corpuscles – further details below). From the sagittal cross sections of the bill-tips, most of the sensory pits appear to be longitudinally sectioned, though some lateral cross sections can be observed (see some examples of lateral cross-sectioned pits in Figures 2.9i & 2.10ce).

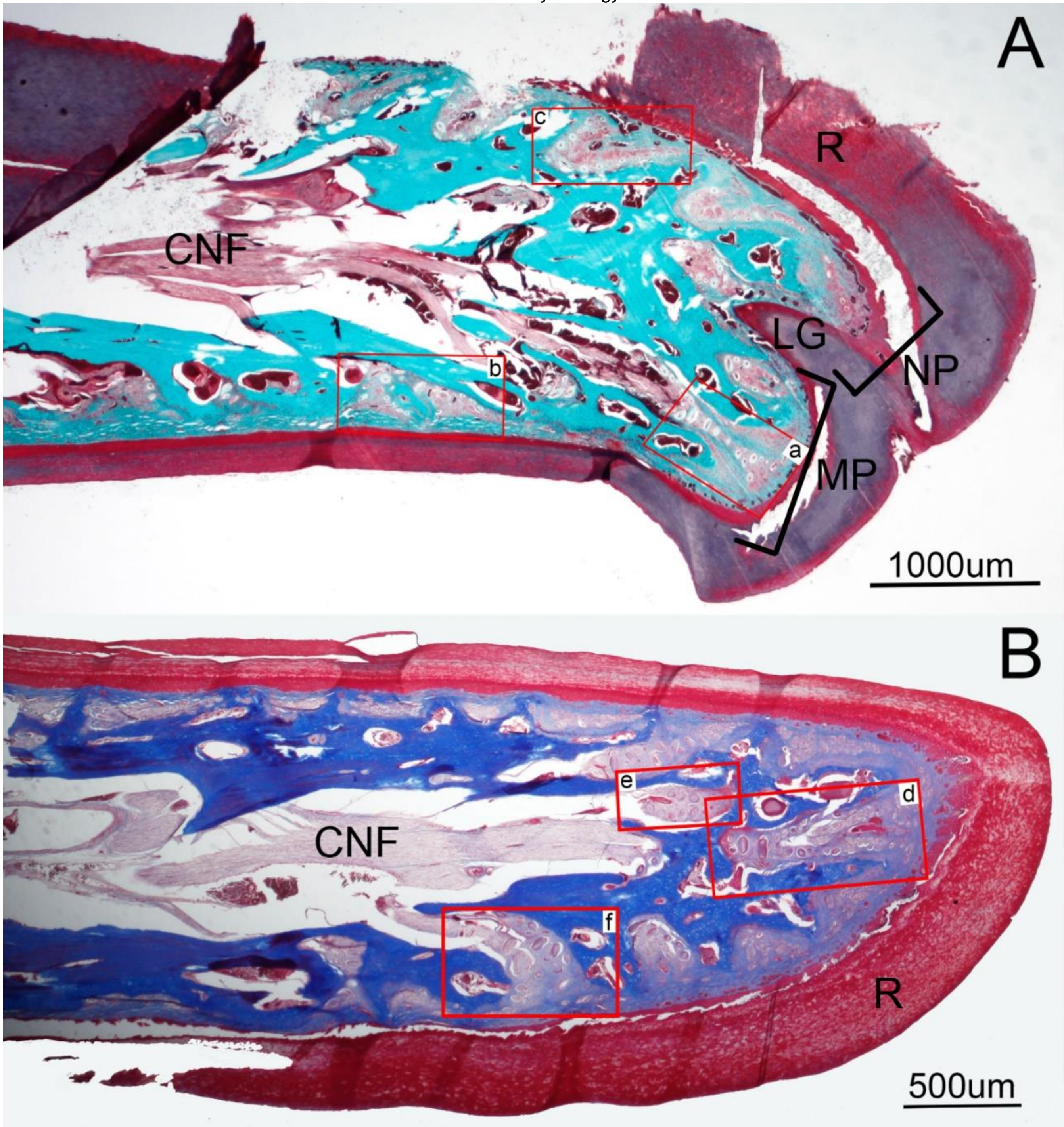

Figure 2.5: Sagittal section of the distal tip of the beak of Hadeda Ibis (*Bostrychia hagedash*) showing the internal histology of the bill-tip organ. A) Premaxilla and B) mandible, both from the same bird (H4, see Appendix 2.2). Several sensory pits have been outlined in red, the letters a-f refer to their high resolution plates in Figure 2.8. Masson’s trichrome stain used: Red outer layer, keratinaceous rhamphotheca; dark blue/turquoise tissue, bone (different dye colors used for the two sections); lighter purple, pinks, blues and greens, soft tissues. “CNF”: central nerve fibre “LG”: lateral groove; “NP”: nasal process; “MP”: maxillary process; “R”: rhamphotheca.

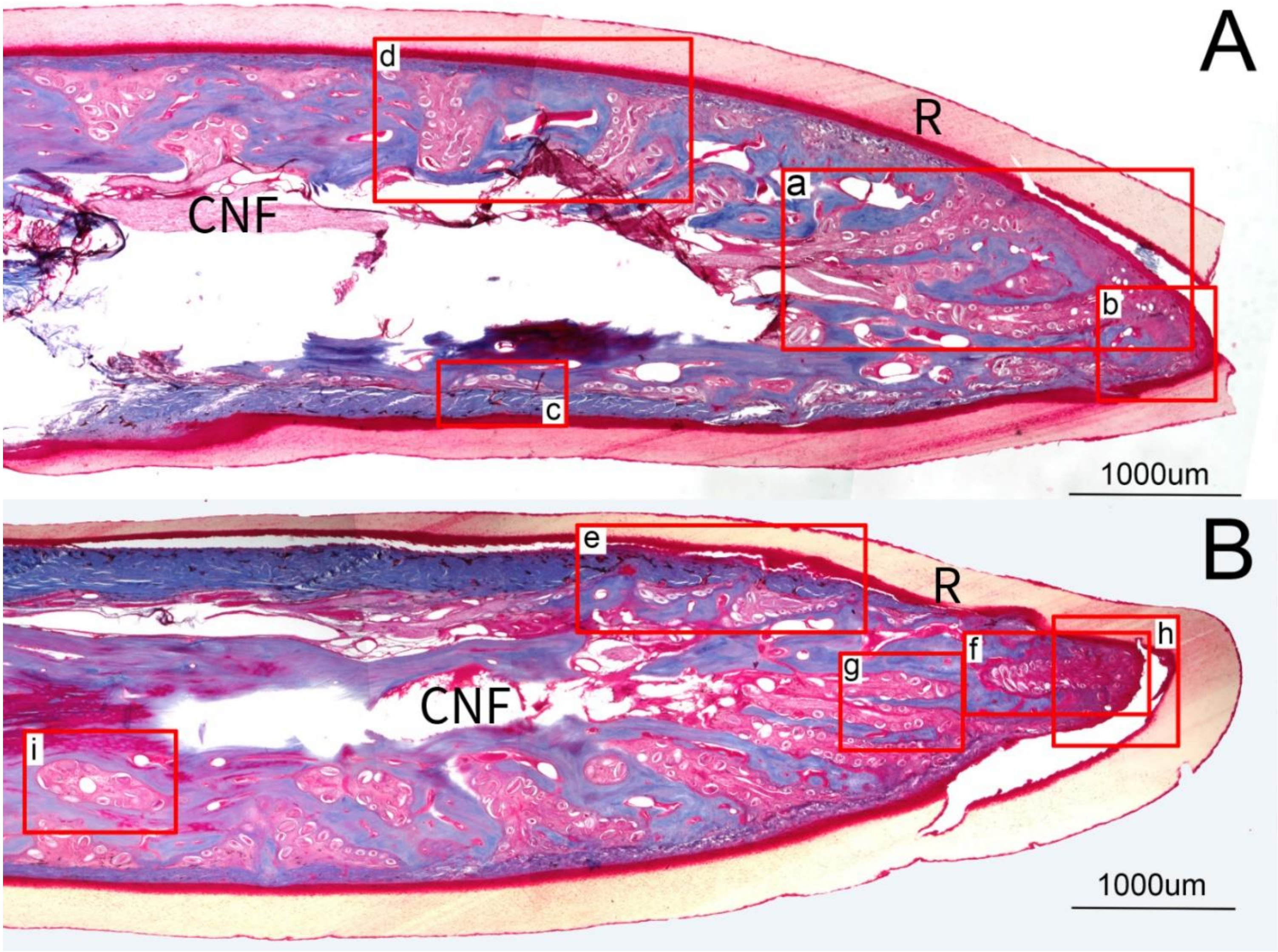

Figure 2.6: Sagittal section of the distal tip of the beak of Sacred Ibis (*Threskiornis aethiopicus*) showing the internal histology of the bill-tip organ. A) Premaxilla and B) mandible (from two different individuals: S22 & S23, see Appendix 2.2). Several sensory pits and regions of interest have been outlined in red, the letters a-i refer to their high resolution plates in Figure 2.9. Masson's trichrome stain used: Red & yellow outer layer, keratinaceous rhamphotheca; dark blue, bone; lighter purple, pinks and blues, soft tissues. "CNF": central nerve fibre; "R": rhamphotheca.

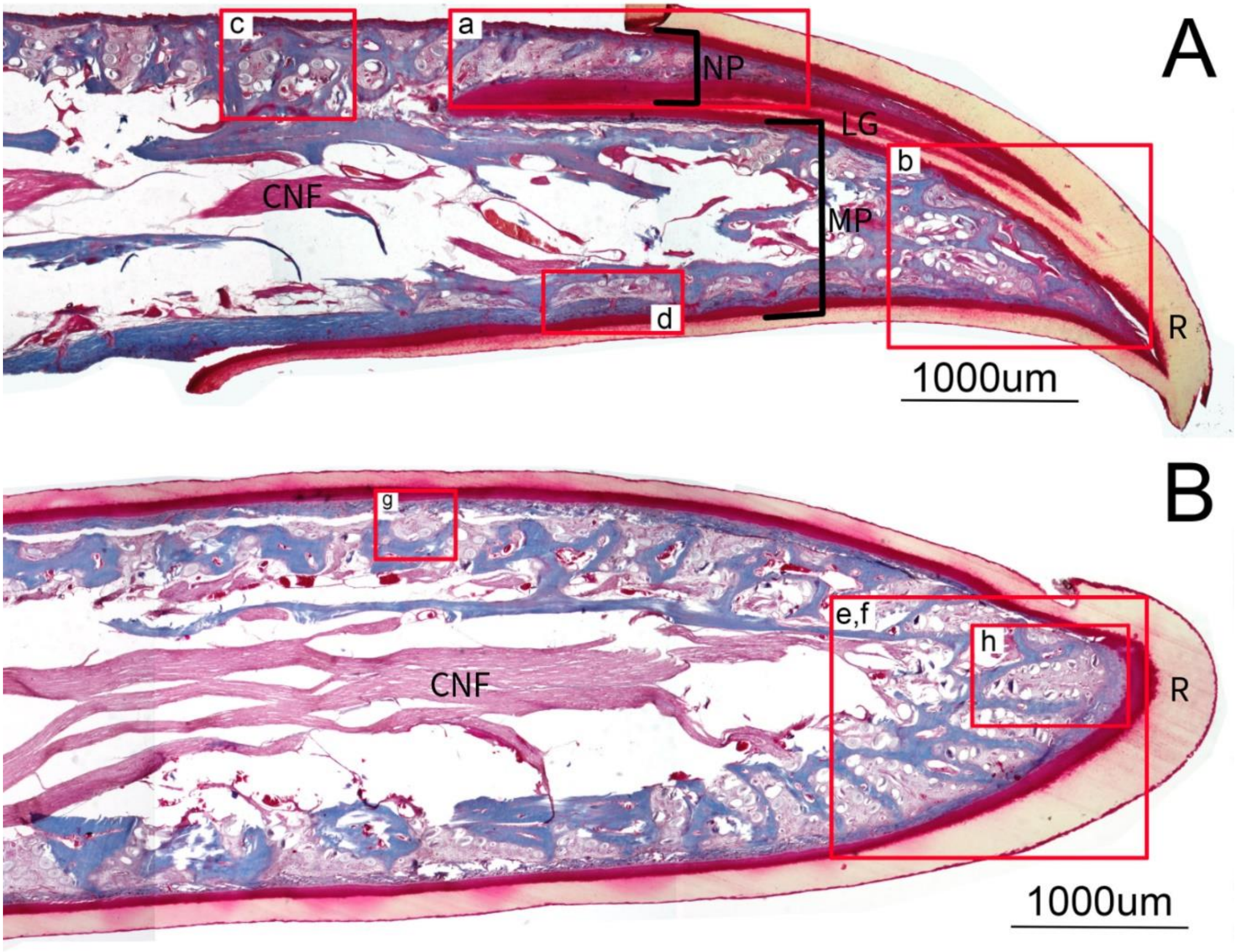

Figure 2.7: Sagittal section of the distal tip of the beak of Glossy Ibis (*Plegadis falcinellus*) showing the internal histology of the bill-tip organ. A) Premaxilla and B) mandible, both from the same bird (G9, see Appendix 2.2). Several sensory pits and regions of interest have been outlined in red, the letters a-h refer to their high resolution plates in Figure 2.10. Masson's trichrome stain used: Red & yellow outer layer, keratinaceous rhamphotheca; dark blue, bone; lighter purple, pinks and blues, soft tissues. "CNF": central nerve fibre; "LG": lateral groove; "MP": maxillary process; "NP": nasal process; "R": rhamphotheca.

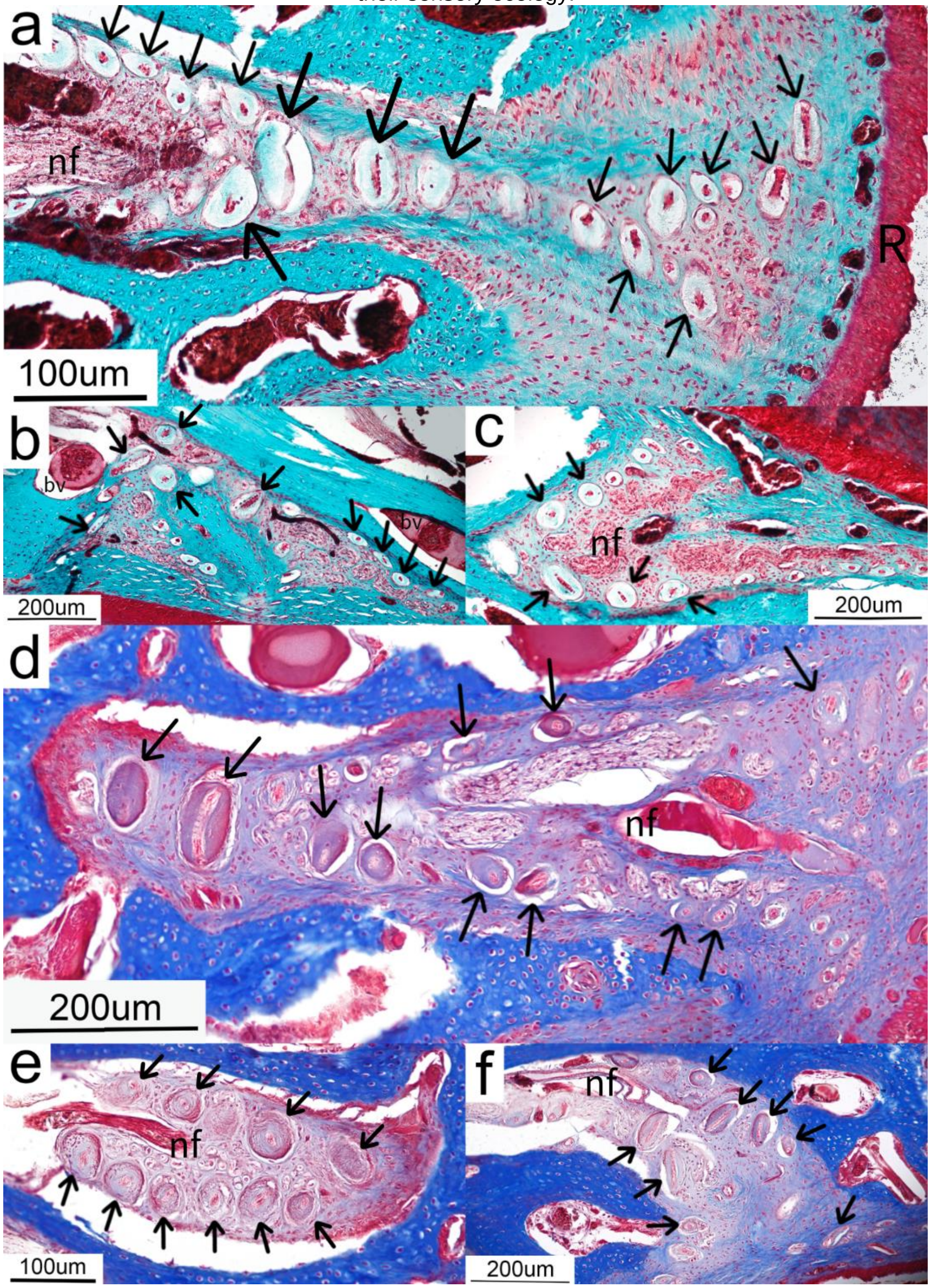

Figure 2.8: Cross sections of various sensory pits from the bill-tip organ of Hadeda Ibis (*Bostrychia hagedash*) showing the arrangement, orientation and number of Herbst corpuscles and other soft tissues within the pits. For relative positioning of the pits on the beak refer to Figure 2.5. [a,d] sections of longest pits, found on the anterior-most surface of the beak. [c,f] pits on the external surfaces of the beak. [b] two adjacent pits on the inside of the beak, note the fusion of the two pits before they connect to the central nerve cavity. [e] proximal section of a sensory pit, showing how some foramina curve along their length. Black arrows showing Herbst corpuscles. “bv”: blood vessel (in b); “nf”: nerve fibre of sensory pit; “R”: rhamphotheca.

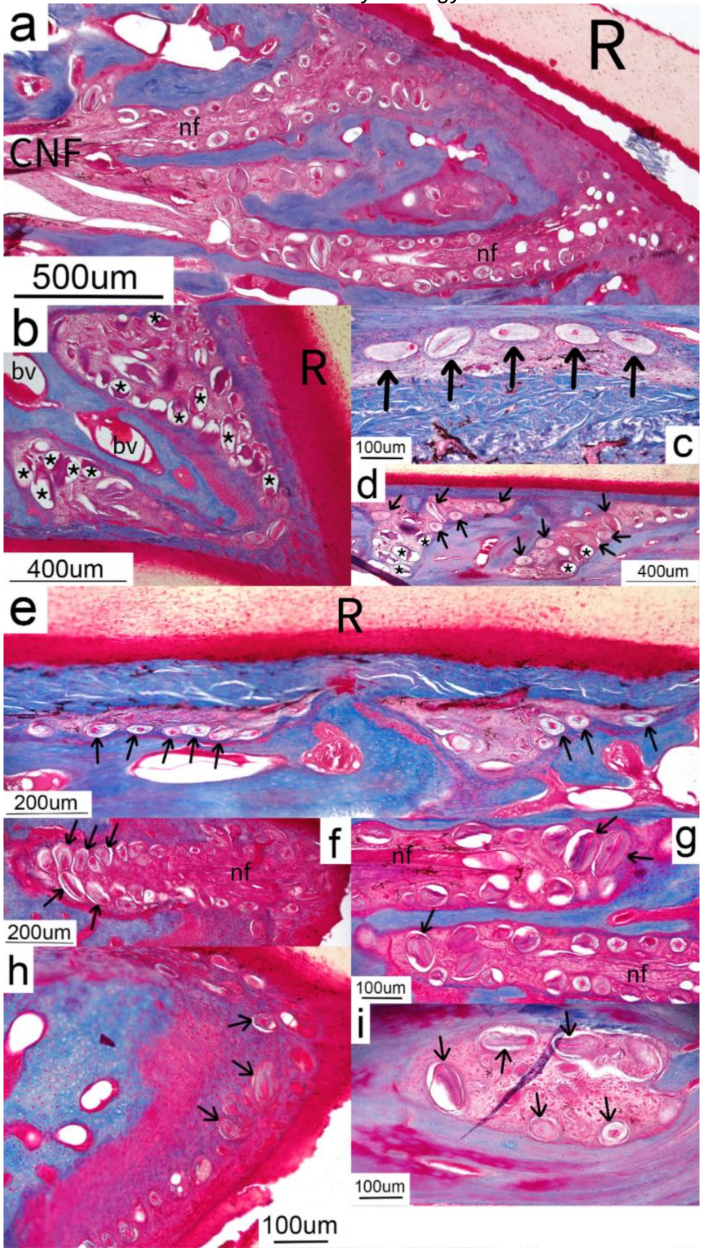

Figure 2.9: Cross sections of various sensory pits from the bill-tip organ of Sacred Ibis (*Threskiornis aethiopicus*) showing the arrangement, orientation and number of Herbst corpuscles and other soft tissues within the pits. For relative positioning of the pits on the beak refer to Figure 2.6. [a,b,f,g] sections of longest pits, found on the anterior-most surface of the beak. [d] pits on the external surfaces of the beak. [c,e] pits on the inside of the beakf. [f,h] same region of the beak (most anterior tip of mandible) from different sections: [f] slices through the centre of a sensory pit, [h] taken between two adjacent pits through the bone. [i] cross section of a pit which runs in medial-lateral plane. Masson’s trichrome stain used: Red & yellow outer layer, keratinaceous rhamphotheca; dark blue, bone; lighter purple, pinks and blues, soft tissues. Black arrows showing examples of Herbst corpuscles. Black asterisks indicate ruptured corpuscles damaged during the sectioning process. “CNF”: central nerve fibre of beak; ‘nf’: nerve fibre of sensory pit; “R”: rhamphotheca.

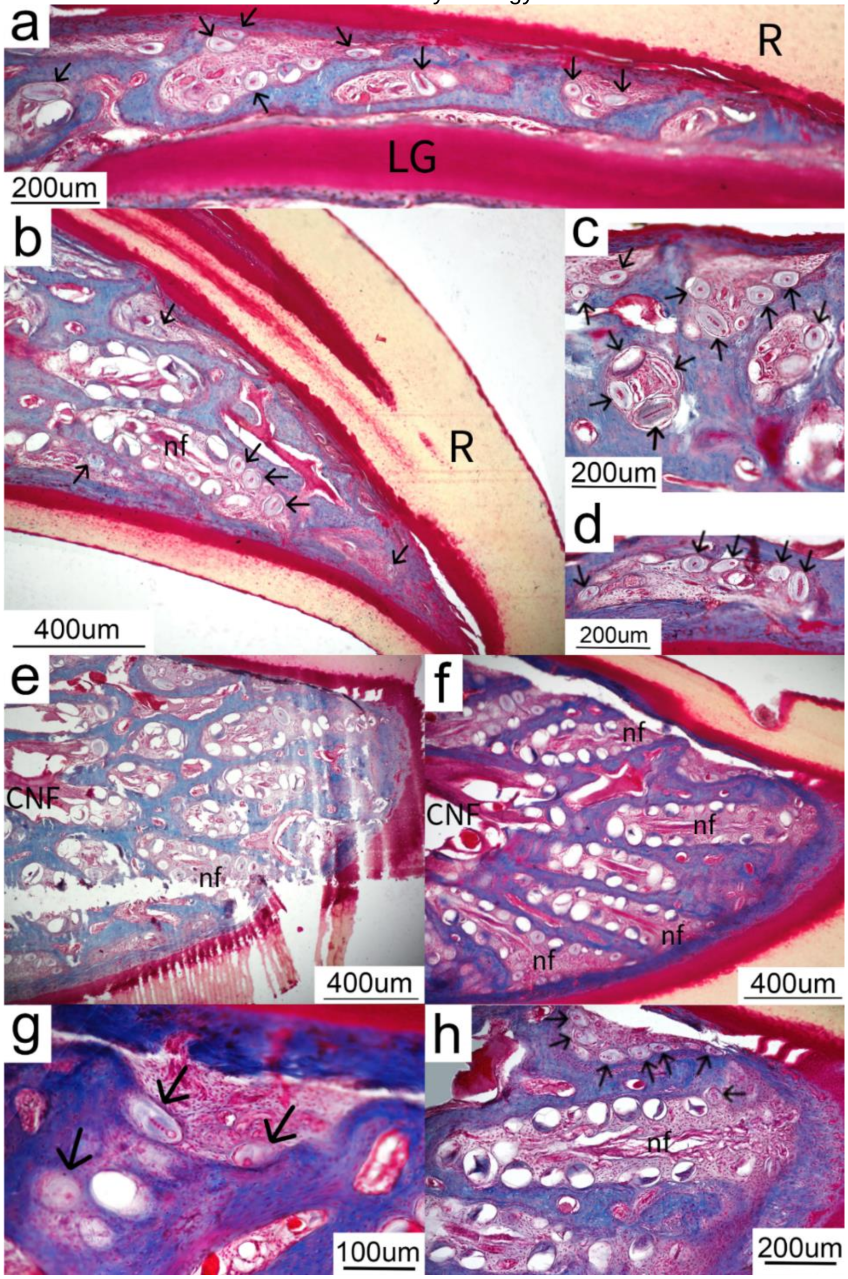

Figure 2.10: Cross sections of various sensory pits from the bill-tip organ of Glossy Ibis (*Plegadis falcinellus*) showing the arrangement, orientation and number of Herbst corpuscles and other soft tissues within the pits. For relative positioning of the pits on the beak refer to Figure 2.7. [a] pits in the section of the premaxilla bisected by the rhamphotheca (the “lateral groove”), forming a separate “lobe” in the beak bone. [b,e,f,h] sections of longest pits, found on the anterior-most surface of the beak. [a,c] pits on the external surfaces of the beak. [d,g] pits on the inside surfaces of the beak. [e,f,h] same region of the beak (most anterior tip of mandible) from different sections. Masson’s trichrome stain used: Red & yellow outer layer, keratinaceous rhamphotheca; dark blue, bone; lighter purple, pinks and blues, soft tissues. Black arrows showing examples of Herbst corpuscles. “CNF”: central nerve fibre; “LG”: lateral groove; “nf”: nerve fibre of sensory pit; “R”: rhamphotheca.

In the large sections of Hadeda and Glossy Ibises' premaxillae (Figures 2.5A & 2.7A), we see how the separation of the processes of the beak bones (nasal and maxillary processes; see Figure 2.2B) extend to form distinct units in the soft tissues as well: the premaxilla is further bisected by the infolding of the rhamphotheca into the grooves where the processes of the bone join, with no nerves or blood vessels running through these lateral grooves. In the Glossy Ibis sections, it can be observed how this arrangement of bone and rhamphotheca creates separate partitioning in the beak, confining the sensory pits to certain areas (as they do not extend through the infoldings of the rhamphotheca or into other processes of the bone – see Figure 2.10a for an enlarged view of this region).

Each sensory pit has a nerve fibre running through it which connects to the central nerve bundles in the neurovascular canals of the beak bones (see multiple examples in Figures 2.5-7, with some detailed sections in Figures 2.9ag & 2.10ef). This nerve fibre is surrounded by numerous Herbst corpuscles. Some adjacent sensory pits appear to merge before joining the central cavity (see Figure 2.8b), though the majority remain separate across their entire length. These sensory pits (or occasional pairs of pits) are separated from each other by thin walls of bone, keeping each pit a distinct unit.

Each Herbst corpuscle is made up of a central nerve axon, which connects on one end to the nerve fibre of the sensory pit and terminates on the other end in a round bulbous "head". The entire corpuscle is surrounded by an outer membrane of collagen, and between this and the central nerve axon we can see numerous lines and stripes indicating the matrix of interconnected lamellae that connect the outer membrane and the inner sheath. The internal structure of the Herbst corpuscles can be best seen when looking at them in longitudinal view (some good examples from each species can be found in Figures 2.8df, 2.9fgi & 2.10cg; compare to the diagram in Chapter 1, Figure 1.2).

The Herbst corpuscles appear to be arranged to maximize their number in each sensory pit, depending on the amount of space. Thus, the highest numbers of Herbst corpuscles were found in the longest/largest sensory pits (predominantly those on the distal/anterior surfaces of the beak – see Figures 2.8ad, 2.9abfg & 2.10befh). The lowest numbers of Herbst corpuscles per pit are found on the interior surfaces of the beaks (the inside of the mouth when the beak is closed), as these are the shallowest pits [see comparison of internal vs external sensory pits in Sacred Ibises (Figure 2.9ce vs. 2.9d) and Glossy Ibises (Figure 2.10d vs. 2.10ah)].

The orientation of the Herbst corpuscles is not consistent across the entire beak tip, but they do tend to be oriented in the same direction within each sensory pit (see Figures 2.8ce & 2.9ce). Where Herbst corpuscles in the same pit appear in different orientations, it is often due to the fact that some of the foramina do not follow a straight line, but bend/curve to reach the external beak surfaces (for examples, see Figures 2.8df, 2.9a & 2.10c).

The size of the Herbst corpuscles do not differ greatly between the three species (see Table 2.2). The only difference is between the Hadeda and Sacred Ibises, in that the Hadeda Ibises have slightly smaller Herbst corpuscles (in both length and width, see Table 2.2).

Table 2.2: Dimensions and counts of the Herbst corpuscles and sensory foramina for the distal portions of the bill-tip organs in three ibis species. Values are quoted as mean ± standard error. Species labels: "Hadeda" = *Bostrychia hagedash*; "Sacred" = *Threskiornis aethiopicus*; "Glossy" = *Plegadis falcinellus*.

Species	Foramina length[1]	n (number of foramina measured)	Number of Herbst corpuscles per pit[2]	n (number of pits counted)	Herbst corpuscle[2]		n (number of corpuscles measured)
					Length	Width	
Hadeda	580 ± 110 µm	50	15.1 ± 1.6	42	79.0 ± 8.6 µm	48.2 ± 4.0 µm	13
Sacred	400 ± 50 µm	90	16.2 ± 1.8	52	111.5 ± 7.7 µm	62.6 ± 4.9 µm	25
Glossy	230 ± 30 µm	53	7.3 ± 0.4	98	94.1 ± 8.6 µm	46.8 ± 6.2 µm	8

[1] measured as the distance from the opening on the external surface of the bone to the opening into the central nerve cavity (measured from micro-CT scans of the distal most ~5cm of beak)

[2] counted/measured from the sagittal histological sections of the distal most ~10mm of the beak

2.3.4: Distribution of sensory pits and Herbst corpuscles

All three species of ibis have high total numbers of sensory pits on their beaks (mean for all species >2000 pits; Table 2.1). Hadeda Ibises have significantly lower numbers of pits (2206 ± 285 pits) than the other two species (Figure 2.11A). There was no significant difference in the total number of pits between Sacred and Glossy Ibises (3155 ± 302 pits & 4354 ± 1092 pits respectively; see Table 2.1 & Figure 2.11A). There are statistically significant differences between all three species when looking at the percentage of the beak length pitted (Figure 2.11B): Hadeda Ibises have the smallest percentage of beak length pitted (23.61 ± 1.95 %), Sacred Ibises have an intermediate extent of pitting (40.54 ± 3.37 %), and Glossy Ibises have the highest pitting extent (52.43 ± 5.19 %). There is a strong significant positive correlation between the extent of pitting and the total number of pits across all three species (R=0.83; Figure 2.11C).

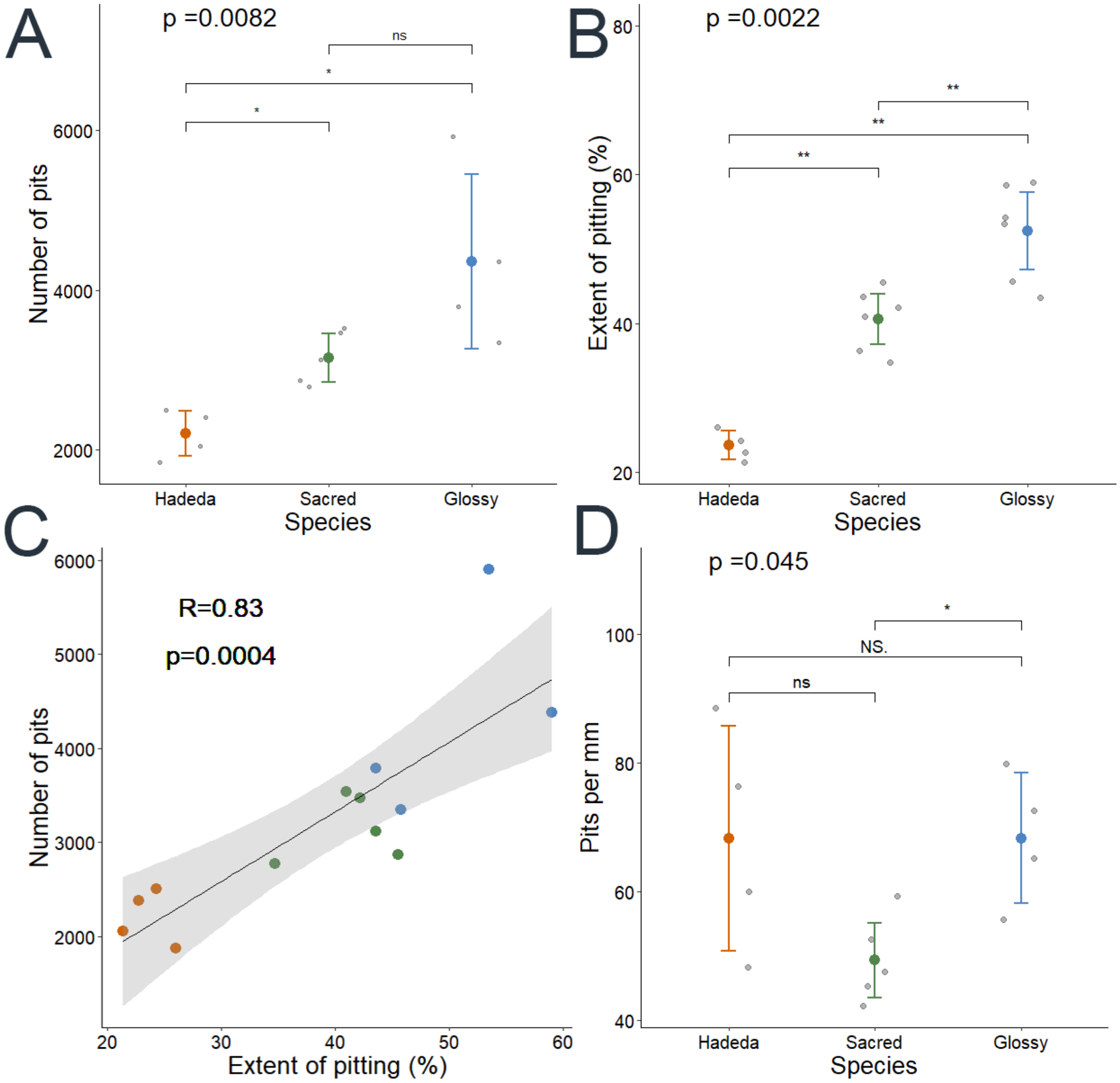

Figure 2.11: Interspecific differences in the patterns of sensory pits in the beak bones of three ibis species. A) Total number of sensory pits on the surfaces of the beak bones. B) Extent of the bill-tip organ as a percentage of beak length. C) Correlation between the extent of pitting and the total number of sensory pits on the beak bones. D) Density of sensory pits in the bill-tip organ (total number of pits divided by bill-tip organ length). (A,B,D): Small grey circles represent individual specimens. Large coloured circles show mean value for species, coloured brackets showing 95% confidence intervals; in (C) coloured circles represent individual specimens (colors representing species), black line showing regression and grey shaded region the 95% confidence interval. Correlation coefficient and significance in (C) calculated using Pearson method. Quoted p-values (A,B,D) from non-parametric Kruskal-Wallis tests; significance codes between species from post-hoc paired Wilcoxon tests: * = $p<0.05$; ** = $p<0.01$; ns = $p>0.05$ (no significant difference). Species labels & colour key: “Hadeda” = *Bostrychia hagedash* (orange)*;* “Sacred” = *Threskiornis aethiopicus* (green)*;* “Glossy” = *Plegadis falcinellus* (blue).

The pattern in the interspecific differences in the density of pitting in the bill-tip organ (measured by the total number of pits divided by the bill-tip organ length – see Figure 2.11D & Table 2.1) indicate that Sacred Ibises have significantly lower pitting densities (49.4 ± 5.8 pits/mm) than Glossy Ibises (68.3 ± 10.1 pits/mm). The Hadeda Ibises have pitting densities that are not significantly different from the other two species (68.3 ± 17.4 pits/mm).

When comparing the average numbers of sensory pits with the average number of Herbst corpuscles per pit (Table 2.2), each species of ibis represents a different morphospace (Figure 2.12A): Sacred Ibises have high numbers of both pits and Herbst corpuscles (HC) per pit (16.2 ± 1.8 HC/pit); Hadeda Ibises have low numbers of pits but high Herbst corpuscles per pit (15.1 ± 1.6 HC/pit); and Glossy Ibises have high numbers of pits but low Herbst corpuscles per pit (7.3 ± 0.4 HC/pit). As the same region of the beak is compared for all three species, these numbers can be used to compare differences between the species, but do not represent the actual values of each species as it was not possible to count the Herbst corpuscles in 3D space.

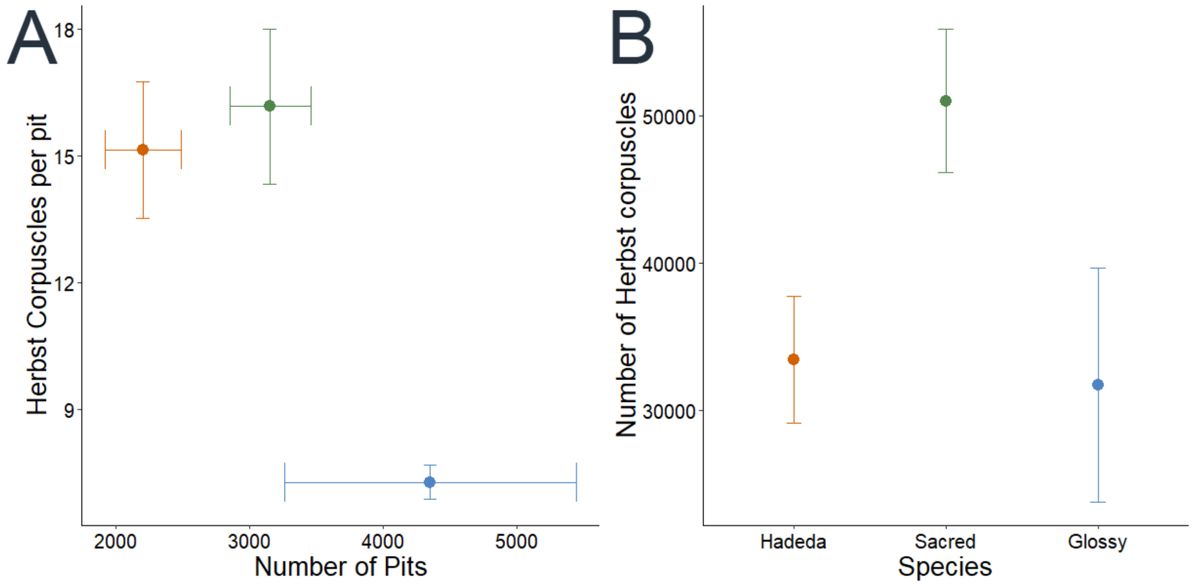

Figure 2.12: Interspecific differences in the patterns Herbst corpuscles in the bill-tip organs of three ibis species. A) The morphospace of the remote-touch bill-tip organs of each species represented by the total number of pits on the beak bones vs average number of Herbst corpuscles within each pit (based on counts from histological sections). B) Estimated total number of Herbst corpuscles in the bill-tip organ (number of pits multiplied by the number of Herbst corpuscles per pit). Large coloured circles show mean value for each species; coloured brackets showing 95% confidence intervals. Species labels & colour key: “Hadeda” = *Bostrychia hagedash* (orange)*;* “Sacred” = *Threskiornis aethiopicus* (green)*;* “Glossy” = *Plegadis falcinellus* (blue).

The estimated total numbers of Herbst corpuscles in the bill-tip organ (calculated by multiplying the total number of pits of each specimen by the average number of Herbst corpuscles per pit for that species) show that all three species have very high numbers of mechanoreceptors in their bill-tip organs (all species total averages estimated >30,000; see Figure 2.12B and Table 2.1). Sacred Ibises have the highest estimated numbers of Herbst corpuscles in the bill-tip organ (51,000 ± 4,900). Glossy Ibises, despite having similar numbers of sensory pits to the Sacred Ibises (Figure 2.11A), have much lower estimated numbers of Herbst corpuscles (31,700 ± 8,000), similar to the numbers calculated for Hadeda Ibises (33,400 ± 4,300). As the total numbers of Herbst corpuscles were calculated using the averaged numbers of Herbst corpuscle per pit visible in the 2-dimensional sections, these values are likely to be underestimates and should be considered proxies used to look at interspecific differences.

2.3.5: A note on challenges of sectioning beak tissues for soft tissue histology

It is quite challenging to successfully section and stain Herbst corpuscles in the sensory pits of the beak. This is due to the delicate nature of the Herbst corpuscles (thin outer membrane enclosing fluid filled sac), the difficulties faced in sectioning beak tissues (due mostly to the differences in density of the tissues – calcified bone tissue, firm keratinized rhamphotheca, soft dermal layers and delicate receptors and blood vessels); and the harsh chemicals used to soften the bone and stain the tissues. Some examples of damage to Herbst corpuscles (while the rest of the beak tissues remain relatively intact) can be seen in Figures 2.9b & 2.10h, where their outer membranes have ruptured and the matrix fluid leaked out, leaving a “deflated” membrane enclosing the central nerve axon. Sometimes, the sectioned corpuscles have fallen out of their cavities in the dermis during the staining procedure (see examples of gaps left behind around the nerve fibres in the sensory pits in Figures 2.9b & 2.10befg). These damaged/lost corpuscles could not be measured, but the deflated corpuscles and characteristic holes left behind in the sections of the sensory pits still allowed me to count them where they would have been.

2.4: Discussion

The beak bone morphology and soft tissue histology of the ibis species sampled indicate the presence of remote-touch bony bill-tip organs. Interspecific differences in patterns of sensory pits in their bill-tip organs are consistent with previous studies (Cunningham, Alley *et al.,* 2010; Cunningham, 2010), which indicate that species living in more aquatic habitats have higher numbers and extents of pitting than those in terrestrial habitats. However, my data do not support the suggestion that higher numbers of sensory pits correlate with greater bill-tip organ sensitivity (Cunningham, 2010). On the contrary, species foraging in drier substrates (Sacred and Hadeda Ibises) had significantly higher numbers of Herbst corpuscles (mechanoreceptors) per sensory pit than the aquatic-foraging Glossy Ibises, suggesting the former two species may possess the more sensitive bill-tip organs (Zweers & Gerritsen, 1997). The number of pits and extent of pitting in bill-tip organs may therefore be related to factors other than bill-tip organ sensitivity, which are discussed below.

2.4.1: Remote-touch bill-tip organs

All three ibis species show similar beak bone structure and soft tissue arrangement as other ibises and remote-touch probe-foraging birds (Bolze, 1968; Gottschaldt, 1985; Nebel, Jackson & Elner, 2005; Cunningham, Corfield, *et al.*, 2013), including Australian White Ibises [*Threskiornis Molucca,* the only other ibis species for which published data exist on beak soft tissue histology (Cunningham, Alley *et al.*, 2010)], and all species for which remote-touch has been experimentally confirmed (Piersma *et al.*, 1995; Piersma *et al.*, 1998; Cunningham, Castro & Alley, 2007; Cunningham, Castro *et al.*, 2010; Onrust *et al.*, 2017). The beaks of all three ibis species have high numbers of foramina densely packed together in the distal regions of their beak bones (typical of the remote-touch bill-tip organ, as shown in Chapter 5, section 5.4.1). These foramina contain clusters of Herbst corpuscles, which as a unit (the foramen, nerves and mechanoreceptors) form the characteristic remote-touch "sensory pit". It is therefore highly likely that all three species use remote-touch to locate buried prey (supported by results of Chapter 3, and confirmed experimentally for Hadeda Ibises in Chapter 4).

2.4.2: Interspecific differences in pitting on the beak bones and their relation to the arrangement of Herbst corpuscles and remote-touch sensitivity

The pattern of increased numbers of foramina and a larger extent of the beak length pitted in more aquatic-dwelling ibis species [previously described by Cunningham, Alley *et al.* (2010)] is reflected in the species sampled here. Hadeda Ibises, which utilize the most terrestrial habitats (Skead, 1951; Hockey, Dean & Ryan, 2005), have the lowest numbers and extent of pitting on their beak bones of the three species. Glossy Ibises, which use mainly aquatic habitats (Matheu & del Hoyo, 1992; Frederick & Bildstein, 1992; Hockey, Dean & Ryan, 2005; Taylor & Taylor, 2015), have the highest numbers and extent of pitting, and Sacred Ibises, which are generalists in their habitat usage (Hockey, Dean & Ryan, 2005; Clergeau & Yésou, 2006; Yésou *et al.*, 2017), show intermediate numbers and extent of pitting. Cunningham, Alley *et al.* (2010) also sampled the beaks of two additional specimens of Glossy Ibises, and their results are consistent with those of my study (when accounting for discrepancies in total pit counts, likely due to advances in digital photograph resolution in the 10 years between the studies).

The previous data from Cunningham, Alley *et al.* (2010) from multiple species of ibises indicated there may be a slight positive correlation between sensory pit density and use of more aquatic habitats in ibises, but my data shows that this is not always the case: pitting density (pits/mm beak length) does not correlate with the number of pits, extent of pitting, density of Herbst corpuscles per pit or aquatic habitat usage in the three species sampled. Therefore, the extent of pitting and number of pits in the bill-tip organ are better osteological correlates than pitting density for estimating aquatic habitat usage in ibises, as the former trends were supported in both studies.

Remote-touch sensitivity may be affected by interspecific differences in bill-tip organ morphology, as hypothesized by Cunningham (2010). Zweers & Gerritsen (1997) determined that the only way remote-touch probing species can increase their sensitivity to vibratory cues is to increase the numbers of Herbst corpuscles within each sensory pit. If it is the case that higher numbers of Herbst corpuscles per sensory pit confer higher sensitivity, then my results would indicate that Hadeda and Sacred Ibises have more sensitive bill-tip organs than Glossy Ibises (see Chapters 3 and 4 for further comments). Species foraging in drier substrates having higher remote-touch sensitivity than species foraging in more saturated substrates is consistent with the poorer transmission of mechanical vibrations in drier substrates (Biot, 1956 & 1962). Furthermore, as my data indicate that the density of Herbst corpuscles does not positively correlate with pitting number/extent (at least in the three study species), the suggestion by Cunningham (2010) that the latter may correlate with higher remote-touch sensitivity is not supported in this study.

2.4.3: Tradeoffs and constraints on bill-tip organ morphology

Though interspecific differences in the density of Herbst corpuscles may be related to substrate-specific sensitivity requirements, there appears to be an effect of overall beak shape on the maximum possible number of corpuscles per sensory pit. All probe-foraging birds have long, thin beaks to minimize resistance while probing (Zweers & Gerritsen, 1997). As the bill-tip organ makes up the distal portion of the beak, this beak shape in probe-foraging birds does restrict the organ's total surface area. The beak tip of a probe-foraging bird cannot expand into a wide disk shape (as seen in spoonbills, for example), as this would prevent birds from probing their beaks into anything except water. Thus, remote-touch probing birds face a trade-off between maximizing the surface area for the bill-tip organ while maintaining the narrow bill profile to reduce the energetic cost of probing (Zweers & Gerritsen, 1997). Furthermore, the size and shape of the foramina in the bone is confined by overall beak shape: i.e., species with wider beaks and thicker beak bones (e.g., Sacred and Hadeda Ibises) can and do have longer foramina (which span the width of the bone from the external surface to the central canal). The length of the foramina in turn appears to limit the maximum numbers of Herbst corpuscles per sensory pit: the species with the smallest foramina (e.g., Glossy Ibises) have the lowest average numbers of Herbst corpuscles per pit.

The mainly aquatic Glossy Ibises do not (and potentially cannot) forage in dry soils [see Chapter 3, section 3.3.1 and previous research (Matheu & del Hoyo, 1992; Frederick & Bildstein, 1992; Hockey, Dean & Ryan, 2005; Taylor & Taylor, 2015)]. This is likely due in part to their very narrow beak shape, which would be more prone to damage than "sturdier" beaks when probing in compacted substrates (Westergaard, 2013). However, their narrow beak profiles are ideal for reducing the energetic cost of probing (Zweers & Gerritsen, 1997). Furthermore, as soft, wet substrates generally allow birds to probe deeply more frequently than hard, dry ones (Kelsey & Hassall, 1989; Cunningham, Castro & Alley, 2007), aquatic foraging ibises may benefit from having a high extent of pitting, as this would increase the surface area of their bill-tip organ in contact with the substrate when probing deeply (Zweers & Gerritsen, 1997; see also Chapter 3 for detailed investigation). The narrow beak shape of Glossy Ibises also means that the foramina in the beak bones are constrained to be shallow, and therefore have space to include only low numbers of Herbst corpuscles. The consequent low densities of Herbst corpuscles per sensory pit in Glossy Ibis beaks may mean that they do not possess the required sensitivity to detect vibrations in drier substrates. Thus, Glossy Ibises appear to have faced an evolutionary tradeoff between bill-tip organ sensitivity and a more streamlined beak shape. They may be able to “afford” this as high remote-touch sensitivity may not be required in very wet substrates, due to the improved transmission of vibratory signals (Biot, 1956 & 1962) (see Chapters 3 and 4 for further comments).

Conversely, species such as Hadeda Ibises that probe in relatively dry, hard substrates (Hockey, Dean & Ryan, 2005; see also Chapter 3, section 3.3.1) face different constraints. The need for a sturdy proximal region of the beak when probing in hard substrates has been described and documented in various studies (Bock, 1966; Zweers, Berkhoudt & Berge, 1994; Zweers & Berge, 1996; Zweers & Gerritsen, 1997; Barbosa & Moreno, 1999). Probing in hard substrates inevitably puts strain on the beak bones, which must dampen the majority of the force in order to prevent damage to the skull. The greatest strain is conferred to the proximal regions of the beak, resulting in selective pressure for this region to be wider and have denser bone (Bock, 1966; Zweers & Gerritsen, 1997), the latter of which would be compromised if carrying a high degree of pitting. Consequently, Hadeda Ibises might be constrained by structural limitations imposed by their foraging environment to have bill-tip organs limited to the tip of the beak bones only. This could place them at an overall disadvantage when foraging in wetter substrates, as their extent of bill-tip organ pitting is relatively low: i.e., even when they are able to probe their beak more deeply (in more penetrable, wetter substrates) they would not increase the contact between the substrate and the sensitive areas of their beaks, as the latter is restricted to the most distal region. This low extent of pitting would likely not hamper them greatly in drier substrates, as birds probing in harder substrates tend to probe more shallowly (Kelsey & Hassall, 1989; Cunningham, Castro & Alley, 2007; see Chapter 3 for further comments). Hadeda Ibises appear to have evolved a sturdy and potentially more sensitive beak (as evidenced by their high densities of Herbst corpuscles, which is likely required for using remote-touch in drier substrates – see Chapter 4) but with a low extent of pitting.

Sacred Ibises seem to have forgone specialising their beaks for a particular substrate type, in keeping with their generalist foraging habits (Hockey, Dean & Ryan, 2005; Clergeau & Yésou, 2006; Williams & Wad, 2006; Chane & Balakrishnan, 2016; Yésou *et al.*, 2017): they have the high densities of Herbst corpuscles, which is likely advantageous for foraging in drier substrates, as well as a large extent of pitting, which is likely useful when probing in softer, wetter substrates (see above). Their beak shape is intermediate between the other two studied species, lacking either the “sturdy” base required for probing in the hardest of substrates (apparent in Hadeda Ibises) or the narrow “energy-efficient” profile seen in Glossy Ibises (which would restrict Sacred Ibises’ Herbst corpuscle densities, and likely in turn their ability to forage in drier substrates; see above).

2.4.4: Morphological patterns in comparison to other remote-touch probing birds

Spoonbills (members of the Threskiornithidae family, nested within the Old World ibises) (Chesser *et al.*, 2010) forage almost exclusively in water. They frequently make use of “head-swinging” while foraging, whereby their open beak is swung side-to-side underwater (Kushlan, 1978). A previous study described the presence of bill-tip organs in spoonbills from the morphology of their beak bones (Swennen & Yu, 2004; supported by my data in Chapter 5). As they do not probe-forage, they are not constrained to a narrow beak shape as the ibises are, and instead have a characteristic disc-shaped expansion of their beak tip. This significantly increases the surface area of their bill-tip organs (Swennen & Yu, 2004). Like Glossy Ibises, they have high numbers of foramina which are densely packed together (see Chapter 5, Figure 5.7B), further indicating that this arrangement is best suited for foraging in saturated substrates. Based on the trends in ibises, I expect spoonbills have relatively low numbers of Herbst corpuscles per pit (similar to or less than Glossy Ibises). They appear to have a highly modified bill-tip organ, potentially representing a further specialization within the groups of remote-touch capable birds. Histological examination of their beaks and behavioural experiments assessing their tactile sesnory capability are thus warranted.

Kiwi follow similar trends to ibises in terms of the relationship between their bill-tip organ morphology and habitat usage: Cunningham, Alley *et al.* (2010) showed that kiwi, which forage in relatively dry substrates, have a similar low extent and number of pits to ibises which forage in similar substrates. They have relatively high numbers of Herbst corpuscles per pit (see Chapter 5, Figure 5.1C), and similar foramen size and pitting density to Hadeda Ibises (see Chapter 5, and Cunningham, Castro & Alley, 2007; Cunningham, Corfield, *et al.,* 2013). This indicates that this type of bill-tip organ morphology is optimal for tactile foraging in drier substrates, and has evolved convergently between ibises and kiwi (Cunningham, Alley *et al.*, 2010). Furthermore, kiwi possess a “sensory pad” – a bulged protuberance at the tip of the premaxilla – which Cunningham, Castro & Alley (2007) hypothesized the birds use to provide general assessment of prey availability and for locomotive guidance (Cunningham & Castro, 2011). None of the ibises sampled possess this sensory pad on their premaxilla, though Hadeda Ibises did show slight overlapping of the premaxilla over the tip of the mandible (best seen in the histological sections), which could suggest that this overlap or bulge of the premaxilla may assist birds (such as Hadeda Ibises and kiwi) which probe in harder substrates.

The shorebirds (family: Scolopacidae) show a lot of interspecific variation in their bill-tip organ morphology. They tend to have lower numbers of sensory pits compared to the ibises [see Chapter 5, Figure 5.7B and Cunningham (2010)]. However, they have mixed extents and densities of pitting and numbers of Herbst corpuscles per pit [see Chapter 5, Figure 5.6 and Bolze (1968)]. It is difficult to generalize descriptions of scolopacid bill-tip organ morphology. Of the families of extant birds which make use of remote-touch, they are the most diverse (91 species of scolopacids, compared to 35 species of ibises and five species of kiwi) (Clements *et al.*, 2019). Most scolopacid species forage close to bodies of water, though the types of substrates they utilize are varied (Gerritsen &

Van Heezik, 1984). Some species also make use of novel foraging techniques, such as surface tension feeding on biofilms (Elner *et al.*, 2005; Ginn & Hamilton, 2007), which may also be affecting their use of remote-touch. To determine whether they follow similar ecomorphological trends as those described in ibises and kiwi, future research would need to look at detailed interspecific differences in substrate usage in relation to their bill-tip organ morphology.

2.5 Conclusions

All three species of ibises sampled possess the beak morphology indicative of remote-touch bill-tip organs. In combination with the data in the subsequent chapters of this thesis, I conclude that all three species are able to use remote-touch. High numbers and extent of sensory pits on their beak bones has previously been suggested to be correlated to ibis bill-tip organs' sensitivity to mechanical vibrations (Cunningham, 2010). However, by comparing the internal soft tissue histology of their bill-tip organs, my study indicates that this is not the case: species foraging in drier habitats have higher numbers of Herbst corpuscles within each sensory pit compared to species foraging in more aquatic habitats, and are therefore likely have more sensitive bill-tip organs (Zweers & Gerritsen, 1997). The main anatomical factor that seems to be limiting the number of Herbst corpuscles per pit are the length of the sensory pits themselves, which in turn are limited by the overall shape of the beak. These results are valuable to sensory ecologists and anatomists studying remote-touch, as they shed light on the relationship between the bone morphology and soft tissue arrangement in the bill-tip organ. The data reaffirms the positive correlation between the number of pits and extent of pitting in the bone and the use of more aquatic habitats for foraging in ibises.

Thus I fully support the previous suggestion (Cunningham, Alley *et al.*, 2010) that these measures are suitable proxies for studying the foraging habitat of various species of remote-touch probing birds, both extant and from the fossil record.

2.6 References

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CHAPTER 3:

Ecomorphology of touch: Ibises' remote-touch bill-tip organs linked to foraging behaviour and substrate usage

Three southern African ibises study species, with backgrounds depicting their natural habitat usage. From mainly terrestrial (Hadeda Ibis), to generalist (Sacred Ibis, middle) to mainly aquatic (Glossy Ibis) [From left to right. See Chapter 1, Figure 1.3 caption for more details]

Abstract

The remote-touch bill-tip organ enables probe-foraging birds, including ibises (Family: Threskiornithidae), to detect prey concealed in opaque substrates at a distance from their beaks by sensing mechanical stimuli in the substrate. The bill-tip organ comprises dense clusters of foramina in the distal portions of the beak bones, which contain numerous mechanoreceptors (as a unit forming the "sensory pits" of the bill-tip organ). Ibis species which forage in aquatic habitats have more extensively pitted beak bones than species from more terrestrial habitats. In the previous chapter, I showed in three species of southern African ibises that the aquatic specialist species had fewer mechanoreceptors per sensory pit on average than the two species which occupy more terrestrial habitats. This chapter investigates how the interspecific differences in bill-tip organ morphology relate to the probing behaviour and substrate use of the same three species of southern African ibises. I hypothesize that the extent of sensory pitting in each species is related to their probing behaviours and the penetrability of their foraging substrates. As wetter substrates transmit vibrations better, I hypothesize that remote-touch foraging birds will have higher prey capture success in these substrates, and that species adapted for drier substrates (in this case, Hadeda and Sacred Ibises) have more sensitive bill-tip organs, as they have higher numbers of mechanoreceptors per sensory pit. Behavioural data was gathered using focal observations of each species at multiple locations in the Western Cape, South Africa. The type of substrate birds were foraging in (among other environmental factors) was recorded, and the substrate penetrability and moisture content were measured. Generalized linear models were used to assess the effects of substrate characteristics on the birds' probing behaviour

and tactile foraging success. Interspecific differences are related to differences in the bill-tip organ morphology and compared to other remote-touch foraging species. The results show strong correlations between probing behaviours (probe depth and open-beak probing), substrate penetrability and bill-tip organ pitting extent between the species. This suggests that the bill-tip organ morphology could be a good proxy for fine-scale behaviour and microhabitat usage, which has implications for both conservation and palaeontological studies. I provide the first tentative behavioural support that there could be interspecific differences in the remote-touch capabilities of ibises linked to their bill-tip organ morphology. Furthermore, the data indicate substrate type and moisture content are significantly correlated with ibises' tactile foraging success, and when other variables are consistent, the effect of increased substrate moisture content appears to be positive. These results suggest that further testing under controlled conditions is warranted, and this is undertaken in Chapter 4.

3.1: Introduction

The foraging behaviour of birds (and indeed all animals) is driven strongly by the habitats in which they forage. Many birds change their foraging behaviour according to the prevailing environmental conditions, with particular regard to changes in the sensory information available to them. Penguins have decreased prey capture success and foraging effort in low light levels (Cannell & Cullen, 1998), and various seabirds change their diving behaviour according to light levels, diving deeper under better light conditions (White *et al.*, 2008; Regular, Hedd & Montevecchi, 2011). Owls change their hunting perch height based on the density of the surrounding vegetation, selecting taller perches when the ground vegetation is less dense (and thus providing less of a visual obstruction) (Tomé *et al.*, 2011). Some wading birds show complete shifts in foraging behaviours under changing sensory conditions, switching from visual to tactile foraging in water when wind speed increases, which in turn increases the turbidity of the water and makes visual hunting more difficult (Cullen, 1994). McNeil *et al.* (1999) showed how various species of waders shift between visual foraging during the day to tactile methods at night.

Studies of ecomorphology show how interspecific differences in behaviour and ecology are often reflected in differences in morphology (McNeil *et al.*, 1999). Links between the morphology of the beaks of birds and their foraging ecology are some of the most well-known examples in ecomorphological literature, with Darwin’s observations on the differences in diet and foraging behaviour in relation to beak shape in Galapagos finches being the most famous example (Darwin, 1859). Differences in beak morphology have been linked to avian foraging ecology across large samples of modern birds (Navalón *et al.*, 2019). Chough species (*Pyrrhocorax* sp.; Family: Corvidae) show differences in beak shape linked to their foraging strategies and foraging microhabitats (Rolando, Laiolo & Formica, 1997), and in flycatchers (Family: Tyrannidae), beak size and shape are good predictors of foraging behaviours (Botero-Delgadillo & Bayly, 2012). Wading birds are well studied in terms of interspecific differences in beak ecomorphology. Stilts and avocets (Family: Recurvirostridae), though closely related, have distinct beak shapes which correlate with their different foraging tactics (Hamilton, 1975). Barbosa & Moreno (1999) described how bill length of different species of shorebirds and waders (Order: Charadriiformes) correlates to their use of visual vs. tactile foraging methods, as well as various smaller scale foraging behavioural changes. Differences in beak morphology have also been linked to foraging success in phalaropes (Genus: *Phalaropus;* Family: Scolopacidae) (Rubega, 1996).

Some groups of birds have evolved "bill-tip organs" in their beaks: highly specialised sensory structures containing high densities of mechanoreceptors, which enable the birds to process fine scale tactile information. At least three types of bill-tip organs are recognised: i) the type found in parrots (Order: Psittaciformes), and probably used for the manipulation of food items (Goujon, 1869; Demery, Chappell & Martin, 2011; Martin & Martin, 2021); ii) the type found in ducks and geese (order: Anseriformes), which likely evolved to facilitate dabbling (Gottschaldt & Lausmann, 1974; Gottschaldt, 1974; Berkhoudt, 1979; Soliman & Madkour, 2017; Avilova, Fedorenko & Lebedeva, 2018; Avilova, 2018; Schneider *et al.*, 2019); iii) the type of bill-tip organ found in three groups of probe-foraging birds: ibises (Family: Threskiornithidae), kiwi (Order: Apterygiformes) and shorebirds (Family: Scolopacidae) (Bolze, 1968; Nebel, Jackson & Elner, 2005;

Cunningham, Castro & Alley, 2007; Cunningham, Alley *et al.*, 2010; Cunningham, Corfield, *et al.,* 2013). This third type of bill-tip organ enables birds to locate their prey at a distance from the beak by detecting vibratory cues in the substrate as they probe their beaks into it. The cues they detect result either from movement of prey in the substrate, or from changes in interstitial pressure gradients generated by the rapid probing action of the birds around the hard-bodies (shells) of prey items (e.g., molluscs) buried in the substrate (Gottschaldt, 1985; Gerritsen & Meiboom, 1985; Zweers & Gerritsen, 1997; Piersma *et al.*, 1998). This sensory modality is known as "remote-touch", as it is a tactile sense that allows the birds to detect their prey "remotely" (i.e., before direct contact with the beak).

The remote-touch bill-tip organ comprises mechanoreceptors clustered in high numbers of foramina in the bones of birds' beaks, with each foramen and its associated mechanoreceptors referred to as a "sensory pit" (Bolze, 1968; Nebel, Jackson & Elner, 2005; Cunningham, Castro & Alley, 2007; Cunningham, Alley *et al.*, 2010; Cunningham, Corfield, *et al.*, 2013). The mechanoreceptors are predominantly Herbst corpuscles, which detect acceleration components of high frequency mechanical waves (Gottschaldt, 1985). The bill-tip organ appears to have evolved convergently at least three times in Neornithes (Cunningham, Castro & Alley, 2007; Cunningham, Alley *et al.*, 2010; see also Chapter 5, sections 5.4.2-3 for details on its evolution in palaeognathous birds). The function of the remote-touch bill-tip organ has been experimentally confirmed in all three groups (ibises, kiwi and scolopacid shorebirds) using anatomical and neurological studies (Cunningham, Castro & Alley, 2007; Cunningham, Corfield, *et al.*, 2013), as well as behavioural sensory assays on living birds (Piersma *et al.*, 1998;

Nebel, Jackson & Elner, 2005; Cunningham, Castro & Potter, 2009; Cunningham, Castro *et al.*, 2010; see also Chapter 4, section 4.4.1 for experiments confirming remote-touch capability in Hadeda Ibises).

Some interspecific differences in the morphology of the remote-touch bill-tip organ have been linked to differences in foraging habitat and behaviour (Bolze, 1968; Zweers & Gerritsen, 1997; Nebel, Jackson & Elner, 2005; Cunningham, Alley *et al.*, 2010). Cunningham, Alley *et al.* (2010) found (in ibises and kiwi) that species from more aquatic habitats have higher numbers of sensory pits in their bill-tip organs and higher extents of pitting (relative to overall beak length) on their beak bones (supported by my data from Chapter 2, section 2.4.2). Cunningham, Alley *et al.* (2010) hypothesized that these interspecific differences in bill-tip organ morphology are related to differences in foraging substrate. I hypothesize that the increase in number and extent of pitting on the beak bones in the more aquatic species are linked to more penetrable substrates in which they probe: if they are able to probe deeply frequently, they would benefit from having a higher extent of pitting, as when probing deeply more of the beak's length would be in contact with the substrate. Furthermore, ibis species foraging frequently in hard, dry substrates need more sturdy beaks (Bock, 1966; Zweers, Berkhoudt & Berge, 1994; Zweers & Berge, 1996; Zweers & Gerritsen, 1997; Barbosa & Moreno, 1999), and could be disadvantaged by having highly perforated proximal regions of their beaks (i.e., extensive pitting in the more proximal regions of the beak would likely weaken the beak structure).

Cunningham (2010) suggested that there may also be interspecific differences in bill-tip organ sensitivity related to the birds' foraging substrates. However, she was not able to comment further on this hypothesis, as the data available (Cunningham, Alley *et al.*, 2010) only dealt with differences in the bones of the beak. The best anatomical measure of bill-tip organ sensitivity has been theoretically calculated to be the number of Herbst corpuscles per sensory pit (Zweers & Gerritsen, 1997). Based on my data from Chapter 2 (sections 2.4.2-3), southern African ibis species foraging in terrestrial habitats (Hadeda and Sacred Ibises) have higher numbers of Herbst corpuscles per sensory pit, indicating that they likely have more sensitive bill-tip organs than species foraging exclusively in aquatic habitats (Glossy Ibises). Substrates with lower water content transmit vibrations relatively poorly (Biot, 1956 & 1962), which suggests that species foraging in drier substrates may need more sensitive bill-tip organs.

Three species of ibises occur commonly in South Africa: Hadeda Ibis (*Bostrychia hagedash*), African Sacred Ibis (*Threskiornis aethiopicus*) and Glossy Ibis (*Plegadis falcinellus*). They are ideal candidates for studying interspecific differences related to foraging habitat, as they use different habitats (Hockey, Dean & Ryan, 2005). Hadeda Ibises are mainly terrestrial, historically living in forests (Macdonald, Richardson & Powrie, 1986), although in the past century they have experienced a large range expansion and now occupy various human-modified environments (agricultural land, urban and suburban gardens) (Duckworth, Altwegg & Harebottle, 2012; Duckworth & Altwegg, 2014). Though they do sometimes forage in wetlands and shallow water, they spend most of their time on relatively dry land (Skead, 1951). Glossy Ibises are almost exclusively aquatic foragers, foraging in bodies of fresh water and in wetlands (Matheu & del Hoyo, 1992; Frederick & Bildstein, 1992; Hockey, Dean & Ryan, 2005; Taylor & Taylor, 2015). Sacred Ibises are generalist in both foraging habitat and behaviour compared to the other two species (Hockey, Dean & Ryan, 2005; Clergeau & Yésou, 2006; Yésou *et al.*, 2017), representing an "intermediate" example in this study. Though they regularly use probe-foraging (Kushlan, 1978), Sacred Ibises will often hunt visually to capture large vertebrate prey (Williams & Ward, 2006), or sometimes even feed on carrion (Chane & Balakrishnan, 2016). Many populations of Sacred Ibises have become urban exploiters, feeding on human refuse (Clark, 1979; Clergeau & Yésou, 2006; Calle & Gawlik, 2011; Yésou *et al.*, 2017).

In this chapter, I document the foraging behaviours and substrate usage of these three ibis species, in order to investigate any links to the interspecific differences in the morphology of their bill-tip organs. I hypothesize that interspecific differences in the extent of pitting on the beak bones is linked to probing behaviours and penetrability of the substrates the birds forage in. I investigate relationships between the moisture content of the substrate and the birds' tactile foraging success, using data from birds observed foraging in a range of substrates. By comparing foraging behaviour between the species, I test my hypothesis that the higher densities of mechanoreceptors in the bill-tip organs of species adapted for drier substrates make them more efficient at using tactile foraging techniques. I predict this would translate to these species having higher foraging success than species with lower mechanoreceptor densities under the same substrate conditions.

3.2: Methods and materials:

3.2.1: Study sites

Ibises were observed at four different sites in the Western Cape Province of South Africa (Figure 3.1), which experiences a Mediterranean climate with winter rainfall (Tyson & Preston-Whyte, 2000). Behavioural observations were carried out at the study sites outside of the breeding season for all three species (Hockey, Dean & Ryan, 2005) during April-July 2018 (autumn to early winter; average monthly rainfall 50-112 mm and average temperatures 12.4-17.0 ºC). I used data from the second Southern African Bird Atlas Project (SABAP2) (Harebottle *et al.*, 2007) to select study sites where all three species are frequently sighted during autumn and winter.

The four sites were (see Figure 3.1): i) "Strandfontein" water treatment park (part of False Bay Nature Reserve) – mainly made up of large pans of water, interspersed with reed beds and dense coastal thicket. There are coastal dunes in the southern portions of the park. The South-West side of the park borders on a refuse landfill, where large flocks of Sacred Ibises (along with other bird species) were observed scavenging. I observed all three species of ibises at Strandfontein in high numbers during April 2018; ii) Berg River Estuary ("Velddrif") – the water in the estuary is brackish, and the vegetation surrounding the edges of the water is made up of seagrasses and other halophytes, with *Sarcocornia* sp. (family: Amaranthaceae) being predominant. The Velddrif study site encompassed saltpans, mudflats and floodplains, with farmlands bordering the site. Sacred and Glossy Ibises were observed there during May 2018; iii) "Scarborough" beach is frequently used by Hadeda and Sacred Ibises, where I observed them foraging in the wet sand around the high tide mark, around piles of washed-up kelp in June 2018; iv) “Kirstenbosch” National Botanical Gardens is only frequented by Hadeda Ibises and was included in order to observe the behaviour of this species in a human cultivated habitat (mostly foraging on stretches of lawn) in July 2018.

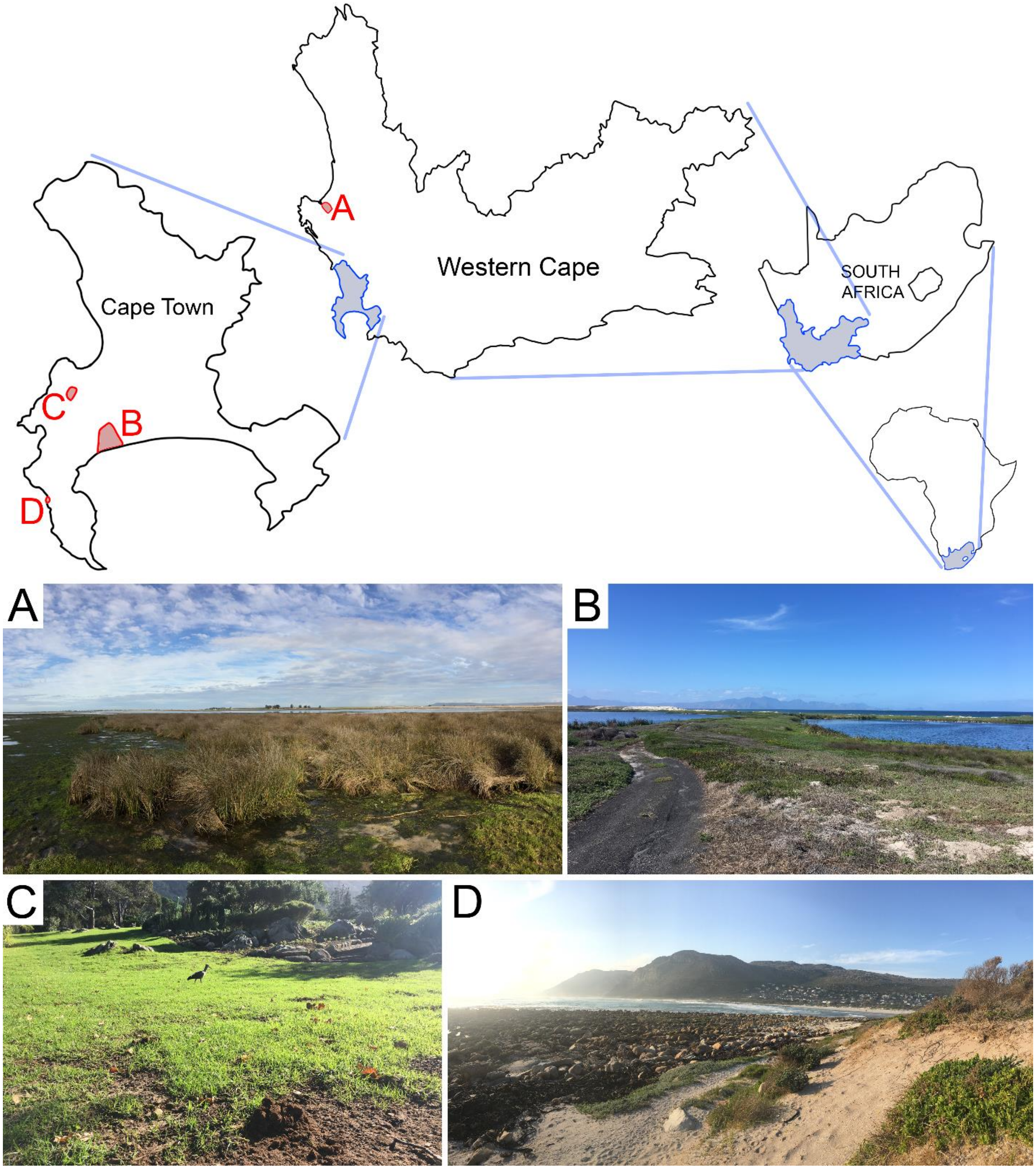

Figure 3.1: Map showing the locations of the four field sites in the Western Cape Province of South Africa, and photographs of the general appearance of each site. Field sites are outlined and shaded in red on the map. A = Velddrif (Berg River Estuary); B = Strandfontein water treatment pans (part of False Bay Nature Reserve); C = Kirstenbosch National Botanical Gardens; D = Scarborough Beach.

3.2.2: Focal observations

Individuals of each species were located by driving, walking or travelling by boat around the field sites between 8-12 am in the morning. Every individual encountered was observed for a 10-15 min interval (target was for 15 min, but I included any focals over 10 min to account for birds flying away, moving out of sight or into a different substrate type, any of which would end the focal), using binoculars or a telescope (depending on the distance between myself and the bird). The three species are easily distinguished from each other, as they have different body sizes and distinct colouring (Hockey, Dean & Ryan, 2005; see cover image at the start of this chapter for a visual example). I watched the birds and dictated the types of behaviour they were displaying in real time, while my field assistant recorded these behaviours in *CyberTracker* (Ansell & Koenig, 2011). *CyberTracker* is a fully customisable cellphone app which allows collection of time-stamped behavioural data. A total of 149 full focal observations were recorded (52 Hadeda Ibises; 56 Sacred Ibises; and 41 Glossy Ibises).

All behaviours during each focal were recorded with time stamps, allowing me to calculate the average relative use of each behaviour [see Table 3.1 for the ethogram, roughly following the categories of Kushlan (1978)]. Every successful prey capture was logged to calculate the relative success of different foraging strategies. Prey capture was easy to note (even when prey items were too small to see clearly from a distance) as all three species capture prey at the tip of their beak and then throw their head backwards to propel the item down their throat.

Table 3.1: Ethogram of different behaviours observed in three species of ibis during focal observations, and descriptions of each. Asterisks indicate tactile foraging behaviours.

Behaviour	Description
Bathing	dipping and shaking body in water to wash self
Comfort	grooming self, including scratching with feet or preening
Drinking	drinking water
Flying	moving location on the wing
Foraging	any method to locate and capture prey (see below)
Interaction	clear interaction with another bird (e.g., fighting, allopreening)
Vigilant	standing up straight and scanning surroundings
Vocalizing	calling out
Walking	moving location on foot (not foraging)
Foraging behaviours:	
Active hunting	visually pursuing mobile prey item out of substrate (prey either on surface of substrate or flying in the air)
Dabbling*	rapid repeated opening and closing of beak in water
Flipping	turning over vegetation or small rocks with beak to expose different surface for visual examination
Gleaning	picking items off surface of vegetation
Headswinging*	moving open beak from side to side in water
Nibbling*	similar to "dabbling", but with the very tip of the beak sifting through the surface layer of soil or sand
Pecking	picking up item from surface of substrate
Probing*	repeatedly moving beak into and out of substrate

When the birds were probe-foraging, more details were recorded. For each probe attempt, the depth of probing was recorded relative to beak length: "quarter" – less than half the beak length inserted into the substrate; "half" – over half the beak length inserted; "full" – entire length of beak inserted into substrate (half and full probes jointly classified as "deep" probes, quarter as "shallow"). I also recorded whether the bird was stationary or walking while probing, and whether the beak was open or closed.

Other factors that were logged at the time of recording included: GPS location; date; field site; cloud cover; rain; water depth that the bird was foraging in (relative to the length of the bird's legs); and numbers of conspecifics and heterospecifics within ~10 m^2 radius. Hourly average air temperatures were provided by the South African Weather Service using data from the nearest weather stations to each field site [these were: Cape Point Station, Climate Number 00048919 (Strandfontein & Scarborough); Langebaanweg AWS Station, Climate Number 00612988 (Velddrif); Kirstenbosch Station, Climate Number 00207804 (Kirstenbosch)]. Vegetation cover surrounding the bird was scored under the following categories: clear (no plant cover – exposed sand/soil or open water); lawn/grass; hyacinth (large mats of the invasive water hyacinth, *Eichhornia crassipes*); and plants (any other plant cover). The substrate the bird was foraging in was visually assessed and also logged, as: sand (course, large-grained substrate), soil (fine-grained substrate), or water. When prey was visible, the size was estimated (judged relative to the bird's beak) and, where possible, a general identification of the prey type was made (e.g., frog, shrimp, worm, etc.).

Using the time stamps of the different behaviours recorded in *CyberTracker*, I put together time budgets for each focal observation in *Microsoft Excel* (2013). I added together the total time spent on each behaviour and calculated the relative use of each behaviour during the focal (as a percentage of the total focal time).

3.2.3: Physical properties of foraging substrates

Two physical properties of the birds’ foraging substrates were recorded: substrate compaction (substrates with higher compaction values have lower penetrability and are harder to probe in) and moisture content (percentage of substrate made up of water, by weight). I attempted to measure these at every position where a bird was recorded during the focal observations. However, this was not always possible, as some of the exact sites were inaccessible (e.g., at Strandfontein, the birds frequently foraged on small islands/mudflats in the middle of the sewage water treatment pans, and I could not enter these due to biohazard risk). Of the total 149 focal observations, I measured substrate moisture content in 93 of them, and compaction from 98.

The compaction of the substrate was measured in situ, using a pocket penetrometer (model 29-3729, *ELE International* 2011 ©). Compaction was measured in kg/cm^2 to two decimal places, with a minimum threshold of 0.50kg/cm^2. Five readings were taken roughly 30 cm apart where each bird was recorded foraging, and the average of these was calculated. When birds were foraging in water, the compaction was recorded as zero.

To measure the moisture content of the substrate, samples were collected using a bucket auger and stored in airtight plastic bags. In the case where the soil was too saturated or loose to remain in the auger, it was scooped into the sample bag using a handheld trowel. The “total mass” of the sample was measured, and then the samples were dried for seven days in a 70ºC oven. Thereafter, the "dry mass" of each sample was weighed, and the "water mass" calculated (i.e., water mass = total mass – dry mass). Moisture content was calculated for each sample using the formula (Gardner, 1986):

$$Moisture\ Content\ (\%) = \frac{\text{Water mass (g)}}{\text{Total mass (g)}}\ x\ 100$$

with zero indicating a completely dry soil sample, and 100% representing open water.

Substrate type (see section 3.2.2 for classifications) is a good proxy for substrate moisture content (see Figure 3.2A), as each type had significantly different moisture contents (based on two-sided Wilcoxon rank-sum tests). However, there was overlap in substrate compaction between sand and soil (Figure 3.2B; no significant difference in compaction between sand and soil).

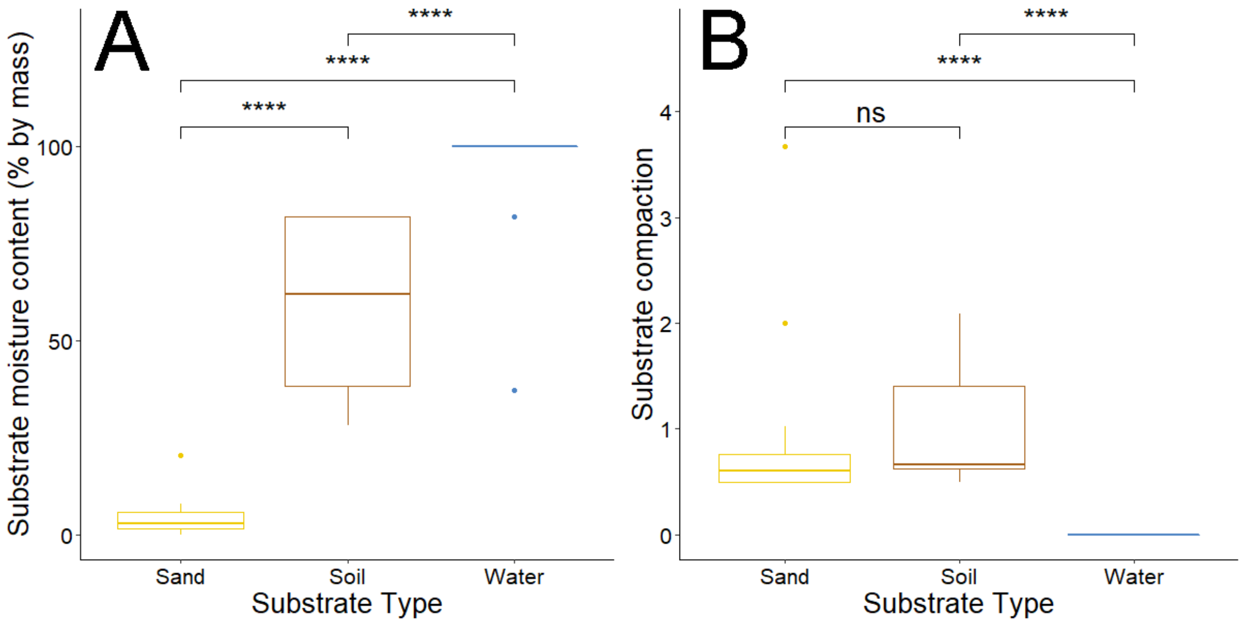

Figure 3.2: Physical properties of foraging substrates used by the three species of ibis during the focal observations. A) Substrate moisture contents (calculated as a percentage: mass water by total mass of substrate [water + solid matter]) of each substrate category. B) Substrate compaction (measured in kg/cm^2, higher values indicating "harder", less penetrable substrates) of each substrate category. Coloured points indicate outliers. Asterisks above brackets represent significance codes from two-sided Wilcoxon rank sum tests: **** $p<0.0001$; "ns" $p>0.05$ (difference not significant).

3.2.4: Statistical analyses

All datasets were compiled and formatted in *Microsoft Excel* (2013), and all data analyses and plotting were done in *RStudio* (RStudio Team, 2016). All plots were generated using the *R* (R Core Team, 2018) package *ggplot2* (Wickham, 2011). When assessing foraging behaviours and success, I only included focal observations where the bird had been foraging for over five minutes, to ensure the bird was sufficiently motivated to forage and not distracted (e.g., by comfort behaviours or vigilance). All data were tested for normality using Shapiro-Wilk tests and by examining quantile-

quantile plots using the *R* (R Core Team, 2018) package *ggpubr* (Kassambara, 2018). As the data were not normally distributed, non-parametric tests were used.

In order to determine what variables (aside from species and substrate properties) should be included in the models, I plotted the measurements of interest against the other variables recorded (see section 3.2.2 for the list) and ran non-parametric tests for significant differences. Based on this initial data exploration stage, the only factors that appeared to be correlated with the birds' foraging behaviours of interest were air temperature, field site location and foraging patch vegetation cover.

3.2.4.1: Differences in foraging behaviour and substrate properties between ibis species and substrate types

Kruskal-Wallace tests were used to assess significant differences between explanatory variable groupings. Post-hoc Wilcoxon signed-rank tests were used to assess any significant differences when the explanatory variables consisted of more than two groups. I used the *R* packages *ggsignif* (Ahlmann-Eltze, 2019) and *ggpubr* (Kassambara, 2018) to plot the P-values and significance codes on the figures.

Wherever behaviour and substrate use data were plotted against measures of bill-tip organ morphology, the latter were obtained from the datasets from Chapter 2. The morphological measures included are: total bill-tip organ pitting extent (average maximum distance of sensory pits from the tip of the beak bone, as a percentage of total beak length) and internal bill-tip organ pitting extent (extent on the inner surfaces of the beak bones; i.e., the inside of the "mouth" when the beak is closed). For methods of how these features were measured and sample sizes, please refer to Chapter 2, section 2.2.

3.2.4.2: Generalized Linear Models assessing differences between species and the effects of foraging substrate properties on ibises' use of probing behaviours and their tactile foraging success

To assess the effects of substrate moisture content and compaction on the probabilities of ibises probing with the beak open or probing deeply, I fitted binomial Generalized Linear Mixed-Effects Models (GLMMs) using the *R* package *lme4* (Bates *et al.*, 2014), with ibis species included as a random effect. Each focal observation was graded 0/1 with regard to open-beak probes: "zero" = bird probed with the beak held open <10 times during the focal; "one" = ≥10 times; and similarly for deep probes [it could not be absolutely binary, as the birds almost always probed deeply/open at least once during each focal. On average, birds probed ~200 times (193 ± 12) in total during each focal, thus using a cutoff of 10 accounted for the occasional (<5%) deep/open probes]. The model predictions and 95% confidence intervals were fitted against the observed ranges of foraging substrate moisture content and compaction. Models were selected based on weighted AICc values (Akaike Information Criterion with correction for small sample sizes; ΔAICc cut-off of 2).

I used Generalized Linear Models (GLMs) to assess the effect of foraging substrate type on the ibises' tactile foraging success [the percentage of total tactile foraging attempts that resulted in a successful prey capture; binomial models fitted using *cbind*(successful attempts, unsuccessful attempts)]. Quasimodels were used to account for overdispersion (Lee & Nelder, 2000). As I could not control for ibis species as a random factor using quasimodels in *R*, the models were fitted for each species separately. The following predictors were used in the three global models (global model for each species): substrate type, air temperature, vegetation cover and field site. Model selections were based on the weighted QAICc values (adjusted AICc for quasimodels, cutoff ΔQAICc < 2). Weightings were obtained using the *R* package *MuMIn* (Barton, 2009) [see Bolker (2020) for the methodology of extracting overdispersion and log-likelihood for quasimodels using *MuMIn*]. I used the *R* package *emmeans* (Lenth *et al.*, 2021) to plot the predicted values and 95% confidence intervals of tactile foraging success from the models against substrate type for each species. Where more than one model was selected that contained substrate type as a predictor (i.e., multiple models per species with ΔQAICc <2), the models' predictions were plotted separately.

In order to compare differences in tactile foraging success between the ibis species, I needed to control for substrate type. As I could not include substrate type as a random factor using quasimodel GLMs (due to overdispersion, see above), models assessing the differences in tactile foraging success between species were run separately for each substrate type. This allowed the comparison of tactile success between species when substrate type was kept constant (e.g., comparing the tactile foraging success of the species when they foraged in water). The predictors included in each of the global models (global model run for each substrate type separately) were: ibis species, air temperature, vegetation cover and field site. Model selection and plotting of predictions followed the same protocols stated in the previous paragraph.

3.3: Results

3.3.1: Foraging behaviour and substrate use

All three species spent on average > 50% of their time during the focals foraging (Figure 3.3A). The birds also spent large amounts of time vigilant, walking and on comfort behaviours. Sacred Ibises displayed the widest range of foraging behaviours (Figure 3.3B). The most frequently used foraging behaviour by all three species was probing, with Glossy Ibises spending the most time probing and Sacred Ibises the least (Figure 3.3B; Hadeda: 76.0 ± 9.0 %; Sacred: 55.1 ± 11.5 %; Glossy: 94.6 ± 5.9 % of total foraging time). Gleaning prey items visually off vegetation was the second most used foraging strategy by Hadeda and Sacred Ibises. Nibbling (a tactile foraging method, see Table 3.1 for description) was the second most used foraging method by Glossy Ibises.

Each species was observed foraging in different subsets of the three substrate types (sand, soil and water; see Figure 3.4A). All three species were observed foraging in soil, and Sacred Ibises were observed foraging in all three substrate categories. Glossy Ibises foraged in the wettest substrates (76.4 ± 8.2 % average moisture content); Hadeda Ibises in the driest substrates (10.5 ± 10.7 %); and Sacred Ibises foraged in substrates with intermediate moisture contents (54.6 ± 16.2 %; see Figure 3.4B). Hadeda Ibises foraged in significantly harder (higher compaction) substrates (0.97 ± 0.52 kg/cm^2) than the other two species (0.33 ± 0.34 kg/cm^2 and 0.34 ± 0.28 kg/cm^2 used by Glossy and Sacred Ibises respectively; see Figure 3.4C).

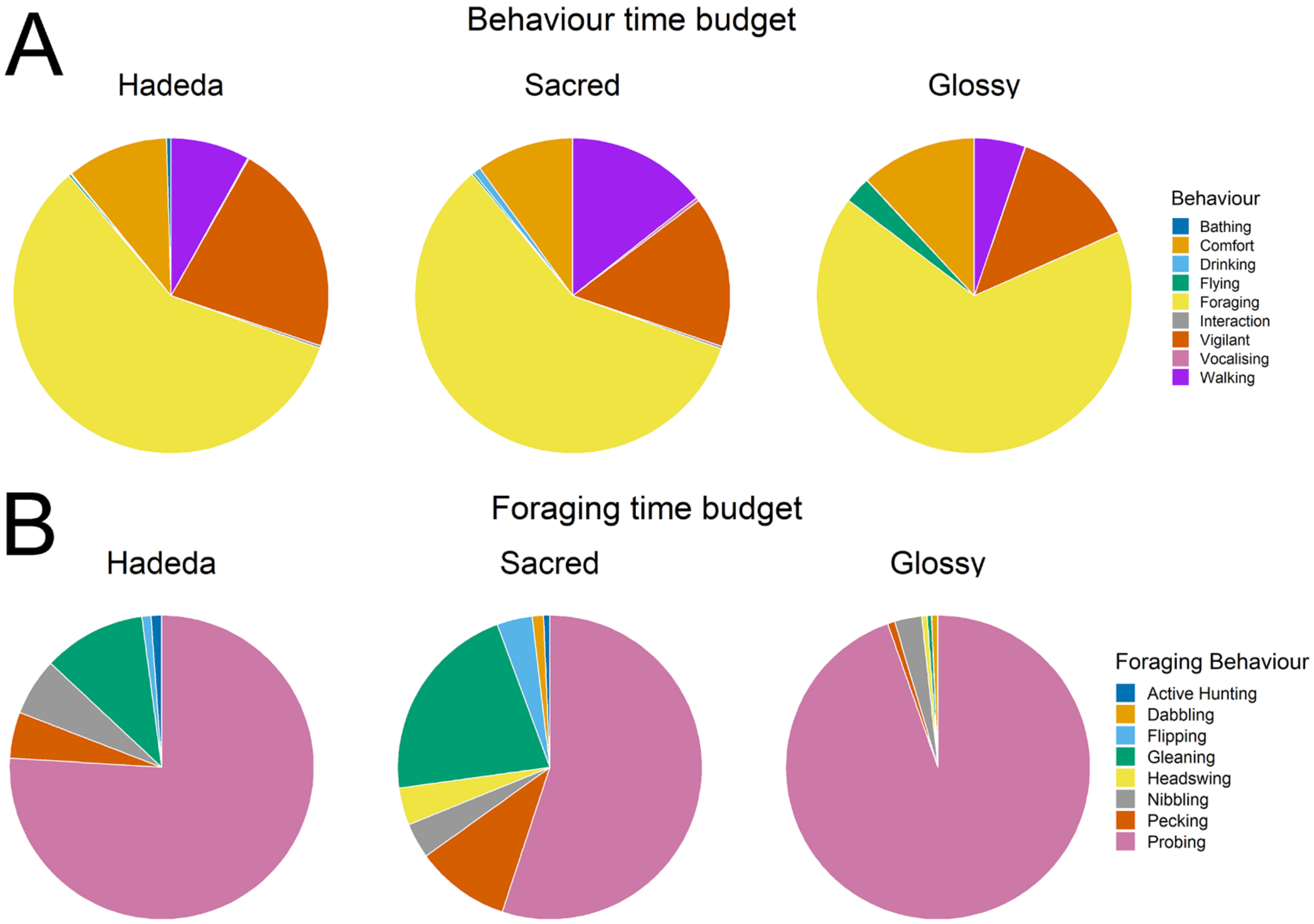

Figure 3.3: Activity budgets for the focal observations for each species of ibis (averaged across focals). A) Total behaviour budget, showing relative proportion of time spent on different behaviours during all the focal observations recorded. B) Breakdown of foraging time (yellow in A) into specific foraging behaviours, showing the average proportion of foraging time spent on each behaviour for each of the three ibis species (from focals where the bird was foraging for over 5 min). "Hadeda" = *Bostrychia hagedash*; "Sacred" = *Threskiornis aethiopicus*; "Glossy" = *Plegadis falcinellus.* For descriptions of behaviours, see Table 3.1.

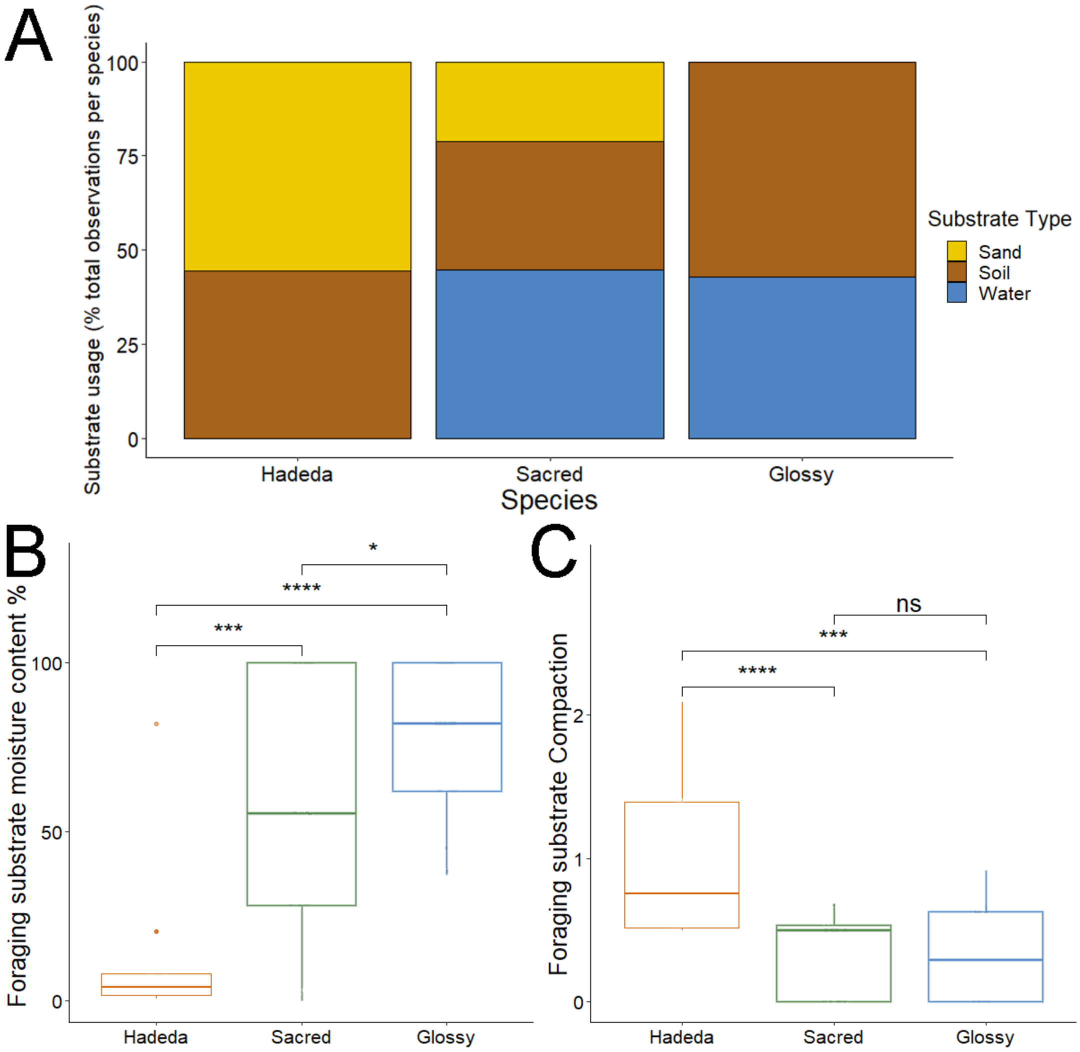

Figure 3.4: Interspecific differences in foraging substrate usage by the three species of ibis. A) Relative usage of the three substrate categories for each species of ibis (as a percentage of the total number of focal observations per species). B) Moisture contents (percentage by mass) and C) compaction (kg/cm^2) of the foraging substrates used by each of the three ibis species. Focals included when the bird spent ≥5 min foraging. "Hadeda" = *Bostrychia hagedash*; "Sacred" = *Threskiornis aethiopicus*; "Glossy" = *Plegadis falcinellus.* Coloured points indicate outliers. Asterisks above brackets represent significance codes from two-sided Wilcoxon rank sum tests: **** p<0.0001; *** p<0.001; * p<0.05; "ns" p>0.05 (difference not significant).

3.3.2: Probing behaviour, beak bone morphology and substrate use

The relative use of deep probing (where over half the length of the beak was inserted into the substrate; relative to the total number of probes attempted during the focal) of each species correlated with the average extent (by beak length) of sensory pitting on the beak bones (Figure 3.5A): the species with the highest extent of pitting on average (Glossy Ibis) probed deeply most frequently (38.8 ± 12.5 % of the total probes were deep), while the species with the lowest extent of pitting (Hadeda Ibis) probed deeply least frequently (2.2 ± 1.4 % of total probes). Sacred Ibises, with intermediate extents of pitting, used deep probing an intermediate percentage of their total probes compared to the other two species (12.9 ± 5.8 % of total probes).

Similarly, the relative use of open-beak probing correlated with the average extent of pitting on the internal surfaces of the beak bones (i.e., "inside" of the mouth; see Figure 3.6A): species with higher extents of pitting on the beak interior (Sacred and Glossy Ibises) probed more frequently with the beak held open (40.9 ± 13.8 % and 48.4 ± 15.6 % of total probes with beak held open, respectively) than the species with lower extents of pitting on the beak interior (Hadeda Ibises; 4.4 ± 2.7 % of total probes).

The average relative use of deep and open-beak probing by the three ibis species also correlated with the type of substrate they were observed foraging in. Deep probing (Figure 3.5B) was used more frequently in water than in sand and soil. Open-beak probing (Figure 3.6B) was used most frequently in water, and more frequently in sand than in soil.

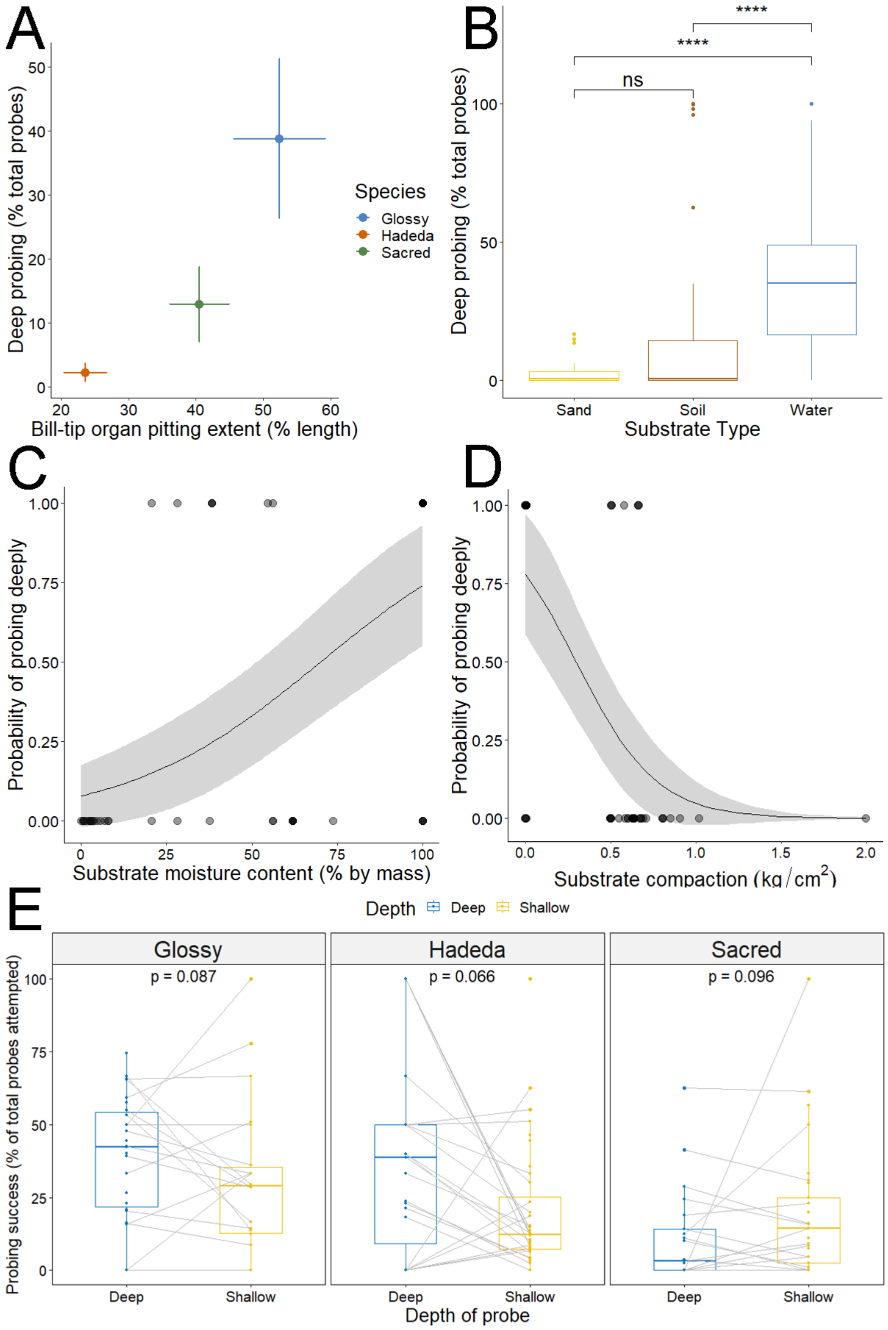

Figure 3.5: Ibises’ relative use of deep probing (over half the beak’s length inserted into substrate, relative to the total number of probes). A) Relationship between the extent of pitting (by length) on the bones of the bill-tip organ of each species of ibis, and the proportion of deep probes. Circles represent the mean for each species, lines the 95% confidence intervals. B) Proportion of deep probes used by ibises in different foraging substrates. Coloured points indicate outliers. C-D) Probabilities of a bird probing deeply ≥10 times during a focal (all three species of ibis), against C) moisture content and D) compaction of the foraging substrate. Circles represent data from each focal. Black lines are predicted values and shaded grey regions indicate the 95% confidence intervals from GLMMs (see Table 3.2 for model summaries). E) Comparison of probing success (percentage of probes that resulted in successful prey capture) between deep and shallow probes, for each species of ibis. Grey lines represent pairwise comparisons within a single focal. P-values from two-sided paired Wilcoxon rank sum tests (no significant differences). “Hadeda” = *Bostrychia hagedash*; “Sacred” = *Threskiornis aethiopicus*; “Glossy” = *Plegadis falcinellus.* Asterisks above brackets in B represent significance codes from two-sided Wilcoxon rank sum tests: **** p<0.0001; “ns” p>0.05 (difference not significant).

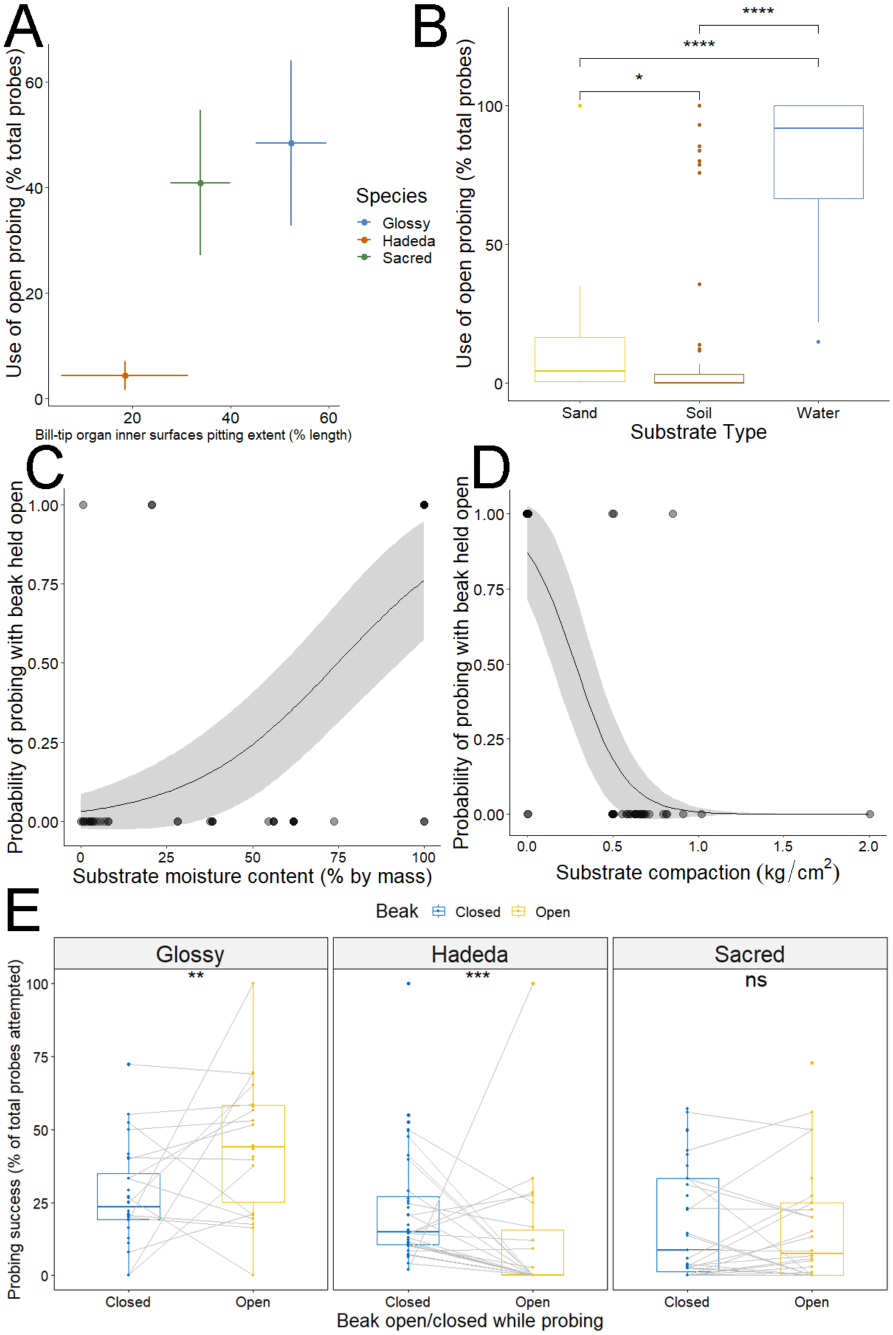

Figure 3.6: Ibises' relative use of open-beak probing (beak held open while probing, relative to the total number of probes). A) Relationship between the extent of pitting (by length) on the inside surfaces of the bones of the bill-tip organ of each species of ibis, and the proportion of open-beak probes. Circles represent the mean for each species, lines the 95% confidence intervals. B) Proportion of open-beak probes used by ibises in different foraging substrates. Coloured points indicate outliers. C-D) Probabilities of a bird probing with the beak held open ≥10 times during a focal (all three species of ibis), against C) moisture content and D) compaction of the foraging substrate. Circles represent data from each focal. Black lines are predicted values and shaded grey regions indicate the 95% confidence intervals from the GLMMs (see Table 3.2 for model summaries). E) Comparison of probing success (percentage of probes that resulted in successful prey capture) between closed- and open-beak probes, for each species of ibis. Grey lines represent pairwise comparisons for data within a single focal. "Hadeda" = *Bostrychia hagedash*; "Sacred" = *Threskiornis aethiopicus*; "Glossy" = *Plegadis falcinellus.* Asterisks in B&E represent significance codes from two-sided Wilcoxon rank sum tests: **** $p<0.0001$; *** $p<0.001$; ** $p<0.01$; * $p<0.05$; "ns" $p>0.05$ (difference not significant).

Table 3.2: Models explaining variation in the probabilities of an ibis probing deeply (over half the beak's length inserted into substrate) or with the beak open more than 10 times during a focal. Ibis species is included in all models as a random effect. Model estimates are shown ± standard errors. Top weighted models shown in bold and null models are shown in italics for comparison. Significance codes: * p < 0.05; ** p < 0.01; *** p < 0.001. Number of observations for all models = 52 (n focals where both moisture content and compaction of the birds' foraging substrate could be measured). Fitted model predictions are shown in Figures 3.5 & 3.6.

Model	Intercept	Effect ± CI	AICc	ΔAICc	Weight
Pr(Deep)					
Null model	*-0.75 ± 0.75*		*67.4*	*11.39*	*0.00*
Compaction	**1.14 ± 0.76**	**-4.20 ± 1.25*****	**56**	**0.00**	**0.73**
Moisture content	-2.42 ± 0.69***	0.03 ± 0.01***	58	2.04	0.27
Pr(Open)					
Null model	*-0.66 ± 0.34*		*71.2*	*33.38*	*0.00*
Compaction	**2.81 ± 1.37***	**-9.23 ± 3.02****	**37.9**	**0.00**	**0.78**
Moisture content	-14.38 ± 8.45	0.22 ± 0.11*	40.4	2.55	0.22

The probabilities of an ibis probing deeply or with the beak held open more than ten times during a focal were both negatively correlated with the birds' foraging substrate compaction, and positively correlated with substrate moisture content (see Figures 3.5CD & 3.6CD), but had no relationship with any other measured variable. For both deep and open-beak probing probabilities, the model including substrate compaction was the best fit (Table 3.2).

None of the species showed any difference in the percentage of successful probes (of total probes attempted) between deep and shallow probes (Figure 3.5E). There were some differences in success between closed- and open-beak probes (Figure 3.6E): Glossy Ibises were more successful when probing with the beak open (probe success: open 44.7 ± 10.1 %; closed 27.2 ± 6.4 %), while the inverse was true for Hadeda Ibises (success: open 11.6 ± 10.1 %; closed 23.0 ± 6.8 %).

3.3.3: Species, substrate type and tactile foraging success in relation to bill-tip organ morphology

The ibises' use of tactile foraging methods (see Table 3.1 for various tactile foraging behaviours) as a percentage of their total foraging time differed between the three species and between foraging substrate types (Figure 3.7).

On average, all three species used tactile foraging more frequently than other foraging methods, with tactile foraging making up a significantly larger proportion of the total foraging time of Glossy Ibises than the other two species (Figure 3.7A).

Pooling the data for all three species (Figure 3.7B), ibises spent significantly lower proportions of their foraging time using tactile methods in sand (80.6 ± 9.34 % of total foraging time) than in soil or water (98.1 ± 1.6 % and 93.3 ± 6.2 % respectively).

To check whether the abovementioned variation in the ibises' relative use of tactile foraging between substrate types was being driven by interspecific differences in the birds' substrate usage (see Figure 3.4A), I split the data by species (Figure 3.7C). Hadeda and Glossy Ibises both followed the "overall" trend (i.e., use of tactile foraging in sand < soil = water, as shown in Figure 3.7B). Sacred Ibises also used tactile foraging methods least frequently in sand, but more in soil than in water (96.7 ± 5.3 % vs. 87.4 ± 12.4 % respectively).

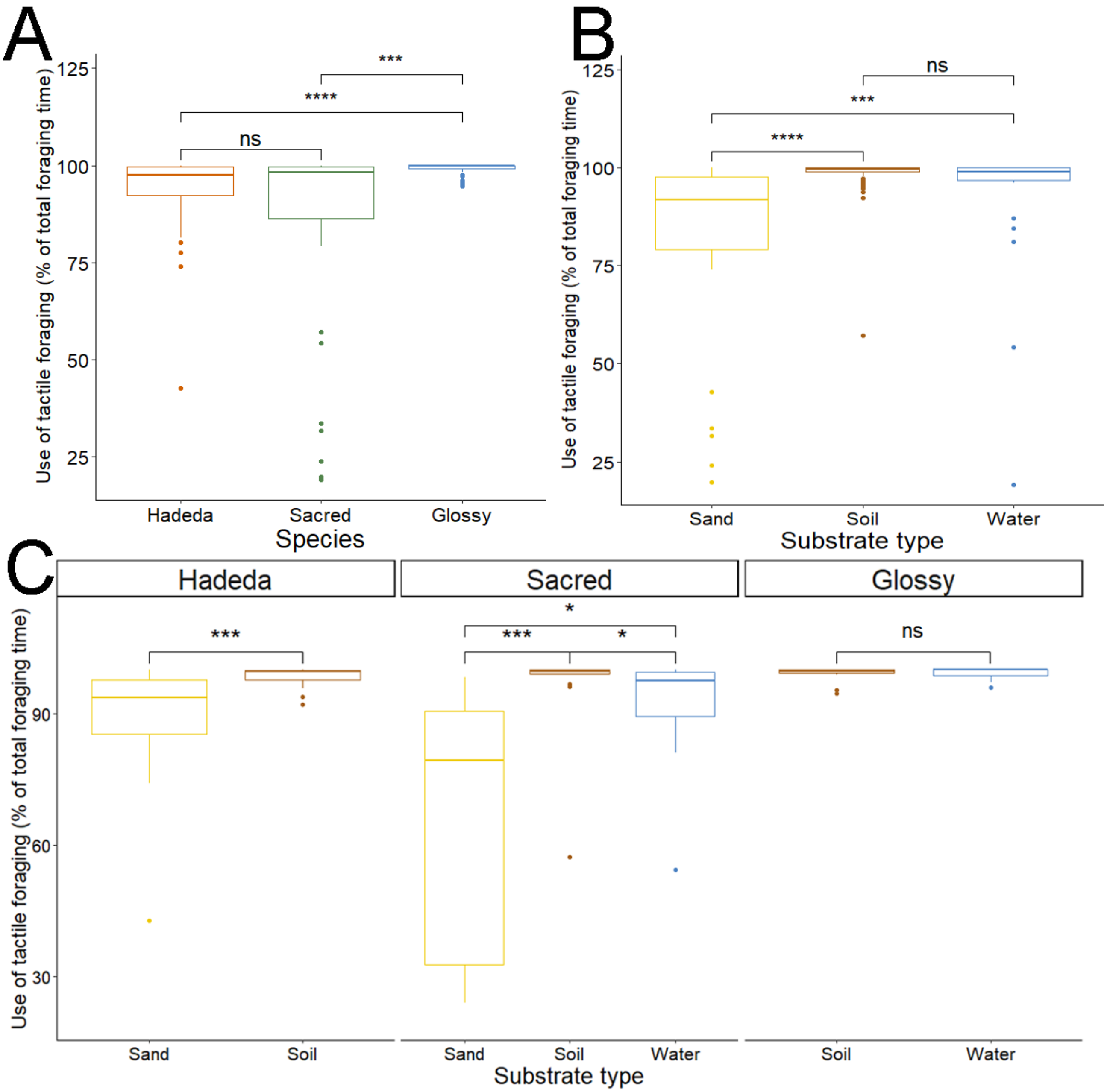

Figure 3.7: Ibises' use of tactile foraging methods, as a percentage of their total foraging time. A) Average for each of the three species of ibis (in all substrates). B) Average (all species) when foraging in each substrate type. C) Averages for each species separately when foraging in different substrate types. "Hadeda" = *Bostrychia hagedash*; "Sacred" = *Threskiornis aethiopicus*; "Glossy" = *Plegadis falcinellus.* Coloured points indicate outliers. Asterisks above brackets represent significance codes from two-sided Wilcoxon rank sum tests: **** $p<0.0001$; *** $p<0.001$; ** $p<0.01$; * $p<0.05$; "ns" $p>0.05$ (difference not significant).

For all three species, substrate type was included as a predictor of tactile foraging success in the model selection outputs in one or more of the top selected models (Table 3.3), and the tactile foraging success of all three species was significantly affected by substrate type (Figure 3.8A). In Glossy Ibises, tactile foraging success in water was higher than in soil (46.6 ± 7.7 % vs. 26.9 ± 5.2 % tactile foraging attempts successful; all predictions from top species-specific models in Table 3.3). Sacred Ibises had their highest tactile foraging success in the mid-range of moisture contents (i.e., in soil: 41.0 ± 6.9 % attempts successful), while in very dry or wet substrates, their tactile foraging success was significantly lower (4.5 ± 5.7 % and 9.5 ± 5.8 % success in sand and water respectively). Hadeda Ibises' tactile foraging success in sand was higher than in soil (28.6 ± 6.2 % vs. 13.2 ± 4.0 % success).

Other variables were also included in the selected models as predictors of tactile foraging success for each species (see Table 3.3). In Hadeda Ibises, temperature had a negative effect on tactile foraging success. Vegetation cover had a significant effect on the tactile foraging success of Glossy Ibises (tactile foraging success higher in substrates clear of vegetation), as did field site (success at Velddrif higher than Strandfontein). Vegetation cover was included in one of the top models for Sacred Ibises, but it had no significant effect on their tactile foraging success.

Table 3.3: Top weighted models (ΔQAICc <2) explaining variation in tactile foraging success (percentage of attempts that result in successful prey capture) for each species of ibis (compared to null models). Global models included foraging patch vegetation cover ("Veg cover"; levelled by "Clear" – i.e., no vegetation), air temperature, field site (levelled by "Strandfontein"), and substrate type (levelled by "Soil"). Model estimates are shown ± standard errors. Models were selected by QAICc values, which represent the Akaike's Information Criterion score adjusted for small sample sizes and quasibinomial models. Top weighted models shown in bold and null models are shown in italics for comparison. Significance is shown: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. "n" = Number of observations for each model set (focals where the bird spent ≥5 min foraging). Model predictions shown in Figure 3.8A.

Model	Intercept	Substrate (Soil)		Veg cover (Clear)		Temperature	Site (Strandfontein)	QAICc	ΔQAICc	Weight
		Sand	Water	Plants	Hyacinth		Velddrif			
SACRED (n=34)										
Null model	*-1.02 ± 0.19****							*95.0*	*46.20*	*0.00*
Substrate	**-0.36 ± 0.14***	**-2.68 ± 0.68*****	**-1.89 ± 0.37*****					**48.8**	**0.00**	**0.52**
Substrate + Veg cover	-0.94 ± 0.63	-2.38 ± 0.74**	-1.55 ± 0.50**	0.58 ± 0.61				50.7	1.94	0.20
HADEDA (n=35)										
Null model	*-1.26 ± 0.16****							*89.7*	*36.25*	*0.00*
Substrate + Temperature	**0.64 ± 0.95**	**0.97 ± 0.24*****				**-0.15 ± 0.05****		**53.4**	**0.00**	**0.66**
GLOSSY (n=34)										
Null model	*-0.71 ± 0.13****							*103.7*	*46.20*	*0.00*
Substrate + Veg cover	**-0.63 ± 0.27***		**0.86 ± 0.22*****	**-0.62 ± 0.24***	**-0.49 ± 0.34**			**57.5**	**0.00**	**0.30**
Veg cover + Field site	0.20 ± 0.16			-0.57 ± 0.25*	-1.32 ± 0.26***		-0.88 ± 0.24***	58.1	0.66	0.21
Substrate	-1.20 ± 0.12***		1.16 ± 0.18***					58.7	1.20	0.16

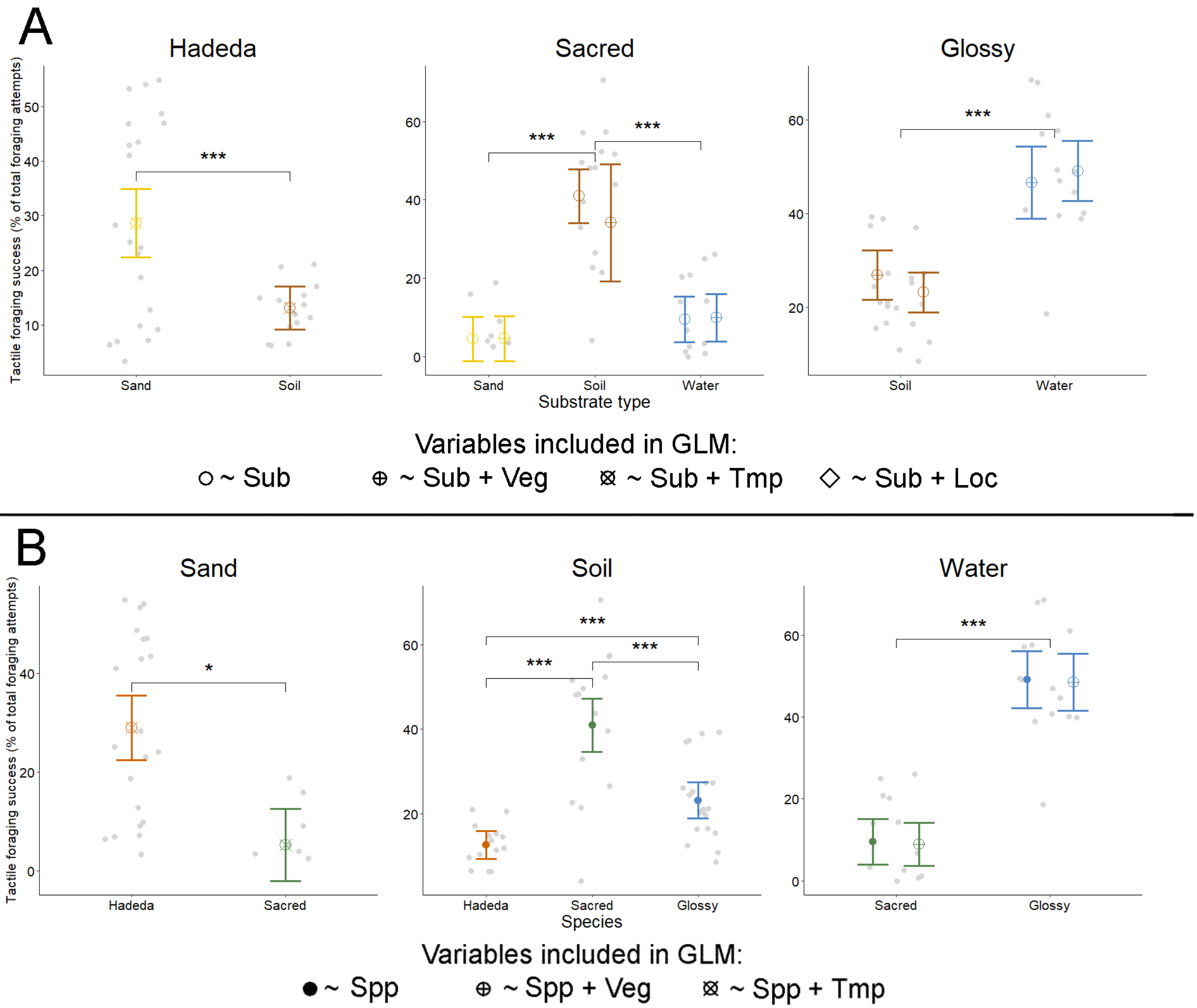

Figure 3.8: Predicted variation in tactile foraging success (as a percentage of total attempts) of ibises from Generalized Linear Models (GLMs), in response to two predictors: A) foraging substrate type and B) species identity. All models were fitted as quasibinomial GLMs. Global models were fitted (A)

per species, and (B) per substrate type. All models with ΔQAICc<2 have been plotted, with the highest weighted model predictions on the left (see Tables 3.3 & 3.4 for model summaries). Small grey circles represent actual data points for individual focals. Coloured shapes are fitted means from the model predictions, with vertical bars indicating 95% confidence intervals. The shape of the fitted mean markers represent which variables are included in the model, which are coded in the keys below the graphs. Asterisks above the brackets indicate statistically significant differences in the fitted values (based on the top weighted model in the case of multiple fitted models per graph). "Sub" = foraging substrate type; "Veg" = vegetation cover of the substrate in which the birds were foraging; "Tmp" = air temperature at the time of observation; "Loc" = location/field site where the birds were observed; "Spp" = species of ibis; "Hadeda" = *Bostrychia hagedash*; "Sacred" = *Threskiornis aethiopicus*; "Glossy" = *Plegadis falcinellus.* Significance codes: *** $p<0.001$; ** $p<0.01$; "ns" $p>0.05$.

When comparing ibises foraging in substrates with similar characteristics and moisture contents (Figure 3.2), there were significant differences in the birds' tactile foraging success between the different species (Figure 3.8B). For all substrate types, species was included in all the selected models (Table 3.4). In sand (lowest moisture contents), Hadeda Ibises were more successful than Sacred Ibises (29.0 ± 6.5 % and 5.3 ± 7.2 % success respectively; all predictions from top substrate-specific models in Table 3.4). In soil (mid-high moisture contents) Hadeda Ibises had the lowest tactile foraging success of the three species (12.7 ± 3.3 % success), and Sacred Ibises had higher success than Glossy Ibises (41.0 ± 6.3 % vs. 23.2 ± 4.3 % success respectively). In water, Glossy Ibises were more successful at tactile foraging than Sacred Ibises (49.1 ± 6.9 % and 9.5 ± 5.6 % success respectively).

In addition, temperature was included as a predictor in the top model for ibises foraging in sand, and had a negative effect on the birds' tactile foraging success (Table 3.4). Vegetation cover was included as a predictor in one of the top selected models for ibises foraging in water, but it had no significant effect on the birds' tactile foraging success (Table 3.4).

Table 3.4: Top weighted models (ΔQAICc <2) explaining variation in tactile foraging success (percentage of attempts that result in successful prey capture) of ibises foraging in each substrate type (compared to null models). Global models included foraging patch vegetation cover (“Veg cover”; levelled by “Clear” – i.e., no vegetation), air temperature, field site (levelled by “Strandfontein”), and ibis species (levelled by “Sacred”). Model estimates are shown ± standard errors. Models were selected by QAICc values, which represent the Akaike's Information Criterion score adjusted for small sample sizes and quasibinomial models. Top weighted models shown in bold and null models are shown in italics for comparison. Significance is shown: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. “n” = Number of observations for each model set (focals where the bird spent ≥5 min foraging). Fitted model predictions shown in Figure 3.8B.

Model	Intercept	Species(Sacred)		Veg cover (Clear)	Temperature	QAICc	ΔQAICc	Weight
		Hadeda	Glossy	Plants				
SOIL (n=49)								
Null model	*-1.19 ± 0.12*					*126.7*	*54.45*	*0.00*
Species	**-0.36 ± 0.13****	**-1.56 ± 0.20*****	**-0.83 ± 0.18*****			**72.2**	**0.00**	**0.70**
SAND (n=28)								
Null model	*-0.94 ± 0.18****					*60.1*	*19.98*	*0.00*
Species + Temperature	**-0.18 ± 1.30**	**1.99 ± 0.76***			**-0.16 ± 0.06***	**40.1**	**0.00**	**0.72**
WATER (n=26)								
Null model	*-0.60 ± 0.20***					*86.6*	*48.15*	*0.00*
Species	**-2.26 ± 0.33*****		**2.22 ± 0.36*****			**38.4**	**0.00**	**0.36**
Species + Veg cover	-2.12 ± 0.34***		2.27 ± 0.36***	-0.43 ± 0.26		38.8	0.39	0.30

3.3.4: Interspecific differences in prey captured

Each species of ibis was seen foraging on slightly different size-classes of prey (Figure 3.9). Glossy Ibises were not seen consuming any prey item larger than 3 cm (Figure 3.9A), and no differences were observed in prey sizes between Glossy Ibises foraging in soil or water (Figure 3.9C&D). One bird was observed catching a larger prey item (~5 cm in length), but it was dropped and the bird did not attempt to pursue it again.

In sand and soil (Figure 3.9B&C), Sacred Ibises were only observed consuming prey items smaller than 3 cm [aside from those classed as “other” - prey items that were larger than 10 cm in length and/or unusual in nature; in this case, pieces of human refuse]. In water however (Figure 3.9D), several birds were observed consuming prey items over 5 cm in length.

In sand (Figure 3.9B), Hadeda Ibises were only observed consuming prey smaller than 3 cm in length (aside from one “other” prey item: a small snake, see below for more details). In soil (Figure 3.9C), the majority of Hadeda Ibises were seen capturing larger prey items than in sand, and several birds were observed capturing prey over 5 cm in length.

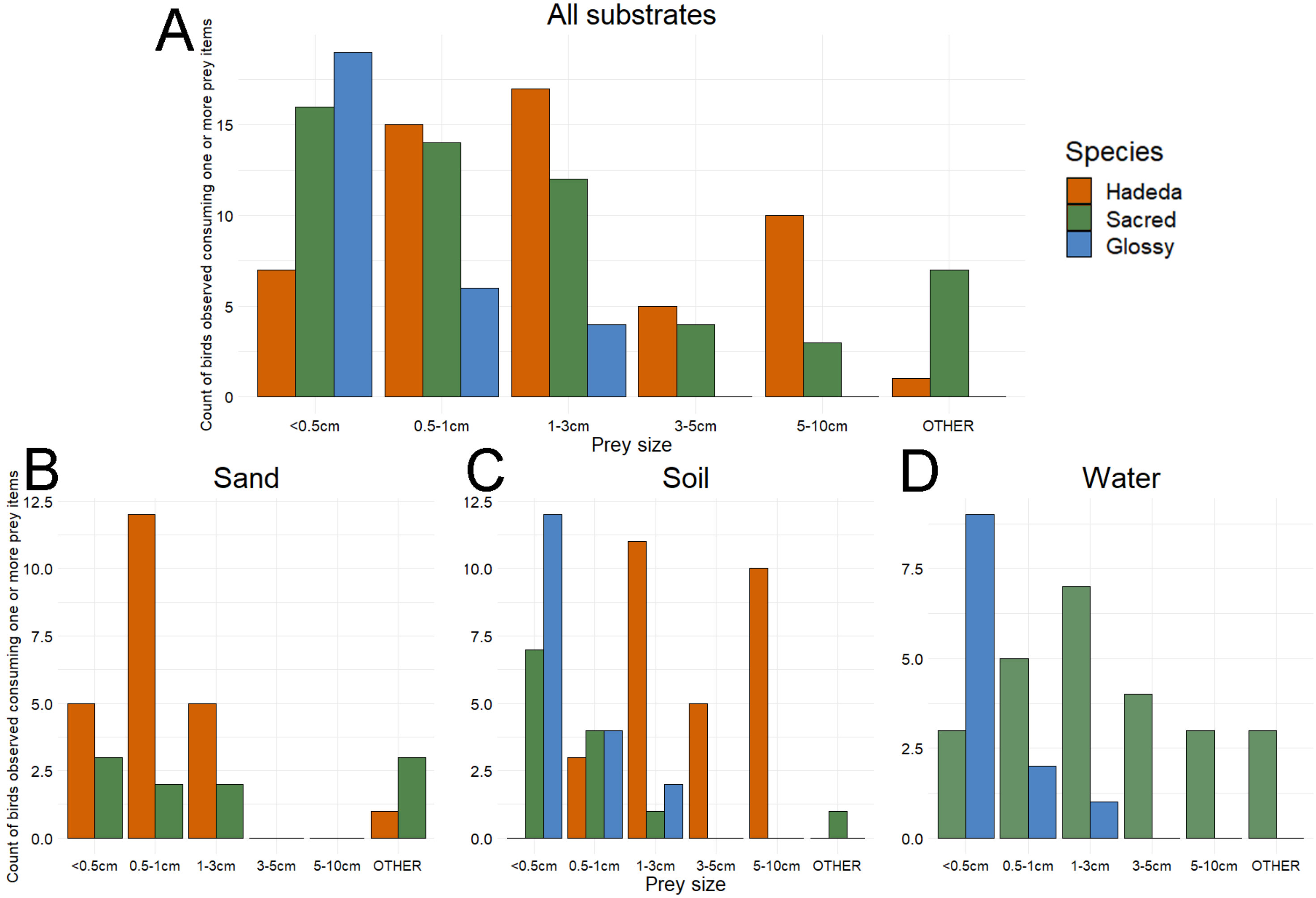

Figure 3.9: Categories of prey sizes observed being consumed by each species of ibis, and the differences between different foraging substrates. A) Combined counts from all substrates. B-D): counts for each substrate type. Counts represent the number of birds recorded during focal observations consuming one or more prey item of each size class (individuals would frequently consume prey items from 2-3 size classes during a 15 min focal period). Size classes were estimated using the size of the prey item in relation to the bird's beak. Only when prey items were clearly visible was their size recorded. "OTHER" refers to prey items that were larger than 10 cm in length, and/or were unusual in nature (e.g., pieces of human refuse). For types of prey items, see Table 3.5. "Hadeda" = *Bostrychia hagedash*; "Sacred" = *Threskiornis aethiopicus*; "Glossy" = *Plegadis falcinellus*.

Table 3.5: Size classes of prey items that could be identified, which were consumed during the focal observations by three ibis species (prey items <0,5 cm were too small to be identified from a distance). "Hadeda" = *Bostrychia hagedash*; "Sacred" = *Threskiornis aethiopicus*; "Glossy" = *Plegadis falcinellus.* For numbers of size classes recorded, see Figure 3.9.

Species	**Prey size**				
	0.5-1 cm	1-3 cm	3-5 cm	5-10 cm	OTHER
Hadeda	insects, snails, beach hoppers	worms, snails, grubs	worms	worms	snake (~20 cm long, 1 cm wide)
Sacred	crabs, snails	snails, worms, shrimp, crabs, fish	worms, crabs	worms, fish, frog, shrimp	refuse carried over from dump
Glossy	crabs, unknown invertebrates	snails, worms, unknown invertebrates			

The majority of the ibises' prey that could be identified were invertebrates (most frequently crustaceans, molluscs, insects and worms; Table 3.5), although Sacred Ibises were observed capturing several small vertebrates (frogs and fish). One Hadeda Ibis captured a small snake, which it located visually on the ground and grabbed by the head. At Kirstenbosch, Hadeda Ibises fed almost exclusively on large earthworms (over 3 cm in length). Sacred Ibises consumed the most diverse range of prey items, and at Strandfontein they were frequently observed feeding on refuse (bones with small pieces of meat still attached) which they had carried over from the landfill adjacent to the field site. They would often hold these items in their beaks and thrash them in the water, and then pick up and swallow the small pieces of meat that detached.

3.4: Discussion

Tactile foraging methods made up the bulk of the time spent foraging by all three ibis species, and all used probing as their main foraging technique. Each of the three species foraged in different subsets of substrate types, though all three species were observed foraging in soil. Sacred Ibises foraged in the most diverse range of substrates (in terms of type, penetrability and moisture content), while Hadeda Ibises tended to forage in harder, drier substrates than the other two species, and Glossy Ibises were restricted to the wettest, softest substrates. The probing depth and use of open-beak probing of all three species were negatively affected by substrate compaction, and were consequently positively related to substrate moisture content. Furthermore, the relative use of deep and open-beak probing differed between the three species, and correlated with the average extents of sensory pitting on their bill-tip organs. When other factors (e.g., prey type and size) were consistent, substrate moisture content appeared to positively affect the tactile foraging success of the birds. Similarly, when comparing birds foraging in the same substrate types, there is some evidence that species of ibises with higher densities of mechanoreceptors in their bill-tip organs (e.g., Sacred Ibises) may have higher tactile foraging success than species with lower densities (Glossy Ibises).

3.4.1: Relationships between probing behaviour, substrate use and beak bone morphology

The different species of ibis showed differences in probing depth and use of open-beak probing that correlate with differences in their bill-tip organ morphology. Use of deep probes correlated with the average total extent of sensory pitting on the bill-tip organ of each species. Likewise, the use of open-beak probing correlated with the extent of pitting on the inside surfaces of the bill-tip organ (which would only be in contact with the substrate if the beak is held open). These findings support my hypothesis that interspecific differences in the extent of pitting evolved in correlation with which surfaces of the bill-tip organ are frequently exposed to the foraging substrate. Furthermore, species probing frequently in harder substrates (such as Hadeda Ibises) require the bases of their beaks be sturdier (Bock, 1966; Zweers, Berkhoudt & Berge, 1994; Zweers & Berge, 1996; Zweers & Gerritsen, 1997; Barbosa & Moreno, 1999). This results in selective pressure for this region to have denser bone (Bock, 1966; Zweers & Gerritsen, 1997), which would be compromised by a high degree of pitting. Thus, they apparently face a trade-off between having a more extensive bill-tip organ and having a beak suited for probing in relatively compact substrates (see Chapter 2, section 2.4.3). Indeed, the morphology of birds' beaks have been shown by various studies to be strongly influenced by a complex set of trade-offs between different factors (Navalón *et al.*, 2019; Friedman *et al.*, 2019; Felice *et al.*, 2019; Pigot *et al.*, 2020).

The probability that an ibis would use either deep or open-beak probing was negatively affected by the birds' foraging substrate compaction and positively by substrate moisture content. Models including substrate compaction appeared to be a better fit (based on weighted AICc values) for predicting whether an ibis would use open-beak or deep probing than models including moisture content. Logically, substrate compaction should be the stronger driver due to the serious consequences of damage to the birds' beaks, though water content likely has an indirect effect as it negatively affects substrate compaction (Vaz *et al.*, 2011) (for this reason, these two effects could not be included in the same models). In more penetrable substrates (i.e., with lower compaction), the energy required for deep probing would be less (Kelsey & Hassall, 1989), which may explain why ibises were more likely to use deep probing in these substrates. Opening the beak increases the surface area of the bill-tip organ exposed to the substrate, which may aid the birds in locating signal direction (Zweers & Gerritsen, 1997). It also transforms the beak from one relatively thick object into two thinner ones, making it more susceptible to breakage [an object with decreased diameter is less able to withstand forces applied against it than a thicker object (Westergaard, 2013)]. Therefore, though it may be beneficial to probe with the beak open, the risk of damage to the beak may deter the birds from doing so frequently in more compact substrates.

Deeper probing was not more successful than shallow probing in any of the three species. The birds likely use each depth of probe in reaction to the location of their prey in the substrate and depending on the substrate properties [as has been observed in other taxa of probing birds (Kelsey & Hassall, 1989; Cunningham, Castro & Alley, 2007)]. Glossy Ibises showed increased success when probing with their beaks held open, potentially supporting the notion that doing so benefits the birds by increasing the surface area of the bill-tip organ exposed to the substrate (Zweers & Gerritsen, 1997). Hadeda Ibises had higher success with their beaks closed, but as they have the lowest internal sensory pitting on their bill-tip organs, opening their beaks would not provide a large increase in bill-tip organ contact with the substrate. Sacred Ibises had no change in success when probing with their beaks open, though this may be due to their varied use of substrate types (i.e., in wetter substrates they do better with their beaks open, but in drier substrates with their beaks closed. Thus overall, their success using both types of probes are balanced).

Based on the above evidence, bill-tip organ morphology, probing behaviour and substrate use appear to be strongly interrelated in these three ibis species. By comparing my results to the morphological data from Cunningham, Alley *et al.* (2010) (who sampled 10 other ibis species), the support for these patterns is greatly strengthened. Kiwi [which show the same trend of decreased bill-tip organ pitting extent in more terrestrial species (Cunningham, Alley *et al.*, 2010)], have been shown to probe more deeply in wetter, more penetrable soils (Cunningham, Castro & Alley, 2007), similar to the ibises sampled in this chapter.

Furthermore, Dunlin [*Calidris alpina*, scolopacid shorebirds which have remote-touch bill-tip organs – see Chapter 5, as well as Bolze, (1968) & Nebel, Jackson & Elner (2005)] have also been shown to preferentially use deeper probing in softer, wetter substrates (Kelsey & Hassall, 1989). Birds changing probing behaviour in response to substrate moisture content and penetrability have been described in multiple other taxa of scolopacid birds (Quammen, 1982; Mouritsen & Jensen, 1992; Danufsky & Colwell, 2003; Granadeiro, Andrade & Palmeirim, 2004; Finn, Catterall & Driscoll, 2007; Finn, Catterall & Driscoll, 2008), indicating this relationship between behaviour and foraging substrate is common to other families of remote-touch foraging birds. There is no published data on whether scolopacids show the same patterns in terms of interspecific differences in bill-tip organ morphology linked to these behavioural and environmental differences. Preliminary data (S.J. Cunningham, *data unpub.*) does indicate that the trend is not as strong in this family as in ibises and kiwi. This could be due to more fine-scale differences in substrate types on the mudflats where most scolopacid shorebirds forage (in comparison to the wide range of habitats utilized by ibises), but this is speculative without further analyses. Therefore, such studies in the future would be of great value.

3.4.2: Bill-tip organ morphology and remote-touch sensitivity

Previous research has indicated that interspecific differences in the morphology of the bill-tip organ could affect the birds' ability to use remote-touch (Zweers & Gerritsen, 1997; Cunningham, Alley *et al.*, 2010). Following work by Zweers & Gerritsen (1997), Chapter 2 (section 2.4.2) provided evidence that ibis species foraging in drier habitats likely have the most sensitive bill-tip organs, based on the density of mechanoreceptors in their bill-tip organs. This trend may be due to the fact that vibratory cues travel poorly in less saturated substrates (Biot, 1956 & 1962).

This chapter provides some support for the hypothesised link between mechanoreceptor density in the bill-tip organ and remote-touch sensitivity. As my results indicate that substrate type and moisture content have a significant effect on the ibises' tactile foraging success (see section 3.4.3), it is important to control for these factors when analysing the differences in tactile foraging success between the species.

When substrate type is controlled for, the birds' tactile foraging success was significantly different between the three species of ibis (a proxy for bill-tip organ structure; see Chapter 2, section 2.4.2). As Hadeda and Sacred Ibises have similar bill-tip organ structures with respect to mechanoreceptor density (see Chapter 2, Figure 2.12A), any differences in the tactile foraging success of these two species are likely influenced by other factors. Thus, it is more valuable to compare these two species to Glossy Ibises, as the latter have lower densities of Herbst corpuscles in their bill-tip organs. If all other factors can be accounted for, we would expect Glossy Ibises to have lower tactile success than Sacred and Hadeda Ibises. The best available comparison in my dataset is between Sacred and Glossy Ibises foraging in soil, as this provides some controls for substrate moisture content and prey size (the two species were foraging on prey items of similar sizes in soil). Furthermore, none of the other factors recorded (aside from species) were included in the top models of tactile foraging success for ibises foraging in soil (Table 3.4). My results show that Sacred Ibises did have higher tactile success than Glossy Ibises in soil, providing the first tentative behavioural support for the hypothesis that mechanoreceptor density in the bill-tip organ could positively affect remote-touch ability. However, there are still numerous other factors that could be affecting this difference between the two species [e.g., prey type, prey density, exact moisture content of the substrate, other differences in substrate and the species' bill-tip organs (Goss-Custard, 1977; Quammen, 1982; Kelsey & Hassall, 1989; Mouritsen & Jensen, 1992; Yates *et al.*, 1993; Botton, Loveland & Jacobsen, 1994; Mouritsen, 1994; Zweers & Gerritsen, 1997; Danufsky & Colwell, 2003; Granadeiro, Andrade & Palmeirim, 2004; Finn, Catterall & Driscoll, 2007; Finn, Catterall & Driscoll, 2008)], and I could not confirm whether the birds were locating prey randomly (i.e., through direct contact with the beak) or via remote-touch.

The need to investigate the pattern under controlled conditions is further supported by further examination of the data. In sand, when substrate moisture content and prey size were relatively consistent between the two species, Sacred and Hadeda Ibises had different tactile foraging success, despite having similar mechanoreceptor densities in their bill-tip organs. The same is true in soil between these two species, but here prey size was different, which would likely have influenced their foraging success (Goss-Custard, 1977). Furthermore, most of my observations of Hadeda Ibises foraging in soil were from a site not used by the other two species (Kirstenbosch Botanical Gardens). Further studies with larger sample sizes per species may also help elucidate whether any effects resulted from or were masked by the relatively low sample size of observations per species. However, this may prove difficult if trying to compare the species in the same habitats, as finding areas where all three species occur in high enough numbers to facilitate these observations was a challenge. Therefore, there are too many other factors that could not be controlled for in my data to comment on the tactile success of Hadeda Ibises in comparison to the other two species. Constraints due to differences in prey size and type also apply to comparing the data from Sacred and Glossy Ibises foraging in water. Thus, future studies should focus on investigating the pattern between species' bill-tip organ morphology and tactile foraging success under more controlled conditions (see Chapter 4 for further comments).

3.4.3: Effects of foraging substrate moisture content on ibises' tactile foraging success

All three species used tactile foraging methods more frequently than other methods on average, as has been previously reported (Skead, 1951; Kushlan, 1978; Hockey, Dean & Ryan, 2005). Hadeda and Sacred Ibises did however use visual methods (capturing prey on the surface of the sand or off vegetation) more frequently in sand (which had the lowest average moisture content) than in other substrate types. Other taxa of probe-foraging birds (e.g., stilts, dunlins, godwits) are known to switch between visual and tactile foraging in reaction to changes in sensory cues (Cullen, 1994; Mouritsen, 1994; McNeil *et al.*, 1999; Jourdan *et al.*, 2021). Furthermore, Piersma *et al.* (1998) showed that Red Knots (*Calidris canutus*) could not detect prey using remote-touch when the sand was dry, indicating that substrate moisture content is indeed a limiting factor for birds sensing vibratory cues. Since highly saturated soils transmit mechanical vibrations better than drier, more porous soils (Biot, 1956 & 1962), ibises may switch to other methods in sand and dry soils to account for this lack of tactile sensory information, though other factors may also influence this behavioural shift (Kelsey & Hassall, 1989; de Fouw *et al.*, 2016).

This study provides behavioural evidence that there may be significant relationships between substrate moisture content and the birds' remote-touch success rate, which warrant further investigation under controlled conditions. In Glossy Ibises, substrates with increased moisture content had a positive effect on the birds' tactile foraging success, and Sacred Ibises showed the same effect when foraging out of water. Though there was a positive correlation between substrate moisture content and tactile foraging success between sand and soil, Sacred Ibises had lower success in water than soil. Prey sizes of Sacred Ibises differed between water and soil, with more birds consuming larger prey items in water. Their higher tactile foraging success in soil could thus have been an artefact of higher prey density [smaller invertebrates tend to have higher densities than larger ones in soil (Turnbull, George & Lindo, 2014)]. When foraging in water, they may have been selectively feeding on larger, less abundant prey items and ignoring smaller ones, which would negatively skew their success percentage. Birds have been shown to ignore more numerous, less desirable prey when other options are readily available (Goss-Custard, 1977; Erichsen, Krebs & Houston, 1980). Glossy Ibises did not shift to larger prey in water as Sacred Ibises appeared to do, potentially due to their beaks and body size being unsuitable for larger prey (Hockey, Dean & Ryan, 2005). Hadeda Ibises had lower tactile foraging success in soil than sand, but captured larger prey items in soil, likely due to an increase in average invertebrate prey size in human modified habitats (Potapov *et al.*, 2019) (where most of the Hadeda Ibises were foraging in soil).

In short: When prey size remained consistent, the ibises' tactile foraging success increased in substrates with higher moisture contents, providing evidence that this trend may be biologically significant. However, as there were several factors I could not control for, and prey may be easier to acquire in wetter substrates for reasons besides signal transmission [e.g., wetter, more penetrable substrates are easier to probe in, or birds could be using other sensory cues in addition to tactile cues (Cunningham, Castro & Potter, 2009; Onrust *et al.*, 2017)], these patterns need to be verified under controlled conditions (see Chapter 4).

3.5: Conclusions

There are strong correlations between probing behaviour, physical properties of the foraging substrate and bill-tip organ morphology in the three species of ibises sampled, which are supported by similar trends in other remote-touch foraging birds (Kelsey & Hassall, 1989; Cunningham, Alley *et al.*, 2010). This could have important implications for understanding some of the environmental needs of these birds regarding their foraging habitats. Furthermore, the results may also be useful in studying the beaks of probe-foraging fossil birds, and could provide information about their behaviour, sensory ecology and evolution (see Chapter 5), as well as providing valuable contextual information about palaeoecology. The results of this study provide the first behavioural support that there are interspecific differences in the tactile capabilities of ibises, which may be linked to interspecific differences in their bill-tip organ morphology, warranting further study. The results support that the tactile foraging capability of ibises is affected by foraging substrate type and moisture content. When other variables could be accounted for, the effect of substrate moisture content on the birds' tactile foraging success seems to be positive. This supports one of the main hypotheses of my thesis, namely that due to the better transmission of mechanical cues in more saturated substrates (Biot, 1956 & 1962), remote-touch probing birds can locate prey in these substrates more easily than in drier substrates. However, to better understand this trend, it is necessary to investigate the changes in remote-touch foraging success in response to soil moisture content under controlled conditions (see Chapter 4).

3.6: References

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Chapter 4:

The effects of foraging substrate water content on the success of remote-touch foraging in birds

Hadeda Ibis, study individual D (knicknamed “Solas”. Photograph by CJ du Toit)

Abstract:

Ibises possess a bill-tip organ enabling them to use “remote-touch” to locate moving buried prey in the absence of all other sensory cues, by detecting mechanical vibrations (‘vibratory cues’) in their foraging substrates. Bill-tip organ morphology varies interspecifically in ibises, correlated with water content of the substrates in which the different species forage. It has been hypothesized that these interspecific differences in bill-tip organ morphology represent adaptations to substrate-specific differences in the detectability of vibratory cues, as wetter substrates transmit vibrations more readily. One prediction of this hypothesis is that prey should be easier to detect in wetter substrates by any remote-touch forager. Here, I test this prediction by examining whether changes in substrate moisture content affected the ability of Hadeda Ibises (*Bostrychia hagedash)* to locate prey using remote-touch. Hadeda Ibises possess a bill-tip organ consistent with the use of remote-touch, but their ability to use this sense has not been previously confirmed. Using a series of sensory assays (in which I manipulated the availability of different sensory cues available to foraging birds under controlled conditions) on a group of captive Hadeda Ibises, I tested: 1) whether the birds can use remote-touch to detect prey in the absence of all other sensory cues; and 2) whether changes in the water content of the foraging substrate affected their success rate using remote-touch. Hadeda Ibises were more successful at locating buried prey when they could use vibratory cues (i.e., when able to use remote-touch) than when only relying only on direct contact with the beak. The availability of auditory or olfactory cues had no effect on their prey capture rates. The rate at which they were able to locate prey using remote-touch was positively correlated with increasing water content

of their foraging substrates; but when only using direct-contact probing (i.e., no vibratory cues available) substrate water content had no effect on their success rate. Thus, I conclude that remote-touch foraging success is positively affected by increasing substrate water content, and this is not simply an artefact of greater ease of probing in wetter substrates. Therefore, foraging substrate moisture content may have influenced the evolution of interspecific variation in remote-touch bill-tip organ morphology. Furthermore, as the recent range expansion of Hadeda Ibises in southern Africa has been linked to increased agricultural and urban irrigation, I suggest that this is in part due to the birds' improved ability to detect prey in wetter substrates. This highlights the importance of considering the sensory ecology of birds when trying to understand their distributions and habitat requirements, which are vital to conservation efforts.

4.1: Introduction

Birds and other animals use various sensory modalities such as sight, hearing and smell to navigate the world around them. One of the most important uses of these senses is in locating food. Prey abundance and accessibility are often believed to be the strongest drivers of birds' foraging success (Holmes & Schultz, 1988; Olsson *et al.*, 2001; Darby, Fujisaki & Mellow, 2012). However, the sensory cues available in an environment can also affect their ability to locate and capture prey (Ontiveros, Pleguezuelos & Caro, 2005; Pierce & Gawlik, 2010), and birds have frequently been shown to alter their foraging behaviours in response to changes in their sensory environments: e.g., visual hunting birds change their foraging behaviour depending on light levels (Cannell & Cullen, 1998; White *et al.*, 2008; Regular, Hedd & Montevecchi, 2011) or visual obstructions (Ontiveros, Pleguezuelos & Caro, 2005; Tomé *et al.*, 2011). Some wading birds shift from visual to tactile foraging methods when water turbidity increases (Cullen, 1994), while others shift from visual hunting during the day to tactile foraging at night (Mouritsen, 1994; McNeil *et al.*, 1999). Birds which forage using non-visual cues, such as olfaction or hearing, suffer reduced success at finding prey when those sensory cues are suppressed (Montgomerie & Weatherhead, 1997; Cunningham, Castro & Potter, 2009).

Probe-foraging birds face the challenge of locating "invisible" prey submerged in opaque substrates. Some probe-foraging birds possess the sensory capability of remote-touch, which enables them to locate prey at a distance from their beaks by detecting high frequency acceleration components of mechanical vibrations in the substrate (Gottschaldt, 1985; Gerritsen & Meiboom, 1985; Zweers & Gerritsen, 1997; Piersma *et* *al.*, 1998). These vibrations result either from the movement of prey through the substrate, or as changes in pressure gradients caused by the birds' repeated probing around hard-bodied sessile prey items (the pressure gradients created by the probing action are distorted by the hard-bodied prey items, e.g., bivalves) (Gerritsen & Meiboom, 1985; Piersma *et al.*, 1998). The specialised bill-tip organ associated with remote-touch is made up of high numbers of mechanoreceptors packed in dense clusters of foramina in the distal portions of the bone of the beak (see Chapters 2 and 5). This bill-tip organ is found in three families of modern probe-foraging birds: ibises (Threskiornithidae) (Cunningham, Alley *et al.*, 2010), kiwi (Apterygidae) (Cunningham, Castro & Alley, 2007) and scolopacid shorebirds (Scolopacidae) (Bolze, 1968; Nebel, Jackson & Elner, 2005). Remote-touch foraging birds also show hypertrophy of brain regions associated with processing of tactile information from the beak (Cunningham, Corfield, *et al.*, 2013).

Remote-touch capability has been demonstrated using behavioural sensory assays in captive birds from all three families possessing the associated bill-tip organ (Gerritsen & Meiboom, 1985; Piersma *et al.*, 1998; Cunningham, Castro & Potter, 2009; Cunningham, Castro *et al.*, 2010). However, there is very little information on how the remote-touch sense is affected by changes in the birds' foraging environment. A single study shows that vegetation cover negatively affects the remote-touch capability of shorebirds, as the root systems of the plants obstruct the pressure fields of buried prey (de Fouw *et al.*, 2016). My data on the foraging behaviour of wild ibises in Chapter 3 (section 3.4.3) indicated that several environmental factors affect their tactile foraging success, including substrate moisture content. However, it was not possible to distinguish whether the birds were foraging using remote-touch or direct contact tactile methods, or to conclusively pinpoint which factors were responsible for variation in foraging success.

Cunningham, Alley *et al.* (2010) observed that interspecific differences in the morphology of the remote-touch bill-tip organs in ibises are correlated with foraging substrate moisture content. Additionally, my data in Chapters 2 & 3 (sections 2.4.3 and 3.3.1) show that ibis species which tend to forage in drier substrates have higher densities of mechanoreceptors in their bill-tip organs, indicative of more sensitive remote-touch capability (Zweers & Gerritsen, 1997). This is consistent with the fact that mechanical vibrations travel less readily in drier substrates than in more saturated ones (Biot, 1956 & 1962), which may necessitate more sensitive bill-tip organs in species foraging in drier substrates. To investigate whether foraging substrate moisture content is an environmental driver of interspecific differences in remote-touch bill-tip organ morphology, it is necessary to establish whether the birds' ability to detect vibratory cues while probing is affected by substrate moisture content.

Hadeda Ibises (*Bostrychia hagedash*) are common probe-foraging birds native to sub-Saharan Africa. Unlike many other ibis species, they spend the majority of their time foraging away from water bodies in more terrestrial habitats (Skead, 1951; Hockey, Dean & Ryan, 2005). Historically, their range was mainly restricted to forested areas, but in the past century they have experienced a significant expansion across southern Africa, now inhabiting various urban and agricultural landscapes (Macdonald, Richardson & Powrie, 1986). This expansion has been linked to increased soil irrigation through human activities (in gardens and agricultural lands) (Macdonald, Richardson &

Powrie, 1986; Duckworth, Altwegg & Harebottle, 2012; Duckworth & Altwegg, 2014). Duckworth, Altwegg & Guo (2010) showed that the foraging success rates of wild Hadeda Ibises increased in wetter soils when they were hunting earthworms. They attributed this to increased prey availability and hypothesized that this increase in foraging efficiency was a key driver of the range expansion of the species. They made no reference to the birds' sensory capacity to detect their prey under different conditions. The data from Chapter 3 (section 3.4.3) showed mixed results regarding the tactile foraging success of wild Hadeda Ibises in relation to soil moisture, but there were various other environmental factors that were likely affecting their foraging success (e.g., prey type and size). Thus, experimental investigation under controlled conditions is warranted.

In this chapter, my aims were: (1) to confirm that Hadeda Ibises are able to use remote-touch to locate buried prey in the absence of all other sensory cues. Based on their bill-tip organ morphology (see Chapter 2, section 2.4.1) and behaviour in the wild (see Chapter 3, section 3.3.1), I hypothesize that they can use vibratory cues to locate prey more successfully compared to direct touch alone; and (2) to test the effect of soil moisture content on their ability to detect prey using remote-touch. Following the observations on the foraging success of wild ibises in the previous chapter, and the fact that wetter substrates transmit vibrations more readily (Biot, 1956 & 1962), I predict that soil moisture content will have a positive effect on their remote-touch foraging success.

4.2: Methods and Materials

4.2.1: Study species, facilities and general set-up

Experiments were run in a free-flight aviary at the World of Birds sanctuary (Hout Bay, Western Cape, South Africa). The experiments commenced in February 2020, but were halted at the end of March 2020 due to COVID-19 restrictions. They recommenced in November 2020 and were concluded in December 2020. Nine Hadeda Ibises were held in the aviary with other species of birds (both exotic and indigenous). Two had broken or damaged beaks and were therefore unsuitable for the study; two were continuously involved in maintaining a nest in both Feb/Mar and Nov/Dec and did not come down to the experimental trays; and one individual was very wary of humans, and would not take part. I thus had four birds participate in the study. The four individuals were easily distinguishable in appearance: “A” had injuries on its feet, such that its nails were either missing or growing upside down; “B” was significantly larger than any of the other three individuals and had no visible defects; “C” was the smallest and had a wing injury; and “D” had a missing toe, and only joined the experiments in November. The sexes of the birds are unknown, though all were adults.

The training and experimental trials were carried out in the aviaries the birds were usually housed in. Trials were run at 8 am, an hour before the park opened to the public and before the birds had been fed, to ensure they were motivated to forage. During the trials, foraging trays were placed in a small open enclosure in the aviary, which the birds walked in and out of voluntarily, allowing me to restrict access to the trays to one bird at a time. During the experiments, each bird performed 1-4 trials each day.

The birds were habituated to me and the trial set up for over a month prior to the commencement of data collection. The birds showed no fear of the foraging trays and probed readily in them almost immediately when I placed them next to their usual plates of food during the initial training period. They consumed both live and dead superworms (*Zophobas morio*) during the training period, showing no marked preference for either. The birds were initially coaxed into the enclosure during training using dead superworms thrown on the ground. After a week of using the enclosure, they no longer required “coaxing” to enter it. The birds were presented with at least 2-4 mock trails of each experimental set up. When trials recommenced in November, I spent a week re-habituating the birds to the set-up.

Ambient noise in the aviary measured between 50-75dB. Hourly air temperature data was provided by the South African Weather Service from the nearest weather station (Kirstenbosch station; Climate Number: 00207804).

4.2.2: Experimental designs

Trial set-up: Each trial consisted of a bird being presented with a soil-filled plastic tray, containing five buried superworms (see section 4.2.3 for more detail). The birds’ prey capture rates (number of worms captured per minute foraging) were recorded under different experimental treatments (13 different treatments in total), which were part of two separate experimental designs (see below). All four birds participated in all treatments, and I attempted to run at least three trials per treatment for each bird. Across all treatments, I recorded 226 successful trials (average of 4 trials per treatment per bird; individual “D” had the fewest replicates of trials, as it only joined the study in

November). Visual cues regarding prey location were absent in all trials as the worms were buried under the soil and the surface of the soil was smoothed over after burying (any trial where worms moved to the surface and caused visual disturbances in the soil were discarded).

Part 1: Effects of different sensory cues on prey capture rate: The first experiment ("Part 1") tested the effects of different sensory cues on the birds' foraging success under constant soil moisture levels (water content of 14.6%, see below). Part 1 contained seven treatments, each designed to isolate various combinations of the following senses: olfaction, hearing and remote-touch, following similar methods to Cunningham, Castro & Potter (2009) & Cunningham, Castro *et al.* (2010) (see Table 4.1 for the combinations of cues used in each treatment). Vibratory "remote-touch" cues were supressed by providing dead superworms instead of live ones (worms were killed by freezing the night before and thawed to ambient temperature before experiments began). Chemical cues (and consequently taste/olfactory cues) were supressed by mixing powdered, freeze-dried worms into the soil [500 ml powdered worms in 5 l soil; shown by Cunningham, Castro & Potter (2009) to successfully supress olfactory prey-detection in kiwi]. Dried worms were obtained from *United Reptiles Pet Supplies* PTY (LTD) and were ground to a fine powder using a *NutriBullet* ® kitchen blender. Auditory cues were supressed when live worms were provided by playing broadband white noise, to reach an ambient background noise of 65 dB at the levels of the foraging trays (using a Bluetooth speaker and cell phone; sound level measured at tray level using a Precision Gold Sound Level Meter, model N05CC), to cover up the sound of the worms burrowing in the soil (around ~30 dB). This was shown to be effective in masking auditory cues of live worms in American Robins (Montgomerie & Weatherhead, 1997). To assess whether the white noise itself had an effect on the birds' prey capture success rate, I ran an additional treatment with dead worms where white noise was played (Treatment 7, see Table 4.1).

Table 4.1: Treatment details for PART 1: Sensory cues available to the birds in each experimental treatment. Visual cues were always excluded, and direct contact (i.e., chance detection) tactile cues with the beak were always available.

Treatment	Prey	Substrate	Background noise	Sensory cues excluded	Sensory cues available
1	live	plain	ambient	none	all
2	live	mealworm-mixed	ambient	chemical	auditory & vibratory
3	live	plain	white noise	auditory	olfactory & vibratory
4	live	mealworm-mixed	white noise	olfactory, auditory	vibratory
5	dead	plain	ambient	vibratory, auditory	olfactory
6	dead	mealworm-mixed	ambient	all	none
7*	dead	plain	white noise	vibratory, auditory	olfactory

*same cues available as Treatment 5, but with white noise added to test its effect on the birds' behaviour as an external stimulus

Part 2: Effect of soil moisture content on capture rate: In the second experiment ("Part 2"), I tested the effects of soil moisture content on the birds' ability to use vibratory cues to locate buried prey. I altered the soil moisture content by adding different amounts of water to the soil in the trays, resulting in four different treatments ["dry" soil (no water added), 250 ml, 500 ml and 750 ml water added (the maximum water level that wouldn't drown the worms; see below)] and tested how this affected the birds' rate of capture of worms.

The amount of water that could be added to the soil without drowning the superworms was determined by mixing 5 l of soil with different amounts of water (starting with no water added, then subsequently adding water in 250 ml increments). Superworms were buried in a glass beaker filled with each soil "treatment", and their activity assessed after 2, 5, 10 and 20 minutes in the soil (repeated using 5 worms for each time interval and water treatment). If 0-500 ml of water had been added to the soil, the worms remained active (moving around in the soil and burrowing away from the tweezers when I tried to extract them) even after 20 min in the soil. When 750 ml of water was added, some of the worms seemed to become sluggish in their movements after 10-20 min. If 1 l water was added, the worms seemed to enter a "torpor" stage after 2-5 min (not dead, as they would recover if removed from the soil at this point); but when left in the soil for 20 min, all five worms had died. Thus 750 ml was determined to be the maximum amount of water that could be added to 5 l of soil without killing the worms.

For each of the four water content treatments (see Table 4.2), birds were given trays with either live worms (to test the effects of soil moisture content on their remote-touch capabilities) or dead worms (to test whether effects of soil moisture content on foraging success could be attributed to ease of probing when the birds could not use remote-touch). Thus, there were eight treatments for this experiment (treatments of live/dead worms at 14.6% soil water content correspond to Treatments 1&5 from Part 1). I did not alter the olfactory and auditory cues in this set of experiments (after having confirmed that the birds were not using these senses to forage during part 1 of the experiments).

Table 4.2: Water contents of soil in the foraging trays (containing 5 l of soil) provided to the birds in PART 2 of the experiments.

Water added to soil (ml)	Total average water volume in 5L soil sample (ml)	Volumetric water content %
0	230*	4.6
250	480	9.6
500	730	14.6
750	980	19.6

*average measured from 10 samples of the “dry” soil (before any extra water added; see Appendix 4.1). All subsequent measures are base water volume (230 ml) + volume water added.

The soil used was organic topsoil, sourced from *Stanler Farms* (Cape Town, South Africa). The water content of the “dry” soil (no water added) was measured using ten 0.5 l samples (Appendix 4.1). These soil samples were weighed, dried for ~48 hr in a 70 ºC oven, and then reweighed to calculate the water mass of each sample (wet weight – dry weight of soil = average water mass of 23.1 ± 3.0 g). Water density is treated as 1 g/ml

(Bilskie, 2001), thus this equated to 23.1 ml of water in a 0.5 l soil sample on average. Therefore, in the 5 l of “dry” soil added to each foraging tray, there was approximately 230 ml water, which translates to a volumetric water content (θ_v) of 4.6% using the formula (Bilskie, 2001):

$$\theta_v = \frac{volume\ of\ water}{volume\ of\ soil\ sample}\ x\ 100\%$$

The water contents of the subsequent three soil treatments were calculated using a total water volume of 230 ml + volume water added (see Table 4.2).

4.2.3: Trial set up

Trials were presented to the birds in randomized order. Every morning, the soil was sieved through a 1 cm sieve and prepared according to the treatments being run. For Part 1: treatments 2, 4 and 6, 0.5 l of powdered worms were added to the soil (see Table 4.1) and the soil was mixed well. The required volume of water was added to the 5 l of soil (always 500 ml water for Part 1, and volume depending on treatment for Part 2 – see Table 4.2). The prepared soil was then placed into the plastic containers (20.5 x 31 x 13.2 cm).

Five superworms were buried ~10 cm deep in the soil at random positions in each tray, and the surface of the soil was smoothed over to remove any visual clues as to the location of the worms. Trays were covered with a plastic lid to ensure that birds could not start probing before the cameras started recording. Trials commenced as soon as the bird first probed its beak into the soil and were considered completed when either all five worms had been caught, or when the bird lost interest and left the enclosure.

4.2.4: Video analyses

All trials were recorded using digital video cameras (*Sony* HDR-XR60E). Data on the birds' prey capture rate (number of worms caught during the trial / time spent probing in the tray) were recorded from the video footage using the program *CyberTracker* (Ansell & Koenig, 2011), a cellphone app used for behavioural data collection with timestamps.

4.2.5: Statistical analyses

All data were compiled in *Microsoft Excel* (2013) and analysed in *RStudio* (RStudio Team, 2016). The behavioural data was paired with air temperature data from the Kirstenbosch weather station using the timestamps of the video recordings, as temperature was shown to have a negative effect on the foraging success of wild Hadeda Ibises in Chapter 3 (Table 3.3). All graph plotting was done using the *R* (R Core Team, 2018) package *ggplot2* (Wickham, 2011), and the figures were combined in *Adobe Photoshop CC* (2018).

Data were tested for normality using Shapiro-Wilks tests and by visually assessing density plots [generated using R package *ggpubr* (Kassambara, 2018)]. Untransformed prey capture success rates were not normally distributed. To assess differences in success rate between the four individual birds, I used non-parametric Kruskal-Wallace and Wilcoxon's rank-sum tests. The relationship between air temperature and success rate (the latter log-transformed to improve normality and enable use of parametric tests) was assessed using Pearson's correlation test.

Linear mixed-models [using *R* package *lme4* (Bates *et al.*, 2014)] were run on log-transformed worm capture success rate data to determine which factors were affecting the birds' foraging success. Bird identity was included as a random factor in all models (to account for differences between individual birds and to avoid pseudo-replication).

In order to assess whether the two "trial seasons" (Feb/Mar and Nov/Dec, see section 4.2.1) had an effect on the birds' success rates, I ran a model on all the trial data, including trial season, sensory cues available and soil water content as fixed effects.

For Part 1 of the experiments (soil moisture content 14.6% in all trials), the fixed effects included in the global model were the presence/absence of the different sensory cues available: vibratory, olfactory, and auditory cues. The effect of white noise played at the levels of the trays was also assessed, to test whether the stimulus itself was altering the birds' foraging success.

For Part 2 (auditory and chemical cues were unaltered), the fixed effects included in the global model were presence/absence of vibratory cues (worms dead/alive) and soil moisture content (four levels, see Table 4.2). This global model was run using the whole of Part 2's dataset. This allowed the assessment of the effect of substrate water content on the birds' foraging success rate.

Two separate models were then run looking only at the effect of substrate water content on the prey capture rate when vibratory cues were kept constant; i.e., using data from subsets of trials with live worms and dead worms respectively. This was done to assess whether the effect of substrate water content on the birds' foraging success rate altered in response to the presence/absence of vibratory cues.

An additional two separate models assessing the effect of vibratory cues on prey capture rate were also run on subsets of trials with dry soil (4.6% water content), and soils with water added (9.6-19.6% water content), respectively. This was done to assess whether the effect of vibratory cues on the birds' foraging success rate altered in response to substrate moisture content.

Statistical significance was assessed using Satterthwaite denominator degrees of freedom post-hoc tests [from *R* package *lmerTest* (Kuznetsova, Brockhoff & Christensen, 2017)]. Data are plotted as model predictions [fitted means and 95% confidence intervals; using *R* package *emmeans* (Lenth *et al.*, 2021)] against the raw data from the trials. Data are presented in the text as pairwise t-values$_{df}$ and p-values from the fitted models (all two-sided), unless stated otherwise.

4.2.6: Ethical Clearance

I obtained permission from the management at World of Birds to conduct my experiments at their facilities using birds already housed in their sanctuary. The study was carried out under ethical approval from the University of Cape Town's Science Faculty Animal Ethics Committee (clearance number 2019/V19/SC).

4.3: Results

4.3.1: General trends in prey capture rates:

There were individual differences in prey capture rate between the four birds included in the study (Figure 4.1A; Kruskal-Wallis χ^2_3= 16.326, P=0.001). Individual "B" had a higher average capture rate (Median: 5.0 captures/min) than the other birds (median captures/min: A=3.2; C=2.8; D=3.4) based on Wilcoxon rank-sum tests, whereas there were no significant differences in capture rate between individuals A, C & D (see Appendix 4.2).

The birds' capture rate did not correlate with air temperature (Figure 4.1B; r= -0.005, t_{218}= -0.069, P= 0.945, from a Pearson's correlation test of temperature vs. log transformed capture rate), and the birds showed no significant differences in their success rates between the two trial seasons (Figure 4.1C; $t_{117.903}$= -1.429, P= 0.156).

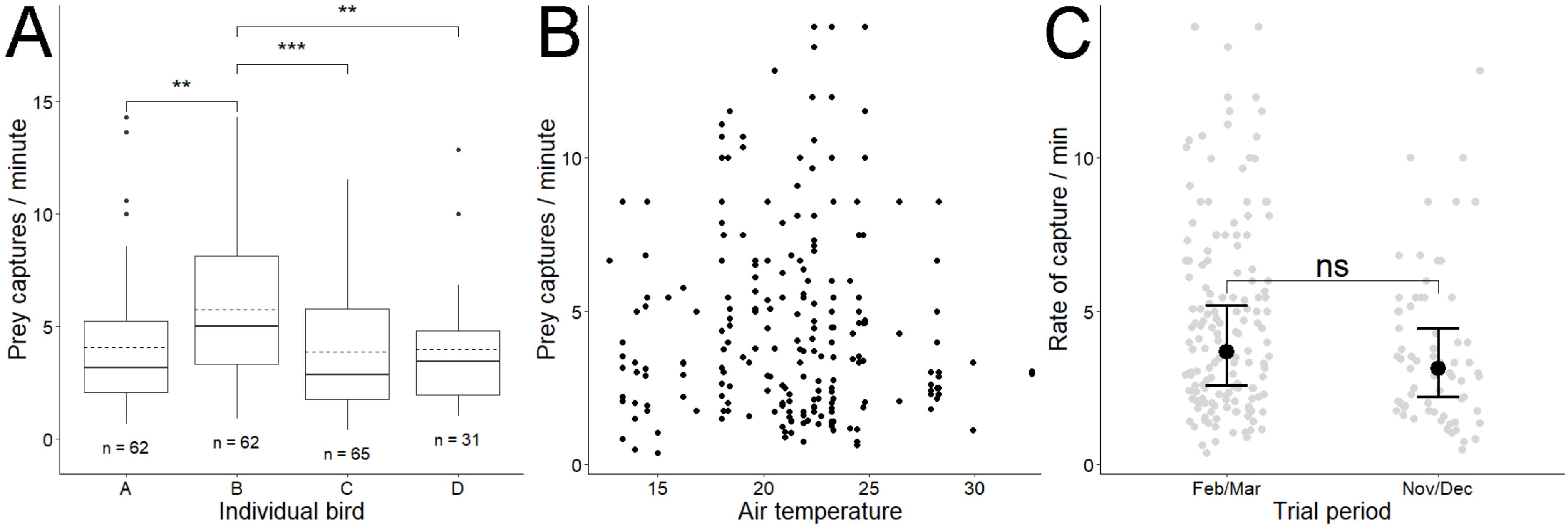

Figure 4.1: Prey capture rates of the Hadeda Ibises during the experiments. A) Prey capture rates of each individual bird; the boxplots indicate ranges and medians (dark horizontal lines) for each individual, with dashed horizontal lines indicating means; points represent outliers of each individual; "n" = number of trials. B) Relationship between air temperature and prey capture rate; each point represents an individual trial. C) Model predictions showing effect of time of year in which trials took place on the birds' success rates from a linear mixed model (model including sensory cues available and soil moisture content as additional fixed effects, and bird identity as a random factor); large black circles indicate model fitted means, with error bars showing 95% confidence intervals; Small grey circles indicate data from individual trials. Asterisks above brackets (A&C) indicate statistically significant differences between groups from (A) Wilcoxon rank-sum or (C) Satterthwaite denominator degrees of freedom tests. Significance codes: *** p<0.001; ** p<0.01; "ns" p>0.05 (i.e., no significant difference). Where brackets have been left out of (A) to avoid cluttering the figure, there are no significant differences between individuals.

4.3.2: Part 1: Effects of different sensory cues on prey capture rate

The ibises' prey capture rates were higher when the birds were presented with live worms (vibratory cues present) compared to when they were capturing dead worms (vibratory cues supressed; Figure 4.2A; $t_{119.018}$= 2.214; P= 0.029). There was no difference in prey capture rate in response to the availability of auditory cues (cues suppressed if worms were dead and/or by playing white noise; Figure 4.2B; $t_{119.364}$= 0.098; P= 0.922). Furthermore, there was no difference in prey capture rate when birds were probing in plain soil (olfactory/chemical cues present) versus soil that had powdered worms mixed in it (olfactory cues supressed; Figure 4.2C; $t_{119.437}$= -0.995; P= 0.322). White noise played at the level of the foraging trays had no effect on the birds' prey capture rate (Figure 4.2D; $t_{121.245}$= 1.065; P= 0.289).

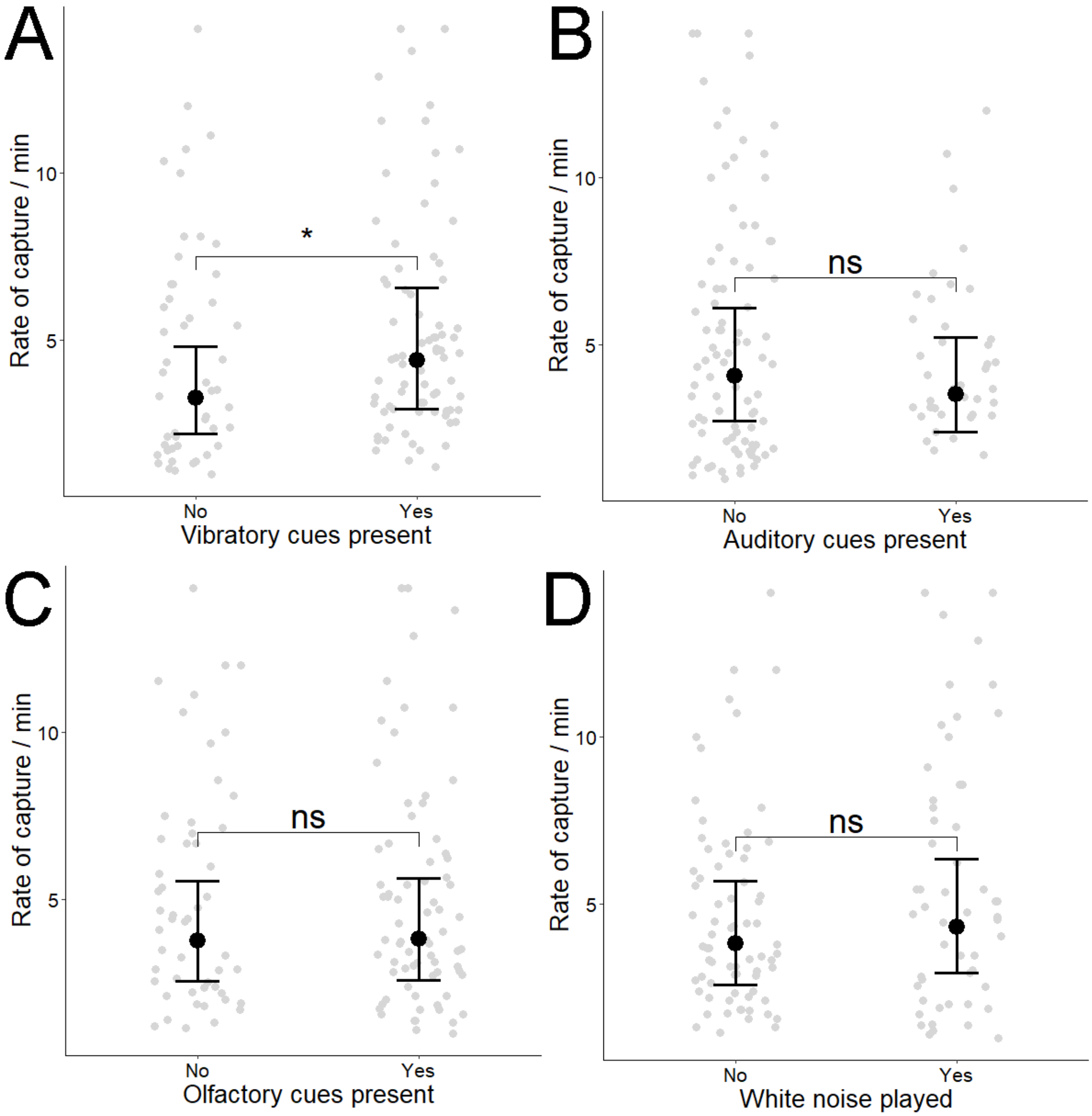

Figure 4.2: Model predictions showing effects of different sensory cues available on Hadeda Ibises' rate of prey capture during Part 1 of the experiments (testing remote-touch capability). A) Effect of vibratory cues (suppressed by providing dead worms); B) effect of auditory cues (suppressed by providing dead worms and/or playing white noise); C) effect of olfactory cues (suppressed by mixing crushed, dried worms in the soil); D) effect of playing white noise. Linear mixed models fitted on log transformed prey capture rates with bird identity as a random factor. Large black circles indicate model fitted means, with error bars showing 95% confidence intervals. Small grey circles indicate data from individual trials. For different treatments used, see Table 4.1. Asterisks above brackets indicate statistically significant differences between two groups from Satterthwaite denominator degrees of freedom tests: * $p<0.05$; "ns" $p>0.05$ (no significant difference between groups).

4.3.3: Part 2: Effect of soil moisture content on capture rate

Based on the global model fitted to the full Part 2 dataset and including vibratory cues (worms dead/alive) and soil moisture content (see Figure 4.3A), the birds had lower success rates in “dry” soil (no water added; average volumetric water content of 4.6%) compared to any of the treatments in which water was added to the soil [t(dry soil compared to 9.6% water content)$_{125.345}$= 3.154, P= 0.002; t(14.6%)$_{125.515}$= 3.544, P= 0.0006; t(19.6%)$_{124.486}$= 2.490, P= 0.014]. There were no differences in capture rates between soil treatments with over 9.6% water content (P-values > 0.05). There was no effect of vibratory cues on success rate when modelling the data from all the water content levels of Part 2 ($t_{124.587}$= -1.916, P=0.058).

Separate models of the effects of the availability of vibratory cues (worms dead/alive) in dry soil versus soil with water added showed differences in the birds’ reactions to vibratory cues (Figure 4.3D&E). If water had been added to the soil (i.e., soil with volumetric water content of 9.6-19.6%), birds had higher prey capture success rates when presented with live worms compared to dead worms (Figure 4.3D; $t_{88.540}$= -2.234, P= 0.028). When no water was added to the soil (“dry” soil, approximately 4.6% water content), there was no difference in the birds’ success rate when presented with dead versus live worms (Figure 4.3E; $t_{33.377}$= -0.446, P= 0.659).

When modelling the effect of soil water content on the birds’ success rate on a subset of the data including only trials were the birds were presented with live worms (i.e., vibratory cues available; Figure 4.3B), the birds had lower success rates when probing in “dry” soil than when probing in soil with water added [t(dry soil compared to 9.6%

water content)$_{70.274}$= 2.836, P= 0.006; t(14.6%)$_{71.737}$= 3.125, P= 0.002; t(19.6%)$_{70.475}$= 2.112, P= 0.038]; and no difference in capture rate between soil treatments with over 9.6% water content (P-values > 0.05).

However, when modelling the effects of soil water content on the birds’ success rate only for data from trials where the birds were presented with dead worms (vibratory cues supressed), the birds showed no significant differences in success rate between “dry” soil or any other soil water content [Figure 4.3C; t(9.6%)$_{50.385}$= 1.570, P= 0.12; t(14.6%)$_{49.38}$1= 1.777, P= 0.08; t(19.6%)$_{49.696}$= 1.396, P= 0.17].

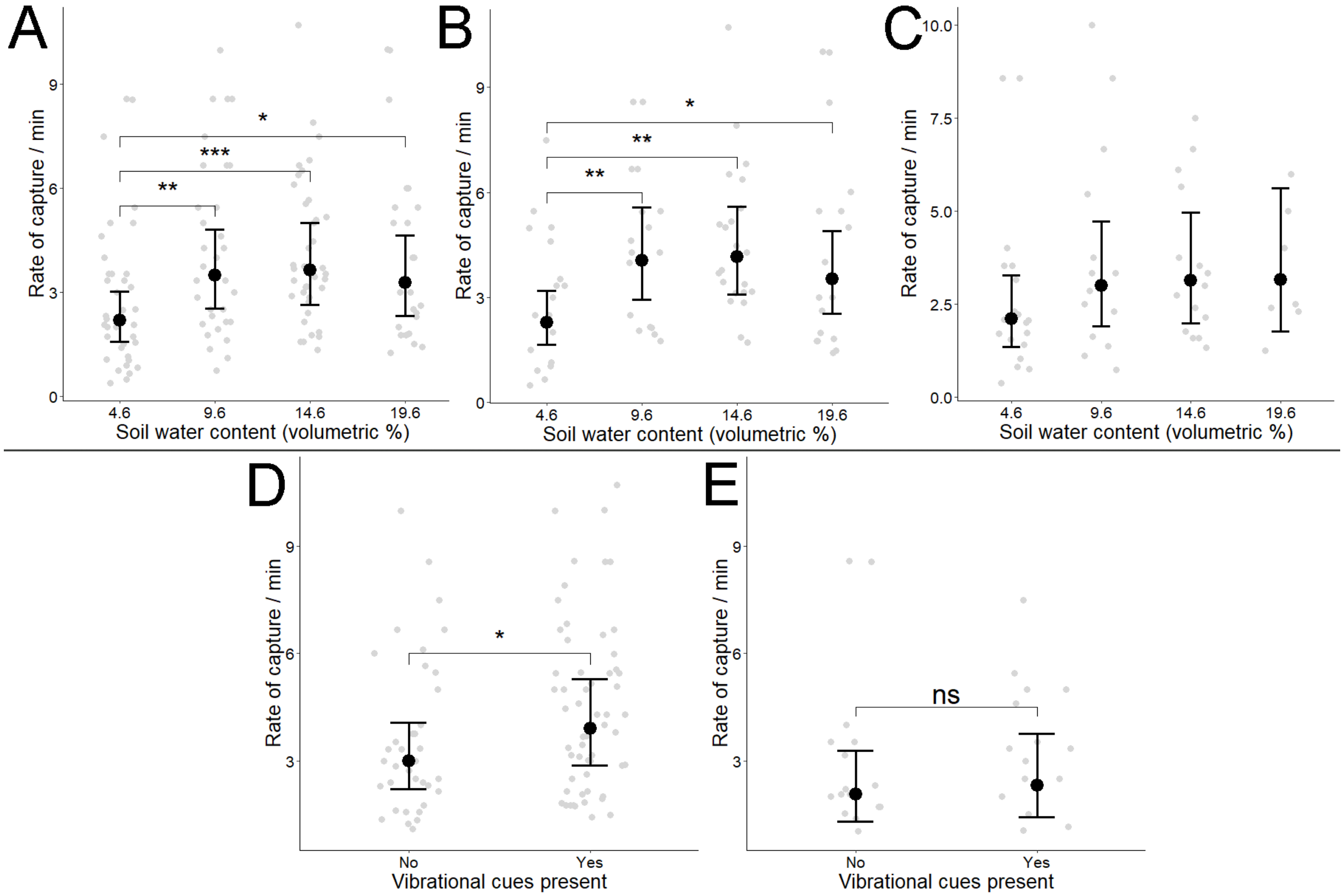

Figure 4.3: Model predictions showing effects of substrate water content (A-C) and vibratory cues available (D-E) on Hadeda Ibises' rate of prey capture during Part 2 of the experiments (testing effects of soil water content on the birds' remote-touch capability). Models run on different subsets of trials: A) all trials; B) trials with worms alive (vibratory cues available); C) trials with dead worms (no vibratory cues); D) trials using "wet" soil (≥250ml water added to 5l soil in tray); E) trials using "dry" soil (no water added to soil trays). Linear mixed models fitted on log transformed success rates, with bird identity as a random factor. Large black circles indicate model fitted means, with error bars showing 95% confidence intervals. Small grey circles indicate data from individual trials. Asterisks above brackets indicate statistically significant differences between two groups using Satterthwaite denominator degrees of freedom tests: *** p<0.001; ** p<0.01; * p<0.05; "ns" p>0.05 (i.e., no significant difference; where brackets have been left out to avoid cluttering the figures, there are no significant differences between the groups).

4.4: Discussion

The experiments indicated that Hadeda Ibises are able to use remote-touch in the absence of all other sensory cues to locate buried prey more successfully than via random direct contact while probing. Furthermore, the data indicate that the birds' success rate of remote-touch probe-foraging is positively affected by substrate moisture content, and that this is not simply an artefact of ease of probing.

4.4.1: Remote-touch capability of Hadeda Ibises

My results from Part 1 of the experiments indicate that Hadeda Ibises are able to use remote-touch in the absence of all other sensory cues (visual, auditory and chemical) to locate soft-bodied moving invertebrate prey buried under the ground. The birds had significantly higher prey capture rates when the worms were alive [and thus moving and creating mechanical vibrations in the soil (Gerritsen & Meiboom, 1985)] than when they were dead (located only by chance direct contact with the beak). This is unlikely to be an artefact of the birds preferring live worms over dead, for three reasons: 1) the dead worms had been killed less than 24 hr before, and thawed out to room temperature before the experiments started, giving them no time to start decomposing; 2) during training, the birds were presented with both live and dead worms as a reward for coming into the foraging enclosure, and showed no marked preference for either; and 3) from Part 2 of the experiments, when the soil was "dry" (and the birds appeared unable to use remote-touch, see section 4.4.2 below) the birds showed no difference in capture rate between live or dead worms.

The birds were not using auditory cues to locate worms, as when auditory cues of live worms were supressed (by playing white noise at the levels of the trays), the birds showed no difference in their prey capture rates. White noise itself as an added stimulus also had no effect on the birds' prey capture rates. Furthermore, the birds were not using chemical (olfactory/taste) cues to locate the worms, as suppressing these (by mixing powdered worms into the soil) had no effect on the birds' capture rates. Thus, during these experiments, the Hadeda Ibises appeared to be only using remote-touch (for live worms) and chance direct contact to locate their prey.

These results are similar to those of Cunningham, Castro *et al.* (2010) on Madagascar Crested Ibises, providing evidence that remote-touch is used by multiple species of ibis. Based on data from my previous chapters on both their behaviour and beak morphology, it is likely all three common southern African ibis species (African Sacred and Glossy Ibises alongside Hadeda Ibises) are able to use remote-touch: both the other species had equivalent or even higher tactile foraging success compared to Hadeda Ibises in the wild (Chapter 3, section 3.4.2), and all three species possess the beak bone morphology and soft tissue histology associated with remote-touch (Chapter 2, section 2.4.1). Examination of the beak bones of multiple other taxa of ibises and spoonbills from all over the world [see Chapter 5, Figure 5.7B and previous research (Swennen & Yu, 2004; Cunningham, Alley *et al.*, 2010)], indicates that the bill-tip organ responsible for remote-touch is common to all extant ibis species sampled.

Based on my data on wild Hadeda Ibises' foraging behaviour in Chapter 3 (section 3.3.1), the birds also use visual methods of foraging. However, most of their foraging time in the wild (>75%, see Figure 3.3B) was made up of probing in sand or soil [this is supported by previous observational studies (Skead, 1951)]. While they likely use visual “clues” to deduce the presence and location of buried prey [e.g., burrows or track marks on the surface of the soil, as other probe-foraging birds have been shown to do (Gerritsen & Meiboom, 1985)], when such clues are unavailable, being able to use remote-touch to locate buried prey would be advantageous. The alternative is locating buried prey only when it comes into direct contact with the beak, which relies on high prey density to be efficient (Hulscher, 1982). Remote-touch is less reliant on high prey densities for efficient prey location, and may even be useful to birds in determining other information about their prey [e.g., prey size and/or density of prey items in a particular patch (Gerritsen & Meiboom, 1985)], which may aid birds in deciding where to forage.

4.4.2: Effect of substrate moisture content on remote-touch foraging

My results indicate that the prey capture rates of Hadeda Ibises are affected by the water content of their foraging substrates. The Hadeda Ibises’ rate of locating live worms (i.e., when vibratory cues were present and the birds were able to use remote-touch, see section 4.4.1 above) was positively affected by adding water to the soil in the foraging trays presented to them. This is unlikely an artefact of ease of probing in wetter soils, as changing the water content of the soil had no effect on their capture rate when the birds were presented with dead worms. As mechanical vibrations are better transmitted by more saturated substrates (Biot, 1956 & 1962), I hypothesize that the increased success rate of the birds in the wetter soils (9.6-19.6% water content, compared to “dry” soils of 4.6% water content) is largely because their prey are easier to detect in these substrates. This is further supported by my results, as in very dry soils (approximate volumetric water content of 4.6%) the birds showed no difference in success rate when capturing live worms compared to dead ones. This suggests that when there is not enough water in the soil, even if prey is present and moving (creating mechanical disturbances in the soil), the transmission of the vibrations in the soil is too poor for the birds to be able to detect them, and thus they had to rely on random direct contact alone.

If the birds' increased success rate between dry and wet soils was an artefact of worm activity, we would expect the worms to be more active in wet soils than in dry soils (and thus producing more vibrations). This was not the case during my initial examination of the worms' activity under different soil moisture levels (see section 4.2.2), as there was no difference in the worms' behaviour between dry soil (4.6% water content) and soils with water content of 9.6 or 14.6%. Some of the worms buried in soils with 19.6% water content actually appeared more sluggish after 10-20 mins. This did not seem to affect the birds' success rate, as their success in very wet soils (19.6% water content) was not significantly different from soils with 9.6 and 14.6% saturation, and they still had higher rates than in the dry soil. This indicates the worms were producing detectable vibrations in the very wet soil (19.6% water content), and if they were lower in threshold as an artefact of decreased worm activity, the positive effect of the high water content on transmission of vibrations may have made up for it.

The fact that there was no significant difference in the birds' success rates between soils with 9.6% and 19.6% water content could indicate that there is a threshold level of water content below which Hadeda Ibises cannot detect the vibrations, and that any water content above the threshold (falling somewhere between 4.6-9.6%) does not affect their success rate. If this is the case, it could be because Hadeda Ibises have such high densities of mechanoreceptors in their bill-tip organs (see Chapter 2, section 2.4.2), indicative of high remote-touch sensitivity (Zweers & Gerritsen, 1997). Alternatively, the difference in water content (between 9.6-19.6% soils) was not great enough to show a significant effect on success rate. As higher water contents would kill prey items such as superworms, a different experimental set-up would be required to test this.

It could be valuable to run similar experiments using a study species such as Red Knots, which can detect hard-bodied static prey using remote-touch (Piersma *et al.*, 1998), to potentially rule out changes in prey behaviour in response to substrate moisture content (and allow for measurements over a greater range of water content levels). The study by Piersma *et al.* (1998) showed that the knots were unable to detect prey using remote-touch when the substrate was dry, but as they only measured dry vs wet sand (no spectrum of water contents), it is not possible to comment on whether in that case it was a threshold effect, or if their success would improve further with increasing water content.

Some interspecific differences in bill-tip organ morphology of remote-touch foraging birds have been hypothesized to be linked to differences in their foraging substrate (Cunningham, Alley *et al.*, 2010). Species which forage in more aquatic habitats tend to have lower densities of mechanoreceptors in their bill-tip organs (see Chapter 2, section 2.4.3) which is indicative of less sensitive bill-tip organs (Zweers & Gerritsen, 1997). I hypothesized that as these species tend to forage in wetter substrates (see Chapter 3, section 3.3.1), they may not need such sensitive organs as vibrations are transmitted more easily in wetter soils (Biot, 1956 & 1962). The data in this chapter provide the first behavioural evidence under controlled conditions to support this hypothesis, as water content did appear to positively affect the ibises' remote-touch capability, suggesting that it could have been a driver of the evolution of the remote-touch bill-tip organ.

Going forward, it would be useful to compare the remote-touch capability of other ibis species with different bill-tip organ morphologies under controlled soil water saturation levels. If the interspecific differences in the mechanoreceptor densities in their bill-tip organs correspond to different sensitivity levels, we would expect ibis species which forage in wetter substrates than Hadeda Ibises (and which have lower densities of mechanoreceptors; e.g., Glossy Ibises) to have a higher threshold of water contents above which they can successfully use remote-touch to locate prey. The effects of increasing water content above this threshold on their success rate may also be stronger in species with lower densities of mechanoreceptors, as the very high densities in the bill-tip organs of Hadeda Ibises may make up for the differences in signal transmission in substrates with different water contents. Using smaller, harder to locate prey items in future studies may also make any effects clearer.

4.4.3: Importance of considering sensory requirements in habitat usage

Over the past century, Hadeda Ibises have experienced a significant range expansion in southern Africa, and studies have shown that this coincided with increased soil moisture due to artificial irrigation in urban areas (Macdonald, Richardson & Powrie, 1986; Duckworth, Altwegg & Guo, 2010; Duckworth, Altwegg & Harebottle, 2012; Duckworth & Altwegg, 2014). Duckworth, Altwegg & Guo (2010) showed that wild Hadeda Ibises' prey capture rate increased with increasing soil moisture. They hypothesized that this increase in capture rate was due to two factors: 1) density of prey items and prey moving closer to the surface in increased soil moisture contents; and 2) ease of accessing prey in more saturated soils. My experiments controlled for the first factor (as prey density was constant, and none of the water treatments used caused the worms to move to the surface of the soil), though it is likely that in the wild this will cause capture rates to increase. But as the captive birds still experienced increased capture rates with increased water content when prey density was kept constant, clearly it is not the only factor affecting their success rate. As the birds experienced no increase in capture rate of dead worms with increasing soil water content, “ease” of probing in wetter substrates does not appear to affect their capture rates. The increase in capture rates of live worms in higher soil water contents suggests that it is easier for the ibises to detect prey in more saturated substrates using remote-touch, and this, along with the effect that artificial human irrigation of soil has had on invertebrate densities (Staley *et al.*, 2007), might explain the pattern observed by Duckworth, Altwegg & Guo (2010). Therefore, it is important to consider substrate moisture content along with the abundance of prey when assessing the suitability of environments for remote-touch foraging birds such as ibises.

The effect that availability of sensory information can have on birds’ foraging efficiency has been shown by multiple studies looking at different sensory systems. Most focus has been on vision (Cannell & Cullen, 1998; Ontiveros, Pleguezuelos & Caro, 2005; White *et al.*, 2008; Regular, Hedd & Montevecchi, 2011; Tomé *et al.*, 2011), though effects of olfactory and auditory cues have been tested under experimental conditions as well (Montgomerie & Weatherhead, 1997; Cunningham, Castro & Potter, 2009).

There is very little information about the sensory requirements of remote-touch foraging birds, with only one published study showing how vegetation cover negatively affects the ability of birds to use remote-touch to locate buried prey (de Fouw *et al.*, 2016). My results indicate that substrate moisture is also an important factor affecting remote-touch capability. Though remote-touch is a relatively "rare" sensory system in birds, its importance as a prey location mechanism (as it is not known to be used for any other function aside from foraging) for the birds which use it cannot be disregarded when considering the habitat requirements and foraging ecology of these taxa (namely ibises, kiwi and scolopacid shorebirds). Furthermore, many birds from these taxa are endangered or even critically endangered, [e.g., Spoon-Billed Sandpipers (*Calidris pygmaea*) & Giant Ibises (*Thaumatibis gigantea*) (BirdLife International, 2018)], thus knowledge about their habitat requirements is crucial for effective conservation efforts.

4.5: Conclusions

Aside from confirming remote-touch in another species of probe-foraging birds which possesses the bill-tip organ associated with this sensory capability, my results show that there does appear to be a link between substrate water content and the birds' ability to locate prey using remote-touch. Foraging substrate water content has been demonstrated to be an important factor to consider when looking at remote-touch foraging birds' habitat requirements (along with prey availability and other environmental factors) as it is likely to have a strong effect on how successful the birds are are at locating prey. In the case of Hadeda Ibises, it may in part be why their range expansion across southern Africa has been closely tied to the increased irrigation of soils in urban and agricultural areas (Duckworth, Altwegg & Guo, 2010). Furthermore, these results provide support for the hypothesis that interspecific differences in the bill-tip organs of ibises and other remote-touch foraging birds are at least in part driven by the moisture contents of the substrates in which the birds forage (Cunningham, Alley *et al.*, 2010), as the sensory capability of remote-touch appears to be affected by water content. The results of this investigation emphasise the importance of including data on the remote-touch sensory requirements of probe-foraging birds in studies of their habitat selection, and highlights a potential gap in previous studies assessing their foraging distributions.

4.6: References

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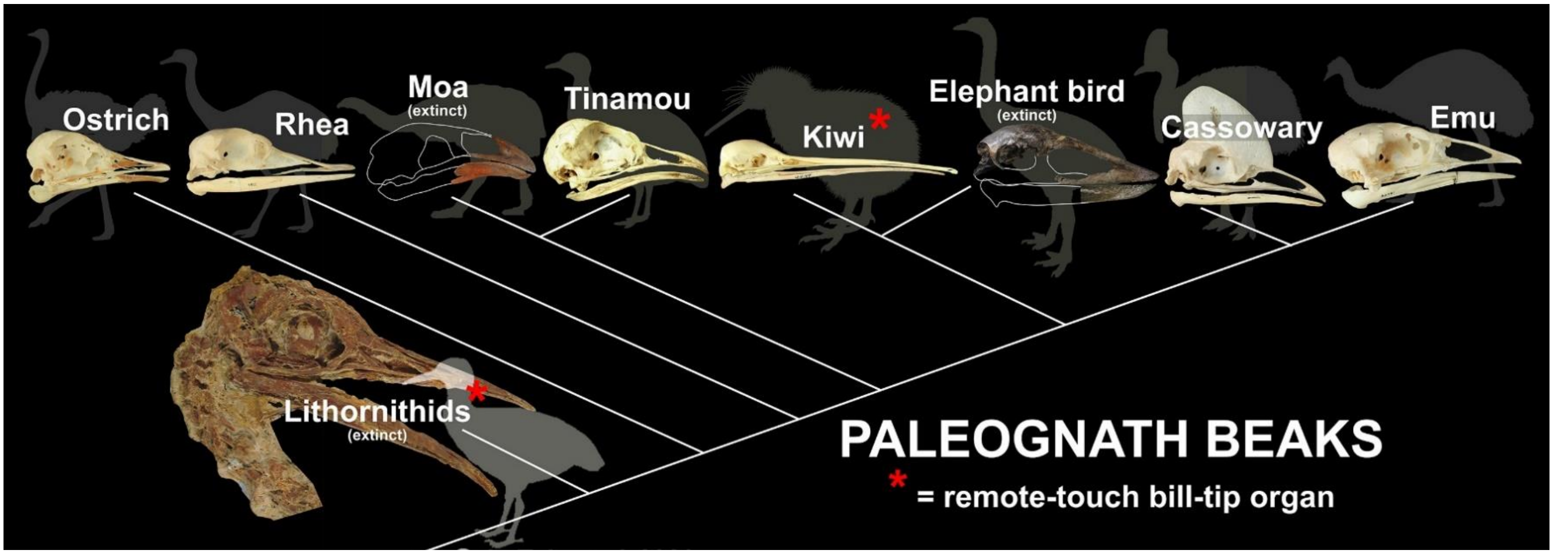
Chapter 5:

Cretaceous origins of the remote-touch bill-tip organ in birds



Graphical abstract for Chapter 5, showing the presence of remote-touch bill-tip organs in the palaeognathous birds and the interrelationships of the members of the clade [based on phylogenies by Mitchell *et al.* (2014) & Yonezawa *et al.* (2017)]. Photographs and image by CJ du Toit.

Abstract:

Some probe-foraging birds locate their buried prey by detecting mechanical vibrations in the substrate using a specialised tactile bill-tip organ comprising mechanoreceptors embedded in densely clustered pits in the bone at the tip of their beak. This remarkable sensory modality is known as “remote-touch”, and the associated bill-tip organ is found in probe-foraging taxa belonging to both the palaeognathous (in kiwi) and neognathous (in ibises and shorebirds) clades of modern birds. Intriguingly, a structurally similar bill-tip organ is also present in the beaks of extant, non-probing palaeognathous birds (e.g., emu and ostriches) that do not use remote-touch. By comparison with my comprehensive sample representing all orders of extant modern birds (Neornithes), I provide evidence that the lithornithids (the oldest known palaeognathous birds which evolved in the Cretaceous period) had the ability to use remote-touch. This finding suggests that the occurrence of the vestigial bony bill-tip organ in all modern non-probing palaeognathous birds represents a plesiomorphic condition. Furthermore, my results show that remote-touch probe-foraging evolved very early among the Neornithes and it may even have predated the palaeognathous-neognathous divergence. I postulate that the tactile bony bill-tip organ in Neornithes may have originated from other snout tactile specializations of their non-avian theropod ancestors.

5.1: Introduction

Probe-foraging birds need to detect prey concealed within opaque substrates. Many of them locate their prey by direct contact with the beak in combination with other sensory cues (Hulscher, 1982), but some possess a unique sensory modality known as “remote-touch”, allowing them to detect buried prey at a distance from the beak through perception of high frequency acceleration components of mechanical vibrations in the substrate (Gottschaldt, 1985). These cues result from either the movement of the prey or changes in pressure gradients in the substrate caused by the birds’ repeated probing action reflected from the surface of hard-shelled prey items (Gottschaldt, 1985; Piersma *et al.*, 1998). Remote-touch probing appears to have evolved independently in three families that are phylogenetically widely separate: ibises (Threskiornithidae), shorebirds (Scolopacidae) and kiwi (Apterygidae) (Gerritsen & Meiboom, 1985; Piersma *et al.*, 1998; Nebel, Jackson & Elner, 2005; Cunningham, Castro & Alley, 2007; Cunningham, Castro *et al.*, 2010).

Three categories of tactile sensory specialisation of bird beaks can be inferred from the published literature: **1:** The remote-touch bony bill-tip organ, used for remote-touch probe-foraging; comprising groups of mechanoreceptors, known as Herbst corpuscles, embedded within densely clustered pits in the bone at the tip of the beak (Bolze, 1968; Gottschaldt, 1985; Nebel, Jackson & Elner, 2005; Cunningham, Alley *et al.*, 2010; Cunningham, Corfield, *et al.*, 2013); **2:** The Anseriform (ducks and geese) bony bill-tip organ, associated with foraging by dabbling (Gottschaldt & Lausmann, 1974; Gottschaldt, 1974; Avilova, Fedorenko & Lebedeva, 2018; Schneider *et al.*, 2019) and also comprising Herbst corpuscles embedded in pits in the bone, but these are organised alongside other mechanoreceptors into cornified structures called touch papillae, which extend through the bone into the rhamphotheca (Gottschaldt & Lausmann, 1974; Gottschaldt, 1974; Berkhoudt, 1979; Soliman & Madkour, 2017; Avilova, Fedorenko & Lebedeva, 2018); **3:** The keratinaceous bill-tip organ of Psittaciformes (parrots), involved in the manipulation of food objects (Demery, Chappell & Martin, 2011), comprising sensory units that are restricted to the keratinized rhamphotheca and not embedded in the bone (Goujon, 1869; Demery, Chappell & Martin, 2011). All other sampled neognathous birds (neognaths) show no tactile specialisation of the bill-tips, though most do possess a number of pits on the surface of the bones of their beaks (Bolze, 1968; Wight, Siller & Mackenzie, 1970; Halata & Grim, 1993; Genbrugge *et al.*, 2012; Van Hemert *et al.*, 2012; Sayed *et al.*, 2014; Soliman & Madkour, 2017).

The palaeognathous birds (palaeognaths) present an enigmatic case with respect to tactile specialisation of the beak. Only the probe-foraging kiwi are known to use remote-touch (Cunningham, Castro & Alley, 2007), yet the soft tissue histology and bill-tip microstructure of the extant herbivorous palaeognaths (e.g., emu and ostrich) indicate the presence of a bony bill-tip organ, similar to that possessed by remote-touch probing birds (Crole & Soley, 2014; Crole & Soley, 2017). This is at odds with the fact that none of the non-kiwi palaeognaths use probe-foraging as a feeding strategy (Tomlinson, 2000; Gussekloo & Bout, 2005), nor do they show enlargement of the brain centres associated with the processing of tactile information from the beak, as seen in specialist tactile-foraging birds (Cunningham, Corfield, *et al.*, 2013). Furthermore, it is unlikely they are using their bill-tip organs for any other sensory function, as no other types of receptors (apart from tactile mechanoreceptors) have been found in their beaks, and studies from other taxa of birds have shown that tactile specialisations in the beak tend to preclude other sensory information from the region (Lallemend & Ernfors, 2012; Schneider, *et al.,* 2019*)*. One possible explanation for this puzzling situation is that the bony bill-tip organ exists as a plesiomorphic trait in this clade, suggesting that a functional bill-tip organ may have been present in their last common ancestor. Furthermore, if the remote-touch bill-tip organ is associated with dense pits in the bone at the tip of the beak, it has the potential to be preserved as an osteological correlate in fossilized specimens (Hieronymus *et al.*, 2009), provided these sensory pits can be reliably differentiated from other types of pits commonly present on birds' beaks. Indeed, an increase in the density of pitting at the tip of the beak might generally indicate the presence of soft tissue structures associated with tactile bony bill-tip organs (Cunningham, 2010), but to date this correlation has not been confirmed by histological examination across a representative sample of modern birds, or with reference to the bill-tip structures of birds that lack a bill-tip organ.

The extinct lithornithids are a group of ancient, volant birds, sister to all modern palaeognaths. Lithornithid fossils have been recovered within or near fossil lake deposits and, together with beak and leg morphology, this has led to hypotheses that they were aquatic wading birds (Houde, 1988; Grande, 2013; Mayr, 2016). They are considered to be the most basal members of the palaeognath clade (Houde & Olson, 1981; Houde, 1988; Clarke *et al.*, 2005; Nesbitt & Clarke, 2016; Yonezawa *et al.*, 2017) which evolved in the Cretaceous and survived the K-Pg extinction (Clarke *et al.*, 2005; Yonezawa *et al.*, 2017). This makes them ideal candidates for understanding the ancestral state of modern palaeognath beaks and investigating a potentially ancient example of remote-touch capable probe-foraging birds.

5.2: Methods & Materials

5.2.1: Institutional Abbreviations used

USNM/NZP – Smithsonian National Museum of Natural History, Washington DC

AMNH – American Museum of Natural History, New York

MNHN – Muséum national d'Histoire naturelle, Paris

SAM – Iziko South African Museum, Cape Town

IRSNB* - Institut royal des Sciences naturelles de Belgique, Belgium

*data was obtained from a published photograph (Mayr, 2009) of a specimen housed at this Institute

5.2.2: Histological sample

My total sample size for histological information was 52 species representing 21 families from 15 different orders of modern birds [36.6% of 41 orders (Clements *et al.*, 2019)]. These data were drawn from published literature and my own sectioning of new material.

My comprehensive literature survey found only 19 published studies at the time of writing describing the soft tissue histology and/or anatomy of bird beaks in sufficient detail to detect the presence and arrangement of Herbst corpuscles or other mechanoreceptors. I searched for any published studies concerning histology of bird beaks in *Google Scholar* and *Web of Knowledge*, including the search terms: ("bird" OR "avian" OR "aves") AND ("beak" OR "bill" OR "maxilla" OR "premaxilla" OR "rhamphotheca") AND ("histology" OR "anatomy" OR "morphology" OR "osteology" OR "tissue") and leaving the date range open. All papers and book chapters which contained images or descriptions of the soft tissue histology of bird beaks were also reviewed to ensure I had included any references cited that were not indexed by the search engines. As I did not find any dispute between different studies of the same species [e.g., multiple studies on *Gallus gallus domesticus* (Wight, Siller & Mackenzie, 1970; Gentle & Breward, 1986; Kuenzel, 2007)] regarding the presence and general arrangement of the mechanoreceptors in the tip of the beak, I have referenced only the first study per species to show sufficient detail for the mechanoreceptor arrangement to be determined. From these studies, I was able to assess the beak histology of 40 species of modern birds, representing 12 families from 10 orders (see Appendix 5.1 for list of species included and data sources).

In order to supplement the gaps in the literature on the beak histology of modern birds, I sectioned the beaks of 10 species (representing 8 families) of birds which died for reasons not related to this study and were donated to the project (see Appendix 5.1 for species included and source of samples). I was also able to study two species from the USNM fluid collection: *Scopus umbretta* (NZP 215513) and *Eudromia elegans* (USNM 344980). My data represent the first published soft tissue histology of beaks of nine of the ten families sampled, with the exception of Threskiornithidae (ibises). The precise methods used for sectioning and staining the beak material are detailed in Chapter 2 of this thesis (section 2.2.5).

5.2.3: Skeletal sample selection

Skeletal samples were sourced from the Smithsonian National Museum of Natural History and the American Museum of Natural History (see Appendix 5.2 for specimens' origins). All 52 species for which I had histology data were included to compare soft tissue histology with the pitting pattern on the bones of their beaks. I additionally sought to sample a representative number of species from every order and as many families as possible of extant birds [according to the latest classifications (Clements *et al.*, 2019)]. Species were selected by first ensuring at least one species per family was included (to account for monospecific families). Thereafter additional species were chosen relative to the number of species per family. This was to account for morphological diversity in families with a high number of species, while setting the total limit of 400 species (due to constraints on the time available for processing museum specimens). Species were selected randomly within each family by assigning each species a number, and then using a random number generator to select a subset for sampling. These selected species were then checked against the museums' online databases, and in the case where specimens were not available, another species from the family would be randomly selected. A small percentage of families were either not represented in the skeletal collections used (4.4%), or the available specimens were unsuitable for the purposes of this study.

I ultimately sampled 353 species of extant birds, representing 210 families (84.3% of 249) and all 41 orders. All five families of extant palaeognaths were sampled, as well as 84.0% of the 244 families of neognaths (205 families sampled); including 96.3% of non-passeriform families (103 of 107). I sampled 75.4% (107 of 142) of passeriform families and found no significant variation in the measurements of pitting on the beak bones within the families sampled, thus I am confident that the coverage for this order is sufficient.

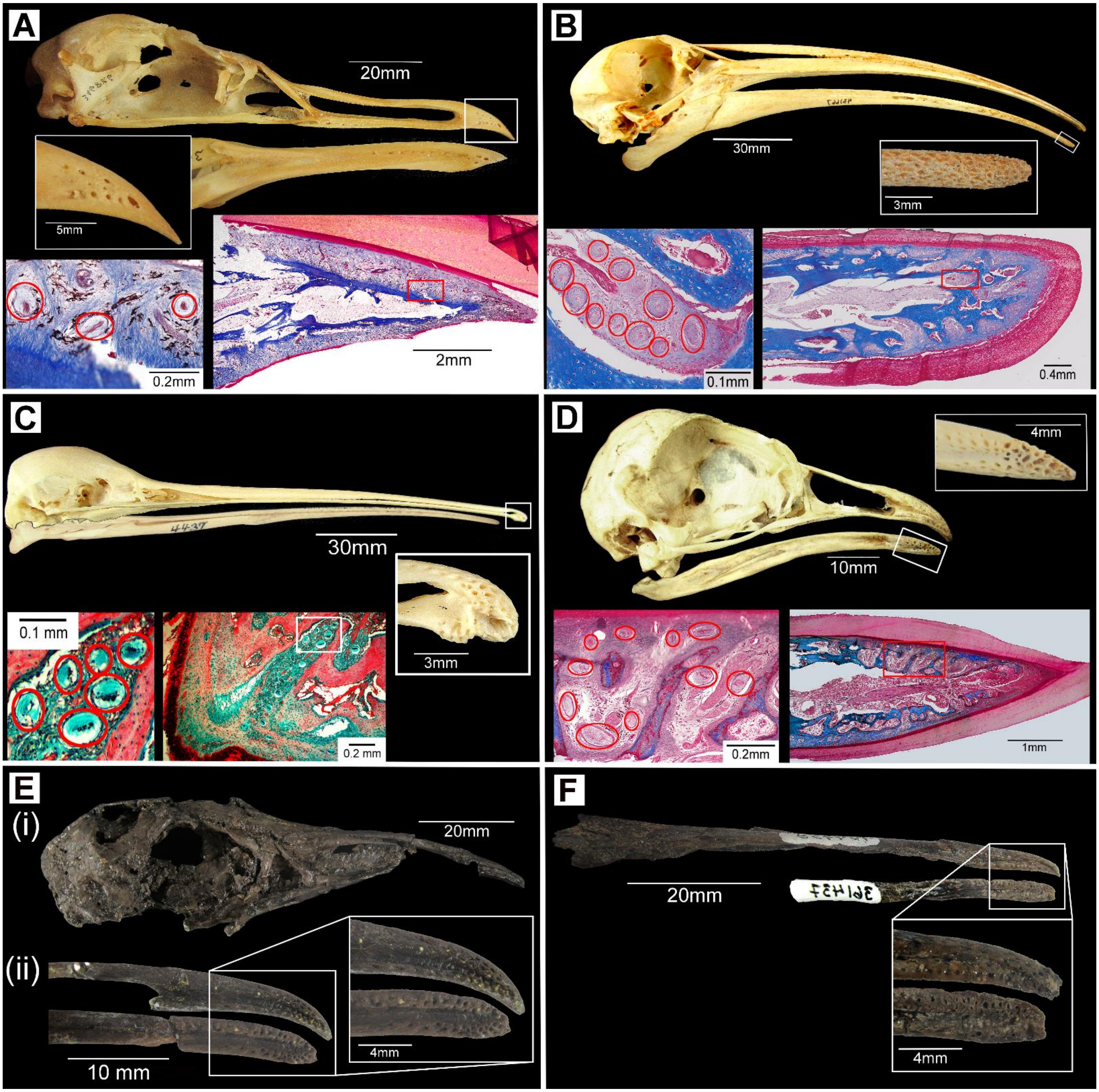


Figure 5.1: Bill-tip organs of various modern birds, and comparisons to analogous regions on the fossilized beaks of lithornithids. A-D: Pitting pattern on the surface of the beak bones and sagittal sections showing the soft tissue histology of the bill-tip for four extant species of birds, illustrating three different types of bony bill-tip organs. **1** – No bony bill-tip organ (most birds): (A) Kelp Gull (*Larus dominicus).* **2** – Remote-touch probing bill-tip organ, in neognathous birds: (B) Hadeda Ibis (*Bostrychia hagedash)*; and in palaeognathous birds: (C) Northern Brown Kiwi (*Apteryx mantelli).* **3** – Non-probing palaeognathous bill-tip organ: (D) Elegant Crested Tinamou (*Eudromia elegans)*. Histology images showing sagittal cross sections of distal portion of the beak (maxilla or mandible; 40X (A, B & D) or 100X (C) magnification) to show soft tissue arrangement. Zoomed in histological images (100X magnification) of the soft tissue matrix surrounding the bone (A), or the soft tissue within bony-pits (B-D), show the arrangement of the mechanoreceptors (Herbst corpuscles – circled in red) relative to the bone. Masson's trichrome stain used for histological sections. See Appendix 5.1 for specimen names & origins. E&F: Cranial fossils of two species of lithornithids, showing high degree of pitting on the

surfaces of their beaks, similar to all extant palaeognathous birds, potentially indicative of a bony bill-tip organ. (E) *Lithornis promiscuus*: (i) skull and attached maxilla (USNM 391983) showing the shape of the beak relative to the skull; (ii) distal portions of maxilla and mandible (USNM 336535). (F) *Paracathartes howardae:* maxilla (USNM 404758) and distal portion of mandible (USNM 361437).

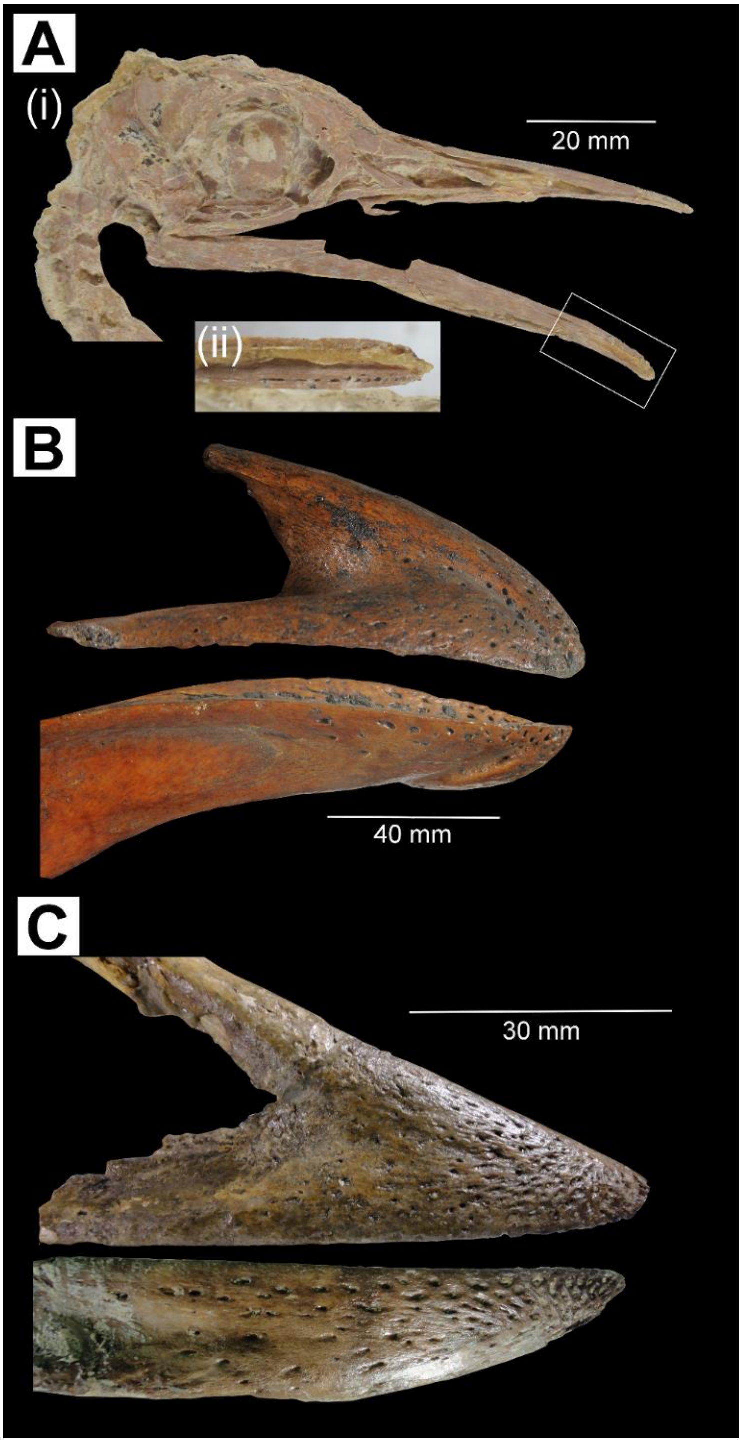


Figure 5.2: Cranial fossils of three extinct palaeognathous birds. (A) The lithornithid, *Pseudocrypturus cercanaxius* (USNM 336103): (i) a lateral view of the complete skull, showing the shape of the beak in relation to the attached cranium; and (ii) a dorsal view of the distal portion of the mandible [section indicated on (i) with white box] showing where some of the surface pitting on the beak bone has been preserved (see Figure 5.1 for well-preserved lithornithid fossil beak surfaces). (B & C) Distal portions of the maxilla and mandible, showing surface pitting pattern similar to all extant palaeognathous birds with bill-tip organs, for (B) the dinornithiform, *Dinornis* sp. (USNM V 6615); and (C) the aepyornithid (MNHN F.MAD8903). All photographs taken under standard light conditions with a digital camera.

5.2.4: Fossil samples

Four lithornithid species (*Paracathartes howardae*, *Lithornis promiscuus, Pseudocrypturus cercanaxius* and a second *Lithornis* species from Messel) and two other fossil palaeognaths (from the orders Dinornithiformes and Aepyornithiformes) were used in this study to assess the presence of a bony bill-tip organ in all clades of modern palaeognaths and in the basal-most known clade of this group.

Fossil specimens' systematics & notes:

LITHORNITHIDS:

Referred specimens:

Paracathartes howardae* HARRISON, 1979: Premaxilla (USNM 404758); rostral portion of mandible (USNM 361437). Elk Creek, Wyoming, USA; Greybullian, middle Wasatchian, early Eocene.

Lithornis promiscuus HOUDE, 1988: Rostral portion of premaxilla & mandible (holotype USNM 336535); skull and premaxilla (USNM 391983). Clark Quadrangle, Wyoming, USA; Clarkforkian, early Eocene.

Pseudocrypturus cercanaxius** HOUDE, 1988: skull, premaxilla and mandible (holotype USNM 336103). Tynsky Quarry, Kemmerer, Wyoming, USA; late Wasatchian, early Eocene.

A **Messel *Lithornis* sp.** MAYR, 2009: skull and premaxilla (IRSNB Av82). Messel, Hessen, Germany; middle Eocene.

* *Paracathartes howardae* was initially described as being a cathartid vulture (Harrison, 1979), but has subsequently been assigned to lithornithids upon further analyses of new material (Houde, 1988; Nesbitt & Clarke, 2016)

***Pseudocrypturus cercanaxius* was initially believed to be a basal sister taxon to other lithornithids, but subsequent analyses have placed it within the Lithornithidae family (Nesbitt & Clarke, 2016)

<u>Comments:</u>

Only the specimens of *Paracathartes howardae* and *Lithornis promiscuus* (USNM 336535) were suitable for analyses of pitting patterns using photographs (see Figure 5.1E-F). The surface of the tip of the beak of the *Pseudocrypturus cercanaxius* specimen is badly eroded preventing a measure of the external pitting pattern although a high degree of pitting is evident (see Figure 5.2A). The Messel *Lithornis* sp. specimen displayed excellent preservation of the lateral external pitting, however dorsal and ventral views of the beak surfaces were not accessible with standard photography due to its preservation in a slab [Fig. 1 in a previously published study (Mayr, 2009)].

All lithornithid specimens, including *Pseudocrypturus cercanaxius* and the Messel *Lithornis* sp., were used in the measure of beak-skull length ratio.

DINORNITHIFORM:

Referred specimen:

***Dinornis* sp.** OWEN (1844): Premaxilla, mandible & posterior portion of the cranium, (USNM V 6615). New Zealand; Quarternary.

Comments:

Both the mandible and premaxilla of this specimen were present, along with the associated posterior region of the cranium (See Figure 5.2B). The beak pitting pattern was measured using this specimen, and the beak-skull length ratio was reconstructed using photographs of the premaxilla and skull and comparing them to other complete specimens from the same genus.

AEPYORNITHID:

Referred specimen:

Aepyornithid sp. MILNE-EDWARDS & GRANDIDIER (1866): Skull, premaxilla & mandible (MNHN F.MAD8903). Madagascar; Quarternary.

Comments:

This specimen was sufficiently well preserved for analyses of the pitting pattern, as well as to determine the beak-skull length ratio (See Figure 5.2C). It was initially classified as *Aepyornis medius* (Milne-Edwards & Grandidier, 1866), but this taxonomic assignment is uncertain (Hansford & Turvey, 2018), and I therefore refer to the specimen as an aepyornithid.

5.2.5: Measures of pitting pattern from photographs

Each specimen, extant or fossil, was photographed using a digital camera (*Canon PowerShot SX620 HS*) and light table. Images were analysed in *Fiji* (Schindelin *et al.*, 2012) [a distribution of *ImageJ2* (Rueden *et al.*, 2017)].

Six images per specimen were analysed (lateral, ventral and dorsal views of both the mandible and maxilla). I observed no noticeable difference in the pitting between the left and right sides of the beak for any of the species measured, and often only one side was suitable for pitting analyses, so lateral pit measurements were conducted on only one side and were duplicated to give estimated total values for the beak.

The measurements extracted for each specimen from the dataset were: total number of pits; average Nearest Neighbour Distance (NND) between pits; average distance of pits from the distal tip of the bill; and maximum distance of pits from the distal tip of the bill. In order to control for beak size, these measurements were transformed by dividing them by the premaxilla length. The length of the premaxilla was measured from the distal tip of the premaxilla to the craniofacial hinge (lateral view). This value was divided by five to give the length of the distal most 20% of the beak, which I used to define the 'tip' of the beak. To measure data on the pitting on the distal most 20% of the beak, only pits with a distance from the tip of the beak less than one fifth of the premaxilla length were extracted to form a separate dataset.

5.2.6: Pitting pattern analyses in Fiji

The distal tip of the beak was selected, and its position in the photograph was measured using the command *Analyze* → *Measure*, to give a pair of coordinates. The origin of the photograph was then reset to the coordinates of the tip of the beak [*Image* → *Properties* → *Origin (pixels)*], so that the tip then represented the 0:0 mark, and further points selected could be measured relative to the tip of the beak. The scale of the image could then be set.

The pits on the beak were selected as individual points. Because no formal description of a ‘sensory’ pit exists, nor is there any published information on how these can be distinguished from other types of foramina in the absence of soft-tissue histology, any foramina along the entire surface of the bone of the beak that was not clearly an artefact of damage was selected. This also allowed me to test the use of pitting pattern as a proxy for the presence of a bill-tip organ in fossils where examination of soft tissue histology is not possible and eliminates individual bias as to the definition of a sensory pit, when further information is lacking.

Where there were many pits on the surface of the beak, the command *Process* → *Find Maxima* was used. The threshold was set for each photograph to ensure that no maxima were selected that did not represent pits on the bone of the beak (eg. representing superficial damage or dust particles on the surface of the beak, or photography artefacts). This meant that often some of the pits were excluded from this initial selection, so these had to be manually selected after the maxima selection.

Once all the pits had been selected, the *Measure* command was used to give a list of the coordinates of each pit (relative to the tip of the beak). These data were then extracted to *Microsoft Excel* (2013), and the distance from the tip of the beak of each pit was calculated using standard trigonometry.

In order to measure the NND of each pit, the plugin *NND* (Mao, 2016) was used. With all the pits selected and the list of their measured coordinates still open, the plugin *NND* was run (*Plugins* → *Analyze* → *NND*). This generated a list of the closest distances between pits for each pit selected. This was then exported across to *Microsoft Excel* (2013) and combined with the coordinates and distance data for each pit.

5.2.7: Relative beak size in relation to skull length

In order to describe variation in the relative length of the beak in relation to the bill-tip pitting pattern, I used the ratio of premaxilla length to skull length (defined from here on as the beak-skull length ratio; see Figure 5.3 for the axes of measurement). The beak-skull length ratios were log transformed in order to satisfy assumptions of normality, which are particularly important in datasets such as this one which contain linear measurements from a broad interspecific sample and which can minimize the skewing of data due to effects of overall body size variation (Harvey, 1982; Kovarovic & Andrews, 2007; Meloro *et al.*, 2013).

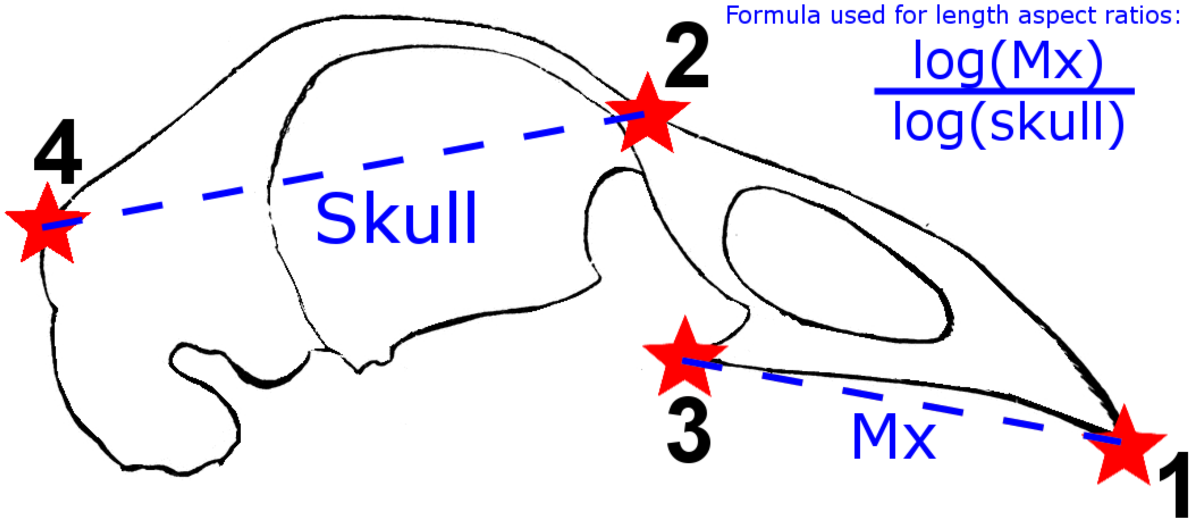


Figure 5.3: Simplified drawing of the lateral view of a bird skull to show the measurements & formula used to calculate the log transformed beak-skull length ratio. The points represent the following anatomical points on the bone: (1) Anterior tip of premaxilla; (2) Craniofacial hinge; (3) Posteroventral tip of premaxilla; and (4) Posterior tip of squamosal region. “Mx” = Premaxilla.

The beak-skull length ratio was measured for all 353 extant bird specimens, as well as the six fossil specimens. For most species, the same specimen was used to measure the pitting pattern and the beak-skull length ratio. A small number of the specimens were unsuitable for both analyses, so another specimen of the same species was used to provide data on beak and skull length. The only exception was the specimen of *Paracathartes howardae* (USNM 404758), for which the associated skull and posterior region of the premaxilla are not present, and no other confirmed specimen of sufficient preservation is known of this genus to use as an alternative. As a proxy I used the skull of the *Lithornis promiscuus* (USNM 391983) specimen and combined the lateral photo of it with that of the *P. howardae* premaxilla, overlaying them digitally to reconstruct its beak shape. In order to overlay the images as accurately as possible, photos from all angles were examined of the *P. howardae* premaxilla and the *L. promiscuus* skull. The best point to overlay them was determined to be the craniofacial hinge, as this is generally easy to locate from a dorsal view and then align laterally. This was the case for the *L. promiscuus* skull, but due to the poor preservation of the *P. howardae* specimen, there is some uncertainty as to the position of the craniofacial hinge. For this reason I used three different reconstructions of the beak & skull shape: one with a longer and one with a shorter beak, to give an idea of the range that it most likely would have fallen within (both created by comparing to other lithornithid fossils to be as realistic as possible), as well as an intermediate beak shape between the other two.

5.2.8: Statistical analyses and plotting of figures

I compiled the raw data measurements in *Microsoft Excel* (2013). I performed all further statistical analyses and plotting of data in *RStudio* (RStudio Team, 2016), and used the photo editing software *GIMP* (The GIMP Team, 2018) to add the clipart and edit some of the axis labels of the figures.

Paired t-tests and Wilcox tests [*R* (R Core Team, 2018) package *ggsignif* (Ahlmann-Eltze, 2019)] were used to assess the statistical significance of differences in the relative number of pits on the distal most 20% of the beak between the different types of soft tissue arrangements found in the extant bird species sampled.

I performed a standard PCA in order to indicate the differences in pitting patterns on the beak, using two variables: number of bony pits in the distal most 20% of the bill, and the average nearest-neighbour distance between those pits. The plots of this PCA data were generated using the *R* (R Core Team, 2018) package *ggbiplot* (Vu, 2011). I drew all shaded regions on the figures in the image editing software *GIMP*. Each coloured area was drawn to minimise the number of vertices (representing the limits of the morphospace), while ensuring all data points from that classification were included in the space.

The phylogeny was constructed and coloured in *RStudio* (RStudio Team, 2016) using the package *ape* (Paradis & Schliep, 2018). Relationships were based mainly on those from a study using targeted next-generation DNA sequencing on a large sample of all extant birds (Prum *et al.*, 2015), but some amendments were made to the palaeognath clade (Mitchell *et al.*, 2014) and the Charadriiformes (Gibson & Baker, 2012), as more detail was required on the relationships within those clades. The branch lengths are not indicative of divergence time or genetic difference and are only meant to indicate the positions of the different clades relative to each other.

5.3: Results

The soft tissue organisation of the distal tips of the beaks of extant birds (Figure 5.1) is visually different between those which do not possess a specialised bony bill-tip organ (example shown of a Kelp Gull *Larus dominicanus,* Figure 5.1A), and birds with a bony bill-tip organ used for remote-touch foraging [examples of a Hadeda Ibis, *Bostrychia hagedash* (Neognath, Figure 5.1B) and a Northern Brown Kiwi, *Apteryx mantelli* (Palaeognath, Figure 5.1C)]. The most obvious difference between these two groups is that Herbst corpuscles are embedded within bony pits in the beaks of birds with bony bill-tip organs used for tactile foraging, while they are either confined to the dermis surrounding the bone of the beak or are absent from the beak altogether in all other neognathous birds. Non-probing palaeognaths have beaks possessing the same soft tissue organisation as remote-touch probe-foraging birds (example shown of a tinamou, *Eudromia elegans*, Figure 5.1D).

5.3.1: Relative number of pits & soft tissue organisation in the beak

Species with Herbst corpuscles embedded in bony pits in their beaks, irrespective of whether palaeognath or neognath, have significantly higher relative numbers of these pits compared to species in which Herbst corpuscles are either altogether absent from the distal portion of the beak or are confined to the soft tissue matrix surrounding the bone of the beak (Figure 5.4). This pattern remains when species with Herbst corpuscles organised into touch papillae (Anseriformes) are separated from all other birds with Herbst corpuscles embedded in bony pits (Figure 5.5).

The beaks of all sampled species of extant palaeognaths have numbers of distal bony pits that are comparable to those of neognath species that have Herbst corpuscles embedded in the bony pits (Figure 5.4). Both species of recently-extinct modern palaeognaths sampled, the aepyornithid and *Dinornis* species (represented as blue dashed lines in Figure 5.4), are also nested within this group, based on the number of distal bony pits preserved in their fossilised beaks; as are the lithornithid species I sampled (Figure 5.4: lithornithids represented by a red-dotted line for *Lithornis promiscuus* & red shaded bar for *Paracathartes howardae* reconstructions).

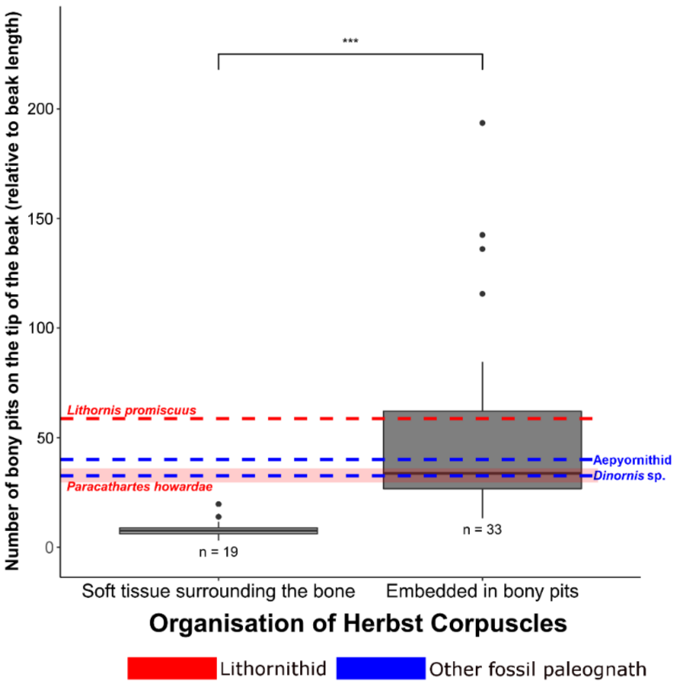


Figure 5.4: Relationship between the number of bony pits (relative to beak length) within the distal-most 20% of the beak, and the arrangement of mechanoreceptors (Herbst corpuscles) relative to the bone of the beak. Coloured horizontal lines indicate the data from fossil palaeognath specimens. The shaded red bar represents the range of measured values for *Paracathartes howardae*, depending on the skull reconstruction (see Figure 5.8). “Soft tissue surrounding the bone”: species where Herbst corpuscles are either absent or restricted to the dermis of soft tissue surrounding the bone at the tip of the beak; “Embedded in bony pits”: species (including Anseriforms) with Herbst corpuscles embedded within the soft tissue in the bony pits, indicating the structural components of a bony bill-tip organ. “n” = number of species. Asterisks indicate statistically significant differences between groups from two-sided Mann-Whitney U test: *** = p-value <0.001. See Figure 5.5 for separate grouping of Anseriformes, & Appendices 5.1 & 5.3 for species included & test statistics.

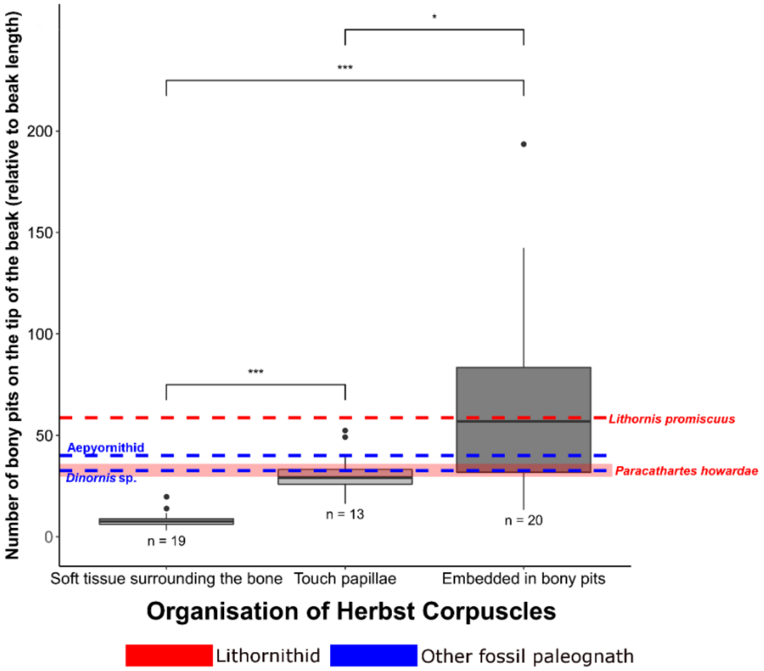


Figure 5.5: Relationship between the number of bony pits (relative to beak length) within the distal-most 20% of the beak, and the arrangement of mechanoreceptors (Herbst corpuscles) relative to the bone of the beak (with touch papillae treated as a separate arrangement). Coloured horizontal lines indicate the data from fossil palaeognath specimens, indicating where they fall in relation to the extant birds. The shaded red bar represents the range of measured values for the lithornithid *Paracathartes howardae*, depending on the skull reconstruction (see Figure 5.8). “Soft tissue surrounding the bone”: species where Herbst corpuscles are either absent or restricted to the dermis of soft tissue surrounding the bone at the tip of the beak; “Embedded in bony pits”: species (excluding Anseriforms) with Herbst corpuscles embedded within the soft tissue in the bony pits, indicating the structural components of a bony bill-tip organ; “Touch papillae”: Anseriform species (ducks & geese) which display a characteristic arrangement of the Herbst corpuscles and other mechanoreceptors into touch papillae, representing a bony bill-tip organ used for dabbling. “n” = number of species. Asterisks indicate statistically significant differences between groups from two-sided Mann-Whitney U tests: * = p-value <0.02; *** = p-value <0.001. See Appendices 5.1 & 5.3 for species included & test statistics.

5.3.2: Pattern of bony pits in different beak types

I found broad variation not only in numbers of distal bony pits, but also in the spatial arrangement of these pits, between birds with different beak types. For example, extant birds without a bony bill-tip organ, (coded “Other” or “Direct-touch probe” on Figures 5.6 & 5.7), tend to have low numbers of bony pits on the distal portion of their beak bones, with small average NND between the pits (see grey shaded region in Figure 5.6).

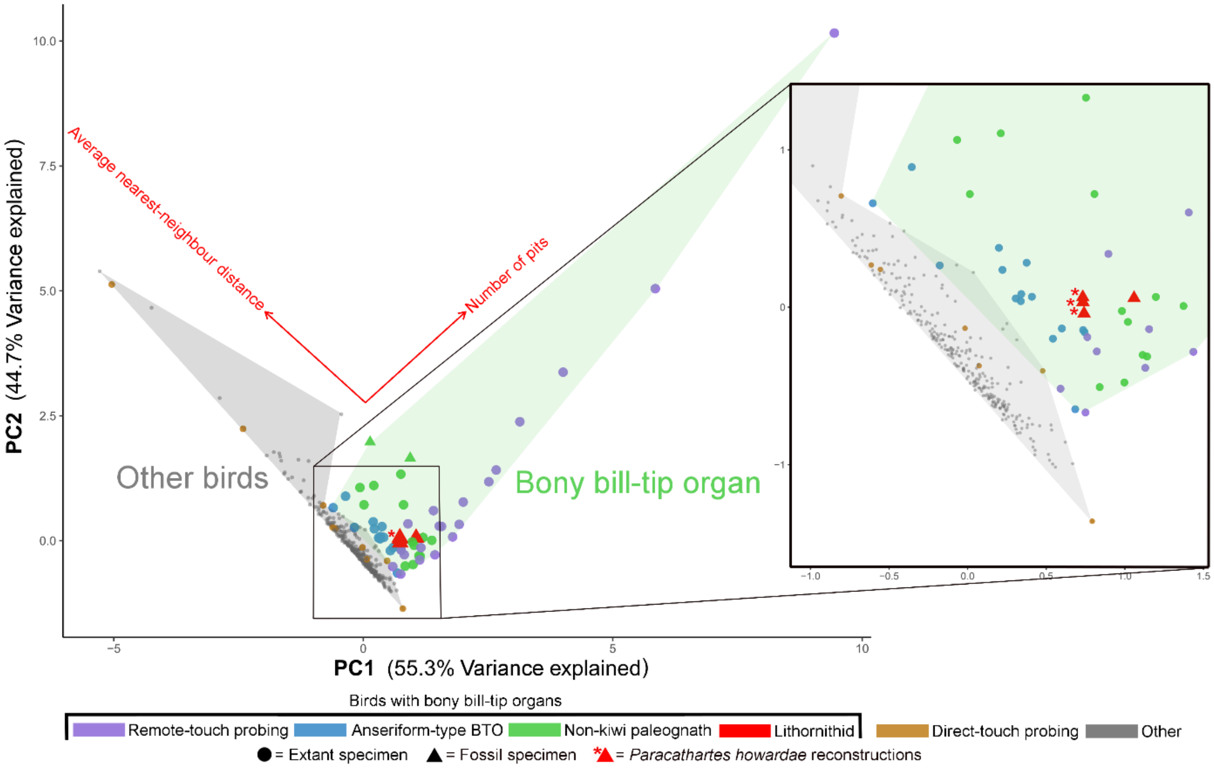


Figure 5.6: PCA of measures of pitting on the surface of the bone at the tip of the beak for extant birds as well as various extinct palaeognaths. Characters included in the analysis are the number of bony pits in the distal-most 20% of the beak and the average nearest-neighbor distance between those pits. Red arrows indicate the effect each variable has on the PCA values. Inset showing zoomed in region to illustrate the small area of overlap between species with a bony bill-tip organ and other birds. Each point represents a different species (n=357). The colours of the data points represent classification of the species in terms of whether it has a bony bill-tip organ, and whether it makes use of remote-touch and/or probing foraging. The shapes of the points represent whether it was an extant or fossil specimens. Asterisks next to the points denote the various *Paracathartes howardae* reconstructions. BTO=Bony bill-tip organ. See Appendix 5.2 for species classifications.

Extant species with bony bill-tip organs, including the remote-touch probe-foraging birds, Anseriformes and non-probing palaeognaths, have beak bones characterised by high numbers of pits which are very close together (see green shaded region in Figure 5.6). This is most obvious in the threskiornithids (ibises & spoonbills), which have very densely pitted bill-tip organs.

Based on the pattern of beak bone pitting, there is no overlap between birds without any bony bill-tip organ and those with the remote-touch probing bony bill-tip organ. None of the palaeognath species sampled, both extant and extinct, overlap with the “Other” birds either (Figure 5.6). Both the extinct *Dinornis* species and the aepyornithid (green triangles in Figure 5.6) group with the extant giant non-probing palaeognaths. They are both most similar to the ostrich (*Struthio camelus*) in terms of their bill-tip pitting patterns.

An examination of the rostral surfaces of the beaks of the fossil lithornithid specimens (Figure 5.1E-F) showed high numbers of distal pits (416-564 pits) and low average NNDs (0.39-0.51 mm). With respect to these pitting patterns on the beak, both *Paracathartes howardae* and *Lithornis promiscuus* fall alongside the other palaeognaths within the group of birds with some type of bony bill-tip organ (lithornithids represented by red triangles in Figure 5.6).

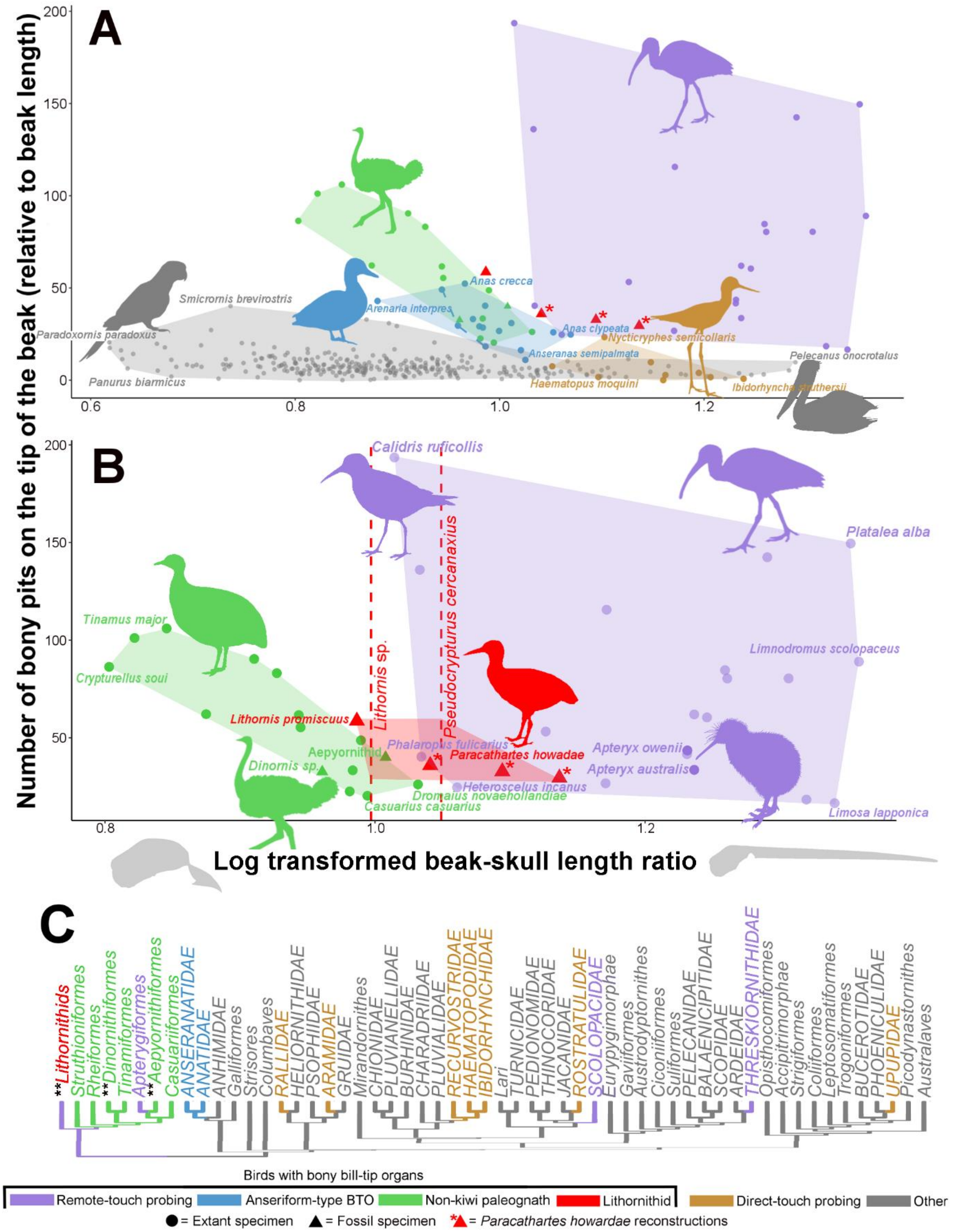


Figure 5.7: Classification of lithornithid beaks in relation to modern birds, based on the relationship between the number of bony pits (relative to beak length) in the distal-most 20% of the beak and the log transformed beak-skull length ratio. Colours represent classification of a species by the presence/absence of a bony bill-tip organ, and whether it makes use of remote-touch and/or probing foraging. (A&B) Log transformed beak-skull length ratio describes the change in beak length in relation to skull length (silhouettes of skulls show extremes of beak shapes). Each point represents a different species (n=357). The shapes of the points represent whether it was an extant or fossil specimens.

Asterisks next to the points denote the various *Paracathartes howardae* reconstructions. The shaded areas represent the space occupied by each group. (A) Data for all orders of modern birds as well as various extinct fossil palaeognaths. Blue dashed line showing separation of Anseriformes from *Arenaria interpres*. (B) Zoomed in version of (A), highlighting where the lithornithids fall in relation to other palaeognaths and remote-touch probe foraging birds. Red dashed lines indicate the beak-skull length ratios for two additional lithornithid species; *Pseudocrypturus cercanaxius* & the Messel *Lithornis* species. The red shaded area has been drawn to account for these additional specimens' proposed positions. (C) Phylogeny showing the occurrence of the different foraging classifications across all modern bird orders. Clades in which all orders are classified as “Other” have been collapsed, and orders which contain more than one classification have been expanded to show the constituent families in capital letters. BTO=Bony bill-tip organ. See Appendix 5.2 for species classifications.

5.3.3: Combining degree of pitting and beak-skull length ratio

When the relative number of pits on the distal surfaces of the beak bones is combined with the log transformed beak-skull length ratio, remote-touch probing birds occupy a unique morphospace characterised by relatively long beaks with very high numbers of distal bony pits. There is no overlap between them and the space occupied by birds without a bony bill-tip organ (Figure 5.7A). Most birds without bony bill-tip organs have much shorter beaks than the remote-touch foragers, and those that do overlap with the remote-touch foragers in terms of beak-skull length ratio have much lower numbers of distal bony pits (generally birds which use direct-touch probe-foraging).

If I use the same criteria to compare the bony bill-tip organs of extant remote-touch probing birds (including kiwi) with the non-probing palaeognaths, the two groups do not overlap (Figure 5.7B). Though both groups have high numbers of distal pits (compared to all other birds), remote-touch probe-foragers tend to have longer beaks. Where there is overlap in beak-skull length ratio, remote-touch probing birds have significantly higher numbers of distal pits than the non-kiwi palaeognaths.

The modern non-kiwi palaeognaths, including the extinct aepyornithid and *Dinornis* species, occupy an area representing a relatively long beak with moderately high numbers of pits on the distal surfaces of the beak bones. They also fall in a separate position compared to birds without any bony bill-tip organ. There is some overlap between the modern palaeognaths and birds with the Anseriform-type bill-tip organ, but this can be clarified by the examination of the other measures of pitting pattern (see Figures 5.4 & 5.6).

All beak shape reconstructions of the lithornithid *Paracathartes howardae* (see Figure 5.8) indicate that this species had a similar beak-skull length ratio to extant remote-touch probing birds, particularly the scolopacids (see starred red triangles in Figure 5.7). Both lithornithid species sampled for pitting pattern (*P. howardae* and *Lithornis promiscuus*) had fairly long beaks with high numbers of bony pits on the rostral surfaces of the bones, differentiating them from the beaks of the other non-kiwi palaeognaths and the Anseriformes. Based on their beak morphologies, all reconstructions of *P. howardae* fall within the group of the extant remote-touch probe foragers, and *L. promiscuus* falls between the remote-touch probing group and the other non-kiwi palaeognaths.

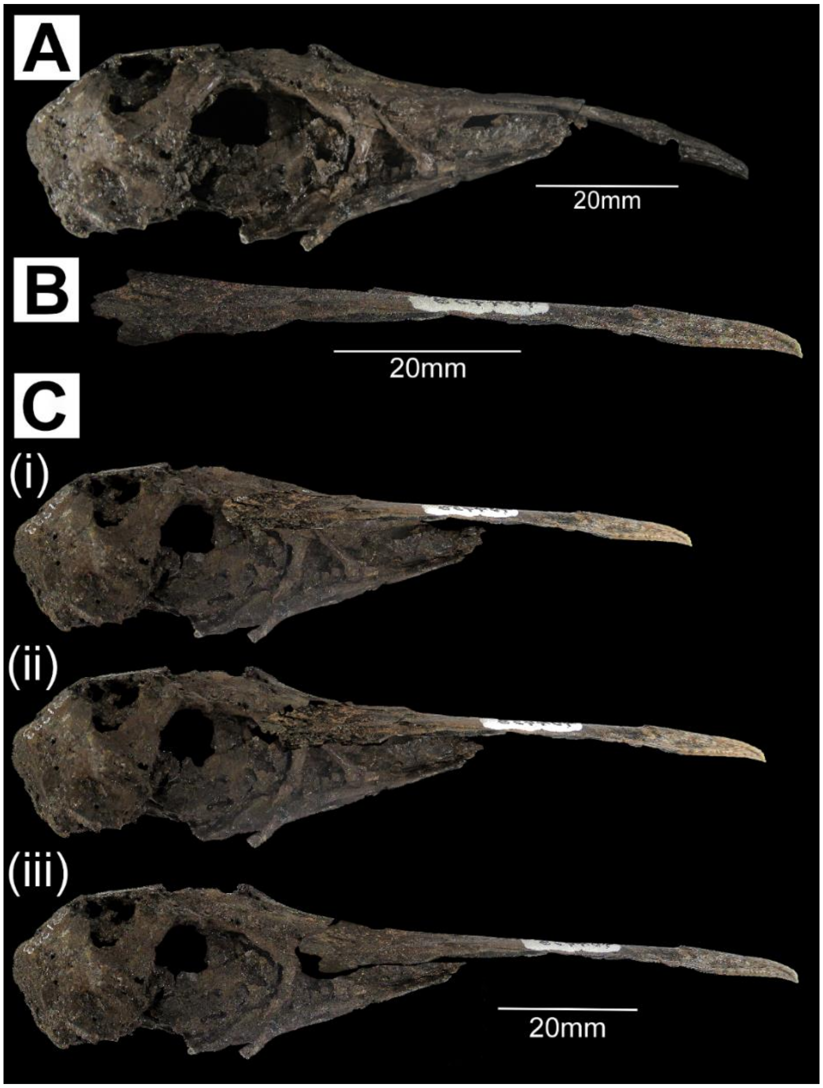


Figure 5.8: Three reconstructions of *Paracathartes howardae* beak shape using (B) the maxilla (USNM 404758) in relation to (A) the skull of another lithornithid species, *Lithornis promiscuus* (USNM 391983). The *P. howardae* maxilla and *L. promiscuus* skull were digitally combined by aligning the craniofacial hinge of the skull with three potential craniofacial hinge points along the maxilla, so as to give a range of possible beak shapes for *P. howardae* (C): (i) represents a shorter beak shape very similar to that of *L. promiscuus*, with a large portion of the dorsal surface of the cranium still attached to the maxilla; (ii) represents an intermediate beak shape, with a small portion of the cranium still attached to the maxilla; and (iii) represents a very long beak shape, with the preserved fossil maxilla having broken off from the skull at the craniofacial hinge. All photographs taken under standard light conditions with a digital camera.

The other two fossil specimens of lithornithids, [representing *Pseudocrypturus cercanaxius* (see Figure 5.2A) and another *Lithornis* species (Mayr, 2009)], were not well preserved enough to allow counting of the bill-tip pits, although the presence of pits was visually detectable. I was able to measure beak-skull length ratios for these two specimens (represented by vertical dashed red lines in Figure 5.7B). I used this alongside the data gathered from *P. howardae* and *L. promiscuus* to estimate a likely range for the number of distal bony pits possessed by *Pseudocrypturus cercanaxius* and the other *Lithornis* species. This gives a conservative morphospace occupied by the lithornithids relative to the sample of modern birds, indicated by the red shaded region in Figure 5.7B.

5.4: Discussion

The remote-touch sensory system, a unique specialised tactile “sixth sense”, allows birds to detect concealed prey at some distance from their probing bill-tips. Here I show that the bone morphology of the beak alone is sufficient to infer the presence of this avian sensory system in fossil material, and I provide evidence that the common ancestor of the modern palaeognaths (including emu, ostriches, and the extinct elephant birds and moa) was likely a probe-foraging bird with this remarkable sense. My analyses corroborate that the basal palaeognaths, the small, volant lithornithids, had a tactile bony bill-tip organ enabling them to use remote-touch to locate buried invertebrate prey items. This finding, combined with our understanding of the evolution of the lithornithids, suggests a Cretaceous origin of the remote-touch sensory system in modern birds before the palaeognath-neognath split.

5.4.1: Beak bone morphologies as osteological correlates for the presence and function of bony bill-tip organs

My comprehensive analysis of beak bone morphology and its links with soft tissue histology in modern birds shows that, despite considerable variation between taxa (Cunningham, Alley *et al.*, 2010), the combination of beak-skull length ratios and bone morphology can be confidently used to infer the presence or absence of a remote-touch-capable bill-tip organ. Thus, the examination of fossilised beaks of extinct avian taxa permits an assessment of the type of bill-tip organ they possessed, directly providing insight into their palaeoecology. The relative ease of acquiring these data and the extensive dataset compiled for extant birds will undoubtedly be invaluable for future studies of the sensory capabilities of beaks of other extinct birds and will enable reliable palaeoecological deductions.

The significant correlation between the high number of bony pits on the distal surfaces of the beak (relative to beak length) and the presence of Herbst corpuscles located within the pits indicates that the relative number of bony pits is a reliable osteological correlate for the presence of a bony bill-tip organ. The ability to distinguish bony bill-tip organs in the absence of soft tissue data in 353 extant species from over 200 families (representing all orders of modern birds) is notable. Using easily obtainable data such as the number of pits in the distal-most portion of the beak, the average NND between the pits and a ratio of premaxilla to skull length, a distinct morphospace is deduced for extant remote-touch probe-foraging birds. Thus, using standard photography with these novel approaches, it is possible to quantifiably differentiate the remote-touch bony bill-tip organ of probe-foraging birds from the non-kiwi palaeognath bony bill-tip organ.

5.4.2: Remote-touch in lithornithids

The basal position of the extinct lithornithids among the palaeognathous birds, combined with their superb fossil record and the fact that they survived the end-Cretaceous extinction event (Houde & Olson, 1981; Houde, 1988; Clarke *et al.*, 2005; Nesbitt & Clarke, 2016; Yonezawa *et al.*, 2017), makes them ideal to study the evolution of remote-touch in the clade. When considering all described measures of the beak bone morphology, the lithornithids repeatedly classify within the group of modern birds which possess some type of bony bill-tip organ. Furthermore, their bill-tip organs are clearly distinguishable from those of both the Anseriformes and, more significantly, the modern non-kiwi (non-probing) palaeognaths. My results indicate that the lithornithid bony bill-tip organs were either very similar to those of modern remote-touch probers, and/or intermediate in form between modern remote-touch probers and all other palaeognaths, suggesting that lithornithids had remote-touch sensory capacity.

My deduction of the sensory capability of remote-touch in lithornithids is supported by multiple lines of evidence: Most lithornithid fossils have been found within or close to fossil lake-bed deposits, and their general morphology is widely accepted to indicate a wading, probe-foraging lifestyle (Houde & Olson, 1981; Houde, 1988; Mayr, 2009; Grande, 2013; Nesbitt & Clarke, 2016; Mayr, 2016). Furthermore, trace fossils from Cretaceous sites show evidence of probe holes alongside tracks of unidentified aquatic birds (Falk, Hasiotis & Martin, 2010), indicating that some birds were already probe-foraging at the time. Combined with my data on the beak morphology of lithornithids, I am confident in my hypothesis that some (if not all) of the lithornithids were able to utilize the unique avian sense of remote-touch to locate buried invertebrate prey. Based on the evidence of the evolution of the lithornithids in the mid-late Cretaceous (Yonezawa *et al.*, 2017), I surmise that this sophisticated sensory modality had evolved by then too.

5.4.3: Plesiomorphy in Palaeognathous Beaks

The presence of a remote-touch capable bony bill-tip organ in the basal lithornithids suggests that this bill-tip organ may be the plesiomorphic state among palaeognathous birds. This explains the intriguing presence of a structurally-similar bill-tip organ in modern non-kiwi palaeognaths, as evidenced by bone morphology and/or soft-tissue histology in both extant [emu & ostrich (Crole & Soley, 2014; Crole & Soley, 2017); tinamou, rhea & cassowary (this study)] and extinct species [elephant bird and moa (this study)], in the absence of a probe-foraging life-style (Tomlinson, 2000; Gussekloo & Bout, 2005).

Only one extant palaeognathous order, the probe-foraging kiwi (Apterygiformes; five species), uses remote-touch (Cunningham, Castro & Alley, 2007; Cunningham, Corfield, *et al.*, 2013). The most parsimonious explanation for the single re-emergence of this sensory capability in kiwi after the extinction of the lithornithids would be a single loss between the lithornithids and all other palaeognaths, and then a re-acquisition of the trait in ancestral kiwi after their split (Mitchell *et al.*, 2014) from the aepyornithids. Alternatively, loss of remote-touch may have occurred independently multiple times among the palaeognaths – evolution within this clade has been shown to take various non-parsimonious pathways. For example, it is well established that loss of flight and gigantism evolved independently multiple times, along with changes in ecological niche (Tomlinson, 2000; Harshman *et al.*, 2008; Haddrath & Baker, 2012; Baker *et al.*, 2014;

Yonezawa *et al.*, 2017). My data alone cannot be used to establish precisely which evolutionary pathway occurred. Either the examination of more fossils or analyses of the genetic pathways responsible for the evolution of the bony bill-tip organ and remote-touch sensation would be required to investigate this further.

The retention of a plesiomorphic bony bill-tip organ among the palaeognaths begs the question: Why keep the structural organ but not the associated sense? It has been demonstrated that extant non-kiwi palaeognaths lack the hypertrophy in the regions of the brain associated with the processing of tactile information from the beak (Cunningham, Corfield, *et al.*, 2013). This could be attributed to the relatively high cost of maintenance of brain tissue (Niven & Laughlin, 2008), compared to the apparent low cost of maintaining their ancestral bony bill-tip organ (as evidenced by its retention across the clade over millions of years). My data indicate that another plesiomorphic bony bill-tip organ may exist in the Ruddy Turnstone (*Arenaria interpres,* see Figure 5.7A); a scolopacid shorebird that uses non-tactile foraging strategies (Whitfield, 1990), which has the high numbers of distal bony pits associated with the remote-touch bill-tip organ, but lacks the relatively long beak. This potential loss of function of the bill-tip organ would have occurred relatively recently (Gibson & Baker, 2012), and further investigations of *A. interpres* could aid in understanding how this might have occurred among the palaeognaths.

5.5: Conclusions

As the lithornithids represent the earliest described birds with a remote-touch bony bill-tip organ, it is possible that this sensory modality evolved within this group in the Cretaceous soon after the palaeognath-neognath divergence. However, as there are various morphological similarities between the palaeognath and anseriform bill-tip organs, it is probable that the bony bill-tip organ evolved in an earlier, more basal common ancestor of palaeognathous and neognathous birds. Similar structures may have evolved convergently in very basal archosaurs, based on the sensory capability and trigeminal nerve morphology of modern crocodilians (Soares, 2002; George & Holliday, 2013). However, further investigation into whether the mechanosensory dome receptors on the facial scales of crocodilians correlate with pitting on their jaw bones is warranted in order to assess their tactile sensibility in relation to remote-touch in birds. Interestingly, there is increasing evidence that some non-avian theropods had specialised sensory structures located on the distal portion of their rostra, based on a high degree of external foramina/pitting preserved on their mandibles (Ibrahim *et al.*, 2014; Barker *et al.*, 2017; Carr *et al.*, 2017). I speculate that perhaps such sensitive snouts in non-avian theropods may have been precursors to the evolution of remote-touch in their avian relatives.

5.6: References

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Chapter 6:

Synthesis of the Research

Palaeognathous birds’ skulls. (photographs by CJ du Toit)

6.1: Overview

Using a range of interdisciplinary methods, this thesis provides support for the ecomorphological links between the remote-touch bill-tip organ and the birds' sensory ecology, and the evolution of the organ in Neornithes. In this chapter, I provide a synthesis of the results and conclusions of the preceding four data chapters (Chapters 2-5), and discuss the broader usage of the methods I developed during this research. I conclude this synthesis with suggestions for future studies.

6.2: Ecomorphology of the remote-touch bill-tip organ

Chapter 2 describes the interspecific morphological context to the differences in sensory and foraging ecology of ibises. I showed that the three species of southern African ibises (Hadeda, African Sacred and Glossy Ibises), representing a spectrum of habitat usage (Hockey, Dean & Ryan, 2005), possess the bone morphology and soft tissue histology of the remote-touch bill-tip organ previously found in other species of ibises and remote-touch probing birds (Bolze, 1968; Cunningham, Castro & Alley, 2007; Cunningham, Alley *et al.*, 2010). My results indicate that pitting extent and number of sensory pits on the beak bones are a good proxy for determining aquatic habitat usage of a species. These findings concur with the results of Cunningham, Alley *et al.* (2010). Contrary to the prediction made by Cunningham (2010), my data showed that the density of mechanoreceptors was not positively correlated with number of sensory pits, and that species living in drier habitats tend to have higher numbers of Herbst corpuscles per sensory pit than species from more aquatic habitats. I hypothesized that species foraging in drier substrates required more sensitive bill-tip organs [indicated by the increased density of Herbst corpuscles (Zweers & Gerritsen, 1997)] as these substrates transmit mechanical vibrations more poorly than wetter substrates (Biot, 1956 & 1962). I further suggest that the interspecific differences in extent of pitting on the beak bones is linked to differences in tactile foraging behaviour, which may in turn be at least partially driven by differences in the substrates the birds forage in. Species foraging in drier, harder substrates also tend to have more robust beaks [to deal with the added force exerted on their beaks while probing (Bock, 1966; Zweers, Berkhoudt & Berge, 1994; Zweers & Berge, 1996; Zweers & Gerritsen, 1997; Barbosa & Moreno, 1999)]. This would also select against having highly perforated proximal regions of their beak bones, restricting pitting to more distal regions of the beak and consequently resulting in lower extents and numbers of pits in the beak as a whole.

Chapter 3 tests the hypotheses that the pitting extent on the bones of the bill-tip organ is correlated with the birds probing behaviour and substrate usage, using the same three species of ibises as Chapter 2. By performing focal observations of wild birds and measuring properties of the substrates they were foraging in, I investigated the link between foraging substrate and probing behaviour and whether these correlated with any of the interspecific differences described in Chapter 2 and previous research (Cunningham, Alley *et al.*, 2010). The results showed that several probing behaviours were strongly affected by the properties of the birds' foraging substrates, as has been shown in other probe-foraging birds (Quammen, 1982; Mouritsen & Jensen, 1992; Danufsky & Colwell, 2003; Granadeiro, Andrade & Palmeirim, 2004; Finn, Catterall & Driscoll, 2007; Finn, Catterall & Driscoll, 2008). Furthermore, my results showed that some of these behaviours were correlated with interspecific differences in bill-tip organ morphology. While it is difficult to comment on the causative interaction between the birds' behaviour and morphology in an evolutionary context, this provides support for the use of the morphological differences in the remote-touch bill-tip organ as proxies for their probing behaviours and substrate usage. This may be useful in studying fossil material of ibises and other remote-touch probing birds in terms of their behavioural ecology and habitat usage, provided that suitably well preserved material is available.

Chapter 3 additionally investigated the hypothesis formulated in Chapter 2, namely that species with higher densities of mechanoreceptors (which tended to forage in drier substrates on average) are more successful at using remote-touch, as they likely have more sensitive bill-tip organs (Zweers & Gerritsen, 1997). This was difficult to validate, as there were multiple other factors that could not be controlled under field conditions, and which have been shown to affect foraging success in other bird species [e.g., prey size, abundance, vegetation cover (Ontiveros, Pleguezuelos & Caro, 2005; Pierce & Gawlik, 2010; de Fouw *et al.*, 2016)]. I also sought to investigate whether increased moisture content of the birds' foraging substrates had an effect on their tactile foraging success. When most other factors could be constrained or accounted for, the results indicated that the ibises were more successful using tactile foraging methods in wetter substrates. However, I could not determine whether the birds were using remote-touch or direct contact to locate prey, and any increased success in wetter substrates could potentially have been attributed to increased ease of probing.

Thus, directly following on from the outcomes of Chapter 3, in Chapter 4 I investigated the effect of substrate moisture content on both direct contact and remote-touch probing under controlled conditions, using behavioural sensory assays on a group of captive Hadeda Ibises. Firstly, these experiments confirmed that they can use remote-touch to locate prey [as has been shown in other birds possessing similar bill-tip organs (Gerritsen & Meiboom, 1985; Piersma *et al.*, 1998; Nebel, Jackson & Elner, 2005; Cunningham, Castro & Potter, 2009; Cunningham, Castro *et al.*, 2010)]. Furthermore, the results support my hypothesis that substrate moisture content positively affects remote-touch foraging success when birds are seeking prey that produce vibratory cues. The notion that this increase in success rate is related to improved propagation of vibratory cues in wetter soil is supported by the fact that substrate moisture content had no effect on the birds' success rate when they were seeking dead worms (i.e., prey that did not create vibrations by moving through the soil). The fact that capture rates of dead worms were not affected by soil moisture content also indicates that increased ease of probing in wetter soils was not responsible for altering the Hadeda Ibises' success rate. This provides support for the hypothesis that the observed increased tactile foraging success of free-living Sacred and Glossy Ibises in wetter substrates (found in Chapter 3) may indeed be due to increased detectability of vibratory cues in these substrates. Taken together, the results of Chapters 3 and 4 indicate that remote-touch foraging in ibises is affected by substrate moisture content, and that it is likely that this could have selected for the interspecific differences in the morphology of the bill-tip organ.

As it is likely prey are easier to detect by remote-touch foragers in wetter substrates, we must consider why some ibis species (like Sacred and Hadeda Ibises) forage in drier substrates. One possibility is that the multitude of easily accessible prey in water and wetter substrates led to greater competition in these foraging habitats. Ibises foraging in these substrates compete with other remote-touch foraging birds and wading birds, as well as other animals which feed on similar prey. Species like Sacred and Hadeda

Ibises which can forage in drier soils have an advantage of being able to occupy other niches in the environment [the kiwi from New Zealand show an excellent example of another remote-touch probe-foraging taxa occupying a similar niche (Cunningham & Castro, 2011)]. While Sacred Ibises frequently utilize both wet and dry types of foraging substrates, the results of Chapter 3 [and the general consensus on Hadeda Ibis ecology (Hockey, Dean & Ryan, 2005)] indicate that Hadeda Ibises have become specialized to probe-forage in drier substrates. Thus, Hadeda Ibises may have evolved from a generalist ancestor (with similar foraging ecology to Sacred Ibises) and subsequently became more specialized over time, or they may have evolved in areas where they could only forage in drier substrates for some parts of the year. It is also possible that the ancestors of all ibises were more similar in ecology to Hadeda Ibises, and the wetter foraging species diverged from this situation. It is uncertain which evolutionary path is more likely as the phylogeny of the ibises is still very ambiguous [see section 6.5(f) for more details], and the ibis fossil record is rather patchy.

6.3: The remote-touch bill-tip organ in the fossil record and its evolution in Neornithes

Chapter 5 of my thesis broadens the focus of the work beyond the Threskiornithidae alone, and concerns the evolution of the remote-touch bill-tip organ in Neornithes as a whole.

The results of this chapter indicated that the remote-touch bill tip organ is a plesiomorphic trait in modern palaeognathous birds, and I hypothesize that it is vestigial in all but kiwi. Through examination of fossil material, I showed that the lithornithids [the oldest known palaeognathous birds, which evolved during the Cretaceous period and survived the K-Pg extinction event (Yonezawa *et al.*, 2017)] had functional bill-tip organs which they used for remote-touch probe-foraging. Whether they were the first palaeognathous birds to possess this bill-tip organ, or inherited it from an earlier ancestor is unclear. As the remote-touch bill-tip organ bears some resemblances to the bill-tip organ of Anseriformes (ducks and geese) (Gottschaldt & Lausmann, 1974; Gottschaldt, 1974; Avilova, Fedorenko & Lebedeva, 2018; Avilova, 2018; see also Chapter 1, section 1.2 for descriptions of other bill-tip organs in birds), it is possible that it may have been a trait common to an even older ancestor of Neornithes, but confirming this would require further examination of additional fossil bird taxa [see section 6.5(j) for potential cadidates]. Regardless, its presence in lithornithids shows that, at the very latest, the remote-touch bill-tip organ had evolved in birds by the Eocene (~55mya), and likely even earlier in the late Cretaceous period (>65mya). This makes remote-touch one of the oldest documented specialized foraging techniques in modern birds.

Previous research has indicated that the earliest Neornithes may have been transitional “shorebirds” (Feduccia, 1995; Field *et al.*, 2020), and probe-foraging was used as a foraging method by various taxa of birds during the Cretaceous (Hou *et al.*, 2004; Falk, Hasiotis & Martin, 2010). Several lines of evidence point towards probe-foraging birds having an advantage following the K-Pg extinction event: 1) the destruction of many forested habitats after the extinction event may have left non-arboreal birds at an advantage (Field, Bercovici *et al.*, 2018); 2) the availability of buried invertebrate prey, which has been documented almost directly following the extinction event (these invertebrates may have been buffered from the harsh climatic conditions by being adapted to live underground under low oxygen conditions and feed on dead plant matter) (Chin, Pearson & Ekdale, 2013); 3) the predominance in many locations of wetlands or "bogs", where before there had been plains or forests (Sweet & Braman, 1992). Furthermore, remote-touch probe-foraging may have conferred an additional advantage over direct contact "random" probing, as it is not as reliant on very high prey densities in order for birds to successfully locate prey (Hulscher, 1982; Gerritsen & Meiboom, 1985).

Studies showing indications of heightened tactile sensation in the snouts of some non-avian theropods (Ibrahim *et al.*, 2014; Barker *et al.*, 2017; Carr *et al.*, 2017) provide evidence that the basis for sensory specialisation of the rostra had already developed by the Cretaceous in this clade (Theropoda) which gave rise to modern birds. Furthermore, the plasticity among Neornithes (shown by the convergent evolution of remote-touch bill-tip organs in at least three distinct clades, as well as the other bill-tip organs seen in ducks and parrots) indicates that maxillary tactile specialisations can evolve under the correct selective pressure.

Exactly which environmental conditions facilitate the evolution or "re-emergence" (as in the case of kiwi, see Chapter 5) of the remote-touch bill-tip organ are unclear. Understanding when remote-touch first evolved in ibises and shorebirds would be important, as both these families (Threskiornithidae and Scolopacidae) appeared after the divergence of neognathous and palaeognathous birds, and after the split between galloanseres and Neoaves (see Figure 6.1). It would seem that the organ first evolved in palaeognathous birds (if it was not already present in a previous ancestor common to all Neornithes). The modern palaeognathous birds indicate that maintaining the morphology of a vestigial bill-tip organ is not developmentally costly, which could suggest that if it was common to the ancestor of all birds, we might expect to see vestiges of it in more neognathous birds. During my study of the beaks of modern birds, I noted novel, undescribed morphologies on the beaks of several taxa of birds, which could indicate some forms of tactile specialisations or osteological signatures of ancestral states. More research is required to investigate these “odd” morphologies, which appear to be different from remote-touch probing bill-tip organs [see section 6.5(e) for details].

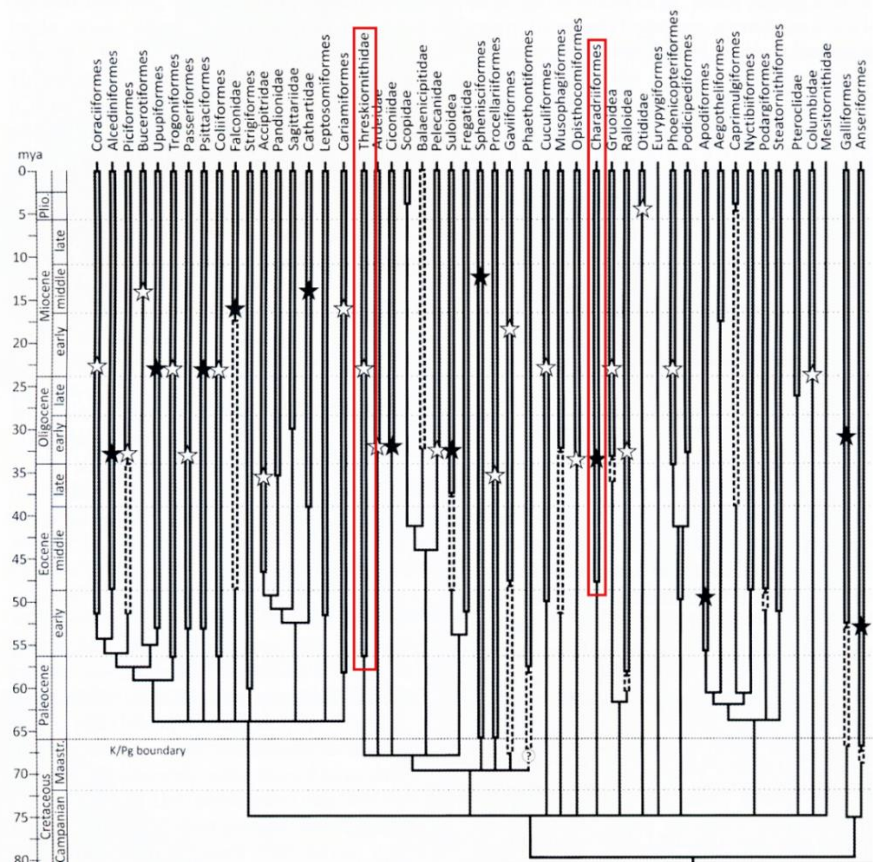

Figure 6.1: The earliest temporal occurrences of Neognathous birds [figure included and adapted from Mayr (2016), with permission]. Gray bars indicate temporal ranges; stars showing earliest occurrences of representatives in the fossil record. Red boxes demarcate the two groups of neognathous birds (ibises and scolopacid shorebirds) confirmed to have remote-touch bill-tip organs (Scolopacidae falls within the Charadriiform order).

One such example of a species of bird with notable bill-tip features is the New Zealand Snipe Raill, *Capellirallus karamu*. I concur with Cunningham (2010) and Olson (1975) that the *Capellirallus* beaks resemble remote-touch bill-tip organs, or at the very least a vestigial version (see Figure 6.2 for an example of the pitting pattern evident on a fossilized *Capellirallus* beak). However, my results from Chapter 5 indicate that no extant rails possess a bill-tip organ, vestigial or otherwise. There are three possible reasons for this: 1) *Capellirallus* has been misidentified as belonging to the family Rallidae, and rather represents an extinct Scolopacid or Threskiornithid; 2) *Capellirallus* represents a clade of Rallidae which went extinct, and the bill-tip organ evolved in this clade after it split from other rails, thus explaining the lack of the organ in extant rails; or 3) the bill-tip organ evolved before *Capellirallus* split from other rails, and the trait has been completely lost in other members of this family. The first option is unlikely, as its identification as a member of Rallidae has been confirmed by multiple different studies on both cranial and postcranial material (Falla, 1954; Olson, 1975; Olson, 1977; Livezey, 1998). I am inclined to tend towards option two, as other extant birds which evolved from a previous common ancestor with a remote-touch bill-tip organ (the non-kiwi palaeognathous birds, and potentially turnstones in Scolopacidae – see Chapter 5) still maintain the morphology of a vestigial organ in their beaks, which extant rails do not, providing evidence against option three. If either option 2 or 3 is the case, then the remote-touch bill-tip organ may have evolved convergently a fourth time in Neornithes in rails, providing even more support for the plasticity of the organ in birds.

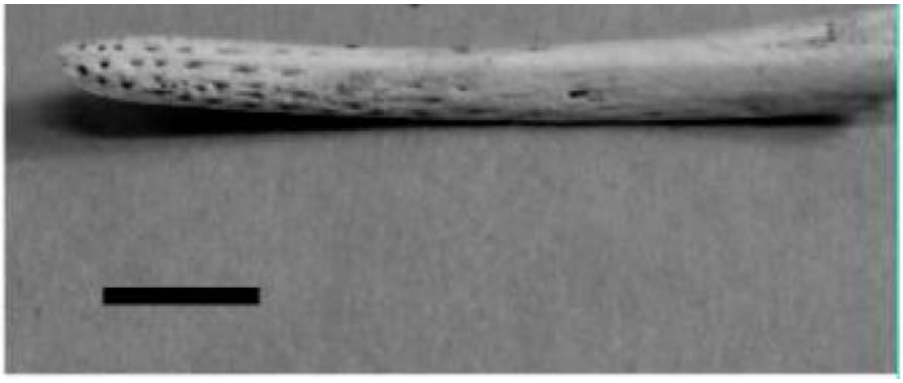

Figure 6.2: Ventral view of *Capellirallus karamu* mandible, showing extensive pitting on the beak bones. Scale bar 5 mm. Image from Cunningham (2010), photograph by S.J. Cunningham (included here with permission).

6.4: Methods for evaluating tactile bill-tip organs in extant and fossil birds

I have shown that the number of sensory pits/foramina on the distal portion of the beak bones (relative to beak length) reliably correlates with the mechanoreceptor arrangement in the beak soft tissues (see Chapter 5, Figures 5.4-5). This is highly significant, as it shows that the arrangements of foramina on the bone are a suitable proxy for studying bill-tip organs in the absence of soft tissues (as would be necessary for the study of fossil birds). In addition, I provided soft tissue histological sections of nine families of birds which had previously been undescribed [before my analyses in Chapter 5, only 12 families of birds had been sectioned for beak histology (see Chapter 5, section 5.2.2 & Appendix 5.1 for details)].

Using digital photography and free-to-use software, I developed methods for differentiating bony bill-tip organs based on their morphology and function, looking at measures of beak dimensions and the arrangements of the sensory pits on the bones. As I used a very broad sample encompassing all orders and most families of extant

Neornithes, this represents a robust analysis and good baseline sample that future specimens can be compared to. Furthermore, these methodologies can be performed remotely [using high resolution (~20MP) photographs of specimens that can be provided by museum curators], and are far cheaper than other analyses (e.g., CT scans). All the photographs of specimens I took are now accessible to other researchers on the museums' databases, and contributed to digitizing museum specimens (e.g., at the Smithsonian Institute, I provided high definition photographs of ~350 different species of extant birds' skulls and mandibles from multiple angles, most of which had not been digitized yet, providing nearly 8000 photographs which are now publicly available as part of their museum's online database).

6.5: Future Directions

I believe that there is substantial scope for further advancing our knowledge of remote-touch and other tactile specialisations in the beaks of modern birds and their relatives. Here are 12 potential areas for further research:

a) Compare the remote-touch capabilities of ibis species with different densities of mechanoreceptors in their bill-tip organs under controlled conditions, to assess whether the average density of Herbst corpuscles in the bill-tip organs of a species correlates with their remote-touch capability (as hypothesized based on the results of Chapters 2-4)

b) Test the remote-touch capability of the bald ibises (genus *Geronticus*), as both species show lower numbers of sensory pits on their beak bones than the other ibises sampled [based on personal examination of skeletal material, results from Chapter 5 and previous study (Cunningham, Alley *et al.*, 2010)]. Furthermore, they do not utilize probe-foraging very frequently, rather capturing prey visually (Manry, 1982; Ali & Ripley, 1983; Matheu & del Hoyo, 1992; Hockey, Dean & Ryan, 2005). This could indicate that these species are less reliant on remote-touch than other ibises, but requires testing on captive birds to assess further. Examination of soft tissue histology would also be valuable, but as both species are endangered or vulnerable to becoming endangered (IUCN BirdLife International Red List 2016) obtaining fresh material of their beaks may be difficult.

c) Test remote-touch capability of Ruddy turnstones (*Arenaria interpres*, family: Scolopacidae), as the data on their beaks from Chapter 5 indicates that their beak morphology [including the arrangement of Herbst corpuscles, as described by Bolze (1968)] resembles that of the non-kiwi palaeognathous birds. Furthermore, turnstones main foraging technique is searching for prey beneath small rocks and stones, often overturning them to expose prey (Whitfield, 1990; Hockey, Dean & Ryan, 2005). This indicates that they do not require remote-touch to locate prey, and having a long, thin highly perforated beak was likely selected against in the turnstones, as handling rocks could damage more fragile beaks (Zweers & Gerritsen, 1997). Thus, Ruddy turnstones may represent another example of a species with a plesiomorphic and potentially vestigial bill-tip organ.

d) Examine the soft tissue histology of the beaks of spoonbills (genus *Platalea*). Both previous research (Swennen & Yu, 2004) and the results from Chapter 5 indicate that they likely possess remote-touch bill-tip organs. I found that they possessed the highest total numbers of sensory pits of any species of birds sampled, and as they have the largest bill-tip organ surface area (due to the wide disc shape of the end of their beaks), they may possess highly a specialised tactile bill-tip organ. This could be confirmed through histological and behavioural studies.

e) During my review of beak bone material in Chapter 5, several groups of birds stood out as having unusual morphologies on their beaks, which may represent novel tactile sensitivities (i.e., not remote-touch, Anseriform or parrot bill-tip organs), vestigial organs (as in non-kiwi palaeognaths) or other sensory functions. My review of the soft tissue histology of birds emphasized how little is known in this area. There are several groups of birds which I believe warrant closer study: flamingos, pelicans, penguins, hornbills, Procellariiformes and certain herons stood out. I believe that there may still be several sensory specialisations in the beaks of multiple clades of birds that are as yet undescribed.

f) Resolve the interrelationships of the Threskiornithidae family using molecular phylogenetics. While the latest genetic evidence establishes that they fall within the Pelecaniform order of birds, and that their closest relatives are the herons (family: Ardeidae), the relationships within the family are less well understood. This will be useful in assessing the relationships between phylogeny and the morphology of their bill-tip organs.

g) A study by Schneider *et al.* (2019) on the expression of Piezo2 [encoding proteins which are involved in adapting mechanically activated currents in mechanoreceptive neurons (Coste *et al.*, 2010)] in the trigeminal ganglia of ducks (which possess a type of tactile bill-tip organ, see Chapter 5 for details) showed that the gene's expression and the number of mechanoreceptor neurons are elevated in species which utilize tactile foraging methods. This is the first study to investigate the molecular mechanism behind tactile sensitivity in the beaks of birds, and it would be extremely valuable to perform similar tests in the remote-touch probing birds, as this could provide a further line of evidence regarding their reliance on tactile foraging. It would also be useful to know whether this molecular mechanism is conserved across the different types of tactile bill-tip organs in birds, which I suspect may be the case, as the action of Piezo2 in mechanoreception is highly conserved in vertebrates (Anderson, Schneider & Bagriantsev, 2017; Zheng *et al.*, 2019).

h) Examine fossil material of ibises and scolopacid shorebirds to investigate the evolution of remote-touch bill-tip organs in these two families of neognathous birds. It may be worth studying fossils of birds from other taxa in the orders Pelecaniformes and Charadriiformes (which contain the ibises and shorebirds respectively, see Figure 6.3) and comparing them to the extant families.

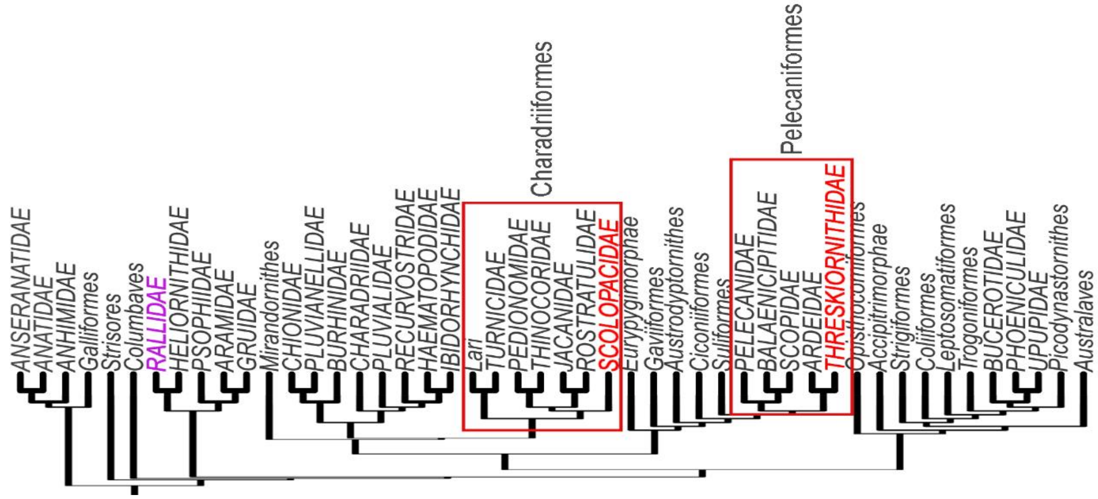

Figure 6.3: Phylogeny showing the confirmed occurrences of the remote-touch bill-tip organ in neognathous birds. In some clades, orders have been collapsed for simplicity (e.g., Australaves), and some orders have been expanded to show the constituent families in capital letters. The two groups which use remote-touch probe-foraging are shown in red. Purple indicates a potential third example of convergent evolution of the organ in rails. (Figure adjusted from Chapter 5, Figure 5.7C)

i) Thoroughly examine the available fossil material of *Capellirallus karamu*, to assess the functionality of the apparent remote-touch bill-tip organ in this species (Olson, 1975; Cunningham, 2010; see also Figure 6.2) and investigation of the evolution of bill-tip organs in Rallidae, as they may represent a fourth example of convergent evolution of remote-touch in Neornithes (see section 6.3 for details).

j) Examination of material of other fossil palaeognathous bird taxa would be valuable in further elucidating the unusual evolution of the bill-tip organs in this clade, as described in Chapter 5. Potentially interesting taxa would be *Diogenornis, Remiornis* and *Palaeotis,* provided suitably well preserved fossils of their beaks exist in order to make morphological comparisons to my dataset (or using alternative methodologies, should these arise in future studies).

k) Examination of fossil material of early neognathous birds may help elucidate whether the bill-tip organs of Anseriformes and palaeognathous birds are inherited with modification from a common ancestor among early Neornithes, or evolved convergently. Fossils from this time are scarce (particularly of well-preserved beaks) but not unheard of (Olson & Feduccia, 1980; Chatterjee, 2000; Field *et al.*, 2020). It may also be valuable to study the beaks of non-neornithine birds [e.g., *Hesperornis* and Ichthyornithiformes (Gingerich, 1973; Field, Hanson *et al.*, 2018)].

l) Other studies have indicated that some non-avian theropods had tactile specializations on their maxilla and mandibles (as evidenced by high numbers of neurovascular canals on the distal regions of their bones, though not nearly as extensive as what is seen in the remote-touch foraging birds, and their function is yet unclear) (Ibrahim *et al.*, 2014; Barker *et al.*, 2017; Carr *et al.*, 2017). The specimens studied thus far have been tyrannosaurs and spinosaurs, though it could be valuable to study specimens of non-avian paravians. It is possible that maxillary-tactile sensitivities evolved convergently among birds and non-avian theropods, or that the bill-tip organs of birds represent a highly specialised trait inherited from a non-avian theropod ancestor. Either may be likely, as the results from Chapter 5 indicate how plastic the bill-tip organ is (having evolving convergently at least three times in Neornithes) and how it can be maintained as a plesiomorphic vestigial trait for millions of years (in palaeognathous birds). Therefore, further study of fossil theropods closer to the origin of birds is clearly warranted.

6.6: Conclusions

Studies of the ecomorphology of birds' beaks are some of the most famous in modern biology, with Darwin's descriptions of the beak shapes of Galapagos finches in relation to their foraging habits being the most well-known (Darwin, 1859). Furthermore, research on avian sensory ecology is a rapidly advancing field. With such a vast diversity of modern birds, it is unsurprising that some possess novel sensory capabilities, such as remote-touch. What is surprising, is that even though this sensory modality and its associated bill-tip organ have been known of in the ornithological community for over 50 years, studies into the sense have been lacking.

Thus, I set out to expand our knowledge regarding this remarkable avian sensory system in this doctoral thesis. By using ibises as a focal group, I have shown that interspecific differences in the remote-touch bill-tip organ are reflected in both the bone and soft tissue, and that these differences appear to be related to differences in the birds' foraging ecology and habitat conditions. Furthermore, the results suggest that substrate moisture content positively affects the birds' ability to detect prey using remote-touch, and that this may play an important role in their foraging habitat selection. It would therefore be invaluable to better understand the remote-touch thresholds of ibises, kiwi and scolopacid shorebirds (several species of which are critically endangered) with regard to their requirements of their foraging habitats, and include these when studying the habitat usage of these birds.

I present the first conclusive evidence that the remote-touch bill-tip organ evolved very early in Neornithes. I also elucidate the mystery of the bill-tip organs found in the beaks of extant, non-probing palaeognathous birds as an organ inherited from a remote-touch probe-foraging common ancestor, which appears to be vestigial in all non-kiwi palaeognathous birds. I suggest that having access to this specialised foraging strategy may have helped some ancient birds survive the K-Pg extinction event. I also tentatively suggest that the remote-touch (and possibly other types of) bill-tip organs in birds may have evolved from other tactile specialisations on the snouts of their non-avian dinosaur ancestors.

Aside from the results and conclusions of my work, I have developed various methodologies that are inexpensive, effective and replicable to analyse the morphology of birds' beaks. I have greatly added to the sample of birds' beaks which have been studied for tactile specializations, created a freely accessible database that future specimens can be compared to [available on *Dryad* (du Toit, Chinsamy & Cunningham, 2020)], and significantly added to the specimen digitization efforts of multiple natural history museums around the world during the work on my thesis.

In writing the conclusion to my thesis, I am reminded of a concept which many aspiring sensory ecologists are taught: The study of sensory ecology must always be from the reference of the studied species and not the observer (humans). Understanding sensory modalities we do not possess can be particularly difficult, as contextualising sensory information we cannot process can be counterintuitive. It is even more challenging when the subjects we are studying have been dead for millions of years, and all we have to work with are remnants preserved in stone. Thus, there is still a great deal we do not know about remote-touch and other tactile specialisations that have evolved in birds' beaks. The interdisciplinary nature of the chapters of this thesis have shown how widely applicable such studies of sensory ecology can be, having substantial implications for our understanding of birds' habitat requirements, ecology and evolution, both in modern and ancient birds.

Hadeda Ibis (photographed by CJ du Toit)

6.7: References

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APPENDIX

CHAPTER 2 APPENDIX

Appendix 2.1: Adult ibis skeletal specimens and sources. “Glossy” = *Plegadis falcinellus;* “Hadeda” = *Bostrychia hagedash;* “Sacred” = *Threskiornis aethiopicus.* Accession number abbreviations: Iziko South African Museum (SAM); Smithsonian National Museum of Natural History (USNM); Natural History Museum at Tring (NHM); University of Cambridge Zoology Museum (UCZM); and Muséum national d’Histoire naturelle (MNHN). “UK” = sex of specimen unknown.

Specimen	Accession number	Source	Sex	Notes
GLOSSY				
G3	NHM 1850.11.14.13	Tring	UK	
G4	UCZM 327.AB	Cambridge	UK	
G5	UCZM 327.AA	Cambridge	UK	
G6	MNHN 1917-25	Paris	UK	
G7	MNHN 1945-170	Paris	UK	
G8	USNM 502698	Smithsonian	Male	
HADEDA				
H15	N/A	Rietvlei Wetland Reserve	UK	Specimen found by reserve manager after being naturally skeletonized
H16	SAM.ZO 58022	Iziko	UK	
H28	MNHN 1927-445	Paris	Male	
H29	USNM 431667	Smithsonian	Male	
SACRED				
S18	SAM.ZO 57162	Iziko	Male	
S21	SAM.ZO 58900	Iziko	UK	
S24	NHM 1860.11.4.8	Tring	UK	
S25	NHM S/2010.1.99	Tring	UK	
S26	NHM S/1952.1.109	Tring	Male	
S28	MNHN 1881-1143	Paris	UK	

Appendix 2.2: Specimens of ibises sectioned for soft tissue histology. "Fitz" = FitzPatrick Institute of African Ornithology, University of Cape Town; "SANCCOB" = Southern African Foundation for the Conservation of Coastal Birds, Cape Town, South Africa; "Eagle Encounters" (EE) = bird of prey rehabilitation centre in Stellenbosch, South Africa.

Specimen	Species	Source	Notes
H4	Hadeda	Fitz	Specimen donated to the department prior to my joining, source unknown
H8	Hadeda	Fitz	Specimen donated to the department prior to my joining, source unknown
H19	Hadeda	SANCCOB	Bird euthanized by SANCCOB as injuries were too severe
S22	Sacred	SANCCOB	Bird euthanized by SANCCOB as injuries were too severe
S23	Sacred	Eagle Encounters	Bird euthanized by EE as injuries were too severe
G1	Glossy	Fitz	Specimen donated to the department prior to my joining, source unknown
G9	Glossy	Fitz	Specimen donated to Fitz by falconer after his bird (Peregrine falcon) caught it

CHAPTER 4 APPENDIX

Appendix 4.1: Masses of 0.5l soil samples used to calculate water content of the "dry" soil (no water added). Measurements in grams. Wet mass measured before oven drying, dry mass after. Water mass = wet mass – dry mass.

Sample	Wet mass	Dry mass	Water mass
1	730.9	701.8	29.1
2	745.1	720.5	24.6
3	739.6	721.6	18
4	761.4	738.6	22.8
5	732.3	714.7	17.6
6	710.7	684.4	26.3
7	704.2	686.8	17.4
8	717.5	692.2	25.3
9	741.5	715.2	26.3
10	721.7	698	23.7

Appendix 4.2: Test statistics for two-sided Wilcoxon rank-sum tests for differences in prey capture rate (captures/min) between individual birds (see Figure 4.1A)

Comparison (Individuals)	n	Median	W-value	P-value	Effect size & CI*
A B	62 62	5.0 3.2	2548.5	0.001753	1.51 (0.52-2.50)
A C	62 65	5.0 2.8	2744.5	0.0004366	1.78 (0.83-2.69)
A D	62 31	5.0 3.4	1297	0.00624	1.47 (0.46-2.70)
B C	62 65	3.2 2.8	2156	0.4979	0.25 (-0.50-0.96)
B D	62 31	3.2 3.4	972	0.9318	0.04 (-0.93-0.90)
C D	65 31	2.8 3.4	917.5	0.483	-0.28 (-1.16-0.69)

*Effect size calculated as the median of the difference between a sample from x and a sample from y, with the associated 95% Confidence Interval (CI).

CHAPTER 5 APPENDIX

Appendix 5.1: Summary of bird species included in the review of soft tissue histology of the beak. Categories indicate arrangement of mechanoreceptors (Herbst corpuscles) in the soft tissue relative to the bone of the beak. Family and order classifications as per (Clements *et al.*, 2019)

Species	Family	Order	Group	Tip pits*	Accession number**	Histology source
Embedded in bony pits						
Apteryx australis	Apterygidae	Apterygiformes	Palaeognaths	33.64	AMNH 4437	Genus sampled: *A. mantelli* (Cunningham *et al.* (2007))
Apteryx owenii	Apterygidae	Apterygiformes	Palaeognaths	43.57	USNM 18279	Genus sampled: *A. mantelli* (Cunningham *et al.* (2007))
Dromaius novaehollandiae	Casuariidae	Casuariiformes	Palaeognaths	26.17	USNM 500379	Crole & Soley (2016)
Arenaria interpres	Scolopacidae	Charadriiformes	Neognaths	42.92	USNM 557596	Bolze (1968)
Calidris alpina	Scolopacidae	Charadriiformes	Neognaths	115.61	USNM 489713	Bolze (1968)
Calidris minuta	Scolopacidae	Charadriiformes	Neognaths	136.06	USNM 603490	Bolze (1968)
Calidris ruficollis	Scolopacidae	Charadriiformes	Neognaths	193.57	USNM 344531	Burton (1970)
Gallinago gallinago	Scolopacidae	Charadriiformes	Neognaths	80.45	USNM 501206	Bolze (1968)
Gallinago stenura	Scolopacidae	Charadriiformes	Neognaths	62.01	USNM 557035	Genus sampled: *G. gallinago* (Bolze (1968))
Limosa lapponica	Scolopacidae	Charadriiformes	Neognaths	16.57	USNM 555156	Bolze (1968)
Numenius arquata	Scolopacidae	Charadriiformes	Neognaths	18.40	USNM 614127	Bolze (1968)

Numenius borealis	Scolopacidae	Charadriiformes	Neognaths	26.74	USNM 7956	Genus sampled: *N. arquata* (Bolze (1968))
Scolopax rusticola	Scolopacidae	Charadriiformes	Neognaths	84.65	USNM 292760	Leydig (1868) & Clara (1925)
Tringa totanus	Scolopacidae	Charadriiformes	Neognaths	53.23	USNM 500649	Bolze (1968)
Bostrychia hagedash	Threskiornithidae	Pelecaniformes	Neognaths	60.48	USNM 431667	Specimen donated to this study
Plegadis falcinellus	Threskiornithidae	Pelecaniformes	Neognaths	142.48	USNM 502698	Specimen donated to this study
Threskiornis aethiopicus	Threskiornithidae	Pelecaniformes	Neognaths	80.40	SAM 57162	Genus sampled: *T. molucca* (Cunningham *et al.* (2010))
Spheniscus demersus	Spheniscidae	Sphenisciformes	Neognaths	13.24	USNM 346396	Specimen donated to this study
Struthio camelus	Struthionidae	Struthioniformes	Palaeognaths	33.41	USNM 557829	Crole & Soley (2016)
Eudromia elegans	Tinamidae	Tinamiformes	Palaeognaths	83.14	USNM 345096	Specimen loan: USNM 344980
Soft tissue surrounding bone						
Charadrius alexandrinus	Charadriidae	Charadriiformes	Neognaths	7.96	USNM 555302	Bolze (1968)
Pluvialis squatarola	Charadriidae	Charadriiformes	Neognaths	5.82	USNM 502630	Bolze (1968)
Vanellus vanellus	Charadriidae	Charadriiformes	Neognaths	9.46	USNM 322391	Bolze (1968)

Larus dominicanus	Laridae	Charadriiformes	Neognaths	7.08	USNM 346876	Specimen donated to this study
Columba livia	Columbidae	Columbiformes	Neognaths	10.80	USNM 346786	Fleissner *et al.* (2003)
Coturnix japonica	Phasianidae	Galliformes	Neognaths	19.66	USNM 344514	Halata & Grim (1993)
Gallus domesticus	Phasianidae	Galliformes	Neognaths	6.96	USNM 500372	Wight *et al.* (1970)
Meleagris gallopavo	Phasianidae	Galliformes	Neognaths	6.12	USNM 556351	Sayed *et al.* (2014)
Anthropoides paradisea	Gruidae	Gruiformes	Neognaths	3.04	USNM 346362	Specimen donated to this study
Anthropoides virgo	Gruidae	Gruiformes	Neognaths	4.20	USNM 555672	Genus sampled: *A. paradisea*
Padda oryzivora	Estrildidae	Passeriformes	Neognaths	7.52	USNM 556082	Genbrugge *et al.* (2012)
Onychognathus morio	Sturnidae	Passeriformes	Neognaths	8.14	USNM 491347	Specimen donated to this study
Scopus umbretta	Scopidae	Pelecaniformes	Neognaths	7.61	USNM 431669	Specimen loan: USNM NZP.215513
Diomedea exulans	Diomedeidae	Procellariiformes	Neognaths	6.68	USNM 559552	Specimen donated to this study
Procellaria aequinoctialis	Procellariidae	Procellariiformes	Neognaths	5.49	USNM 559787	Specimen donated to this study
Amazona ochrocephala	Psittacidae	Psittaciformes	Neognaths	13.89	USNM 343132	Goujon (1968)
Ara macao	Psittacidae	Psittaciformes	Neognaths	7.89	USNM 226164	Goujon (1968)

Psittacula eupatria	Psittaculidae	Psittaciformes	Neognaths	6.20	USNM 343237	Goujon (1968)
Morus capensis	Sulidae	Suliformes	Neognaths	11.66	USNM 292389	Specimen donated to this study
Touch papillae						
Anas acuta	Anatidae	Anseriformes	Neognaths	26.68	USNM 502680	Avilova *et al.* (2018)
Anas clypeata	Anatidae	Anseriformes	Neognaths	24.72	USNM 489915	Avilova *et al.* (2018)
Anas crecca	Anatidae	Anseriformes	Neognaths	52.36	USNM 431532	Avilova *et al.* (2018)
Anas falcata	Anatidae	Anseriformes	Neognaths	33.13	USNM 647319	Avilova *et al.* (2018)
Anas penelope	Anatidae	Anseriformes	Neognaths	49.12	USNM 431541	Avilova *et al.* (2018)
Anas platyrhynchos	Anatidae	Anseriformes	Neognaths	30.95	USNM 288486	Berkhoudt (1970)
Anas querquedula	Anatidae	Anseriformes	Neognaths	40.35	USNM 499910	Avilova *et al.* (2018)
Anas rhynchotis	Anatidae	Anseriformes	Neognaths	25.95	USNM 554699	Genus sampled: (Avilova *et al.* (2018) & Berkhoudt (1970))
Anser albifrons	Anatidae	Anseriformes	Neognaths	29.58	USNM 489754	Gottschadt & Lausmann (1971)
Anser anser	Anatidae	Anseriformes	Neognaths	18.25	USNM 290322	Gottschadt & Lausmann (1970)
Aythya ferina	Anatidae	Anseriformes	Neognaths	29.17	USNM 431534	Avilova *et al.* (2018)
Mergellus albellus	Anatidae	Anseriformes	Neognaths	28.52	USNM 502415	Avilova *et al.* (2018)

Tadorna tadorna	Anatidae	Anseriformes	Neognaths	16.24	USNM 614124	Avilova *et al.* (2018)
Fossil specimens						
†Aepyornithid sp.		†Aepyornithiformes	Palaeognaths	40.05	MNHN F.MAD8903	N/A
†*Dinornis* sp.	†Dinornithidae	†Dinornithiformes	Palaeognaths	32.63	USNM 6615	N/A
†*Lithornis promiscuus*		†Lithornithiformes	Palaeognaths	58.66	USNM 336535	N/A
†*Paracathartes howardae*		†Lithornithiformes	Palaeognaths	29.53-35.96***	USNM 404758 USNM 361437	N/A

* pits/mm: Number of bony pits on the tip (distal most 20%) of the beak, relative to tip length;

** Museum accession number of skeletal specimen used for analysis of pitting pattern;

*** Range based on reconstructions of beak shape (see Figure 5.8);

† Extinct

Appendix 5.2: Data for all extant and fossil species included in the pitting pattern and geometric morphometric beak shape analyses, with accession numbers of specimens used. Family and order classifications as per (Clements *et al.*, 2019)

Species	Family	Order	Bill-tip category*	Accession number
Accipiter nisus	Accipitridae	Accipitriformes	Other	AMNH 27050
Buteo magnirostris	Accipitridae	Accipitriformes	Other	USNM 289803
Circus melanoleucos	Accipitridae	Accipitriformes	Other	USNM 289863
Haliaeetus leucocephalus	Accipitridae	Accipitriformes	Other	USNM 489276
Pandion haliaetus	Pandionidae	Accipitriformes	Other	USNM 614480
Sagittarius serpentarius	Sagittariidae	Accipitriformes	Other	USNM 431491
†Aepyornithid sp.		†Aepyornithiformes	Fossil	MNHN F.MAD8903
Anas acuta	Anatidae	Anseriformes	NPBTO	USNM 502680
Anas clypeata	Anatidae	Anseriformes	NPBTO	USNM 489915
Anas crecca	Anatidae	Anseriformes	NPBTO	USNM 431532
Anas falcata	Anatidae	Anseriformes	NPBTO	USNM 647319
Anas penelope	Anatidae	Anseriformes	NPBTO	USNM 431541
Anas platyrhynchos	Anatidae	Anseriformes	NPBTO	USNM 288486
Anas querquedula	Anatidae	Anseriformes	NPBTO	USNM 499910
Anas rhynchotis	Anatidae	Anseriformes	NPBTO	USNM 554699
Anser albifrons	Anatidae	Anseriformes	NPBTO	USNM 489754
Anser anser	Anatidae	Anseriformes	NPBTO	USNM 290322
Aythya ferina	Anatidae	Anseriformes	NPBTO	USNM 431534
Mergellus albellus	Anatidae	Anseriformes	NPBTO	USNM 502415
Tadorna tadorna	Anatidae	Anseriformes	NPBTO	USNM 614124
Chauna torquata	Anhimidae	Anseriformes	Other	USNM 344619
Anseranas semipalmata	Anseranatidae	Anseriformes	NPBTO	AMNH 1772
Apteryx australis	Apterygidae	Apterygiformes	RTP	AMNH 4437
Apteryx owenii	Apterygidae	Apterygiformes	RTP	USNM 18279
Buceros hydrocorax	Bucerotidae	Bucerotiformes	Other	USNM 613076
Tockus erythrorhynchus	Bucerotidae	Bucerotiformes	Other	USNM 321102
Bucorvus cafer	Bucorvidae	Bucerotiformes	Other	USNM 429091
Phoeniculus purpureus	Phoeniculidae	Bucerotiformes	DTP	USNM 558552
Upupa epops	Upupidae	Bucerotiformes	DTP	USNM 603638
Aegotheles cristatus	Aegothelidae	Caprimulgiformes	Other	USNM 227841
Apus affinis	Apodidae	Caprimulgiformes	Other	USNM 642848
Chaetura andrei	Apodidae	Caprimulgiformes	Other	USNM 561284
Cypsiurus parvus	Apodidae	Caprimulgiformes	Other	USNM 347445
Antrostomus vociferus	Caprimulgidae	Caprimulgiformes	Other	AMNH 26577
Caprimulgus macrurus	Caprimulgidae	Caprimulgiformes	Other	USNM 557183
Hemiprocne mystacea	Hemiprocnidae	Caprimulgiformes	Other	USNM 560828
Nyctibius grandis	Nyctibiidae	Caprimulgiformes	Other	USNM 623085

Podargus papuensis	Podargidae	Caprimulgiformes	Other	USNM 614956
Steatornis caripensis	Steatornithidae	Caprimulgiformes	Other	AMNH 22733
Anthracothorax mango	Trochilidae	Caprimulgiformes	Other	USNM 556857
Calypte anna	Trochilidae	Caprimulgiformes	Other	USNM 19782
Klais guimeti	Trochilidae	Caprimulgiformes	Other	USNM 559997
Cariama cristata	Cariamidae	Cariamiformes	Other	AMNH 1722
Casuarius casuarius	Casuariidae	Casuariiformes	NKP	AMNH 963
Dromaius novaehollandiae	Casuariidae	Casuariiformes	NKP	USNM 500379
Cathartes aura	Cathartidae	Cathartiformes	Other	USNM 345340
Brachyramphus brevirostris	Alcidae	Charadriiformes	Other	USNM 288086
Fratercula corniculata	Alcidae	Charadriiformes	Other	USNM 610510
Uria lomvia	Alcidae	Charadriiformes	Other	USNM 502361
Burhinus vermiculatus	Burhinidae	Charadriiformes	Other	USNM 430630
Charadrius alexandrinus	Charadriidae	Charadriiformes	Other	USNM 555302
Pluvialis squatarola	Charadriidae	Charadriiformes	Other	USNM 502630
Vanellus vanellus	Charadriidae	Charadriiformes	Other	USNM 322391
Chionis alba	Chionidae	Charadriiformes	Other	USNM 489489
Dromas ardeola	Dromadidae	Charadriiformes	Other	USNM 488404
Cursorius temminckii	Glareolidae	Charadriiformes	Other	USNM 431709
Haematopus moquini	Haematopodidae	Charadriiformes	DTP	USNM 558480
Ibidorhyncha struthersii	Ibidorhynchidae	Charadriiformes	DTP	USNM 292767
Actophilornis africana	Jacanidae	Charadriiformes	Other	USNM 488156
Anous stolidus	Laridae	Charadriiformes	Other	USNM 431405
Larus dominicanus	Laridae	Charadriiformes	Other	USNM 346876
Rynchops flavirostris	Laridae	Charadriiformes	Other	USNM 430835
Pedionomus torquatus	Pedionomidae	Charadriiformes	Other	USNM 555614
Pluvianus aegyptius	Pluvianidae	Charadriiformes	Other	USNM 491870
Himantopus himantopus	Recurvirostridae	Charadriiformes	DTP	USNM 558481
Recurvirostra avosetta	Recurvirostridae	Charadriiformes	Other	USNM 610452
Nycticryphes semicollaris	Rostratulidae	Charadriiformes	DTP	USNM 612032
Rostratula benghalensis	Rostratulidae	Charadriiformes	DTP	USNM 431501
Arenaria interpres	Scolopacidae	Charadriiformes	NPBTO	USNM 557596
Calidris alpina	Scolopacidae	Charadriiformes	RTP	USNM 489713
Calidris minuta	Scolopacidae	Charadriiformes	RTP	USNM 603490
Calidris ruficollis	Scolopacidae	Charadriiformes	RTP	USNM 344531
Gallinago gallinago	Scolopacidae	Charadriiformes	RTP	USNM 501206
Gallinago stenura	Scolopacidae	Charadriiformes	RTP	USNM 557035
Heteroscelus incanus	Scolopacidae	Charadriiformes	RTP	USNM 557551
Limnodromus scolopaceus	Scolopacidae	Charadriiformes	RTP	USNM 638734
Limosa lapponica	Scolopacidae	Charadriiformes	RTP	USNM 555156
Numenius arquata	Scolopacidae	Charadriiformes	RTP	USNM 614127
Numenius borealis	Scolopacidae	Charadriiformes	RTP	USNM 7956
Phalaropus fulicarius	Scolopacidae	Charadriiformes	RTP	USNM 491732
Scolopax rusticola	Scolopacidae	Charadriiformes	RTP	USNM 292760

Tringa totanus	Scolopacidae	Charadriiformes	RTP	USNM 500649
Xenus cinereus	Scolopacidae	Charadriiformes	RTP	USNM 488337
Stercorarius parasiticus	Stercorariidae	Charadriiformes	Other	USNM 613810
Attagis malouinus	Thinocoridae	Charadriiformes	Other	USNM 490853
Turnix suscitator	Turnicidae	Charadriiformes	Other	USNM 562149
Ciconia ciconia	Ciconiidae	Ciconiiformes	Other	USNM 605011
Colius macrourus	Coliidae	Coliiformes	Other	USNM 499815
Chalcophaps stephani	Columbidae	Columbiformes	Other	USNM 615023
Columba livia	Columbidae	Columbiformes	Other	USNM 346786
Drepanoptila holosericea	Columbidae	Columbiformes	Other	USNM 562454
Goura cristata	Columbidae	Columbiformes	Other	USNM 345081
Macropygia amboinensis	Columbidae	Columbiformes	Other	USNM 557039
Ptilinopus superbus	Columbidae	Columbiformes	Other	USNM 558284
Chloroceryle americana	Alcedinidae	Coraciiformes	Other	USNM 560164
Dacelo novaeguineae	Alcedinidae	Coraciiformes	Other	USNM 620232
Brachypteracias leptosomus	Brachypteraciidae	Coraciiformes	Other	USNM 223863
Coracias caudata	Coraciidae	Coraciiformes	Other	USNM 638622
Merops bulocki	Meropidae	Coraciiformes	Other	USNM 322418
Momotus momota	Momotidae	Coraciiformes	Other	USNM 428664
Todus angustirostris	Todidae	Coraciiformes	Other	USNM 554620
Centropus superciliosus	Cuculidae	Cuculiformes	Other	USNM 430785
Crotophaga major	Cuculidae	Cuculiformes	Other	USNM 430932
Piaya melanogaster	Cuculidae	Cuculiformes	Other	USNM 622314
†*Dinornis* sp.	†Dinornithidae	†Dinornithiformes	Fossil	USNM 6615
Eurypyga helias	Eurypygidae	Eurypygiformes	Other	USNM 492379
Rhynochetos jubatus	Rhynochetidae	Eurypygiformes	Other	USNM 18994
Caracara plancus	Falconidae	Falconiformes	Other	AMNH 888
Falco peregrinus	Falconidae	Falconiformes	Other	AMNH 31659
Malacoptila rufa	Bucconidae	Galbuliformes	Other	USNM 562213
Nystalus maculatus	Bucconidae	Galbuliformes	Other	AMNH 25636
Ortalis canicollis	Cracidae	Galliformes	Other	USNM 345802
Penelope superciliaris	Cracidae	Galliformes	Other	USNM 345798
Megapodius freycinet	Megapodiidae	Galliformes	Other	USNM 557003
Numida meleagris	Numididae	Galliformes	Other	PRIVATE
Dendrortyx leucophrys	Odontophoridae	Galliformes	Other	USNM 343477
Coturnix japonica	Phasianidae	Galliformes	Other	USNM 344514
Gallus domesticus	Phasianidae	Galliformes	Other	USNM 500372
Meleagris gallopavo	Phasianidae	Galliformes	Other	USNM 556351
Tragopan temminckii	Phasianidae	Galliformes	Other	USNM 491867
Gavia pacifica	Gaviidae	Gaviiformes	Other	USNM 346557
Aramus guarauna	Aramidae	Gruiformes	DTP	USNM 614588
Anthropoides paradisea	Gruidae	Gruiformes	Other	USNM 346362
Anthropoides virgo	Gruidae	Gruiformes	Other	USNM 555672
Heliornis fulica	Heliornithidae	Gruiformes	Other	USNM 345807

Psophia viridis	Psophiidae	Gruiformes	Other	USNM 320971
Aramides ypecaha	Rallidae	Gruiformes	Other	USNM 614592
Fulica americana	Rallidae	Gruiformes	Other	USNM 19781
Porphyrio poliocephalus	Rallidae	Gruiformes	Other	USNM 343212
Rallus limicola	Rallidae	Gruiformes	DTP	USNM 489973
Leptosomus discolor	Leptosomidae	Leptosomiformes	Other	USNM 291845
†*Lithornis promiscuus*		†Lithornithiformes	Fossil	USNM 336535 USNM 391983
†Messel *Lithornis* sp.		†Lithornithiformes	Fossil	IRSNB Av82
†*Paracathartes howardae* (i)				
†*Paracathartes howardae* (ii)		†Lithornithiformes	Fossil	USNM 404758 USNM 361437
†*Paracathartes howardae* (iii)				
†*Pseudocrypturus cercanaxius*		†Lithornithiformes	Fossil	USNM 336103
Monias benschi	Mesitornithidae	Mesitornithiformes	Other	USNM 290927
Tauraco porphyreolophus	Musophagidae	Musophagiformes	Other	USNM 490282
Opisthocomus hoazin	Opisthocomidae	Opisthocomiformes	Other	USNM 344065
Choriotis kori	Otididae	Otidiformes	Other	USNM 631759
Smicrornis brevirostris	Acanthizidae	Passeriformes	Other	USNM 559031
Acrocephalus scirpaceus	Acrocephalidae	Passeriformes	Other	USNM 604151
Hippolais icterina	Acrocephalidae	Passeriformes	Other	USNM 490515
Aegithalos concinnus	Aegithalidae	Passeriformes	Other	USNM 318034
Aegithina tiphia	Aegithinidae	Passeriformes	Other	USNM 562095
Alaemon alaudipes	Alaudidae	Passeriformes	Other	USNM 502120
Mirafra assamica	Alaudidae	Passeriformes	Other	USNM 343274
Artamus fuscus	Artamidae	Passeriformes	Other	USNM 557514
Strepera versicolor	Artamidae	Passeriformes	Other	USNM 103690
Bombycilla cedrorum	Bombycillidae	Passeriformes	Other	USNM 610542
Buphagus erythrorhynchus	Buphagidae	Passeriformes	Other	USNM 431826
Calcarius ornatus	Calcariidae	Passeriformes	Other	USNM 320795
Creadion carunculatus	Callaeidae	Passeriformes	Other	USNM 18289
Coracina pectoralis	Campephagidae	Passeriformes	Other	USNM 489443
Lalage leucopyga	Campephagidae	Passeriformes	Other	USNM 561831
Passerina amoena	Cardinalidae	Passeriformes	Other	USNM 561906
Pheucticus ludovicianus	Cardinalidae	Passeriformes	Other	USNM 428016
Certhia familiaris	Certhiidae	Passeriformes	Other	USNM 491694
Chaetops frenatus	Chaetopidae	Passeriformes	Other	USNM 558651
Chloropsis cyanopogon	Chloropseidae	Passeriformes	Other	USNM 292230
Cinclus cinclus	Cinclidae	Passeriformes	Other	USNM 499067
Cisticola juncidis	Cisticolidae	Passeriformes	Other	USNM 603270
Eremomela icteropygialis	Cisticolidae	Passeriformes	Other	USNM 430696
Orthotomus atrogularis	Cisticolidae	Passeriformes	Other	USNM 613174
Conopophaga castaneiceps	Conopophagidae	Passeriformes	Other	USNM 491523
Corcorax melanorhamphos	Corcoracidae	Passeriformes	Other	USNM 227842
Corvus albicollis	Corvidae	Passeriformes	Other	USNM 620703

Gymnorhinus cyanocephalus	Corvidae	Passeriformes	Other	USNM 614439
Ptilostomus afer	Corvidae	Passeriformes	Other	USNM 291459
Lipaugus vociferans	Cotingidae	Passeriformes	Other	USNM 429369
Pipreola formosa	Cotingidae	Passeriformes	Other	USNM 344560
Dicaeum australe	Dicaeidae	Passeriformes	Other	USNM 562833
Dicrurus ludwigii	Dicruridae	Passeriformes	Other	USNM 431912
Donacobius atricapilla	Donacobiidae	Passeriformes	Other	USNM 346088
Dulus dominicus	Dulidae	Passeriformes	Other	USNM 555861
Emberiza calandra	Emberizidae	Passeriformes	Other	USNM 431290
Lonchura striata	Estrildidae	Passeriformes	Other	USNM 343368
Padda oryzivora	Estrildidae	Passeriformes	Other	USNM 556082
Psarisomus dalhousiae	Eurylaimidae	Passeriformes	Other	USNM 557509
Formicarius rufipectus	Formicariidae	Passeriformes	Other	USNM 613308
Haemorhous mexicanus	Fringillidae	Passeriformes	Other	AMNH 32231
Loxia curvirostra	Fringillidae	Passeriformes	Other	USNM 489848
Cinclodes patagonicus	Furnariidae	Passeriformes	Other	USNM 491018
Lepidocolaptes albolineatus	Furnariidae	Passeriformes	Other	USNM 346003
Nasica longirostris	Furnariidae	Passeriformes	Other	USNM 562241
Phacellodomus rufifrons	Furnariidae	Passeriformes	Other	USNM 559143
Synallaxis erythrothorax	Furnariidae	Passeriformes	Other	USNM 344905
Syndactyla ruficollis	Furnariidae	Passeriformes	Other	USNM 643969
Grallaria ruficapilla	Grallariidae	Passeriformes	Other	USNM 614859
Hylopezus macularius	Grallariidae	Passeriformes	Other	USNM 637111
Petrochelidon ariel	Hirundinidae	Passeriformes	Other	AMNH 18728
Hyliota flavigaster	Hyliotidae	Passeriformes	Other	USNM 322540
Hypocolius ampelinus	Hypocoliidae	Passeriformes	Other	USNM 645975
Cacicus leucoramphus	Icteridae	Passeriformes	Other	USNM 428817
Icterus chrysater	Icteridae	Passeriformes	Other	USNM 428273
Pseudoleistes virescens	Icteridae	Passeriformes	Other	USNM 614732
Icteria virens	Icteriidae	Passeriformes	Other	USNM 560449
Irena cyanogastra	Irenidae	Passeriformes	Other	USNM 613152
Lanius collurio	Laniidae	Passeriformes	Other	AMNH 31558
Alcippe striaticollis	Leiothrichidae	Passeriformes	Other	USNM 319314
Heterophasia capistrata	Leiothrichidae	Passeriformes	Other	USNM 347624
Locustella luscinioides	Locustellidae	Passeriformes	Other	USNM 604060
Sphenoeacus afer	Macrosphenidae	Passeriformes	Other	USNM 558700
Laniarius leucorhynchus	Malaconotidae	Passeriformes	Other	USNM 347507
Tchagra australis	Malaconotidae	Passeriformes	Other	USNM 430668
Malurus lamberti	Maluridae	Passeriformes	Other	USNM 620249
Oedistoma iliolophum	Melanocharitidae	Passeriformes	Other	USNM 489217
Melanopareia elegans	Melanopareiidae	Passeriformes	Other	USNM 500591
Myza celebensis	Meliphagidae	Passeriformes	Other	USNM 226226
Philemon eichhorni	Meliphagidae	Passeriformes	Other	USNM 615066
Menura novaehollandiae	Menuridae	Passeriformes	Other	USNM 632118

Melanotis hypoleucus	Mimidae	Passeriformes	Other	USNM 343485
Mimus thenca	Mimidae	Passeriformes	Other	USNM 318416
Mitrospingus oleagineus	Mitrospingidae	Passeriformes	Other	USNM 504883
†*Moho braccatus*	†Mohoidae	Passeriformes	Other	USNM 19125
Clytorhynchus pachycephaloides	Monarchidae	Passeriformes	Other	USNM 561680
Hypothymis azurea	Monarchidae	Passeriformes	Other	USNM 613179
Anthus rubescens	Motacillidae	Passeriformes	Other	USNM 489803
Macronyx croceus	Motacillidae	Passeriformes	Other	USNM 321656
Ficedula parva	Muscicapidae	Passeriformes	Other	USNM 344031
Phoenicurus phoenicurus	Muscicapidae	Passeriformes	Other	USNM 603894
Saxicola torquata	Muscicapidae	Passeriformes	Other	USNM 605104
Aethopyga gouldiae	Nectariniidae	Passeriformes	Other	USNM 319352
Arachnothera longirostra	Nectariniidae	Passeriformes	Other	USNM 559119
Nesospingus speculiferus	Nesospingidae	Passeriformes	Other	USNM 501895
Nicator gularis	Nicatoridae	Passeriformes	Other	USNM 634576
Oriolus brachyrynchus	Oriolidae	Passeriformes	Other	USNM 347466
Sphecotheres viridis	Oriolidae	Passeriformes	Other	USNM 612639
Colluricincla megarhyncha	Pachycephalidae	Passeriformes	Other	USNM 489093
Pachycephala pectoralis	Pachycephalidae	Passeriformes	Other	USNM 557434
Panurus biarmicus	Panuridae	Passeriformes	Other	USNM 621229
Paradisaea apoda	Paradisaeidae	Passeriformes	Other	AMNH 4101
Oreocharis arfaki	Paramythiidae	Passeriformes	Other	USNM 489229
Pardalotus striatus	Pardalotidae	Passeriformes	Other	USNM 632126
Setophaga ruticilla	Parulidae	Passeriformes	Other	USNM 555887
Atlapetes torquatus	Passerellidae	Passeriformes	Other	USNM 643888
Junco hyemalis	Passerellidae	Passeriformes	Other	USNM 621176
Spizella pallida	Passerellidae	Passeriformes	Other	USNM 491156
Hypocryptadius cinnamomeus	Passeridae	Passeriformes	Other	USNM 488947
Passer montanus	Passeridae	Passeriformes	Other	USNM 641818
Drymodes superciliaris	Petroicidae	Passeriformes	Other	USNM 489081
Petroica goodenovii	Petroicidae	Passeriformes	Other	USNM 614827
Peucedramus taeniatus	Peucedramidae	Passeriformes	Other	USNM 555657
Microligea palustris	Phaenicophilidae	Passeriformes	Other	USNM 555890
Phylloscopus ruficapilla	Phylloscopidae	Passeriformes	Other	USNM 558677
Picathartes oreas	Picathartidae	Passeriformes	Other	USNM 291348
Pipra fasciicauda	Pipridae	Passeriformes	Other	USNM 346065
Pitta angolensis	Pittidae	Passeriformes	Other	USNM 491222
Pityriasis gymnocephala	Pityriasidae	Passeriformes	Other	USNM 559905
Platylophus galericulatus	Platylophidae	Passeriformes	Other	USNM 613279
Batis moliter	Platysteiridae	Passeriformes	Other	USNM 558715
Euplectes hordeaceus	Ploceidae	Passeriformes	Other	USNM 429166
Ploceus bojeri	Ploceidae	Passeriformes	Other	USNM 556958
Microbates cinereiventris	Polioptilidae	Passeriformes	Other	USNM 491172
Pomatostomus ruficeps	Pomatostomidae	Passeriformes	Other	USNM 612730

Promerops cafer	Promeropidae	Passeriformes	Other	USNM 558756
Prunella rubeculoides	Prunellidae	Passeriformes	Other	USNM 319260
Ptiliogonys cinereus	Ptiliogonatidae	Passeriformes	Other	AMNH 2350
Amblyornis macgregoriae	Ptilonorhynchidae	Passeriformes	Other	AMNH 31740
Chlorocichla flaviventris	Pycnonotidae	Passeriformes	Other	USNM 492010
Pycnonotus xanthorrhous	Pycnonotidae	Passeriformes	Other	USNM 292149
Regulus calendula	Regulidae	Passeriformes	Other	USNM 490142
Rhagologus leucostigma	Rhagologidae	Passeriformes	Other	AMNH 31763
Pteroptochos megapodius	Rhinocryptidae	Passeriformes	Other	USNM 318406
Scytalopus femoralis	Rhinocryptidae	Passeriformes	Other	USNM 491515
Rhipidura threnothorax	Rhipiduridae	Passeriformes	Other	USNM 489173
Rhodinocichla rosea	Rhodinocichlidae	Passeriformes	Other	USNM 562707
Urosphena squameiceps	Scotocercidae	Passeriformes	Other	USNM 344033
Sitta pusilla	Sittidae	Passeriformes	Other	USNM 499704
Spindalis zena	Spindalidae	Passeriformes	Other	USNM 554543
Acridotheres fuscus	Sturnidae	Passeriformes	Other	USNM 611231
Onychognathus morio	Sturnidae	Passeriformes	Other	USNM 491347
Streptocitta albicollis	Sturnidae	Passeriformes	Other	USNM 226204
Paradoxornis paradoxus	Sylviidae	Passeriformes	Other	USNM 321830
Sylvia nisoria	Sylviidae	Passeriformes	Other	USNM 603246
Teretistris fornsi	Teretistridae	Passeriformes	Other	USNM 555211
Drymophila caudata	Thamnophilidae	Passeriformes	Other	USNM 490038
Dysithamnus plumbeus	Thamnophilidae	Passeriformes	Other	USNM 560043
Formicivora grisea	Thamnophilidae	Passeriformes	Other	USNM 345981
Thamnophilus caerulescens	Thamnophilidae	Passeriformes	Other	USNM 555971
Catamenia inornata	Thraupidae	Passeriformes	Other	USNM 428755
Conirostrum leucogenys	Thraupidae	Passeriformes	Other	USNM 347201
Dacnis venusta	Thraupidae	Passeriformes	Other	USNM 610294
Poospiza torquata	Thraupidae	Passeriformes	Other	USNM 645271
Tangara cayana	Thraupidae	Passeriformes	Other	USNM 621092
Tichodroma muraria	Tichodromidae	Passeriformes	Other	USNM 292842
Dumetia hyperythra	Timaliidae	Passeriformes	Other	USNM 346832
Pomatorhinus ruficollis	Timaliidae	Passeriformes	Other	USNM 611898
Pachyramphus homochrous	Tityridae	Passeriformes	Other	USNM 555508
Schiffornis turdinus	Tityridae	Passeriformes	Other	USNM 620163
Campylorhynchus griseus	Troglodytidae	Passeriformes	Other	USNM 347183
Troglodytes aedon	Troglodytidae	Passeriformes	Other	USNM 611150
Turdus fuscater	Turdidae	Passeriformes	Other	USNM 428064
Zoothera mollissima	Turdidae	Passeriformes	Other	USNM 319296
Myiopagis viridicata	Tyrannidae	Passeriformes	Other	USNM 610275
Xolmis irupero	Tyrannidae	Passeriformes	Other	AMNH 6687
Vidua regia	Viduidae	Passeriformes	Other	USNM 430442
Pteruthius flaviscapis	Vireonidae	Passeriformes	Other	USNM 559875
Vireo modestus	Vireonidae	Passeriformes	Other	USNM 558948

Zeledonia coronata	Zeledoniidae	Passeriformes	Other	USNM 555590
Yuhina diademata	Zosteropidae	Passeriformes	Other	USNM 319253
Zosterops atriceps	Zosteropidae	Passeriformes	Other	USNM 557467
Ardea melanocephala	Ardeidae	Pelecaniformes	Other	USNM 558410
Egretta garzetta	Ardeidae	Pelecaniformes	Other	USNM 291653
Ixobrychus minutus	Ardeidae	Pelecaniformes	Other	USNM 645892
Tigrisoma mexicanum	Ardeidae	Pelecaniformes	Other	USNM 343840
Balaeniceps rex	Balaenicipitidae	Pelecaniformes	Other	USNM 344963
Pelecanus onocrotalus	Pelecanidae	Pelecaniformes	Other	USNM 555612
Scopus umbretta	Scopidae	Pelecaniformes	Other	USNM 431669
Bostrychia hagedash	Threskiornithidae	Pelecaniformes	RTP	USNM 431667
Platalea alba	Threskiornithidae	Pelecaniformes	RTP	USNM 558417
Plegadis falcinellus	Threskiornithidae	Pelecaniformes	RTP	USNM 502698
Threskiornis aethiopicus	Threskiornithidae	Pelecaniformes	RTP	SAM 57162
Phaethon aethereus	Phaethontidae	Phaethontiformes	Other	USNM 18555
Phoenicoparrus andinus	Phoenicopteridae	Phoenicopteriformes	Other	USNM 502130
Capito aurovirens	Capitonidae	Piciformes	Other	USNM 560881
Indicator variegatus	Indicatoridae	Piciformes	Other	USNM 428639
Lybius guifsobalito	Lybiidae	Piciformes	Other	USNM 322303
Psilopogon pyrolophus	Megalaimidae	Piciformes	Other	USNM 612223
Campephilus magellanicus	Picidae	Piciformes	Other	USNM 488264
Celeus flavescens	Picidae	Piciformes	Other	USNM 562765
Picumnus olivaceus	Picidae	Piciformes	Other	USNM 500590
Sphyrapicus thyroideus	Picidae	Piciformes	Other	USNM 489964
Aulacorhynchus prasinus	Ramphastidae	Piciformes	Other	USNM 428855
Ramphastos toco	Ramphastidae	Piciformes	Other	USNM 622394
Podiceps major	Podicipedidae	Podicipediformes	Other	USNM 227355
Diomedea exulans	Diomedeidae	Procellariiformes	Other	USNM 559552
Oceanodroma melania	Hydrobatidae	Procellariiformes	Other	USNM 498406
Garrodia nereis	Oceanitidae	Procellariiformes	Other	USNM 559030
Macronectes halli	Procellariidae	Procellariiformes	Other	USNM 553248
Procellaria aequinoctialis	Procellariidae	Procellariiformes	Other	USNM 559787
Amazona ochrocephala	Psittacidae	Psittaciformes	Other	USNM 343132
Ara macao	Psittacidae	Psittaciformes	Other	USNM 226164
Pionus sordidus	Psittacidae	Psittaciformes	Other	AMNH 1267
Charmosyna placentis	Psittaculidae	Psittaciformes	Other	USNM 614243
Loriculus amabilis	Psittaculidae	Psittaciformes	Other	USNM 557142
Prioniturus platurus	Psittaculidae	Psittaciformes	Other	USNM 224918
Psittacula eupatria	Psittaculidae	Psittaciformes	Other	USNM 343237
Nestor notabilis	Strigopidae	Psittaciformes	Other	USNM 226487
Strigops habroptilus	Strigopidae	Psittaciformes	Other	USNM 18275
Pterocles orientalis	Pteroclidae	Pterocliformes	Other	AMNH 1580
Pterocnemia pennata	Rheidae	Rheiformes	NKP	USNM 631762
Rhea americana	Rheidae	Rheiformes	NKP	USNM 631153

Eudyptula minor	Spheniscidae	Sphenisciformes	Other	USNM 614343
Pygoscelis antarctica	Spheniscidae	Sphenisciformes	Other	USNM 488305
Spheniscus demersus	Spheniscidae	Sphenisciformes	Other	USNM 346396
Aegolius acadicus	Strigidae	Strigiformes	Other	USNM 431077
Otus atricapillus	Strigidae	Strigiformes	Other	USNM 555942
Pulsatrix perspicillata	Strigidae	Strigiformes	Other	USNM 18350
Tyto capensis	Tytonidae	Strigiformes	Other	USNM 560528
Struthio camelus	Struthionidae	Struthioniformes	NKP	USNM 557829
Anhinga anhinga	Anhingidae	Suliformes	Other	USNM 499385
Fregata minor	Fregatidae	Suliformes	Other	USNM 289133
Microcarbo melanoleucos	Phalacrocoracidae	Suliformes	Other	AMNH 23374
Phalacrocorax auritus	Phalacrocoracidae	Suliformes	Other	USNM 560565
Morus capensis	Sulidae	Suliformes	Other	USNM 292389
Crypturellus parvirostris	Tinamidae	Tinamiformes	NKP	USNM 612015
Crypturellus soui	Tinamidae	Tinamiformes	NKP	USNM 430716
Crypturellus tataupa	Tinamidae	Tinamiformes	NKP	USNM 345742
Eudromia elegans	Tinamidae	Tinamiformes	NKP	USNM 345096
Nothoprocta ornata	Tinamidae	Tinamiformes	NKP	USNM 620751
Nothura maculosa	Tinamidae	Tinamiformes	NKP	USNM 635711
Rhynchotus rufescens	Tinamidae	Tinamiformes	NKP	USNM 612018
Tinamus major	Tinamidae	Tinamiformes	NKP	USNM 621693
Pharomachrus mocinno	Trogonidae	Trogoniformes	Other	USNM 428246
Trogon clathratus	Trogonidae	Trogoniformes	Other	USNM 613995

* Based on soft tissue histology, foraging behaviour & taxonomy

† = extinct

Abbreviations:

NPBTO = non-probing bony bill-tip organ (including Anseriformes); RTP = remote-touch probing bony bill-tip organ; DTP = direct touch probing; NKP = non-kiwi palaeognath; Fossil = extinct palaeognath; Other = all other birds

Appendix 5.3: Test statistics for two-sided Mann-Whitney U tests [see Figures 5.4 & 5.5]

Comparison	n	Median	U-value	P-value	Effect size & CI*
Soft tissue surrounding bone	19	7.52	621	$7.86\text{x}10^{-13}$	27.44 (21.02-45.26)
Embedded in bony pits	33	33.64			
Soft tissue surrounding bone	19	7.52	254	$2.30\text{x}10^{-8}$	21.49 (17.80-25.46)
Touch papillae	13	29.17			
Touch papillae	13	29.17	197	0.0128	26.62 (2.69-53.72)
Embedded in bony pits	20	56.85			
Soft tissue surrounding bone	19	7.52	376	$3.48\text{x}10^{-10}$	47.57 (26.56-73.72)
Embedded in bony pits	20	56.85			

* Effect size calculated in *R* (using function “wilcox.test”, with “conf.int = TRUE”) as the median of the difference between a sample from x and a sample from y, with the associated 95% Confidence Interval (CI).